Proceedings of the conference

Broadening the Genetic Base of Crops

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Introduction

In 1972 a conference on European and Regional Gene Banks was organized jointly by the Section Wild Species and Primitive Forms and the Gene Bank Committee of the European Association for Research on Plant Breeding (Eucarpia) in Izmir, Turkey. About 70 scientists attended this conference.

Early in 1975 the Director of the Institute of Plant Breeding (I.v.P.) of the Agricultural University of Wageningen, Prof. J. Sneep, was approached by Prof. J.G. Hawkes, chairman of the above mentioned section of Eucarpia with the request to organize a next meeting on genetic resources for 1976 in Wageningen. Although the proposal was accepted immediately, the date had to be reconsidered for a number of reasons. The meeting was first postponed till 1977. It was postponed again till 1978 because of the General Meeting of Eucarpia planned for 1977 in Madrid. Finally, July, 1978 was chosen, at which time the rebuilding activities at the Institute of Plant Breeding would be terminated.

An Organizing Committee was set up and started to work. It was checked that the subject of the Conference would be 'Broadening the Genetic Base of Crops'. The title chosen indicates that the domestication of wild plants and consecutive evolution of crops into the present forms leads to a reduction of the genetic variation, which in a number of cases may cause an unacceptable narrow base. This entails a considerable risk for future food production.

It was the intention to reach with this conference botanists working on subjects like domestication and crop evolution, population geneticists and 'gene bankers' looking for the best way to maintain genetic variation in collections, and also plant breeders facing the practical consequences of genetic erosion in their crops.

Because of the rather wide field covered by the conference subject, it was agreed not to concentrate this time on the much discussed role of wild species and primitive cultivars in breeding for resistance and also to leave out the contribution of induced mutations to increase genetic variation.

For the final programme the Committee chose to begin with an assessment of the present situation with respect to the vulnerability of some major crops in Europe, followed by a session on present collecting activities mainly within Europe – dealing with specific regions as well as with specific crops – and by reports on especially European gene banks. A logic continuation was found in a session concerning the contribution of wild species and primitive forms to the performance of modern cultivars.

The problems encountered when making crosses between plants of the just mentioned groups were to be discussed consecutively and the conference should be finished with an idea of the prospects offered by (potential) new breeding methods like protoplast fusion and transfer of genes by bacterial messengers.

The Conference started on Monday, July 3rd at I.v.P. with an opening address by Prof. Hawkes, honorary president of the meeting. In all, 86 scientists participated, of whom more than 50% came from outside the Netherlands. Among them two distinguished guest lecturers, Prof. J.M.J. de Wet from Urbana, Illinois, U.S.A. and Prof. Ch.M. Rick from Davis, California, U.S.A. Their invitation was made possible by a generous grant from the Board of the Agricultural University of Wageningen. The participation from East-European colleagues was restricted, because of a most unfortunately coinciding meeting on genetic resources in Prague, Czecho-Slovakia. The Agricultural University also made a substantial donation to enable publication of the Conference proceedings at reasonable costs. Financial support was further received from the Board of Eucarpia. For these contributions the Organizing Committee is most grateful.

About 50 lectures were presented, most of which were on subjects and by speakers suggested by the Organizing Committee. The scientific level of the majority of these contributions was impressively high.

In retrospect it appears that the intention of the organizers of this Conference have been fully met. Botanists, population geneticists, gene bank employees and breeders from Government Institutions and private breeding companies have obtained more knowledge and have met each other in a very relaxed atmosphere.

At the end of the Conference the chairman of the respective sessions, together with the scientific secretaries, met in order to reach some conclusions and recommendations, which have been included in the Proceedings.

The participants were received one evening by the Dean of the Faculty of the Agricultural University, visited two other plant breeding institutes in Wageningen (Institute for Horticultural Plant Breeding, IVT and Foundation for Agricultural Plant Breeding, SVP), and made a touristical tour to a beautiful but rather unknown part of the country, not far from Wageningen. On Thursday evening, July 6, a farewell dinner was organized.

Finally, we as scientific organizers, secretaries and editors, must say that we greatly enjoyed our job, not only because of the excellent support from a competent administrative staff, but also because of the enthousiasm expressed by the participants with respect to the scientific programme as well as to the further arrangements during the Conference.

We hope and believe that this meeting has contributed considerably to making a proper use of the botanical wealth in nature and in collections, in order to ensure for a long time the supply of food for all inhabitants of our world.

A.M. van Harten,
A.C. Zeven.

Wageningen, August 1978.

Genetic poverty of major crops in Europe

Chairman: prof. dr J. Sneep (Wageningen)

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The breeder's point of view on broadening the genetic base

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Index words

History of plant breeding, genetic variation, wide crosses, old varieties, man-made species, protoplast fusion, resynthesis.

Summary

The first phase of plant breeding was characterized by selection in phenotypic variation that had arisen spontaneously. Later, in the 18th Century, scientists applied themselves to interspecific and intergeneric crosses. A more realistic basis was found at the beginning of the 19th Century in intraspecific crosses and at the end of that century in backcrosses. This resulted in a conservation of favourable gene-blocks, but not much was done to create new ones.

Gene collection started with the gathering of 'distant' material. In the mean time, many land races and old varieties were left to disappear. In addition, the local genotypic variation received too little attention.

Contrary to what is sometimes suggested, the man-made species are rather difficult to make into cultivated crops. A short-term solution is more likely to be found in resynthesis of existing amphidiploid crop species. The interspecific and intergeneric derivatives should also be included in the gene-banks.

The primitive forms and relatives of our crop plants are often used to introduce genes for resistance and quality into crop species. But broadening the genetic base can also have many indirect advantages for breeding, e.g. introduction of male sterility, broadening the cytoplasmic variation, induction of haploids, enhancing interspecific crossability, better pre-breeding in parents for 'distant' crosses, breaking incongruity and changing the mating system.

Composites, when left to the influence of natural selection, offer little prospect. They must undergo a further man-guided selection after some generations.

When ready to be put into practice, gene-transfer will demand a great effort from the breeder to convert the product into a useful variety.

History

In the first phase of plant breeding man selected from available phenotypic variation. The suitable variants were maintained for further propagation. And then with a big jump scientists applied themselves to wide crosses, in other words matings between species or genera, ignoring the wealth of intraspecific variation. Examples of these are the crosses between species of *Dianthus*, as described by Fairchild in 1719, and the crosses between species of *Nicotiana*, which Koelreuter reported in the period 1761–1766. There are also reports that Linnaeus carried out interspecific and intergeneric crosses already about 1760 (Sneep, 1966).

However, it was not until the beginning of the 19th Century that we find descriptions of interspecific crosses, such as the reports of Thomas Andrew Knight (England) about 1820 on his work with peas and wheat. In 1845, Lecoq (France) already wrote: 'It is to be regretted that so far so little attention has been paid to the crossing of agricultural crops, and that so much in the acquisition of new varieties is left to chance, whereas the crossing of crops will certainly create a new source for prosperity in agriculture'. His words are a clear indication of how things stood at that time.

In the course of the 19th Century, with the development of real breeding on the part of private companies, we notice an increasing use of existing varieties of a certain crop species for crosses. People soon became aware that good varieties carried favourable gene blocks that did not allow interference. If one meddled with these gene blocks, it took a great deal of effort and time to recover a good variety. So by the end of the 19th Century breeders started backcrossing to existing varieties and the method is still often used in improvement of self-fertilizing species. If a rather 'distant' parent were used, for instance to obtain genes for resistance, breeders would (and will) soon introduce backcrosses, and recurrent backcrosses to an existing top-variety. This method is extremely efficient, but does not much broaden the wealth of genes. One may say that the method of backcrossing conserves what there is, or even that it is a conservative way of improvement.

Let us not forget that in this way plant breeding has made large advances. For many crop species, the annual increase in yield is as much as 0.5–0.75%, merely because of varietal improvement. Building up useful gene blocks has borne rich fruit.

Collecting genes

In the history of the systematic collection of genes for the benefit of our crops, we can find analogies with what happened in plant breeding taken in the strict sense of the word. The interests have often been in finding the more remote type of foreign regions instead of preserving the variation at home, which has long adapted to the environment. On the other hand, breeders now possess valuable remote material. In this context we can think of the potato varieties, which not only have become enriched with absolutely needed genes for resistance, but which have, as a result, also shown a considerable increase in yield.

On the other hand, we did not do enough to prevent many useful European land races of several crop species from being lost. This is regrettable, especially since these land races were adapted to our climate.

In self-justification, it is sometimes said that the useful landraces were once used as parents in earlier crosses and that they therefore continue in their progeny. However, nobody can ascertain the loss of genes from the progeny.

As for crop species for which north-west Europe is the centre of diversity (as for some grasses), we should discuss the conservation of the genotypic variation. Some questions are: Should a central collection be established? Are the habitats in danger? Are they to be preserved? Is the range of our varieties so varied that it reflects the wealth of genes?

Even if the answer to the last question should be yes this wealth must be saved in gene banks, because breeding of grasses is at the treshold of an era in which collecting must give prominence to other breeding techniques in which narrowing of diversity may occur. Such a situation occurs already in some Brassicas. Plant breeders used the rich genotypic variation in the landraces as a source for their starter material. Now the number of hybrid varieties is increasing at a fast rate and there is imminent danger that the variation for the Brassicas will be reduced to the stock of inbred lines available at the breeding stations.

Loss of old varieties

A great loss of genes is caused in many instances by the disappearance of old varieties as more productive ones take their place. The former should be maintained with the landraces in gene banks. Only when we know that all characteristics of the old varieties are available in new varieties may we regard conservation as less urgent. But, who will take the trouble to check this?

Vegetatively propagated crops such as potato are particularly difficult. Hermaphrodite varieties could be converted to seed, if need be. For male-sterile varieties such procedure cannot be adopted. In their case the only choice is between vegetative maintenance and seed preservation after crossing.

When converting varieties to seed, one should be aware of the dangers that lie in small numbers. Small numbers could cause a drift of the genes that are carried by the clones. Inbreeding should be avoided.

Man-made species

The plant breeder wonders now and again about the significance of man-made species. For ornamentals and particularly for vegetatively propagated plants several successes have been recorded. In crops propagated from seed, especially those grown for their seed or fruit, such successes have been less apparent and, at any rate, much slower in coming. An example of this is triticale. Wheat and rye happen to cross spontaneously in nature, but the progeny of this combination has not progressed much with evolution.

Octaploid triticales were already produced artificially in 1875. However, they were sterile. In 1819, Rimpau succeeded in making an amphidiploid with a certain degree of fertility. When it became relatively easy in 1938 to produce amphidiploids with colchicine, the outcome looked more promising for research. Since 1948, researchers have concentrated on obtaining hexaploid triticales (Canada, Hungary). But there too, they were initially confronted with problems of fertility. Research on triticales was also started by CIMMYT in 1964. But by 1967, these man-made species yielded a mere 80% of what good wheat lines used to yield. The problems still to be solved are many, even though yields are reasonable on moderately fertile soils. So far breeding has required much work and patience, but much remains to be done before the triticales are grown on a wide scale for feed as grain.

occur, or some disorder which may upset the evolutionary balance.

How great the technical triumph of gene manipulation may be, the receptor plant will only reap success after extensive breeding.

We feel fortunate in having so many experts willing to deal with all the problems that plant breeders have. And they have many! Even more than I have mentioned.

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Genetic poverty of the potato in Europe

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Index words

Solanum tuberosum, potato, wild *Solanum* species, cultivated *Solanum* species, genetic poverty, crop vulnerability, collecting.

Summary

The narrow genetic base of the potato in Europe has been a cause for concern for over a century and a half, particularly after the disastrous late blight (*Phytophthora infestans*) epidemics of the 1840s. For about 250 years after the introduction of the potato into Europe all varieties seem to have been derived from only two introductions (Spain, ca. 1570; England, ca 1590). No grave disease epidemics were recorded during this period, however, probably because of the limited extent to which it was grown until the early 19th century and the lack of understanding of disease at that time.

Attemps to broaden the genetic base were made from the middle of the 19th century, and especially in breeding *Phytophthora* resistance into the European varieties. However, these attempts were not successful until the second decade of the 20th century.

From the 1930s onwards, resistance to fungi, viruses, bacteria, insects and nematodes, and adaptation to environmental extremes began to be widely looked for. Setbacks were encountered when more than one pathotype of each pathogen appeared, and for this reason more and more primitive cultivated and wild species were collected and evaluated for resistance to these diseases.

At present a high proportion of potato varieties used in European countries contains 'wild' germplasm, but the battle between host and pathogen is by no means over. Much genetic diversity is still needed to combat newly discovered pathogens and pathotypes and most of this diversity still needs to be incorporated into good commercial varieties.

Historical notes

Many potato breeders from the early years of the 19th Century onwards have commented on the dangerously narrow genetic base of the potato in Europe. In those days, they used different phrases, of course, referring to the need for 'new blood' and lamenting its 'degeneration'. Although we can never be sure that unrecorded introductions were not made, the state of the European potato in the 19th Century is evidence enough of several centuries of selection on the basis of only two initial introductions, with a few additional accessions to the European gene-pool in the 19th Century.

The history of those first introductions has been frequently discussed (Roze 1898; Wight 1916; Salaman 1937; 1946; 1949; 1954 Salaman & Hawkes 1949; Hawkes 1956; 1966; 1978). It seems fairly clear that there was a first introduction into Spain in about 1570 and a second one into England in about 1590. From these, the potato spread gradually throughout Europe as a botanical curiosity rather than a crop plant. This was due to its derivation from the short-day adapted Andean *S. tuberosum* subspecies *andigena*, rather than the long-day adapted subspecies *tuberosum* from Chile. Since short-day adapted potatoes can only begin to tuberize from late September when the natural day-length is reduced to 12 hours, and need until November or December to mature a crop, the potato could at first only be grown in the southern and western regions of Europe where the winters were mild and frost-free. There is, indeed, abundant documentary evidence for this.

Little by little, through selection for yield and earlier maturity in materials grown from true seed, the European potato became more long-day adapted and hence could be grown more widely in areas with a more severe climate. However, it only began to be grown on a large scale after about 1780 and did not become really popular until the early 19th Century.

Up to 1851, there is no evidence of further introductions, but in that year C. Goodrich (quoted by Hawkes 1967 introduced a variety known as 'Rough Purple Chili' into the United States from which he bred the first early variety 'Early Rose'. Two comments may be made here. Firstly it is now considered that 'Rough Purple Chili' really did come from Chile and must therefore have been already adapted to yield well in North American and European latitudes. Secondly, from its granddaughter Early Rose, some 300 European first and second early cultivars were derived, evidently using the long-day adaption of their Chilean grandmother (Ross 1958; Hawkes 1967).

What other genetic material was added to the European potato gene-pool in the 19th Century? The variety 'Daber' is said to have been introduced about 1830 from South America, and this gave rise to many well-known varieties such as 'President Krüger', 'Industrie' and 'Wohltmann' (Ross 1958).

There is also evidence that potatoes were taken from Peru to the Canary Isles in 1622 (Zubeldia et al. 1955), and it seems that derivatives from those introductions are still grown there. However, no records of early transference of material from the Canary Isles to Spain or elsewhere in Europe are known, though of course unrecorded introductions might have taken place.

The concept of making fresh introductions of the cultivated potato into Europe to help 'strengthen' and 'renew' it after the disastrous *Phytophthora* epidemics of the 1840s started with Lindley (1848) who caused large quantities of potatoes to be brought to London from Peru and Colombia in 1846 and 1847 and tried them out in the grounds of the Royal Horticultural Society. Amongst these was the cultivated diploid *S. goniocalyx* but all the introduced samples quickly became diseased, presumably with viruses, and none seems to have been used for breeding purposes. In any case, the yields of this short-day potato in London would have been very low or even non-existent.

In a similar way, importations of wild potato tubers were made by Lindley from

Mexico and by Schlechtendal at Halle (Schlechtendal 1841), who also introduced *S. verrucosum*, *S. stoloniferum* and *S. oxycarpum* from Mexico. Other early collections of wild potatoes were made from Argentina and Uruguay of *S. commersonii* and from Chile of *S. maglia*.

None of these early introductions of wild species was used for breeding, since they were either displayed as horticultural curiosities or considered to be wild forms of the cultivated potato and thus suitable for cultivation in Europe in their own right. However, Klotzsch (1849; 1852) introduced *S. demissum* (called by him *S. utile*) from Mexico and began using it for breeding. Lindley too had obtained this same species from Mexico in 1846 but unfortunately the stock he received happened to be a *Phytophthora* – susceptible clone. Nevertheless, from Klotzsche's material, it is said that the German W races may have been derived, since they were used in the breeding of potato cultivars with some *Phytophthora* resistance. Ross (1958) considers that the W races may have originated from *S. edinense*, which was later shown to be an F_1 hybrid of *S. tuberosum* × *S. demissum* (Broili 1921). Salaman's success in 1909 and 1910 in establishing resistance to *Phytophthora* in a hybrid of *S. demissum* × *S. tuberosum* (as '*S. etuberosum*') also deserves mention (Salaman 1911). From then onwards, until the 1920s, breeding for resistance to potato blight was the only objective, apart from the standard basic activities of selecting for yield, quality and general disease resistance. Small samples of wild species were brought in from time to time but in a rather haphazard manner, with no real attempt to screen them for disease resistance or to breed their useful characters into commercial varieties.

Collecting work

In 1925, a new era dawned with the fully scientific expedition to collect potatoes in Middle and South America, conceived by N.I. Vavilov and led by S.M. Bukasov. Vavilov's objectives are so well known that it is hardly necessary to describe them afresh. His inspired idea of making detailed collections throughout the entire range of a cultivated species seems obvious enough to us now yet in his day it was revolutionary. Carefully planned scientific expeditions were sent to the gene centres of cultivated plants in order to capture as much as possible of the genetic variation of the crops and their wild relatives. The material thus collected was evaluated and made available to breeders so that they would have as wide a genetic base as possible available to them. These early expeditions of Vavilov and his Russian colleagues to South and Middle America were followed by similar though generally not such extensive collecting missions from other countries which are listed in Table 1.

This table takes no account of the collections made by North American botanists and breeders, such as Correll, Reddick and others (Correl 1962). Nor does it deal with the Japanese collections of Matsubayashi or the very fine work of South American botanists, breeders and agronomists such as C. Ochoa (Peru), M. Cárdenas (Bolivia), N. Estrada (Colombia), and K. Okada (Argentina), all of whom have added considerably to our knowledge of potato materials in the living state.

Ochoa, in particular, has not only gathered and described very many wild species (Ochoa 1962) but, together with Huamán and other colleagues at the International

Potato Center in Peru, has collected the largest amount of cultivated materials ever to be assembled (Ochoa 1975).

So far, so good; or so it would appear. Much material has been collected from the United States southwards to Chile, of the seven cultivated and many of the 160 wild potato species, by a series of mission-oriented expeditions by professional

Table 1. Potato Collecting Expeditions from 1925 onwards (European expeditions only)

Date	Collector(s)	Areas Visited	Material collected	Sponsoring country
1925-6	S.M. Bukasov	Mexico, Guatemala, Colombia	Mainly cultivated species	Soviet Union
1927	S.W. Juzepczuk	Peru, Bolivia, Chile, Argentina	Mainly cultivated species (some wild)	Soviet Union
1930	Bauer and Schick	Peru, Bolvia, Argentina	Cultivated species	Germany
1932	N.I. Vavilov (and Kesselbrenner)	Mexico, S. America	Mainly cultivated species	Soviet Union
1933-4	C. Hammarlund	Peru, Bolivia	Cultivated species	Sweden
1938	E.K. Balls and W.B. Gourlay	Mexico (Central regions)	Wild species	United Kingdom
1939	E.K. Balls, W.B. Gourlay and J.G. Hawkes	Colombia, Ecuador, Peru, Bolivia, Argentina	Wild and cultivated species	United Kingdom
1949	J.G. Hawkes	Mexico	Wild species	United Kingdom
1955	J.H. Toxopeus	Peru	Cultivated species	Netherlands
1956	J.P. Hjerting E. Petersen and K. Rahn	Argentina, Chile	Wild species	Denmark
1958	J.G. Hawkes, J.P. Hjerting,and R.N. Lester	U.S.A., Mexico, Central America	Wild species mainly	United Kingdom, Denmark
1958	P.M. Zhukovsky	Latin America	Wild and cultivated species	Soviet Union
1959	H. Ross et al	Peru, Bolivia, Argentina	Wild and cultivated species	Germany
1960	K.S. Dodds and G.J. Paxman	Andes of S. America	Cultivated diploid species	United Kingdom
1960	J.P. Hjerting	Peru, Chile	Wild species	Denmark
1962	K.S. Dodds and N.W. Simmonds	Andes of S. America	Cultivated species	United Kingdom
1964	J.G. Hawkes, C. Ochoa and C. Vargas	North and South Peru	Wild species	United Kingdom
1965	H. Ross	Coastal lomas of Peru	Wild species	Germany
1966	J.G. Hawkes, J.P. Hjerting and K. Rahn	Argentina	Wild species	United Kingdom, Denmark
1971	J.G. Hawkes, J.P. Hjerting and P. Cribb	Peru, Bolivia	Wild and cultivated species	United Kingdom
1975	J. Hermsen, A. van Harten J.G. Hawkes and A. Okada	Peru, Bolivia, Argentina	Wild and cultivated species	Netherlands

scientists, all anxious to obtain the maximum amount of genetic diversity. A great deal of this material is still available in the living state, though there is probably little left of the very early materials.

Germplasm collections of potatoes exist in Peru (International Potato Center, Lima), the Soviet Union (N.I. Vavilov Institute of Plant Industry, Leningrad), the United States (Potato Introduction Station, Sturgeon Bay, Wisconsin), the Federal Republic of Germany (Collaborative German/Dutch Potato Gene Bank, Braunschweig), the German Democratic Republic (Gross Lüsewitz), United Kingdom (Commonwealth Potato Collection, Scottish Plant Breeding Station Edinburgh), Argentina (Balcarce, provincia Buenos Aires) and Colombia (Tibaitatá, Bogotá), as well as smaller working collections in other countries. There is thus no shortage of material. The question is, how much of this material is really being used by the breeders?

Breeding work

As all breeders know, potato breeding is not an easy task; it is time-consuming, labour-intensive, and therefore expensive. A balance must therefore be struck between the cost of breeding a series of disease-resistant cultivars and the saving on insecticide and fungicide sprays together with the higher yields it is hoped to obtain.

Assuming for the moment that favourable decisions are made, the wild and primitive cultivated material needs to be evaluated for disease resistance and suitable parental material needs to be selected. Some 35 years ago, there was not so much to choose from and the problem of breeding for resistance to different pathotypes of the same pest or pathogen was hardly envisaged. Resistance to fungi (chiefly *Phytophthora*), viruses (chiefly X, Y and leaf-roll) bacteria (*Pseudomonas*, *Erwina*), insects (Colorado beetle) nematodes (*Heterodera rostochiensis*) and frost were considered as the chief objectives. Some screening was undertaken for higher content of protein, vitamin C and starch (Hawkes 1945).

By 1958, some 20 years ago, breeding objectives were more clearly defined in one sense, but were becoming more complex in another, since new pathotypes of fungal and bacterial diseases were being discovered, thus necessitating further screening of a wider range of wild and primitive cultivated material to look for appropriate resistances. At that time, most breeding for *Phytophthora* resistance had switched from vertical (single-gene) resistance to horizontal (multiple-gene) resistance, so as to provide protection against a wide range of races or pathotypes.

During the last 20 years this trend has continued. The establishment of the International Potato Center in Peru in 1972 was a landmark in this work, especially in research on viruses, viroids and nematodes, both round-cyst and root-knot groups. Workers in Peru and Europe had identified two species of potato-cyst nematodes and a number of distinct races. More viruses of the potato, including viroids, some of them transmissable through true seed, had alerted the quarantine authorities to the need for stricter measures. Resistance to bacterial wilt (*Pseudomonas*) had been discovered in the diploid cultivated species *S. phureja*, and several pathotypes also appeared, thus complicating the situation. The cold war, between pests and pathogens on the one hand and the alliance of host plant and plant breeder on the other, continues in full force, even though a great deal of 'wild' germplasm has already been built into most of the new potato cultivars bred in Europe during this 20-year period.

The relative importance of various breeding objectives in 1978 may be judged by looking at the papers on potato breeding to be presented at the 7th Triennial Conference of the European Assocation for Potato Research in Warsaw this year: viruses (6), nematodes (2), bacteria (1), *Phytophthora* (1), quality (2), adaptation (1); other papers dealing with breeding methods and general matters (16). Obviously this is too small a sample on which to base general conclusions, but it certainly indicates the importance attached to virus resistance breeding and breeding methods by those attending the conference.

Present situation

It would be valuable at this time to take stock of the present situation in potato breeding. Are we making the fullest possible use of our basic breeding material? Do we need more? Are our methods as good as they should be?

First of all – the material. Breeders can use only what is available to them, and can do so only on the basis of a good understanding of its nature. This means that studies of an evolutionary, biosystematic, cytogenetic and 'taxogenetic' nature are needed, to provide a framework of thought and knowledge useful to the breeder. We in Birmingham, together with colleagues in Europe, North America and South America, have been attempting to provide such information and understanding during the last three decades. This work has meant also that much plant exploration work has been stimulated in addition to that arising from the need to save genetic resources because of the threat of genetic erosion (Hawkes 1973; 1976).

Thus, taxonomy with a view to helping plant breeders provides the following services:

- The pattern of variation of a sequence of populations can be studied, and species boundaries can be elucidated.
- Crossability studies can throw light on the ease or otherwise by which transfer of useful qualities can be made to *S. tuberosum*.
- The presence of natural hybrids between two or more species can be identified, and gene flow and introgression of useful genes can provide information of value to the breeder.
- Ploidy levels and genome analyses can be investigated.
- Biochemical and immunological studies can be carried out (alkaloids, phenolics, proteins) not only to provide an aid to identification but also to be of use in research on breeding for resistance.
- The geographic range of each species can be mapped, thus making it possible to sample it adequately throughout its entire range (see distribution maps, from Hawkes 1966 and Hawkes & Hjerting 1969).
- Distribution mapping need not be confined to species but can also be extended to the mapping of resistance genes. For an early and admittedly rather out-of-date attempt at this see Hawkes (1958). Furthermore, if the collection data are stored in computer memory, the maps can be recalled at will, in whatever form desired.

So, what of the species themselves? The problem here is to give any kind of meaningful overall view of the results and significance of screening for resistance in a short paper of this nature. Even in 1958 twenty years back, Ross's excellent attempt (Ross 1958) was extremely complex to say the least. It would be impossible now to include all the information available to us in a single table. Indeed information in such a form is perhaps hardly necessary. With the sophistication of electronic data processing now available to us, we can surely call up all the information we require, at a moment's notice. Or, can we?

This is an area in which theoretical possibilities run far ahead of practical reality. If Eucarpia, together with The United Nations Development Programme, can really establish a data base, or several data bases, for the crops of interest to European breeders, and if the funding and collaboration to run them are available, using agreed standard descriptors and descriptor states, then, and only then, will it be possible to call up such information by telephone or on-line terminal. This may be all just around the corner, but still much remains to be done. In particular it needs to be done by Eucarpia members through their crop sections, linked into the Eucarpia Gene Bank Committee. In this way the results of screening on a cooperative basis can be stored and made available to breeders, easily and quickly.

The final point I wish to deal with here is that of actually utilizing potato genetic resources in plant breeding. We have much information on which species should be used in breeding for qualities of resistance, adaptation, yield and so on. Undoubtedly, we need more initial material to help with solving problems of resistance to newly discovered pathogens or new pathotypes of the old ones. This can be collected, and in fact is often already present in existing gene banks, awaiting evaluation.

Again, interspecific crosses are not too difficult and genome differentiation hardly exists, though there are exceptions. We shall be hearing about how to overcome these incongruous species crosses later in this conference, by somatic hybridization and the like. Yet the genetic base within our existing potato cultivars is still rather narrow. Why is this?

In my opinion this is undoubtedly due to an understandable reluctance on the part of the breeders to embark on the stormy seas of wild-species breeding. We need, it seems, more 'link-men', able to take promising wild species and produce good breeding lines from them. This can be done, for example, by developing 'column resistance' (CIP 1974), as described by Hermsen (1974) where lines combining different types of resistance to a pathogen are made available to the breeder. Another method is to combine resistance to a whole series of pests and pathogens in a group of promising parental lines (Swiezynski 1971; 1978), which are already developed up to only a few generations distant from a new and promising cultivar. The parental line breeder, or whatever we like to call him, fills the gap between the cytogeneticist, biosystematist or pathologist on the one hand and the breeder on the other. Furthermore, he provides the desired characters of the wild species already set into a 'cultivated' genetic background.

Despite many successes in the Netherlands, Germany and elsewhere, this, I would submit, is where our weakness still lies. The narrow genetic base in European potato breeding is not so much in lack of initial material but in its evaluation and complete incorporation into our breeding programmes.

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Genetic poverty of the sugarbeet in Europe

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Index words

Beta vulgaris, sugarbeet, *Beta maritima*, sea beet, genetic poverty, cytoplasmic poverty, crop vulnerability.

Summary

Sugarbeet is one of our youngest crop plants, which is believed to have originated from a relatively limited range of fodder beet types less than 200 years ago. Although it is likely that spontaneous hybridizations with cultivated leaf-beet types, as well as with wild *B. maritima*, have contributed additional genetic variation, the genetic base of sugarbeet is probably narrower than that of most other cross-fertilizing crop species.

Until very recently, however, sugarbeet varieties were bred and adapted to a wide range of ecological conditions by employing breeding methods that are likely to have retained most of the original variation.

Although the introduction of hybrid breeding methods has probably involved the loss of many populations of less immediate value, I still do not believe that the present gene pool of sugarbeet in Europe is dangerously narrow. Moreover, I believe that the nuclear genetic variability in our present diploid and triploid top-cross hybrid varieties is sufficiently large that there need not be reason for concern, even if some of these hybrids are grown over quite extensive areas.

But the almost complete cytoplasmic uniformity, irrespective of variety, is disturbing. Though a measure of cytoplasmic diversity may be provided by nuclear genes controlling mitochondrial or chloroplast components, only the simultaneous use of a number of male sterilizive cytoplasms and regular tests for susceptibility to cytoplasm-specific diseases or insect pathogens would offer adequate protection.

Irrespective of what has been said about the present sugarbeet gene pool and varietal situation, in the long run, a broadening of both the cytoplasmic and the nuclear genetic variation, with breeders as well as on a farm-to-farm basis, may be of decisive importance in saving the crop against epidemics and in securing continued breeding progress.

Breeding history

The section *Vulgares* of the genus *Beta* L. includes all cultivated beets, as well as a wide range of wild forms. With few exceptions (Buttler 1977) the *Vulgares* beets are diploid ($2n = 18$), sexually compatible with each other and the hybrids set an abundance of seed. So taxonomic treatment is difficult and, although *B. Vulgaris* L. is usually considered to be a composite species embracing all taxa of the section, the division of *B. vulgaris* L. into subspecies and varieties has varied greatly.

Recently, Ford-Lloyd & Williams (1975) have revised the taxonomy of the *Vulgares* beets for a study of the affinities between the wild species and the origin, from them of the cultivated types.

By field studies of many types of cultivated beets in Turkey, Ford-Lloyd & Williams identified a form that they considered to be the remains of an ancestral subspecies, ssp. *provulgaris*. This subspecies is believed to have given rise to two other subspecies, ssp. *cicla*, representing leaf-beet types, and ssp. *vulgaris*, representing beets with swollen roots. In the opinion of Ford-Lloyd & Williams, ssp. *provulgaris* has most likely originated by simple selection from primitive maritime beets.

Whereas the first cultivated beets are believed to have been vegetables similar to Swiss chard and to have originated in the eastern Mediterranean area around 500 BC, sugarbeet is one of our youngest crop plants, whose history goes back less than 200 years.

Although, as early as 1608, Olivier de Serres referred to the sweet juice from red beets as 'semblable à syrop au sucre' and farmers used fodder beets to produce syrup for feeding their bees, it was not until 1747 that the German chemist Marggraf studied the sweet substance in beet and found that it was in fact sucrose, which until then was thought to exist only in cane. Marggraf is said to have extracted only 1.56% sugar from the best of the beets he studied (Knapp 1956), but even so he produced a small amount of sugar.

At the end of the Eighteenth Century, Achard, a student of Marggraf, began to study beet growing and sugar manufacture, and in 1799 he produced a few tons of raw sugar. Some of the refined product was presented to Friedrich Wilhelm III of Prussia, who became interested in Achard's work and gave him the Cunern Estate in Silesia, where Achard built a sugar factory in 1802. Observing the very wide variation in the existing fodder-beet, Achard also began to select beet types, mainly from the 'White Silesian' beet, that were more suitable for sugar manufacture.

After Achard, several others attempted to improve the existing beet and the cultivation methods. One of these, Louis de Vilmorin, started selection work based on the Silesian beet, and in 1837 he introduced progeny testing as a selection method. As a consequence of all this early selection work, the sugar content in the beet gradually increased and about 1830, it varied from 7 to 9%. With the introduction of the polarimeter in 1850, the sugar content increased still further and in 1858 the German breeder Knauer had developed the 'Beta Imperialis', usually considered to be the mother of modern sugarbeet. This beet had a sugar content of 11 to 13% and several sugarbeet researchers, among others van Heel (1947) and Rasmusson (1951), have suggested that it originated from spontaneous crosses between the Silesian beet and a North Atlantic form of *B. maritima*. In support of this hypothesis, van Heel (1947) described a sugarbeet population that he had developed from a cross between the fodder beet 'Kirches Ideal' and a *B. maritima* biotype, and that compared favourably with the then existing 'Kuhn' sugarbeet. A similar cross at Hilleshög, and now in the F_6 generation, has resulted in well shaped roots with quite acceptable sugar content and quality characteristics.

However, others like Zossimovich (1940), believe that the sugarbeet is the result of repeated selection in populations of the Silesian beet, which Zossimovich considered to be the result of natural hybridizations between various types of cultivated root and leaf beets. Knapp & Mündler (1957), who studied F_1 and F_2 generations from crosses between a fodder-beet and a mangold variety, found the F_2 generation to vary widely both in root weight and in content of dry matter. Like Zossimovich, they suggested that such crosses may have played a role in the evolution of sugarbeet.

With no barriers to hybridization between the cultivated and the wild members of the section *Vulgares*, there is reason to believe that both *B. maritima* and various leaf-beet forms contributed to the genetic variation that made possible the development of 'Beta Imperialis', as well as the rapid increase in sugar content that followed. Thus, around 1900, the best cultivars contained about 16% sugar; in 1920 as much as from 16 to 19%.

The rate of progress made during this period suggests the availability of source populations with a wide range of genetic variation. However, the intense and rather one-sided selection for high sugar content must have resulted in a gradual narrowing of the gene pool of sugarbeet. To what extent this might have restricted later progress in sugarbeet breeding is difficult to ascertain.

Similarly, around 1 900 European sugarbeet breeders began to select and market cultivars representing different combinations of sugar content and root yield. This differentiation into E, N, Z and ZZ types, together with a growing number of sugarbeet breeding organizations, undoubtedly conserved more genetic variation than would have been the case had selection been for one type of beet only.

The method of producing first-generation synthetic varieties – developed in the 1930s and soon adopted by most European sugarbeet breeders – contributed also to the maintenance of a reasonably wide gene-pool and broadly based commercial varieties. This method requires breeders to develop and continuously reselect many more or less close-bred populations. After testing these selected populations for their general combining ability, a limited number of the best combiners are intercrossed to form the cultivar. To obtain maximum advantage of the effects of heterosis, the commercial seed is always produced anew from a mechanical mixture of basic seed of the constituent populations. Also the introduction of autopolyploidy in development of sugarbeet cultivars in the late 1940s has probably resulted in conservation of more genetic variation in sugarbeet.

Thus, although during the period from 1930 to 1960 the increase in sugar yields in most European countries was smaller than yields in other major crop plants, this was probably not due to lack of genetic variation, but more likely to the many populations used by each breeder and the resultant infrequency with which these populations were reselected.

Genetic poverty

Similarly, I do not believe that the rather modest yield increases, which can be credited so far to hybrid breeding are necessarily an indication of an exceptionally narrow range of genetic variation in sugarbeet. It is sometimes argued that a major reason for the success of hybrid maize is the wide racial diversity in this crop and that similar results must not be expected from crops that have more limited genetic resources, e.g. sugarbeet. Although in its entirety the germplasm of sugarbeet is, no doubt, much narrower than that of maize, it does not necessarily follow that the difference between the germplasm presently utilized by maize breeders in the United States and that available to European sugarbeet breeders is of the same magnitude. Even though the genetic variation in the Corn Belt dents was very great in the days of open-pollinated maize, the dents still represented but a fraction of the total germ plasm of maize (Lonnquist 1974). Since then, the replacement of open-pollinated cultivars by hybrid cultivars, based on a limited number of superior inbred lines, has not only drastically reduced the genetic diversity of the maize crop on a farm-to-farm basis, but most likely has also affected the breeding material by favoring breeding work with highly adapted rather than more 'exotic' types (Lonnquist 1974).

This also applies, to a certain extent, to sugarbeet. In breeding conventional sugarbeet, the development and maintenance of many populations of diverse origin were a major part of the breeding work. Today, the search for maintainer genotypes, the development of numerous inbred lines and the assessment of their combining ability have limited the number of open-pollinated multigerm populations that can be subjected to selection, and has undoubtedly resulted in the loss of many such populations of less immediate value.

Present-day hybrid cultivars are, of course, also based on a more restricted germplasm than were the conventional cultivars. However, because most hybrids have as yet been either triploid or diploid top-cross hybrids, they still have much genetic variation.

Thus there is no substantial evidence that the present gene pool of sugarbeet is particularly narrow or in immediate danger of being exhausted. However, in the long run, a broadening of the genetic variation, both on a farm-to-farm basis and with the breeders, may be decisive in saving the crop from epidemics and in maintaining progress in breeding.

Although of minor economic consequence, the recent widespread occurrence of powdery mildew on sugarbeet in north-western Europe should be viewed as a timely warning.

In maize, similar outbreaks of maize dwarf-mosaic virus, yellow leaf blight and southern corn leaf blight have alerted breeders to this problem and have created considerable interest in the introduction and utilization of exotic maize germplasms.

Although it is an old idea that hybridization between sugarbeet and various wild *Beta* species, especially *B. maritima* and other members of the section *Vulgares*, might provide the sugarbeet breeder with new valuable genetic variation, most attempts along these lines have not been of sufficient intensity and duration to be of much practical value (Coons 1975). An important exception is the work of the well-known Italian sugarbeet researcher Munerati (1932). From crosses between sugarbeet and selected *Cercospora*-resistant *B. maritima* biotypes, collected in the estuaries of the Po River, he developed, by repeated selection and recombination in an area with severe leaf-spot attacks, sugarbeet populations that were highly resistant to *Cercospora*. Munerati later gave his material to Italian sugarbeet breeders and today most leaf-spot resistant varieties in Europe as well as in the United States are considered to be derived from the Munerati selections.

Though the importance of the pioneering work of Munerati was generally recognized, few serious attempts to isolate additional resistant *B. maritima* biotypes have been made since (Coons 1975).

However, with the more efficient methods of populations improvement that are now available and the experience that has been gained from handling exotic germplasms in other crops, sugarbeet breeders should consider the long-term view and create broad-based breeding populations derived from outstanding sugarbeet populations and selected new wild-beet germplasm. If given ample opportunity to recombine under random mating with only mild selection for a few generations before more intense selection is initiated, such populations may yield not only a broad spectrum of *Cercospora*-resistant lines but also lines that may contribute to higher sugar content, greater drought resistance and increassed yield.

What has been said about genetic variation in sugarbeet up till now has concerned only chromosomal genetic variation.

Cytoplasmic uniformity

In sugarbeet, however, we now use a cytoplasmic-genic system of male sterility (CMS) for the production of hybrid cultivars and thus we no longer have the cytoplasmic variation that existed in the old cultivars. Since the discovery and introduction of CMS (Owen 1945; 1948) we have learnt that cytoplasmic inheritance results from DNA molecules in such cytoplasmic organelles as plastids and mitochondria and that these DNA molecules can undergo changes, in much the same way as nuclear genes, that result in plastid and mitochondrial mutations. Above all, we have learnt, from the devastating effect of a new race of *Helminthosporium maydis* (southern corn leaf blight) on maize hybrids with the Texas (T) sterile cytoplasm in 1970, and the increased virulence of *Phyllosticta zeae* (yellow leaf blight) on genotypes carrying the same cytoplasm that, at least for disease resistance, cytoplasmic diversity may be as important as genetic diversity.

In sugarbeet, we now depend on a single cytoplasmic-genic system. Some breeders claim that they have found new sterile cytoplasms; but to my knowledge, nobody has as yet used these cytoplasms in commercial hybrid production. Although so far no sugarbeet disease-producing organism appears to have shown specificity for the Owen sterile cytoplasm, it cannot be excluded that new races of these organisms, specific for this cytoplasm could develop in the future if we continue to use this cytoplasm on virtually the entire sugarbeet area in Europe.

Even if we judge the risk of a repetition of the maize disaster in sugarbeet to be small, there is reason to be concerned, because with the perfect flowers in sugarbeet we have no alternative resort, like detasselling in maize, if we can no longer use male sterility. In such a dire situation, we would have to return to the earlier diploid or polyploid synthetic varieties – a slow and difficult process that would undoubtedly result in considerable losses to both farmers and industry.

As a safety measure, I have, for some years, been working with three new male sterile cytoplasms that were discovered in wild *Vulgares* beets collected in the 1950s in Morocco, Yugoslavia and Turkey, respectively. A fourth male-sterile type from Yugoslavia turned out to be strictly genic. Because all the male-sterile plants were found in the original seed samples, there can be no doubt that they constitute new and independent origins of cytoplasmic male-sterility. It is not yet possible to say with certainty to what extent they differ from the Owen sterile cytoplasm but al least two of them appear to require quite different maintainer genotypes.

At present, we are converting these cytoplasmic male-sterile wild beets to sugarbeet by repeated backcrossing to male sugarbeet parents. Concurrently, we are studying their respective maintainer genotypes and are checking for environmental stability.

Conclusions

Our studies have not yet progressed to the point where we can draw conclusions about the usefulness of the new cytoplasmic-genic male-sterility systems in hybrid-seed production. However, our goal is to produce hybrids based on these new cytoplasms that are at least as good as those now in produuction. This would allow simultaneous marketing of a range of hybrids representing three or four different cytoplasms. Together with regular tests for susceptibility to cytoplasm-specific diseases or insects, such a system would minimize the vulnerability of the crop and ensure a considerable protection for the producer, as well as for the consumer (Duvick 1972).

In conclusion, although a broadening of the genetic variation of sugarbeet is, no doubt, highly desirable, I do not believe that the present gene pool is exceptionally narrow. I also believe that the nuclear genetic variation in our present diploid and triploid top-cross hybrids is sufficiently large. There is thus no reason for concern, even if some of these hybrids are grown over quite extensive areas.

However, the almost complete cytoplasmic uniformity, irrespective of cultivar is disturbing. Even if a measure of cytoplasmic diversity can be provided by nuclear genes controlling mitochondrial or chloroplast components, only the simultaneous use of a number of sterile cytoplasms and regular tests for susceptibility to cytoplasm-specific diseases or insects would offer adequate protection.

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Strategy of preventing crop vulnerability

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Enriching the potato gene-pool using primitive cultivars

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Index words

Solanum tuberosum, potato, *Solanum* sp., Neo-Tuberosum, genetic environment, cytogenetic enrichment.

Summary

Primitive tetraploid cultivars of Group Andigena extend from Colombia to northern Argentina, with geographical variation. A related population occurs in temperate Chile. European potatoes probably derived mainly from Colombian Andigena. Diploid cultivars, Groups Phureja and Stenotomum, occur alongside Andigena, Phureja being more northern. The tetraploids probably combine diploid-cultivar and wild species ancestries. The immense variation observed by early collectors is being reduced as modern cultivars displace traditional ones.

A population based on Andigena mainly from Bolivia and Peru has been mass-selected for adaptation to our climate and requirements and is now termed Neo-Tuberosum. In yield and tuber size it compares with commercial potatoes (Tuberosum), and the variation in the population at least equals that in the Tuberosum population. Selected Neo-Tuberosum clones have been used as parents in our cultivar breeding programme in most years since 1969, with promising results.

A population based on Phureja has also been developed, and selections from it are being crossed with Tuberosum dihaploids. Ways of using diploid parents in tetraploid breeding programmes are being investigated.

Most seed from natural pollination of tetraploids is selfed. The resultant inbreeding probably facilitated the rapid development of Neo-Tuberosum from Andigena, but must have reduced the genetic base. A new broad-based population is being established by crossing Neo-Tuberosum with Andigena, and ancestries within it are being randomly combined by manual intercrossing in preparation for future inbreeding and selection.

The Chilean population has some useful characters but is infertile, probably due to inbreeding, and appears to be Andigena-derived. Neo-Tuberosum may be a more fruitful source of those characters.

Introduction

Professor Hawkes has described the genetic poverty of the potato in Europe, and later I discuss the contribution of wild species and primitive cultivars to cultivar performance. Inevitably these contributions will overlap; I hope not excessively.

The primitive cultivars of South America are diploid, triploid, tetraploid and pentaploid; the triploids and pentaploids may be ignored in the present context. The tetraploids are more widespread; Group Andigena occurs at high altitudes from Colombia and Venezuela to northern Argentina and a related population occurs at sea-level in temperate Chile. The diploids occur alongside Andigena (not in Chile) but are scarcer. The European potato, Group Tuberosum, is tetraploid and probably evolved mainly from Colombian Andigena though with some introductions from elsewhere, including at least one of significance from Chile.

Modern collecting commenced in the 1920s. Initially the variation in the material was immense, and single market-stall purchases sometimes included several cultivars, even different ploidies, due to plantings being mixed. Modern cultivars are replacing the traditional ones rapidly but the results of recent collecting (Ochoa 1976) suggest that well over a thousand distinct Andigena cultivars still exist, and many diploids also.

Andigena appears to form a single population without major discontinuities, but there is geographical variation. Leaf shapes vary, the leaflets tending to be fewer and broader in the north, and there is variation in the frequencies of pigmented plants and in other characters (Salaman 1946; Simmonds 1964; Rothacker & Junges 1965; Glendinning 1968). Some scarce characters may be local, for example eelworm resistance has, I believe, been found only near the Bolivia-Peru border.

The diploids are classed into two main groups, Phureja which has low dormancy, needing to be replanted soon after harvest, and tending to occur at low altitudes where more than one crop can be grown each year, and the more dormant Stenotomum which is grown at higher altitudes. Phureja has a more northern distribution. The distinction between these Groups seems rather arbitrary. One diploid form, Ajanhuiri, appears to be derived from hybridization with a wild species, *S. megistacrolobum* (Huamán 1975).

The relationship between the diploids and the tetraploids has not been fully clarified. The tetraploids do not appear simply to be doubled-up diploids. One suggestion is that Andigena involves Stenotomum together with *S. sparsipilum*, a common weed of cultivation (Hawkes 1956; Cribb 1972; Woodcock & Howard 1975); another involves the wild species *S. vernei* (Brücher 1958). One suspects that the origin of so variable a population as Andigena will be complex, involving various wild species and diploid cultivars.

Development of Neo-Tuberosum

Two experiments were initiated at the John Innes Institute, England in the early 1960s. One was an attempt to adapt a population derived from Andigena to British day-lengths in order, firstly, to show that Andigena could have been the origin of our cultivated potato, Tuberosum, and secondly, to provide material for use in breeding. It seemed desirable to show that Andigena could be adapted because some considered that the Chilean population, which is adapted to somewhat longer days than Andigena, was both independent of Andigena in origin and more likely to be the source of Tuberosum. The second experiment was an attempt to adapt a population based on diploid cultivars of the Phureja Group to our conditions.

The procedure was simple. Several thousand seedlings were planted out, and at harvest tubers were kept from virtually every plant that had given any. At first, only few plants tubered. The tubers were planted next season, isolated from other potatoes, and naturally set berries were taken. This seed was used to plant another field of seedlings, which gave better yields; tubers were again kept and replanted to give further seed. This was repeated again and again, the selection criteria being modified as the populations improved, first to favour higher yields rather than just any yield, and later, as yields became adequate, to favour, for instance, better tuber shapes or colours. A re-selection was made in the tuber-planted seed-production plots, tubers being kept for inclusion in the following year's seed-production plots. And for many years about half the seedlings were grown each year in Cornwall, in the extreme south-west of England, where severe blight usually occurs, selections from the survivors being included in the same seed-production plots as those from the 'home' site.

The experiments were transferred to the Scottish Plant Breeding Station in 1967, the Andigena-based experiment becoming my responsibility and the diploid population that of my colleague mr. C.P. Carroll (= P. Sudheer). Procedures have diverged somewhat, partly due to differences in the nature of the material and partly due to our different approaches, but the underlying aims remain unchanged.

Yields of the diploid population are now well within the commercial range. The site in Cornwall remains in use for exposure to blight. In sub-programmes the material is being interbred with dihaploids obtained from Tuberosum, and means of using diploids or diploid-dihaploid hybrids as parents for tetraploid varieties are being explored. There are alternatives to the obvious one of doubling the chromosomes with colchicine, since diploids often produce a proportion of diploid gametes, so that tetraploid offspring can be obtained in crosses between diploids and tetraploids. This was discussed at the 8th Congress of EUCARPIA (Sudheer 1977).

Both the diploid and tetraploid projects have been impeded, in recent years, by an unfortunate occurrence. The seed-transmissible disease Spindle Tuber was detected in the Commonwealth Potato Collection, which had provided the source material. The populations therefore fell under suspicion, especially the tetraploid one as I and my assistants also look after the Collection. This happened at a time when it would have been appropriate to have commenced using our high-altitude site, Blythbank, which is relatively virus-free, for long-term maintenance of selected material. Blythbank is also used for the generation of blight epiphytotics and I had just discontinued use of the site in Cornwall, intending to use Blythbank for blight-resistance selection, when this problem arose. We could not transfer anything to Blythbank for some years. A succession of mild winters led to severe problems with leaf-roll and virus Y at our low altitude sites and it has been difficult to maintain selected clones for any length of time.

No spindle tuber disease was found in either population, and use of Blythbank has now commenced.

The initial tetraploid population was based on about 300 Andigena accessions and was about 45% Bolivian, 35% southern Peruvian, with about 10% each from northern Peru and from Colombia. Chilean material was not included. The material was from the Commonwealth Collection and derived from the 1939 collecting expedition and subsequent donations by South American workers. There would undoubtedly be some duplications among the accessions but probably not on the scale found during recent collecting; there was much greater variation in the material grown in those days, and the South American workers, who could maintain their collections in the field, had doubtless sorted them in advance.

Evaluation of Neo-tuberosum

By 1969, we had coined the term Neo-Tuberosum for the population which had fewer stems and larger, more closely placed leaves, commenced tubering earlier, and gave greatly increased crops of fewer but much larger tubers than Andigena (Glendinning 1975b). In these respects, the population approached Tuberosum. Neo-Tuberosum yields equalled those of Tuberosum at an early harvest and, due to later maturity and continued bulking of crop, exceeded them finally. Neo-Tuberosum resembled Andigena and exceeded Tuberosum in plant size and in flowering, but these seemed likely to decline with selection for earlier maturity. Leaf shape differed from that of Tuberosum, this probably reflecting the essentially Bolivian-Peruvian origin of Neo-Tuberosum and the probably Colombian origin of Tuberosum.

Comparisons of batches of clones selected from the population with a small but varied batch of commercial cultivars have shown that the variation in Neo-Tuberosum is at least as wide and often wider than in Tuberosum (Glendinning 1975c). When wider, it tends to extend into commercially unacceptable ranges, e.g. some clones are excessively late maturing or are low-yielding or show excessive after-cooking blackening, but in each of these respects some other clones are exceptionally good. In some properties, such as crisp quality, after-cooking blackening, and blight resistance, the proportion classifiable as good is higher than in the commercials. Only in tuber appearance, for which modern cultivars are highly selected, were the best commercials clearly superior to the best Neo-Tuberosum clones, but even there, most clones were superior to one commercial cultivar, 'Golden Wonder'. This is a very old cultivar which persists in commerce due to its unusual quality, associated with an exceptionally high specific gravity.

Neo-Tuberosum is free-flowering and very fertile. Of 94 clones studied in 1970 all but two had flowers and 81 bore naturally set berries. This provides a contrast with commercial varieties for which special techniques are often required to get flowers on plants wanted for breeding, and among which the choice of male parents is limited by frequent lack of fertile pollen.

These observations were made some years ago before the Spindle-Tuber scare and the virus epidemics. Since then, clones have been viewed as potential parents for the commercial breeding programme and have been rigorously re-selected over two years on yield and visual criteria, only the few surviving both this selection and the severe roguing for virus infection being submitted for a limited range of disease-resistance tests. Now that clones have been established at Blythbank, more detailed assessment is again becoming possible. A batch of clones last year appeared to outyield commercial varieties in an adjoining planting at comparable maturities. This year, they have been laid out in a replicated trial with commercials, which I hope will not be spoiled by roguing. They are being tested for resistance to several diseases.

The first significant use of Neo-Tuberosum in the breeding programme was in

1969 when seven clones, selected as seedlings from 1965 to 1967, were crossed with a wide range of Tuberosum parents. After a year under glass, about 7 000 selections, each as a single tuber, were planted out in 1971. About 5 000 derived from crosses with 'established' Tuberosum parents, and about 1½% of them survived selection then and in the next two years to be planted as sixteen-tuber plots in 1974. Survival ranged from 0.3 to 4.8% over derivatives of the seven Neo-Tuberosum parents. From the other 2 000 singles, which derived from crosses with less-developed breeding lines, only few survived. Survival in the accompanying Tuberosum X Tuberosum material averaged about 3%. Thus, in crosses with 'established' parents, survival to the '16s' stage averaged about half that in the general through-put, but progenies from the best Neo-Tuberosum parents were distinctly superior.

Neo-Tuberosum was also used in pollinations in 1970 and 1971 but on a smaller scale, giving about 1 400 and 3 400 'singles' in 1972 and 1973; results to the '16s' stage were similar, about 1% survival, about half that of the general through-put, but with progenies of some individual Neo-Tuberosum clones markedly superior.

A two-year hiatus followed due to the Spindle-Tuber scare, then in 1974 Neo-Tuberosum was again used on a major scale, giving about 11 000 'singles' in 1976, about 56% of the 'singles' of that year. It is too early to report results on this batch. Small-scale use of Neo-Tuberosum continued up to 1977.

Beyond the '16s' stage, surviving clones are submitted to tests for disease resistance. Such tests on the first wave of material have revealed higher frequencies of gangrene-resistant and of scab-resistant clones than in the general through-put of the programme, even though these first Neo-Tuberosum parents had been chosen on tuber appearance only, and nothing was known of their resistances when they were used. Some selections from this batch of material remain under consideration as potential cultivars.

Interest in Neo-Tuberosum is not restricted to Britain. Professor R.L. Plaisted of Cornell University in the United States was stimulated by the heterosis observed in Andigena x Tuberosum in eelworm resistance breeding, and commenced work independently in 1964. He then heard of the experiment started by dr N.W. Simmonds at the John Innes Institute and obtained material from him (Plaisted 1972). Neo-Tuberosum has also been supplied to dr J.G.Th. Hermsen, dr C. Mastenbroek and dr W. Prummel in the Netherlands, to K.G. Proudfoot initially in Northern Ireland and subsequently in Canada, to T.R. Tarn in Canada, and to various others. Various people are selecting within the population, crossing with cultivars, investigating heterosis in such crosses, or looking at disease resistances.

Effect of self-fertilazation

Diploid potatoes are self-incompatible but set seed readily when pollinated by bumble-bees. It was therefore initially assumed that the tetraploids, also pollinated by bumble-bees, were substantially cross-pollinated although they are self-compatible. But suspicions arose both in the United States where Plaisted found that his retained population traced, in the female line, to only few original parents, and in Britain where I found that the associations of characters in the population were not random. Observations indicated that a bumble-bee visit results in a lot of self-pollen getting onto the stigma, and a marker-gene experiment indicated about 80% selfing.

A comparison of crossed and selfed progenies showed that crosses were more vigorous and more likely to be selected, but only to the extent that perhaps 30 instead of 20% of selections from naturally-produced seed would derive from crossing. Perhaps even more striking was the fact that 57% of the selections made in this experiment derived from only 14% of the progenies, and 53% of the progenies contributed nothing. It was clear that the gene-pool was being severely reduced (Glendinning 1976).

As inbreeding will progressively reduce vigour and yield, it is unlikely to proceed far in any one line. But favoured lines could displace many others from the population then be replaced, as they become too inbred, by new lines derived by crossing between them. A better system for improving the population, for the limited range of characters on which selection could be operated, could hardly have been devised. But the fate of characters not subjected to selection, characters for which tests would have to be applied to each plant – impossible when working with a large population – would depend purely on chance, and many such characters may have been lost.

Expanding the Neo-Tuberosum gene-pool

Similar processes must have operated in the evolution of Tuberosum, and the Neo-Tuberosum gene-pool, derived from a probably larger number of accessions and selected over fewer generations than Tuberosum, is probably still much larger than that of Tuberosum. Nevertheless, it has seemed desirable to add to Neo-Tuberosum variation. What has been done so far can be viewed as pilot work in which a technique has been tried out with reasonable success; the next stage is to refine the technique and apply it to making available as much of the Andigena gene-pool as possible.

Plaisted has recommenced with a larger sample of Andigena obtained from the Commonwealth Potato Collection, the Wisconsin Collection and South American sources, and with the intention of selecting within rather than between progenies so as to maintain the genetic base (Plaisted 1972). We have taken a different approach, and have crossed Neo-Tuberosum with Andigena from the Commonwealth Potato Collection, including material obtained subsequent to the initial setting up of the Andigena experiment. (Other sources were closed to us during the Spindle Tuber scare, quarantine facilities being fully occupied.) The initial crosses were made with bulk Neo-Tuberosum pollen so that each berry involved many male parents. A second generation was obtained by mass-interpollinating within the F_1 so that Andigena as well as Neo-Tuberosum ancestries were randomly combined. Two third generations are being produced, one by mass interpollination within the F_2 and the other using Neo-Tuberosum pollen on the F_2; the latter will be nearer to the required standard for instance of yield but will have a reduced 'new' contribution of Andigena genes. This work has been done without selection, in a greenhouse, but the third generation will be field-grown and natural pollination, involving much selfing, will subsequently be relied upon for some years. Many undesirable dominants will have been introduced from Andigena, and selfing will be required to expose their recessive counterparts and thus permit selection against them. The rapid progress we made, in terms of generations, in developing Neo-Tuberosum was probably due largely to inbreeding. In our diploid population, where progress, though satisfactory, has been slower, inbreeding will not be significant.

Chilean material

Having discussed Andigena and the cultivated diploids, I should briefly mention the Chilean tetraploid population. At least one Chilean importation, 'Rough Purple Chili', obtained by Goodrich in 1852, has contributed significantly to our modern potatoes and another, 'Villaroella', is in the ancestry of some important cultivars. A study of a sample of Chilean material (Glendinning 1975a) led me to conclude that it is probably a highly inbred population derived from Andigena, so inbred as to be highly infertile. It did seem to contain some some useful genetic characters but, as those characters were probably from Andigena, there seemed no point in using infertile Chilean material while Neo-Tuberosum was available.

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Collecting within Europe

Chairman: dr ir A.C. Zeven (Wageningen)

funds and the interests are limited. (Let us invite the Ethiopians to come and collect the Dutch beans!).

As breeding work of the principal crops started early in Europe, the landraces have been replaced by newer ones, and these have had to make room for still newer cultivars. Lack of knowledge at that time about the usefulness of obsolete cultivars as genitors allowed most of them to be lost. Since 1945, the Netherlands has lost the Dutch land races of rye and spelt (*Triticum spelta* L.). In our Institute's Triticinae collection we find accessions of Dutch landraces of bread wheat: Zeeuwse Witte, Gelderse Ris and Limburgse Kleine Rode. However, after some 60 generations of maintenance only one genotype of each landrace, which was a mixture of genotypes, is left. The conserved genotype may even not be the most frequent one of the landrace. Many genetic resources are still available in Europe, even in the industrial areas (Hammer et al. 1977; see also below). Fortunately much collecting work is going on.

If we accept Zhukovsky's view on megacentres, much of Europe belongs to either the European Centre of diversity or to the Mediterranean Centre (Zeven & Zhukovsky 1975). Collecting is proceeding in both centres. For instance, in the Mediterranean Centre, diploid, tetraploid and hexaploid wheats, maize, barley and oats are sufficiently collected, but this is not yet true for beans, chick pea, vetch, one-flowered tare and ervil. Collection work is also being done for pomaceous and stone-fruits (Sanchez-Monge 1978) and for Cruciferae. The gene bank of Bari in Italy is collecting for both sides of the Mediterranean (Porceddu, this volume).

Collections are also taking place in the European Centre of diversity. For instance Szucs (1974), Hanelt & Hammer (1975), Kühn et al. (1976), Hanelt (this volume) and Bares (this volume) describe collecting work in east central Europe. Other examples are Alpine wheats (Mayr 1964; Köck 1973; Schachl 1973), barley and oat (Schachl 1975) in Austria, and seabeets in west France (Cleij et al. 1970) and southern England (Zeven, see below), and common beans of the dry type in the Netherlands (Zeven, see below).

Varekamp (unpublished) compiled a list of species collected in Europe and used in the breeding program of the Dutch Institute for Horticultural Breeding (IVT) (Appendix 1). In this list, he also indicates present use.

Collecting a wild species: sea beet

Sugarbeet (*Beta vulgaris* ssp. *saccharifera*) derives from seabeet (ssp. *maritima* (L.) Thell.) from the Mediterranean Basin. When the sugarbeet came into contact with the west European seabeet the latter proved tolerant to beet yellows virus (BYV) in contrast to sugarbeet. From the western seaboard of Europe, BYV spread over Europe. Apparently the European sugarbeet cultivars all carry the same genotype for BYV susceptibility. This demonstrates a genetic poverty of the crop for this disease. It was to be expected that sugarbeet breeders looked to the west European seabeet as a source of tolerance genes. So Cleij et al. (1970) collected seabeet on the Atlantic coast of France. The collected material included genes for tolerance to BYV, male sterility (for hybrid sugarbeet breeding), monogermy (for easy cultivation), red and green pigmented plants, and erect and flat plants. At one site, fodder beet was growing near seabeet. This may have resulted in a bilateral exchange of genes (Tjebbes 1933). Likewise the seabeet of Norfolk seems to possess genes of cultivated beet (Watson & Witts 1959).

I collected seabeet on the coast some 10 km east of Hastings (southern England) in 1974 and sent this material to three institutions, including the Plant Genetics and Germplasm Institute at Beltsville, Maryland, USA. The previous introduction of British seabeet into the United States was in 1921. These seabeets could be an important source of tolerance genes to various virus diseases in California, but could introduce BYV into disease-free areas.

This material I collected during a holiday, grew in the low lying Romney Marshes behind a high sea-dike. This material also contained genes for male sterility and monogermy, and perhaps for tolerance to BYV. The beets may be either high (Tjebbes 1933) or low in sucrose (Mayer Gmelin 1925), but it is not known whether high content is genetically controlled or whether it results from the thinner roots of wild beet.

West European seabeet may broaden the genetic base of sugarbeet, also for other characters than BYV tolerance.

Collecting semi-wild species: grasses in the Netherlands

Although crossing work is applied, most of the breeding work with various grasses in the Netherlands (*Agrostis* spp., *Dactylis* spp., *Festuca* spp., *Lolium* spp., *Phleum* spp., and *Poa* spp.) consists of collecting wild and semi-wild material and selecting within it. Apparently there is a great diversity among these grass species and so the Netherlands is called a primary centre of diversity for grasses. It is included in Zhukovsky's European Megacentre (Zeven & Zhukovsky 1975). According to what some Dutch grass breeders have told me (C. van den Bogaert, P. Bom, J.J. Hintzen and M. Kamps), the diversity of the Dutch pasture and meadow grasses is largely exhausted, but of lawn grasses (lawns, sport fields, road sides, paths) it is not. So the Dutch breeder collects within the Netherlands if he is looking for a new variety of a lawn grass and goes abroad (even crossing the Atlantic), to collect material for production grasses.

However, no material has been collected for a gene bank as it is believed that the diversity available in wild and semi-wild plants for field grasses will remain for the time being, conserved in permanent grassland (pastures, meadow, marshes and orchards) and for lawn grasses in lawns, in edges of forests, in open forests and verges.

If a certain grass species or ecotype is not grown, the breeder may store seed in the hope that it will regain attention in the future.

There is no need to collect grasses for a Dutch gene bank since sufficient genetic diversity remains. Old and modern cultivars and some special rare types should be maintained in a collection. The knowledge breeders have of phenotypic variation in Dutch and foreign species of grass must be enormous, but little has been published. This is a great omission.

Collecting cultivars in the Netherlands: cole crops, bulbs, potato and dry bean

Cole crops

By breeding of F_1 hybrid varieties of cole crops (*Brassica oleracea* L.), landraces and local selections are in danger of becoming extinct. Breeders maintain only inbred lines, and with the extinction of the old varieties the genetic variation will disappear. This will result in an enormous genetic impoverishment. Fortunately N. van Marrewijk, formerly of the Institute for Horticultural Breeding (IVT) and his colleagues have collected some 160 accessions of Brussels sprout, as the breeding of F_1 varieties of this crop is the most advanced and some 80% or more of the area under Brussels sprouts is covered by hybrid varieties. He obtained material of the main group varieties like Bredase and Roodnerf (Groninger type, Kennemer type, Westlandse type and Barendrechtse type) and several other varieties (van Marrewijk, pers. commun., June 1978). Probably nondescript selections can still be found in backyard gardens where private growers cultivate Brussels sprouts for home use.

The next subspecies in danger is the cabbage, for which F_1 varieties are being produced and the old varieties and selections will be superseded in the near future. Plans exist to collect these, and here again back gardens should not be neglected.

A start has been made with the breeding of F_1 varieties of cauliflower and kale, but it will be some years before they are grown on a large scale (Roelofsen, this volume).

Hortus bulborum

In 1924, P. Boschman started to collect old cultivars of tulips, hyacinths, and put them together in a 'Hortus bulborum' (van Eijk 1973). This collection includes old cultivars like the tulip cv. Semper Augustus, which dates from 1637. This collection is not yet protected by law and like all private and semi-private collections their maintenance is insecure because of the high costs. Perhaps one of the first tasks of the future Gene Bank of the Netherlands should be to take over responsibility for this praiseworthy genetic resource.

Collecting potato cultivars

Fortunately a large collection of potato cultivars of Dutch and foreign origin exists in the Netherlands. Part of this collection – some 400 cultivars – is included in the 'Genitor Collection' (Zingstra & Scheijgrond 1976), which ensures their survival. However, not all maintained cultivars are in the Genitor Collection and some may be removed every year. The maintenance of these cultivars is not protected by law and from time to time their maintenance comes under discussion. The high costs and the increasing number of cultivars to be conserved make regular pruning of the collection necessary. If it be decided to discard a cultivar, it should first be checked whether it is maintained by a gene bank. If not, it should be sent to a gene bank or if it be a Dutch-bred cultivar it should be maintained in the Netherlands. If sufficient funds could be raised for a Dutch expedition to South America in 1974 to collect highly desired tuberous *Solanum* material, similar funds should also be made available to maintain Dutch-bred cultivars.

Other crops in danger

In the Netherlands silage maize is increasing at the expense of fodder beet, rye and turnips (*Brassica rapa* L. var. *rapa* (L.) Thell). Breeders and farmers are losing interest in these crops and much of the genetic variation will be lost. As far as I know, only limited attempts have been made to collect the turnip varieties (Roelofsen, this volume), but I do not know of a collection of fodder beet and rye.

Similarly spelt (*Triticum aestivum* spp. *spelta* (L.) Thell.) has been replaced by bread wheat, and fodder carrots by other crops. In 1951, a last collection of Dutch spelt cultivars was discarded and only a few cultivars of forage carrots, described by Wijbrans (1953), still survive as seed in the cold store at the Government Institute for Research on Varieties of Cultivated Plants (RIVRO) in Wageningen. At present, the seed is being renewed and the material is being sent to gene banks abroad.

Many cultivars and selections of ornamentals disappear because the costs of trading in many (colour) types have become too high (Ruys 1978).

The cultivars of different willow species will also disappear. A nuclear collection is being maintained.

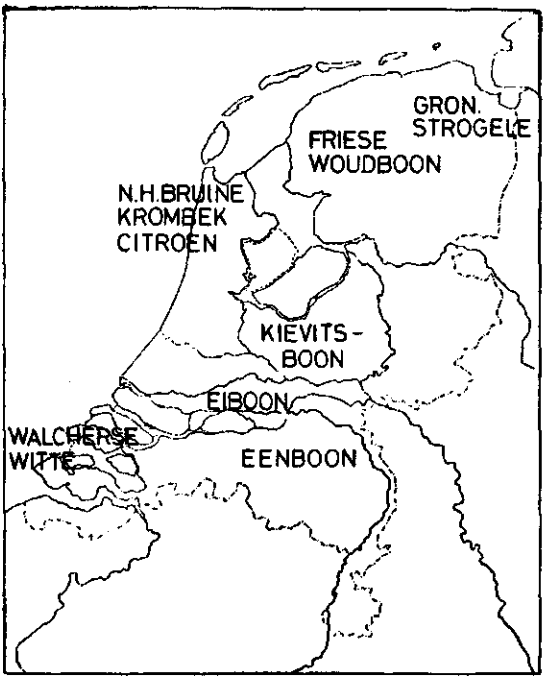

Fig. 1. Geografic distribution of dry beans in the Netherlands.

Dry beans

To demonstrate that it is worthwhile to collect local and obsolete cultivars in the Netherlands, a search was made for dry bean (*Phaseolus vulgaris* L.) types. This bean is grown as a field crop for commerce and by private growers for home use. Some landraces – often somewhat improved by mass or line selection – are still offered by seed companies, which also trade in bred cultivars. (Zeven, in preparation).

The farmer growing dry beans on an agricultural scale cultivates, in general, bred cultivars, but with demand from consumers some old types are grown for sale too. Such bred varieties are 'Berna' and 'Narda', both being brown beans.

In 1942 to 1946, Nijdam (1947) collected some 1 600 accessions of dry beans. These beans were collected to learn about the bean cultivars in the Netherlands and compare land races with types offered for registration. Nijdam described the types concisely. At that time, there were no facilities to maintain the collection. In fact there was no need to do so as the beans were cultivated widely. Unfortunately, no herbarium material was preserved. Of part of the collection, dead seed samples were maintained by RIVRO and these have been transferred to the Institute of Plant Breeding (I.v.P.) as a reference collection.

Nijdam (1947), indicated that there was a geographic distribution for seed colour and seed shape (Fig. 1). In Groningen, south-east Friesland and north Drenthe, there is a local bush bean variety called in Groningen and North Drenthe 'Groninger Strogele' (Groningen Straw Yellow) and in Friesland 'Friese Woudboon' (bean of the Friese Wouden area). This variety is grown as both a field and a garden crop. In the field crop, there are several types for colour, shape, hilum ring and corona in the accessions. The same types may be found with private growers, but since few beans are needed for the next generations the 'selection' of each grower is quite pure.

In North Holland are bush bean landraces like Noord-Hollandse Bruine (North Holland Brown), (white) Krombek and Citroen (Lemon). These three races are still traded and some 'breeders' maintain their 'purity'. The cv. Beka was selected from North Holland Brown.

Formerly the landrace Noordhollandse Kleine Bruine (North Holland Small Brown) was grown but is probably extinct. Its selection 'Dévé' may still be grown somewhere.

Up to the early 1940s, the bush bean Walcherse Witte (Walcheren White), susceptible to bean common mosaic virus, was grown as a field crop on Walcheren and some other islands in the Province of Zeeland. It could be grown there as aphids – vectors of the virus – were not common on these islands. However, it is said that after the introduction of gladioli as a crop tolerant to the bean yellow mosaic virus, Walcherse Witte and the cultivars Rona and Walcheria selected from it, which already suffered badly from bean common mosaic succumbed. The farmers quickly replaced these cultivars with less susceptible brown-seeded cultivars. This replacement was complete and no seeds could be obtained during a visit to farms in 1976. One accession was received from a private grower and some material was already present in collections. Whether these accessions derive from the local variety or from Rona or Walcheria is not known.

On Walcheren, the Walcherse Kogelboon was formerly cultivated, but this type has been lost. Only its selection 'Aka' is present in our collection.

On the sandy soils of Utrecht and the north of Gelderland, we find large-seeded and small-seeded types of the Kievitsboon (purple-striped Horticultural Bean). The same seed type is grown around Roelofarendsveen, Prov. of South Holland but as a climbing bean. It is cultivated around gardens to protect vegetables from wind damage. These types and also climbing runner beans (*Phaseolus coccineus* L.) were used on the Veluwe to protect tobacco plants against wind damage (Nijdam 1947). They probably disappeared with tobacco, as such types have not been found near Wageningen. The 'Tabaksbonen' (Tobacco Beans) were sold (Roessingh 1976) for extra income by the tobacco growers. It is not known whether the climbing Kievitsboon was developed locally, for instance from a cross between Kievitsboon and a climbing bean. A similar or perhaps identical type was also obtained from France.

The Kievitsboon crosses fairly easily with *Ph. coccineus* and it has a similar pattern of resistance or susceptibility for halo blight to that species. Therefore, N. Hubbeling suggested to me recently that the Kievitsboon might derive from an interspecific hybrid *Ph. vulgaris* × *Ph. coccineus*. The *coccineus* genes probably introgressed into *vulgaris* in South America where such hybrids are found.

In the river clays along the distributaries of the Rhine and Meuse, and in North Brabant and Limburg, there is the white-seeded bush bean Eiboon ('Egg Bean'), so called for its resemblance to a white egg. One private grower informed me that his type 'resisted hybridization', i.e. it did not hybridize with neighbouring haricot beans. The cause of the 'resistance' to hybridization was the earliness of his selection of the Eiboon.

South of the Rivers, Nijdam found the white-seeded 'Eenboon'. Its seed shape suggests relationship with Krombek from North Holland.

Further south, one occasionally meets black-seeded bush beans. A sexton who did not like the sight of black-seeded beans on his plate mixed them with brown beans. In general such black-seeded types or dark brown types are grown in the same way as haricot beans.

Other types are found scattered about the country. These are blackish, brown and purple types of the 'Soldatenboontje' (Soldier Bean), so called for its peculiar eye around the hilum. Such colour types may be grown in mixtures. The type is grown as a curiosity.

It would be interesting to know whether similar geographic distribution of bean type exists in other European countries.

A brown-marbled type has come from Gelselaar near Borculo (Gelderland). The farmer's wife called it 'Bakkenhagen' as the farmer's grandparents and parents had used that name. She did not know how the name had originated. This type is not grown by her neighbours.

I have now collected some 110 accessions of bush and climbing beans and in only a few cases a mixture of major colour types was received. So there is not the mixture of various colour types nor even mixtures with runner beans, as was found in Czechoslovakia (Hanelt & Hammer 1975).

The geographic distribution for major colour and seed shape types that we observed was the same as observed by Nijdam (1947). Such a distribution may reflect a local preference.

The 53rd Dutch List of Cultivars (1978) mentions for dry-bush beans that these are sporadically grown for home use. This is not correct as they are quite commonly grown in gardens.

Lie (1978) showed that the pea cultivars of Europe have an identical or very similar genotype for the symbiosis with the European strain of *Rhizobium pisi*. He also indicated that the primitive pea cultivars from outside Europe have a different association with this European strain; they are resistant to it. This may have been caused by human selection for yield, which may have selected for a pea–*Rhizobium* association giving the highest yield. This may have happened during the introduction of peas in Europe, after which only those pea genotypes remained that yielded well with the adapted 'European' strain under the pressure of human selection. Lie also showed that the pea cultivars of Europe are genetically poor for their association with *Rhizobium*.

It is not known whether something similar has happened for beans during their evolution under domestication in South and Central America, or during their spread in Europe. However, Graham & Rosas (1977) found for Colombia that the highest yielding types were inoculated landraces with a local strain of *Rhizobium phaseoli*. This may indicate that a co-evolution exists between the local cultivars and the local *Rhizobium* strain. Something similar may have happened in the Netherlands. Perhaps after the introduction of beans to Europe, only that genotype for symbiosis survived that had an optimum association with a *Rhizobium* strain. Other genotypes disappeared from cultivation because of their lower yield. If so, the present dry bean cultivars must be genetically poor for their association with *Rhizobium* strains, and reciprocally the *Rhizobium* strain or strains must be genetically poor for their association with different bean genotypes.

It would be worthwhile to test for any genotypic similarity of the Dutch bean collection by determining electrophoretic patterns of iso-enzymes, and resistance patterns to diseases and to *Rhizobium* strains.

Conclusion

Collecting of dry beans has shown that many genetic resources remain. The same is probably true for vegetables, fruit trees and ornamentals found in private back gardens in the industrial Netherlands. These should be collected. Each year of delay means a loss of irreplaceable material. The Bakkenhagen bean serves as an example. For some reason, this rare type was not grown for two years and this land race is lost now.

An attempt is being made to describe all the types collected, to multiply the material, to send it to various gene banks of the world, and to include plants in the Wageningen Herbarium. This should be done too for other crops.

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Appendix 1. Species collected from various habitats in Europe (as delimited in Flora Europaea) and their uses in breeding programmes of the Institute for Horticultural Plant Breeding (IVT) at Wageningen.

Species	Use
Anemone coronaria hortensis *hortensis* *palmata* *pavonina*	progenitors for production of cut flowers, the present cultivars showing inbreeding deficiencies; new colour patterns
Dianthus spp.	shortening of the growth cycle; low bushy plants with long flowering stems (saving labour); new colour patterns
Hyacinthus orientalis	breeding for resistance to protozoal yellows (*Xanthomonas hyacinthi*)
Iris (bulbous, subgenus *Xiphion*)	suitability for forcing; new colour patterns
Lactuca spp.	response to light and temperature; resistance to *Bremia lactuca*; gene bank
Lilium spp., e.g. L. *candidum*	suitability for forcing; new colour patterns
Tulipa spp., e.g. *T. bocotica* *T. hungarica* *T. praecox* *T. rhodopea* *T. scardica*	shortening of the juvenile phase; suitability for forcing; resistance to *Fusarium* rot; flower quality; gene bank

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Collecting in Czechoslovakia and Poland

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Index words

Land races, gene erosion, cereals, grain legumes, vegetables, collecting work, gene bank, Czechoslovakia, Poland, Europe.

Summary

Long-term research programs for collecting and evaluation of gene resources had been devoted mainly to the gene centres of cultivated plants; only recently has it become evident that similar investigations are also an urgent necessity in regions outside of these well known centres.

During the last decade, Czech botanists have drawn attention to a refuge of cultivation of local races and old varieties in the eastern parts of Moravia and Slovakia and afterwards a similar situation proved to be true for the southern parts of Poland. In a joint undertaking colleagues from these countries and the Gatersleben institute, organized three collecting trips. So far more than 700 samples of cereals, leguminous crops, vegetables and medicinal plants have been collected.

Geographic isolation, ecological factors, infrastructure of agriculture, tradition among the farmers and reluctance to cultivate pure lines have favored the persistence of these indigenous varieties, although recently a rapid decrease in their cultivation was observed in most of the region. Complete loss could be expected in the near future.

The local races are often rather heterogeneous phenotypically and represent populations characterized by a great morphological variation often containing up to 10 botanical varieties (e. g. in wheat, beans, peas and poppies). Often natural crosses contribute – also in basically autogamous species – to the striking variation in the material.

In some areas the cultivation of relic crops, like naked barley, emmer wheat, chickpea, vetchling and safflower was still found, some of which were thought to have completely disappeared from the Central European agriculture; in the easternmost part of Slovakia, a kind of shifting cultivation is still practised with a typical spectrum of crops including buckwheat and millets.

The material of these collections will be propagated and maintained at Gatersleben, Radzików and in Czechoslovakian institutions. First observations point to a high variation within this material for characters like disease resistance, winter-hardiness, protein content, earliness, which may be used by the plant breeders. Its practical value could be proved already in preliminary studies when very winter-hardy types of rye resistant to lodging from Poland, resorcinol-free Slovakian ryes, early ripening peas and beans and low-stemmed barleys could be found among the material.

Introduction

For a long time, the study of the gene resources of cultivated plants has been mainly devoted to the classical and well known gene centres, in which a fairly intensive but still insufficient collecting work has been done. Only in recent times has it become evident – as evidence this meeting – that other regions outside these centres offer similar opportunities for collecting and studying indigenous plant material, too and that there is also an urgent necessity for such work. This is true for different parts of the world and also for Europe. Here earlier the common opinion was widespread that north of the Alps all local races and autochthonous material of agricultural and horticultural crops had completely disappeared some decades ago. The incorrectness of this assumption could already be seen from early reports of Austrian botanists and agronomists, who observed cultivation of relic local races of cereals, even recently not only in the mountainous regions of this country but also in the foreland of the Alps.

In spite of this, it was rather surprising that by the activities of Czech colleagues during the last 10 years a refuge of local races and rare cultigens could be discovered in eastern Moravia and in Slovakia; also from the Tatra districts in Poland, there were records of local races of cereals at least for the first years after World War II (Sawicki 1958; 1960). For a more thorough study of these regions and for collection of this material since 1974, three collecting trips were organized, participants were Dr F. Kühn from the Agricultural College in Brno, Dr W. Kulpa, Mag. Ing. M. Górski and other colleagues from IHAR (Instytut Hodowkli i Aklimatyzacji Roślin) in Radzików and Dr K. Hammer and I myself from Gatersleben. The academies of our three countries and the institutes in Prague-Ruzyně and Radzików have been responsible for the official arrangements and for financial funding.

The travels went through many parts of the western range of the Carpathian Mountains, in Czechoslovakia for instance to the Moravian-Silesian and the Slovakian Beskids, the White Carpathians, Krupina Mountains, the Slovakian Ore Mountains (Erzgebirge), the south-west Slovakian lowland and to the Lower Beskids in the easternmost part of Slovakia. In Poland the main collecting activity was hitherto devoted to the Pienine Mountains, the foreland of the Tatra and different parts of the Polish Beskids and the Bieszczads in south-east Poland. There are still unexplored regions, for instance the foothill regions of the High Tatra, the Lower Tatra and the Tatra in Slovakia to which we hope to extend the collecting in future. So far more than 700 seed samples of cereals, legume crops, vegetables and medicinal

Table 1. Collections from Czechoslovakia and Poland (1974-1977).

wheats	75	peas	46	buckwheats	24
barleys	86	field beans	31	poppies	30
ryes	69	*Phaseolus* beans	23	*Brassica*	13
oats	112	others	14	*Lactuca*	11
others	8	legume mixtures	3	*Allium*	11
cereal mixtures	41			other cultigens	48
				weeds, wild plants	59
	391		117		196

plants have been collected, fruit crops and potatoes had to be excluded, although old and primitive varieties of them seemed to be widespread (Table 1).

Several factors strongly favor the maintenance of the local races in the agricultural system of the area mentioned:

– it is geographically mostly a mountainous rather isolated region whose communications have developed to a more modern level only in recent decades since World War II;

– the ecological conditions – arable land available only in orographically well differentiated territories with rather steep slopes and so on – do not allow the introduction of a highly mechanized agricultural system with industrial methods of production;

– this is connected with the infrastructure of the agriculture in these area in which so far private ownership and small or medium-sized farms prevail enabling formerly a kind of subsistence culture;

– there is a strong tradition especially among the older generation of farmers, their aversion to cultivation of pure lines – very often met in Poland – and their liking for cultivation of a diversity of crops or crop types in the fields and in gardens.

Nevertheless the area of local races is rapidly decreasing in recent years and in some parts these have been already completely lost. This process is caused also by several factors. Agricultural infrastructure is changing; arable land now no longer profitable to manage is abandoned or converted on a large scale to grassland for animal husbandry; strong official recommendations exist for switch of seed (Poland) in order to replace the old races by modern cultivars and finally often the small farms in the mountains are only part time managed (the owners are working in factories some distance away and spend only their weekend on the farm) or only the older farmers feel responsible for the fields. In a relatively short time, that material will almost certainly be lost, which is still cultivated in these fields.

The speed of disappearance of local races may be illustrated by their complete replacement by modern varieties in districts for which Sawicki (1958; 1960) had still indicated old wheat and barley varieties and by the observations in one village of the Pienine Mountains where old forms of sugar pea were seen frequently in the gardens in 1975 (also a characteristical garden plant in Slovakian montane regions) but were wholly lost by the seed switching a year later. Therefore we are convinced that these very years offer the last opportunity to collect and maintain this indigenous material.

Cultivation of local races in the mentioned regions is often connected with a very intensive ecologically well based use of the available land. Thus intercropping (such as maize and bean, potatoes and field beans, beets and poppies) is frequently practised and even very small patches within a potato field originated by the decay of one or several potato plants are used afterwards and resown with some bean or pea seeds. In the home gardens or garden plots along the fields, a high diversity index is the result of such cultivation and on a small area many cultivated species or different strains of the same taxon can be found.

Description of collected crops

In the following the collected material will be characterized by some examples.

Slovakian barleys (Hordeum vulgare)

Barley cultivation is common through all the explored regions, it reaches to the upper limit of cropping and here we often find stands of barley mixed with oats. Most barleys are two-rowed forms of the *nutans*-type with narrow grains typical of local races. Frequently are to be seen together different colour variants of glumes and awns, forms with different basal setae, with different size and grain number of the ears. Multi-rowed varieties were rare; they were collected mostly in the Beskids of Slovakia and Moravia and – with one exception – grew only as admixtures in convar. *distichon*. Of the formerly rather often cultivated naked barley only few relicts could be observed in northern Moravia in the mountains (var. *nudum*); its low resistance to lodging was the reason for a mixed cultivation of naked barley and the stiff summer wheat by some farmers. Grains of naked barley were used for porridge and the preparation of a special sausage; in the Polish Lower Beskids, another refuge of these barleys, they were fed mainly to horses. The Slovakian material of *Hordeum* was varied more than the Polish; some of the Polish local races, often known under special names, apparently came originally from more southern regions.

Polish wheats (Triticum aestivum)

Within the area visited, wheat cultivation – almost exclusively winter types – was important in the foreland and the lower parts of the Beskids and eastwards. Often the local races are maintained only as tolerated components of older cultivars (like 'Eka', 'Blondynka') or even recent cultivars ('Mironovskaja') in which they represent more or less abundant admixtures. To avoid yield depressions, a cultivation of morphologically and ecologically different types is often preferred (as in Slovakia sometimes a mixture of rye and wheat) and so the local races are still to be seen and often exhibit an extremely diverse array of lines, in the basin of Sącz for instance consisting of the botanical varieties *ferrugineum, ferrugineumcompactoides, milturum, erythrospermum, lutescens* and *lutescenscompactoides*. Awned varieties are frequent within the stands of local races; generally the farmers avoid cultivation of homogenous awnless populations thought to be susceptible to damage by wild boar. There is a rich variation (much more expressed than in the Slovakian material) for colour variants, different compactness and length (up to 18 cm) of the spikes and so on. Apparently the local races show a comparable strong tendency to cross-pollinate (at least in distinct years) which can be demonstrated by the frequent segregations within offsprings of single spikes and which may contribute significantly to the high variation of Polish wheats. First observations demonstrate also a great variation in resistance to mildew, leaf rust and cold winters within the material.

Rye (Secale cereale)

Rye was everywhere widespread. In Poland, it replaces wheat in the Tatra and Pienine mountains nowadays almost completely; sometimes it reaches also to the upper limit of agriculture (900 m) and, at higher altitudes, it is often sown mixed with oat or barley. Winter rye dominates; the populations especially in Slovakia are often very heterogeneous, for a cross-pollinating species rather strikingly. For instance, plants with very long and short spikes, greenish or yellowish grains and with two size-classes of grains (thousand-grain mass more than 29 g or less than 26 g) were often observed within one field.

A special local race with very long stems is cultivated in southern Poland to obtain twine for the sheaves, the use for grain being only a secondary one.

We learnt that 10–20 years ago, there was still some cultivation of the perennial rye *S. cereale* var. *multicaule* especially in the Beskids. It was a crop of a small-scale shifting cultivation and was sown immediately on the burned ground of the former forest land. We tried to collect this variety in vain but afterwards Dr Kühn still obtained two samples of this old rye variety from the Slovakian Beskids. Interesting for the breeders are the high winter-hardiness, the large grains and almost self-compatibility of some Polish ryes.

Slovakian peas (Pisum sativum)

Peas are in Slovakia the second most important pulse; they are sown in gardens and small field plots, sometimes intercropped with other legumes or in other crop stands. Mostly the different convarieties (such as convar. *speciosum*, field pea, convar. *axiphium*, sugar pea) are grown in pure stands but almost always consist of several botanical varieties. A collection from central Slovakia, for instance, had 7 varieties of convar. *speciosum* and one of convar. *sativum*. Conspicously were sugar pea races with extremely broad green pods used as fresh vegetable, also the convar. *medullare*, vegetable peas with wrinkled seeds, have shown considerable variation; half of the botanical varieties described in the classification of Lehmann (1954) could be recognized in the Slovakian material. Most variable are the field peas, some of them used as shelled peas in the dry state for soups and porridge and partly grown together with the white-flowered convar. *sativum*. Especially interesting have been field pea races collected as admixtures in fields of spring cereals (oat, barley, rye) at higher altitudes till to the upper limit of agriculture. These peas (seen also in the mountainous regions of southern Poland) were extremely small-flowered, had very small pods (3.5–5 cm lang) and very small grains (diameter 3.5–5.5 mm, weight of 1 000 seed 50–62 g). The populations varied distinctly also in other respects e.g. the flower color and possessed some primitive characters, e.g. very long inflorescences typical for wild peas. The plants are rather sparsely distributed in the cereal fields and seemed to be tolerated by the farmers; sometimes they eat the unripe pods. Maybe they represent relics of former cultivation of these primitive types.

Slovakian beans (Phaseolus spp.)

These are the most important grain legumes in the visited area and they must contribute considerably to the nutrition of the inhabitants. The genus is represented by *Ph. vulgaris* in the lower and somewhat warmer zones; at higher, more humid and cooler altitudes it is mostly replaced by *Ph. coccineus*, although very often mixtures of both species can be seen. They are sown in all gardens, small fields, at the edge of other crops, sometimes in intercropping, for instance with maize. Mostly the dry seeds are consumed, less often the green pods as a vegetable. The *Phaseolus* beans were the most variable legume plants, 10 samples could be separated into 70 lines differing in seed size, form and color pattern and one sample contained already 16 seed variants. Within the material, pole beans were dominant, but nearly all samples had also some bush types (var. *nanus*). Other characters were highly polymorphic too, the length of the pod, for instance, ranges from 8 to 19 cm. Interesting are pole beans with a distinct anthocyanin coloration of the leaves, the stem and the young pods which become finally dark violet. Such beans much resemble varieties known as 'Blaue Speck' in our country 40–50 years ago. They were observed several times also in southern Poland. The very late maturing *Ph. coccineus* too shows a distinct variation in seed and flower color. For breeding, some early ripening lines of *Ph. vulgaris* with a high fertility may be of interest.

Poppy (Papaver somniferum)

The poppy is a very popular crop widespread everywhere in gardens, field plots or intermixed with other species. Only at higher altitudes is it impossible to cultivate the poppy; the seeds are used in baking and for preparation of special macaronis but not for oil. The variation in most samples was striking, cross-pollination of the different types within the fields and between neighboring fields and gardens contributes significantly to the heterogeneous picture of the populations. Even the offspring of selected material (for seed color and capsule characters) have a wide range of segregations of the mentioned characters and a wide variation in others like plant height, flower color, waxiness of the stems and branching. Interestingly enough, some samples contain or consist almost exclusively of plants with shattering capsules, often possessing some other primitive characters too (small capsules, branched stems). Large poppy populations were mostly characterized by the occurrence of these shattering types; in one place we observed them representing a kind of semi-cultivation where the plants propagated themselves and were only harvested together with plantings of non-shattering types nearby in the garden.

Cabbage(Brassica oleracea)

A special race of cabbage is grown in the mountains with a high precipitation. It seems to be limited to the western part of the Carpathians. The race is characterized by a rather long main stem (60–70 cm), by much foliage along the stem and around the comparatively small heads, by an intermediate color of the heads varying between typical red to white cabbage and by long-stalked stem leaves. It is a rather late-maturing variety and under Gatersleben conditions unsatisfactory but seemed to be preferred by the local farmers partly because one can use the stem leaves for forage or as vegetable long before the heads could be cut.

Relic crops

During travels in Slovakia and Poland, we discovered also the cultivation of some relic crops thought to have already disappeared at least within this part of Europe. First should be mentioned emmer wheat (*Triticum dicoccum*), which Kühn (1970) reported from a few localities in Slovakia and which was seen still in cultivation in two villages in the White Carpathians and the Krupina mountains. The emmer fields had an area of at most 5 000 m^2, the few farmers still cultivating the species used the grains for porridge, soups and sausages. Some decades ago the cultivation was much more common, older people knew this crop well and informed us often about its former cultivation. The Slovakian emmer belings to the ssp. *volgense*, which was also observed in archeological remains within this area (Kühn 1960). Other relic crops for the whole of central Europe were chickpea (*Cicer arietinum*) and vetchling (*Lathyrus sativus*) grown still in some places in southern and western Slovakia, either in gardens or on small field plots. The dry seeds are used for soups. The chickpea samples were rather uniform, but *Lathyrus* shows distinct variation in flower color, seed size and seed coat spots. They sometimes resemble the Mediterranean types, sometimes the European or even the Asiatic ones of this species. After World War II, the cultivation of these two crops was also much more widespread than now.

In the same regions some safflower (*Carthamus tinctorius*) is still cultivated, only as patches of a few plants, their flowers being used for food dyeing.

These are some examples of floristically interesting parts of the collection. Worth mention are also the small-scale shifting cultivation, which still continues in the eastern-most parts of Slovakia by the Ukrainian minority in those districts. The former forest ground is cultivated for a period of 4–5 years, and buckwheat and millets (*Setaria italica*) are characteristic species of these fields (Hammer 1978).

Multiplication, maintenance and evaluation

All the material from the different trips, we are now propagating and it will be maintained in Gatersleben and in institutions in Poland and Czechoslovakia. Preliminary observations proved a high variation within these collections for characters like disease resistance, winter-hardiness, protein content, earliness and other economically important features as has already been mentioned. We are sure that some of the strains could be immediately incorporated into breeding programs, for instance resorcinol-free ryes from Slovakia, very winter-hardy ryes from Poland, apparently also resistant to lodging, early ripening peas and beans.

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Preservation of genetic resources in grasses

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Index words

Diversity of grasses, river valleys, world distribution of *Poa pratensis* clones.

Summary

Preservation of genetic diversity in grasses requires both long-term storage of seeds in gene banks and conservation of suitable natural and semi-natural vegetation. Conservation is necessary because only a small proportion of the genetic resources is yet available as seed. The genetic diversity in grasses is correlated with the diversity of the environment, above all with the microhabitat. Identification of apomictic seed-clones in *Poa pratensis* on the basis of detailed morphological characteristics showed that some seed clones are spread over Europe and the United States.

Introduction

Some information is presented on the genetic diversity of grasses in the Netherlands. As in other countries, gene banks are being set up and the question arises what priority grasses should have among the crops to be included in these gene banks. The economic importance of grasses is evident. In the Netherlands, a third of the farmed area is under arable crops and two-thirds is under grassland. A considerable area more is covered with grass for other purposes such as lawns. It is not easy to establish exactly to what extent the genetic variation is being reduced by the introduction of newly bred varieties. But certainly much of the natural and semi-natural vegetation changes with modern agricultural management or intensive use for recreation; and much gives way to cities and highways.

Diversity on a microscale

Biosystematic studies on plants of *Agrostis* spp. and *Festuca* spp. collected in the wild showed that a remarkably large variation can sometimes be found over a small distance. The greatest genetic variation was found in the river valleys. This is probably attributable to two causes which act together: a rich supply of genotypes from upstream and a great diversity of environmental factors like soil type, moisture, microclimate and management.

Recently an evaluation study of the river flats of the Meuse and Rhine distributaries has been published (de Soet et al. 1976). Their maps give a good impression of the environmental and botanic diversity.

This evaluation was based on general criteria; neither detailed studies of the grass species nor the genetic diversity within the grass species were taken into account. It is likely that many of these grasslands contain a wealth of genetic variation, only a very small part of which has reached the plant breeder.

I therefore believe that for grasses we have not only a duty to store genetic resources in gene banks, but also to preserve rich natural and semi-natural vegetations. This is true also for the limestone grasslands in the south of Limburg, for the dunes, the coastal washes and all other areas that contribute to the genetic diversity of grasses in our country. We should compile data to indicate these areas more precisely.

Lack of diversity on a macroscale

At our institute, many provenances of *Poa pratensis* have been tested over the years. They propagate by apomixis: instead of a fertilized egg cell, an undivided cell of the parent plant develops into an embryo. Apart from occasional aberrants, the offspring of one plant is genetically uniform. Such an offspring is called a seed clone. Among the material tested in the Netherlands, no other way of reproduction has been met as yet.

Since the species *Poa pratensis* is widespread throughout the world and since each plant represents a potential cultivar, it was to be expected that the number of genotypes would be practically unlimited. To establish distinctness many detailed morphological attributes are compared, fifteen of which are listed in appendixes 1–3.

As far as we can judge, seed clones identical in these fifteen attributes are identical also in other respects such as morphology or phenol reaction of the seed and probably electrophoresis patterns. In recent years, plant breeders have collected thousands of seed clones, among which those were selected that gave a dense, healthy and persistent vegetative growth and a good seed yield.

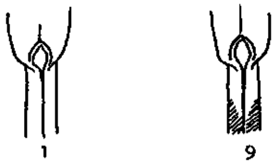
It is now becoming clearer that only a few seed-clones show this combination of properties to a satisfactory degree and that these 'successful' seed clones have a wide distribution. They turn up repeatedly in collections both from Europe and the United States. In 1977, 12 out of 24 applications for registration of *Poa pratensis* cultivars had to be rejected through lack of distinctness from already registered cultivars. Evidently the worldwide variation is much more restricted than one would suppose. Collecting far away, therefore, is no guarantee of greater diversity than collecting on one's doorstep.

Reference

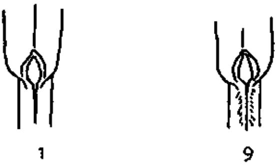
Soet, F. de, 1976. De waarden van de uiterwaarden. (The values of the river flats). Pudoc, Wageningen.

Appendix 1. Characters used for identification of seed clones of *Poa pratensis.*

1. Leaf sheath : anthocyanin coloration
   (seedling; 6–10 leaves)

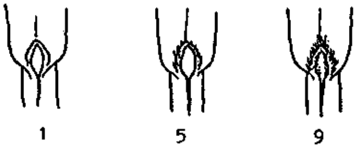


2. Leaf sheath : hairs on margin
   (as for 1)

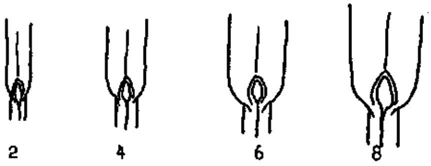


3. Leaf sheath : hairs on both sides just beneath the leaf blade
   (as for 1)

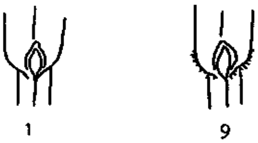
4. Leaf sheath : hairiness of the ligule
   (as for 1)



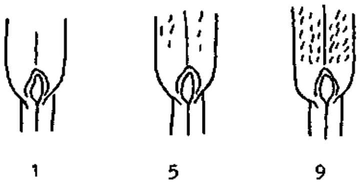
5. Leaf blade : width
   (autumn of year of sowing)



6. Leaf blade : fringe of hairs on margin of base
   (as for 1)

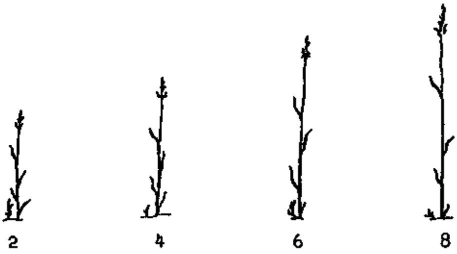


7. Leaf blade : hairs on upper side
   (as for 1)

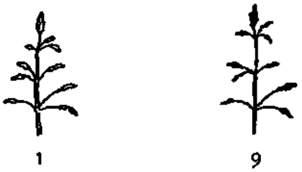


8. Leaf blade : hairs on lower side
   (as for 1)

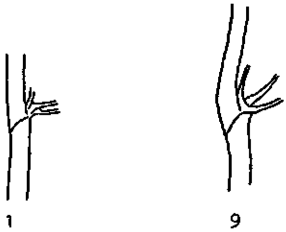
9. Stem length (including flowering head at end of flowering)



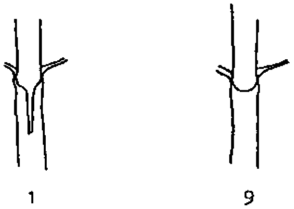
10. Flowering head : anthocyanin coloration (at flowering)



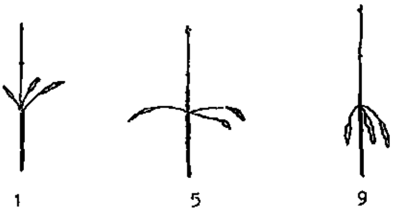
11. Flowering head : shape of rachis opposite lower side-branches
(as for 10)



12. Flowering head : form of collar of rachis opposite lower side-branches
(as for 10)



13. Flowering head : attitude of lower side-branches
(after anthesis)



14. Seed : size

15. Time of heading (mean date in second year)

early	medium late	medium early	late
2	4	6	8

Appendix 2. *Poa pratensis*. Classification of cultivars by three attributes.

Anthocyanin-coloration in leaf sheath	Hairs on upper side of leaf blade							
	Ligule without hairs (1)			Ligule with short hairs (5)			Ligule with long hairs (9)	
	absent (1)	sparse (5)	dense (9)	absent (1)	sparse (5)	dense (9)	absent (1)	dense (9)
absent or very weak (1)	Fylking Prato Enmundi Encrona Kimono Enwarto Turnier	Merion Windsor Captan Enoble		Golf Baron Parade Entensa Birka Sydsport Pion Pac Mosa	Geronimo Enprima		Enita Nugget Ardita	
present (9)	Atlas Delft Baronie Monopoly Ensema Enaldo	Delta Adorno Pondorosa Dynamo Arena	Park Apoll Bartense	Späth's Newport Minimo Trampas Taisetsu	Aquila Campina	Barones Entopper	Barkenta Arista Olymprisp Continental Orna Barzan	Modena

Appendix 3. Characteristics of *Poa pratensis* cultivars.

cultivar	*leaf sheath*				*leaf blade*					*flowering head*					
	anthocyanin coloration	hairs on margin	hairs beneath leaf blade	hairiness of the ligule	width	hairs on margin	hairs on upper side	hairs on lower side	stem length	anthocyanin coloration	shape of rachis	form of collar	attitude of lower side branches	seed size	heading
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Adorno	9	1	1	1	2	1	5	1	4	1	1	9	5	1	2
Apoll	9	1	1	1	8	9	9	1	8	1	1	1	5	9	4
Aquila	9	1	9	5	6	9	5	1	2	9	1	9	5	9	6
Ardita	1	9	9	9	6	9	1	1	4	1	1	1	5	9	6
Arena	9	1	1	1	4	9	5	1	8	1	1	1	5	9	4
Arista	9	9	9	9	6	9	1	1	2	1	1	9	5	9	6
Atlas	9	1	1	1	8	9	1	1	8	1	1	9	5	9	2
Barkenta	9	9	9	9	8	9	1	1	4	9	1	9	5	9	4
Baron	1	9	9	5	8	9	1	1	2	9	9	1	5	9	8
Barones	9	9	9	5	6	9	9	1	2	9	1	9	5	9	6
Baronie	9	1	1	1	4	9	1	1	2	9	1	1	5	9	6
Bartense	9	9	9	1	8	9	9	1	4	9	1	1	1	9	6
Barzan	9	9	9	9	6	9	1	1	2	9	1	9	5	9	6
Birka	1	9	9	5	6	9	1	1	2	9	1	1	5	9	8
Campina	9	9	9	5	6	9	5	1	2	9	1	9	5	9	8
Captan	1	1	1	1	6	9	5	1	4	1	1	9	9	9	4
Continental	9	9	9	9	6	9	1	1	2	1	1	9	5	9	8
Delft	9	9	9	1	6	9	1	1	4	1	1	1	5	9	4
Delta	9	1	1	1	4	1	5	1	6	1	1	1	5	1	2
Dynamo	9	1	1	1	8	9	5	1	8	9	9	1	5	9	4
Enaldo	9	9	9	1	8	9	1	1	6	9	1	1	5	9	4
Encrona	1	1	1	1	4	9	1	1	2	9	1	1	5	9	4
Enita	1	1	9	9	6	9	1	1	2	9	1	1	5	9	8
Enmundi	1	1	1	1	6	9	1	1	2	1	1	1	5	9	8
Enoble	1	1	1	1	8	1	5	1	2	9	1	1	5	9	4
Enprima	1	9	9	5	4	9	5	1	4	1	1	1	5	9	4
Ensema	9	1	1	1	8	9	1	1	8	9	1	1	5	1	4
Entensa	1	9	9	5	6	9	1	1	6	9	1	1	5	1	8
Entopper	9	9	9	5	8	9	9	1	4	9	1	1	5	9	8
Enwarto	1	9	1	1	6	9	1	1	4	9	9	1	5	9	8
Fylking	1	1	1	1	6	9	1	1	2	1	1	1	5	9	4
Geronimo	1	9	9	5	6	9	5	1	4	9	9	1	5	9	6
Golf	1	9	9	5	8	9	1	1	2	9	9	1	5	9	8
Kimono	1	1	1	1	6	9	1	1	2	1	1	1	5	9	6
Merion	1	9	1	1	6	9	5	1	4	1	9	9	5	1	8
Minimo	9	9	9	5	6	9	1	1	2	9	1	9	5	9	4
Modena	9	9	9	9	6	9	9	9	2	1	1	1	5	9	6
Monopoly	9	9	1	1	8	9	1	1	8	1	9	1	5	1	8
Mosa	1	1	9	5	8	9	1	1	4	9	1	1	5	9	8
Newport	9	9	1	5	6	9	1	1	6	1	1	1	5	9	6
Nugget	1	9	9	9	4	9	1	9	2	9	1	1	5	9	4
Olymprisp	9	9	9	9	4	9	1	1	4	1	1	1	5	9	6
Orna	9	9	9	9	8	9	1	1	4	9	1	9	5	9	4
Pac	1	9	9	5	6	9	1	1	2	1	1	1	5	9	4

Appendix 3. (*continued*)

cultivar	*leaf sheath*				*leaf blade*					*flowering head*					
	anthocyanin coloration	hairs on margin	hairs beneath leaf blade	hairiness of the ligule	width	hairs on margin	hairs on upper side	hairs on lower side	stem length	anthocyanin coloration	shape of rachis	form of collar	attitude of lower side branches	seed size	heading
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Parade	1	9	9	5	6	9	1	1	6	1	1	9	5	9	8
Park	9	1	1	1	4	9	9	1	6	1	1	1	5	9	4
Pion	1	9	9	5	8	9	1	1	8	1	9	1	5	1	8
Pondorosa	9	1	1	1	2	9	5	1	6	9	9	1	5	1	2
Prato	1	1	9	1	6	9	1	1	2	1	1	1	9	9	6
Späth's	9	9	1	5	8	9	1	1	4	9	1	9	5	9	6
Sydsport	1	9	9	5	8	9	1	1	4	9	1	1	5	1	8
Taisetsu	9	9	9	5	8	9	1	1	4	9	1	1	5	9	6
Trampas	9	9	9	5	6	9	1	1	2	9	1	9	5	9	4
Turnier	1	1	1	1	4	9	1	1	4	9	1	1	5	9	6
Windsor	1	1	9	1	6	9	5	1	6	1	1	1	5	9	4

Proc. Conf. Broadening Genet. Base Crops, Wageningen, 1978. Pudoc, Wageningen. 1979.

Wheat collecting in the Mediterranean region

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Index words

Triticum sp., collections of wheats, genetic resources, genetic erosion, wheat distribution, Mediterranean region.

Summary

After a short description of the possible routes that wheats may have taken in their migration through the Mediterranean Basin, the situation of the genetic resources of this crop in the region is reviewed. Short reports are given for Sicily and Cyprus already described by several scientists and for Libya and Morocco, while much more attention is paid to the situation in Greece, Algeria, Tunisia and Spain, where the author has been active in exploration and collection missions.

The urgent need for further exploration and collection trips is stressed.

Spread of wheats

The Mediterranean has been one of the centres of diversity of wheats, with tetraploids attaining a high degree of genetic variability (Vavilov 1951). A number of studies have been made on these wheats. Many of them concentrated on the botanical description of different forms, and very little attention was paid to frequencies of forms in populations from different areas; in addition, many intermediate forms were ignored. In spite of this limitation, these studies allow some considerations about the migration of wheats in the Mediterranean Basin.

From the analysis of wheat forms from Italy, in comparison to those from other Mediterranean parts, Ciferri & Bonvicini (1960) confirmed the hypothesis of Schreiber (1934), who states that the two main Italian islands, Sicily and Sardinia, represent a bridge for tetraploid wheats displacing from east to south and west.

A very large number of botanical forms of *T. turgidum* were displaced from Rhodos and Anatolia, Turkey and concentrated in Sicily, from where many of them spread to the southern part of the Italian peninsula and to North Africa. On the contrary, a few botanical forms of *T. durum*, characterized by a very large number of cultivars, originated from the Sirio-Palestinian area, crossed Cyprus and

Crete and concentrated in Sardinia. From there they preferable passed to the Iberian peninsula and the western part of North Africa, to constitute a western geographical unit, very different from the eastern one.

Other migrations were direct from central Greece to the Balkan region and to the Italian peninsula, where they spread, pushing back the wheats coming up from Sicily.

The introduction of common wheats in the Mediterranean basin seems much more recent and not yet completely clarified. The natural hybridization, extensive in the past, prevents the verification of any hypothesis.

Breeding wheat varieties

Breeders started to utilize this variation at the end of the last century by selecting and putting into cultivation the best lines from the original land races, and since the very beginning of the present century by crossing good lines with introduced germplasm.

Also genetic erosion started at that time. New introductions occurred at that period: displaced from Asia Minor, Greek populations brought with them many Turkish cultivars; extensive exchanges of seeds occurred, for instances, between Greece and Italy, the Mediterranean islands and North Africa; North Africa with France and Spain; wheats were also introduced from the Black see area and from Australia.

Initial collections

In the 1920s and 1930s, there were exploration and collection missions, but unfortunately the material was directly used in classification studies and no attempt was made to preserve it in the original form. A few 'purified' lines or selections from the original populations remain preserved in some institutions, but most of the material, classified as useless, has long since been discarded.

Recent collections

The danger of genetic erosion, in spite of repeated appeals from some scientists, was not fully appreciated until the mid-sixties, when several researchers, careful observers of evolution in agriculture, launched an urgent appeal for measures to be taken to preserve the existing natural genetic diversity (Frankel & Hawkes 1975). As a result of these new appeals, gene banks have been established, whose purpose it is to explore and preserve remaining germplasm.

The situation of wheat germplasm in some Mediterranean areas was summarized by Bennett (1973); those situations will be briefly reviewed in order to draw attention to areas that were unexplored then.

Sicily (Italy)

Sicily occupies a central position in the Mediterranean, very close to the mainland of Italy, which has experienced enormous economic and social changes in the past 30 years. In this period, agricultural techniques have changed, new types of seed have been distributed and an efficient road network has opened up the island. It was assumed, therefore, that genetic erosion would be extensive. Indeed, many extensive areas only grow uniform modern Italian cultivars. However, a surprising amount of variation still exists. In addition to forms that can be found mixed with modern cultivars, landraces still formed the bulk of the crop in the interior of the island, outside the major wheat-growing areas.

Cyprus

In Cyprus, in spite of the energetic and successful wheat-breeding program by the Ministry of Agriculture, the most important landraces were still widely cultivated, though undoubtedly they now possess a much more uniform aspect than previously.

Greece

In Greece, in spite of the former diversity of Greek wheats, and their widespread distribution within the country, genetic diversity is much reduced. The plains of Macedonia, Thessaly and the south-eastern part of the Peloponnesus – are the areas of more intensive wheat cultivation and the whole wheat crop now grown consists of modern cultivars. In Crete, the main plains, like the Messara one, are almost completely planted to vegetable crops and fruit trees, and wheat is no longer cultivated. The same happened in the plains of Arta and Preveza in Ipiros. As a consequence, existing genetic resources of wheat have been driven back to isolated mountain areas. Of 11 landraces reported by Papadakis (1929) to be present in Ipiros, 9 were found and collected in the provinces of Igoumanitsa and Ionnina close to the Albanian border. In this rugged mountainous region, in which cropping is usually in small plots, covering only 15% of the total surface, farmers still use landraces, although they are probably continuously evolving mixtures determined by threshing machines. In many cases, however, the finding of an old cultivar was the result of a long and patient search from village to village for hours and days. A few landraces have in fact replaced the former rich spectrum of variation, constituting severe genetic erosion.

Quite similar is the situation on mountains of Crete, where landraces are present on fields at altitudes of 300–700 m. Recently introduced cultivars are still relatively rare in these mountains but they are increasing rapidly because considerable amounts of seed have been and continue to be distributed by the agricultural banks and credit organizations. Although names like Asprostaro, Kondrostaro, Deves, Grinias, Katranitsa, Kokkinostaro, Koutrulias, Mavragani, Roussias, Mevroteris, Rovakis and Levendis can still be heard in Ipiros and on Crete, the mountain wheats tend to consist of improved or introduced cultivars.

North-Africa

As said before, the wheats of North-Africa derive from the contacts that these regions have had with the two major Mediterranean islands, Sicily and Sardinia, in one direction and with people south of the Sahara in the other, especially the caravans and the nomads who travelled from the Red Sea to Morocco with their commerce and raw materials. Testimony of this wealth of forms is found in Orlov (1922), who classified more than 22 botanical forms of Algerian durum wheat, each of which included many types.

This great variation is disappearing, being replaced by the uniformity of the new selections. This is a rather common fact tied to agricultural progress. Rather peculiar is the way with which genetic erosion is taking place.

Plant breeding started in North Africa during the first decade of this century utilizing indigenous germplasm with the aim of saving gene blocks that, for instance, conferred adaptation to local conditions and resistance to the various plant diseases. The new selections spread rather quickly, occupying vast areas at the expense of the old local populations.

This vast diffusion even to zones for which they were not selected, soon brought to light the limits of the new cultivars. After World War I, the first losses occurred due to septoriosis, a disease well tolerated by the previous landraces.

The spread through cultivation of cultivars imported or obtained with introduced germplasm made even greater the danger of genetic erosion and, although the usefulness and indispensability of original material would seem obvious for progress in wheat breeding, nobody collected and preserved the indigenous germplasm.

Algeria

After World War II and, especially after Tunisia and Algeria gained their independence, local wheats started to disappear. In addition to the danger from importation and cultivation of selected foreign varieties, an even greater danger is that, according to forecasts, wheat germplasm will completely disappear within a few years.

The reasons are different from region to region. In the *Atlas Mountains* at te time of the country's independence, the lands left by the French became State properties and their cultivation was entrusted, in various forms, to cooperatives made up of farmers who had worked the land previously. A single cooperative administers several square kilometers. It furnishes the machinery, seeds and other supplies and harvests the crop. In this way, enormous areas are planted with one or only a few cultivars. The seed comes from a single stock and is multiplied and maintained in varietal purity.

In fact, in this area only three indigenous cultivars of durum wheat are now cultivated: Hebda 3; Mohamed ben Bachir 8037; Oued Zenati 368. They were selected from landraces and their seed is produced under the supervision of a technical commission which is particularly stringent about varietal purity.

The genetic resources are further endangered by the fact that many farmers who cultivate small pieces of land of their own are tempted to use seed from the cooperative to be sure to sell their crops. In this way, the landraces are confined to small areas situated in marginal agricultural zones, where the harvest is mainly utilized for family consumption.

In the Constantine Highlands, the replacement of the landraces has gone on for a long time and so are rather difficult to find.

Only north of Setif, in a mountainous and agriculturally marginal zone was it possible still to find populations reproduced by the farmers since time immemorial. They are made up of mixtures of lines, some very different from others, from which it was possible to identify Mohamed ben Bachir (but the ear is generally smaller), Hebda, Oued Zenati and Biskri. Nineteen forms of ears were identified among the 26 samples obtained, some with a very low frequency. Very similar is the situation on the Chelif Plain between Algiers and Oran.

In the *oasis* during the last few years, a reduction in the area under some crops has occurred to the advantage of some vegetables used as a cash crop. The cultivation of wheat has decreased notably since 1965 and today has almost completely disappeared from the oases around Ouargla, El Oued and Adrar, while it is still relatively important around Touggourt, Timimoun and especially in Biskra. In the past, these represented the typical zones of supply of cereals to caravans that crossed the desert.

On the eastern oasis around Touggourt, Ourgla, El Oued, where the wheat is grown only under the palms, 11 samples of durum and common wheat were collected. Some of these are supposed to be local selections of the 'Bel Mabrouk' population.

On the oasis of Biskra, where the flora of the steppes becomes richer and denser and where cultivation without protection of palms is more frequent, 9 samples of wheat were obtained.

In the Timimoun region, only 3 populations of wheat were found, not many when compared with the 18 landraces which, it is said, existed in the past. On the Tarhit and Adrar oases, the cultivation of wheat and barley has almost completely disappeared.

Remarkably different is the situation around Laghouat, a region situated immediately south of the eastern Atlas at an average altitude of 600 m and with an average annual rainfall of about 200 mm. There wheats proved to be rich in variation and made possible the identification of several landraces.

Completely different is the situation in the *Hoggar* region. Contacts of the Hoggar with the rest of the Sahara (including Libya, Sudan and Niger) depend mostly or entirely on caravans while those between Tamanrasset and Algiers are established by lorries and a few tourist flights. Because of the nature of the mountains and the economic conditions of the region, internal communications are very poor.

In these conditions genetic erosion cannot be considered very dangerous. In particular for wheat, the Touaregs prefer to cultivate the landraces instead of the new ones provided by the Ministry of Agriculture, because the former have a shorter cycle and other not well defined advantages. What happens then, is, that Touaregs eat the seeds given from the State and continue to cultivate their own cultivars.

In spite of this, there are two aspects which have to be emphasized:

– From the time of the Algerian independence, many young people have left and continue to leave the Hoggar or the oasis for work in the National Societies, in the northern part of the country.

– As a consequence of a dry cycle started about 15 years ago and probably because of the emigration mentioned above, some oasis were abandoned.

In these cases, some farmers again became nomads and the most probable fate of their seeds and plants was to be lost for ever.

Tunisia

As far as Tunisia is concerned, three different sections can be considered. The Northern mountains and coastal ranges, where field crops have been replaced by fruit trees and vegetables; the El Kef province, a continuation of the Atlas mountains, where old local varieties still exist and have the same form as on the Algerian side. The Oasis sections, where the surface area under wheat is rather limited as most of the cultivated land is grown to fruit trees and vegetables, especially in the coastal zone; wheat crops are often utilized for sheep grazing. Along side new varieties, like Inrat 69 or Cocorit, however, one can still find old local varities like Mahmoudi, Biskri, Hamra, Bidi, or other varieties that were introduced during the French period, as Provence, Hile Hirondelle, Florence-Aurore, etc.

Libya

In Libya, the distribution of wheats is determined by their adaptability to the prevailing climate and soil conditions. Durum wheat is predominantly cultivated in the heavy clay soils of the plateaux, in Cyrenaica and Tripolitania, where rainfall is relatively high (> 700 mm), and the growing season is cooler and longer. The leading varieties are Mahmoudi and Hamra, followed by Obaica, Flaiha, Meezko, Mogherabiya, Tawaliya. Common wheats are generally grown either under rainfall conditions in the coastal plains, or under irrigation in the oases. The main variety is Ithkair, followed by Aourig, Baush, Farina, Fratizza, Khresi and Soukni (Al Jibouri 1966). Apparently, genetic erosion is not too strong though some has been introduced with the seeds of foreign released varieties.

Egypt

An exploration and collecting expedition to Egypt was organized by members of the Laboratory's staff in spring this year (1978). It was found that genetic erosion is extremely strong in the northern sections of the country. In the southern section, land races still supply a remarkable source of genetic variation which has not yet been eroded. Old bread wheat populations can still be found in Upper Egypt, more readily so in the New Valley where the varieties were introduced only a few years ago.

With durum wheat, the situation is altogether different and much more favourable. Durums can be traced quite easily since their genetic heritage has not yet suffered from any severe erosion due to the fact that very few new varieties, if any at all, have been hitherto introduced into the country. Of special interest is the possibility of collecting land races in the Upper Nile Valley.

Morocco

Little is known about the state of wheat genetic resources in Morocco. Still, whatever breeding programmes have thus far been started and the rather unfrequent and relatively scanty introduction into the country, and distribution to farmers, of seeds from varieties released abroad, seem to suggest that here again a remarkable number of old cultivated forms could be found and collected.

Spain

The presence of wheats in Spain dates back to about 4 500–4 000 B.C. as shown by the findings of Muñoz-Arnilibia in the caves around Alicante and Cordoba (Salazar 1978). *T. aestivum* appears to be the oldest, and dicoccums and turgidums a more recent form, about the 3rd century B.C. (Tellez & Ciferri 1954). At the beginning of the 19th century, Clemente and Lagasca carried out an intensive survey on wheats present at that time, many of which are now to be regarded as obsolete. At the beginning of this century, Arana initiated the search of samples of *Triticum* which by that time showed a great variety of forms; this collection was latter enriched by Alonso Peña, Tellez, Pozuelo and Salazar (Salazar 1978). The majority of these collections have been maintained at INIA and were described by Sanchez-Monge (1957) and Gadea (1954). At present, most of the indigenous forms are disappearing. Among diploids, *T. boeoticum* can still be found along the banks of the Jucar river in the province of Cuenca, and *T. monococcum* in the fields of the same province, whereas formerly it used to be cultivated in at least ten different provinces.

Among tetraploids, the dicoccums are restricted to the mountains of the Asturias and Navarra. No evidence has been left of the once cultivated *T. polonicum*, *T. durum* and *T. turgidum* populations. Formerly widely grown all over the country with numerous local forms, they are now practically restricted to the mountains of Estremadura, Andalusia and Valencia.

Hexaploids are represented by *T. spelta* and *T. aestivum*. The former has been found mainly in the Asturias, whereas the latter was and is still present all over Spain, though old local varieties are mainly confined to Galicia and Costa Vasca. The general impression reported by scientists from the Laboratory, who visited Spain in 1977, was that irrespective of the species, variability in each field is rather, limited, since crops are dominated by one spike form, while other forms can be found through painstaking searching.

Since seed of improved varieties is at present being distributed by official centres and private firms, genetic erosion is very much advanced and very little time is left for exploration and collection activities in this region.

Portugal

Portuguese wheats were carefully described by Vasconcellos (1933) who identified 71 indigenous forms of which about 80% were tetraploids. From information received, it would seem that in very remote districts of the country there are still being cultivated some 40 varieties of durum wheat, 16 of bread wheat and one of *T.*

polonicum. Those varieties that existed were carefully picked in the past, particularly by the Elvas Plant Breeding Station, which was established immediately after World War II as a Germplasm Station and had started, in this capacity, an accurate work of exploration and gathering both inside and outside the country. Thanks to this work, Portuguese wheats are fairly well represented in today's world collections. According to Portuguese experts, the possibility of finding old forms of wheat which are not adequately represented in the world collections is at present restricted to the mountain areas north-east of the country where tetraploid wheat predominates.

General conclusion

As a general conclusion to the surveys made and exploration and collection work carried out to date, one can say that the situation with regard to the genetic resources of wheat in the Mediterranean region varies considerably from one country to another and is closely related to the migrations of peoples and to the development of agricultural research in general, and of breeding work in particular. Furthermore, things are different depending on whether tetraploids or hexaploids are considered. It goes without saying, however, that the overall situation is extremely serious, that genetic erosion is by now very much advanced or is becoming so very rapidly in certain areas, and that there is very little time left for whoever is going to collect these precious resources which nature and earlier civilizations have bestowed upon us and which it is our duty to save and hand down to future generations.

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Collections of forage grass in Europe

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Index words

Lolium, Dactylis, Festuca. grasses, plant collection, genetic variation, Europe.

Summary

Improved grass varieties for intensive grassland systems have a relatively short history of development. Initially genetic variation for breeding was obtained from natural pastures in the U.K., but in the last two decades extension of the variation for seasonal distribution of yield, herbage quality and resistance to climatic stress has increasingly been sought by plant collecting expeditions to continental Europe.

The relationships between environment of origin and plant growth potentials both in the field at Aberystwyth and in controlled environments has proved invaluable in predicting suitable collection areas. Based on these predictions valuable collections of primitive cultivars of *Lolium multiflorum* have been obtained, and of *L. perenne* from Eastern France and Switzerland. The Iberian peninsular and the mountainous regions of southern Europe have proved to be important sources of the wild relatives of cultivated *Dactylis* and *Festuca.*

Collection strategy was, in the first place, largely determined by considerations of macroclimate which resulted in extensive sampling over considerable distances. However, examples of heritable ecotypic differentiation in closely adjacent but contrasting environments suggest that intensive collection, including some degree of sub-sampling when relevant, could maximize genetic variation.

Introduction

Grass has the distinction of being one of the earliest plants used by man, but one of the most recent to be regarded as a crop, i.e. sown as part of a designed farming system. Temperate forage grasses are outbreeders so that sown grassland can revert to a wild state by introgression with adjacent uncultivated grasses, particularly if the standard of management is relaxed. Thus the distinction between cultivated and natural grassland can, in practice, be ill defined. Indeed a gradation exists from wild relatives of cultivated species in non-agricultural habitats, through natural and semi-natural grassland of cultivated species, to sown grassland. However it has been possible in general terms, to group our collections into two of the three categories proposed by Frankel (1973). Firstly, primitive or traditional cultivars, which have not been deliberately altered by man, and secondly wild species related to domesticated species.

Genetic variation for breeding

Breeding of forage grass in the United Kingdom in the 1920s relied largely on the range of variation present in indigenous pastures. However the advances in grassland farming in the late 1950s and the early 1960s required the breeder to incorporate novel combinations of characters into the new grass cultivars. Characteristics considered to be of primary importance were improvements in the seasonal distribution of yield and herbage quality and resistance to climatic stress.

Breeders soon became aware that genetic variation for the growth rhythm required were not expressed in indigenous ecotypes nor in the cultivars available in commerce.

However a considerable body of evidence was then available showing the genetic basis of physiological adaptations to contrasting climates (Turesson 1922; Clausen et al. 1940; Cooper 1941, 1962a) and it was considered that collections of climatic ecotypes would provide the necessary variation to initiate programs.

Prediction of collection areas

In order to optimize our relatively limited resources for plant collection, we attempted to predict the most likely climates that would provide the required growth attributes. This was done initially by reference to work on physiological adaptation in herbage grasses and later by a consideration of the relationships of the growth rhythms of our own collections to environmental origin. Our first objective was a search for populations exhibiting late autumn and early winter growth in order to extend the growing season in the British Isles. It had been demonstrated that a high negative correlation existed between increase in leaf area at 5 °C and latitude of origin in populations of *Lolium perenne* and *Dactylis glomerata* (Cooper 1962b) which suggested that from regions with a Mediterranean or quasi-Mediterranean climate, ecotypes with winter growth under conditions usual for Britain could be obtained. Evaluation of populations obtained on expeditions to these climatic regions confirmed our predictions. In fact populations from the true Mediterranean climate, although of the general growth type anticipated, were too extreme in their summer dormancy and low in productivity; populations from less extreme Mediterranean climates proved more suitable.

Screening of collections has enabled us to predict in a general way suitable collection areas for other characters. Relationships between plant reaction at Aberystwyth and gross environmental parameters at place of origin, have been obtained for the following characters: positive correlations of increase in rust severity with altitude and latitude, and winter greenness with winter temperature; negative correlations of autumn growth with altitude, and winter survival with winter temperature (Tyler 1970; Borrill 1961). Such relationships can form a working hypothesis for selection of collection areas but, as more populations are examined, modifications are sometimes required, for instance in the study of cold tolerance where the influence of autumn irradiation modified the relationship with winter temperature (Lorenzetti et al. 1971).

Many of the above relationships appear to be biologically logical and simply explained. However certain features of considerable importance in the herbage grasses were not so readily resolved. Studies on early spring growth suggested that a positive relationship with spring temperature at origin only existed with populations from regions with mean minimum January temperature range about −2 to +3 °C. The evidence of these empirical relationships does not necessarily imply causality, although it can often give pointers to more detailed investigations, but has been used with some success in identifying collection areas which in turn have provided raw material for the breeding of broader based varieties.

Plant collections

Since the inception of the Plant Introduction Unit at Aberystwyth in 1963, there have been nine European expeditions to climatically characterized areas. Both primitive cultivars and wild relatives of cultivated grasses have been collected and the expeditions will be discussed under these categories.

Primitive cultivars

Within this category, a wide range of types can be recognized related to the intensity of cultivation imposed on them. At one extreme is natural grassland only sporadically browsed by livestock and at the other is semi-natural grassland, which has been intensively and expertly managed. Although not deliberately sown, all these have been influenced to some degree by man and his livestock. Populations from intensive semi-natural grassland are adapted to cultivated situations and are likely to be more amenable to breeding, and as such have been more fully exploited by collectors and breeders. The collections to be described are of this latter type.

Lolium multiflorum from northern Italy. The objective of this expedition was to obtain genetic variation that would maximize growth during the period from late spring to late summer. Prediction based on evaluation of other collections suggested that maximum summer growth would most likely be found in populations from regions of high irradiation in summer, high temperature with no water limitations in summer. These conditions were found in the irrigated meadows on the north Italian plains. Examination of this material showed it to be of considerable value in extending the range of variation in this species particularly for large and rapid summer regrowth, nutritive quality, persistency and canopy features. Susceptibility to fungal diseases and poor seed retention at harvest were serious disadvantages of this material, and with restricted variation for these characters offered little scope for selection. However hybridization with ecotypes of north European origin, which have higher disease resistance and no problems in seed retention, promises to be more successful in combining the desired characters.

These irrigated meadows of northern Italy, situated in the centre of the area of maximum diversity for the Gramineae (Hartley & Williams, 1956) have now been recognized by a number of breeding institutes as a promising source of variation particularly in the genus *Lolium*. The more valuable meadows in this area are the summer and winter irrigated marcita which have an intricately graded system of ridges and channels. Construction and renewal is very labor-intensive and thus expensive, so gradual deterioration and consequent change in botanical composition is a distinct possibility. This and the continual demands of industry and commerce for building land resulting in the loss of some of the finest meadows, point to the need for collection and conservation of seed before a crisis is reached.

Lolium perenne from eastern France and Switzerland. The objective was to extend our pool of genetic variation for winter hardiness and early spring growth in *L. perenne.* Based on the relationships of cold tolerance to winter temperatures and autumn irradiation and spring growth to winter and spring temperatures, collection along a latitudinal transect from Belgium through Luxembourg and eastern France to Switzerland, with altitudinal variation, was carried out. Evaluation showed a similar relationship of spring growth to spring temperature of origin as was obtained previously with *Dactylis* with the exception of populations originating from the Zürich uplands. These gave considerably higher yields than would be anticipated from a consideration of spring temperature. No completely satisfactory explanation of this deviation was found. A significant relationship was observed between spring growth and flowering date over all maturity groups (r = –0,83; $P < 0{,}001$), although small and statistically insignificant within groups (very early, r = –0,47; early, r = 0,33; medium, r = 0,01).

Similar relationships were obtained in a study of cold tolerance and flowering date. This apparent lack of relationship within maturity groups offers hope that winterhardy genotypes with good spring growth without extreme earliness of flowering can be selected.

Wild relations of cultivated grasses

In this category are included species, subspecies and cytological races closely related to the species cultivated in Britain and Western Europe. These were usually species occurring in non-agricultural habitats, although in the more primitive agricultural situations they might form a considerable proportion of the available forage.

Iberian Collections. This region has yielded a number of wild relatives of *Dactylis glomerata* that have given variation outside the range exhibited by the cultivated tetraploid subspecies *glomerata.* In 1964, the diploid relative *D. glomerata* subspecies *lusitanica* was collected in Central Portugal and used as the winter-growing parent in the hybrid cultivar Saborto (Borrill 1961). In the same expedition, populations of another relative *D. marina* were collected from coastal cliffs. Its value only became apparent on screening a wide range of *Dactylis* for digestibility, the main measure of nutritive quality in forage grasses. *D. marina* had higher digestibility well outside the limited range of variation exhibited by the cultivated species, but was extremely unproductive. A breeding program to combine the high digestibility of *D. marina* with the higher yields of *D. glomerata* types has produced a number of synthetics by using a range of *D. glomerata* parents. Agronomic trials have shown a mean annual advantage of three digestibility units over control cultivars with yields of dry matter about approximately 10% lower. However, perhaps more significantly, the normally rapid decrease in quality with maturity was slower in the *marina* hybrids resulting in the high-quality (66% digestibility of dry matter) stage being maintained for 2½ weeks longer than existing cultivars of the same maturity group.

During the same expedition, a diploid *Dactylis*, indistinguishable in the field from the tetraploid subspecies *glomerata* was collected in Galicia. In the United Kingdom, this ecotype has thin soft pale-green leaves, large number of tillers per plant; highly competitive properties and flexibility under contrasting managements, particularly grazing, and has provided variation previously unknown in this genus. This variation could give a new dimension to the use of *Dactylis* in grassland systems.

Festuca collections. Objectives in this group were of a long-term nature, being concerned with the collection and study of the distribution of relatives and possible progenitors of cultivated fescues for a cytotaxonomic study of phyletic relationships within the broad-leaved fescues and *Lolium*, involving the construction of new fertile polyploid types.

Distribution maps were produced, based on some 400 *Festuca* accessions, and have proved useful in corroborating cytotaxonomic data (Borill et al. 1971, 1976). *F. arundinacea* var. *genuina* $2x = 42$) and *F. pratensis* ($2x = 14$), the cultivated species of the broad-leaved fescues (Bovineae) have a very wide distribution in Europe and have been found in most collection sites from Scandinavia, southwards to northern Italy and eastwards to Hungary. *F. arundinacea* appears to be more restricted than *F. pratensis*, being absent in the more northerly regions of Scandinavia, the high Alps and in upland Turkey, whereas *F. pratensis* seems well adapted to these regions of extreme winter cold. In southern Europe the two species were less common, *F. arundinacea* in particular showing ecological preference for infrequent defoliation and wet habitats. The octoploid ($2x = 56$) and decaploid ($2x = 70$) chromosomes races on *F. arundinacea* have only been found in the Atlas Mountains of Morocco, again showing preference for wet habitats in an otherwise summer dry region. The tetraploids ($2x = 28$), *F. arundinacea* var. *glaucescens* and *F. pratensis* var. *apennina* have a mountainous distribution in the French, Italian and Swiss Alps, the former usually associated with disturbed habitats and the latter replacing diploid *F. pratensis* at high altitudes (Tyler et al. 1978). Although the relationships of this polyploid series to the cultivated fescues and to the genus *Lolium* is complex and not yet resolved (Borrill 1976), the potential for broadening the genetic pool is considerable. Intergeneric tetraploid *Festuca*/*Lolium* hybrids (Lewis 1966) and hexaploid *F. pratensis*/*F. pratensis* var. *apennina* hybrids (Chandrasekharan 1968) show considerable promise in this direction and their agronomic value is being intensively studied.

Collection strategy

The heritable variation obtained on the expeditions summarized above has largely been the result of extensive collections where differences in macro-climate have been the major consideration in determining collection area. Our experience on these expeditions and the subsequent characterization of the material, however, has impressed on us the importance of micro-environment. A comparison of the growth rhythm of two adjacent north Italian *L. multiflorum* populations collected from the same macro-climatic zone, one irrigated and the other unirrigated, showed the former to have twice the summer growth at Aberystwyth, where water was not limiting in summer (Tyler 1970). Heritable differences have also been observed within the same meadow. Progeny of *L. perenne* from a heavily trodden path running diagonally through a hay meadow in Valtellina in north Italy was one month later flowering, had considerably less spring growth, and had shorter and more densely tillering seedlings than progeny of plants from the surrounding hay meadow (Tyler & Chorlton 1976). Similar large and significant differences have often been found when subsampling ecologically variable meadows. Examples such as these and more detailed studies of micro-environmental differentiation in forage grasses (Bradshaw 1960) suggest possible modifications of collection strategy for forage grasses. In practice, it is not possible to determine, on site, if selection pressure has been sufficiently strong to establish heritable change. In fact, heritable differences cannot always be demonstrated in all phenotypically variable situations (Tyler & Chorlton 1976). However its occurrence is sufficiently frequent to suggest that such subsampling would be valuable in maximizing the collection of genetic variation where ecologically different situations are encountered, particularly if the general macroclimate and management in the region is otherwise relatively uniform.

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Collecting pyrethrum, Chrysanthemum cinerariaefolium Vis. in Yugoslavia for Kenya

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Index words

Chrysanthemum cinerariaefolium, pyrethrum, collecting, flower yield, pyrethrins content.

Summary

Pyrethrum, grown for the insecticidal pyrethrins extracted from the dried flower heads, is indigenous along the coast and on the islands of Yugoslavia. In 1971, pollen and seed were collected from 18 and 20 sites, respectively. The pollen, sent by airmail to Kenya, was used to fertilize flowers of some clones known to have a good general combining ability. The seed obtained was sown together with the collected seed. Compared with Kenyan varieties, the flower yield of the wild pyrethrum populations was very low, the pyrethrins content considerably reduced, the flower heads smaller, the flower stalks shorter and lodging more severe. Of the seed-derived populations, a large proportion of the plants often remained vegetative, restricting gene exchange. Of the pollen-derived populations, most plants formed flowers. Introgression of genes from wild pyrethrum into cultivated Kenyan pyrethrum may be more effective through the introduction of pollen than through introduction of seed.

Introduction

Pyrethrum, *Chrysanthemum cinerariaefolium* Vis., is a small perennial herb, tending to become woody at the base. The flower heads are produced on branched leafy stems rising from a crown of foliage (Fig. 1). Due to an effective sporophytic incompatibility system, out-crossing is the rule (Brewer & Parlevliet 1969). Multiplication is done by means of seed (cultivars) or splits (clones). The seedlings, 5 to 6 months old from sowing, or splits obtained by dividing old plants (Cormack 1935; Delahye 1968) are planted at the onset of the rains. The flowers are harvested at intervals of two to three weeks over a period of two to four years. The picked flowers are dried and processed to extract the pyrethrins, a group of related compounds with a marked insecticidal action.

Pyrethrum is a relatively recent crop. It was brought into cultivation along the Dalmatian coast of Yugoslavia, where it is indigenous, in the first half of the Century. In Kenya, now the largest producer, it was introduced in 1928 (Beckley

Figure 1. Flowering pyrethrum plant.

1938). Most of the Kenyan pyrethrum can be traced back to two introductions, one from cultivated pyrethrum in Dalmatia (1928) and one from selected material from the Plant Pathological Laboratory of the Ministry of Agriculture, Harpenden, England (1931). To broaden the genetic basis of the Kenyan breeding material, it was thought to introduce wild pyrethrum genes from Dalmatia. For this purpose, pollen and seed were collected in May and July 1971, respectively.

Area and habitat

Pyrethrum occurs along the coast of Yugoslavia from the Italian border into northern Albania. It is restricted to a rather narrow coastal zone, the islands and peninsulas where it is found in the evergreen (wintergreen) plant communities. Some populations, however, may be found at slightly higher altitudes or more inland in deciduous (summergreen) plant communities. The climate is typically Mediterranean with dry and warm summers and wet and mild winters, the mean temperatures of the coldest month ranging from 4 to 8 °C.

Pyrethrum grows predominantly on shallow, rocky or stony sites, slopes or outcrops, fairly exposed or in light shade. It often occurs next to the tarmac roads in the disturbed zone consisting of stones, rocks and sand. It tends to grow in small or large clusters; isolated single plants rarely occur.

Collecting

The area from which populations were collected extended from the island Krk in the north to Hercegnovi about 40 km south-east of Dubrovnik. In May 1971, pollen was collected from 18 sites in the morning from the moment pollen was shed from the anthers. The pollen was tipped into small vials. It was thoroughly dried by placing the open vials directly after collecting in a closed jar over NaOH pellets. After drying for 12 to 14 h, the pollen was packed in small plastic bags, sealed with as little air as possible and dispatched by airmail to Kenya. There the pollen was applied to flowers of certain clones, known to have an excellent general combining ability (Parlevliet & Contant 1970).

In July of the same year, seed was collected from 16 of the 18 sites sampled for pollen and from an additional four sites (Fig. 2). Per site, seed heads were collected from as many plants as possible. Most sites represented natural populations, a few, however, showed signs of cultivation. Site Z5 was presumably a pyrethrum field abandoned several years ago, and run wild. At Z10, Z11 and Z14, the pyrethrum occurred as a cultivated weed, kept clean from other weeds after natural emergence. At Z15 the pyrethrum was planted and cultivated, but no longer harvested. In the

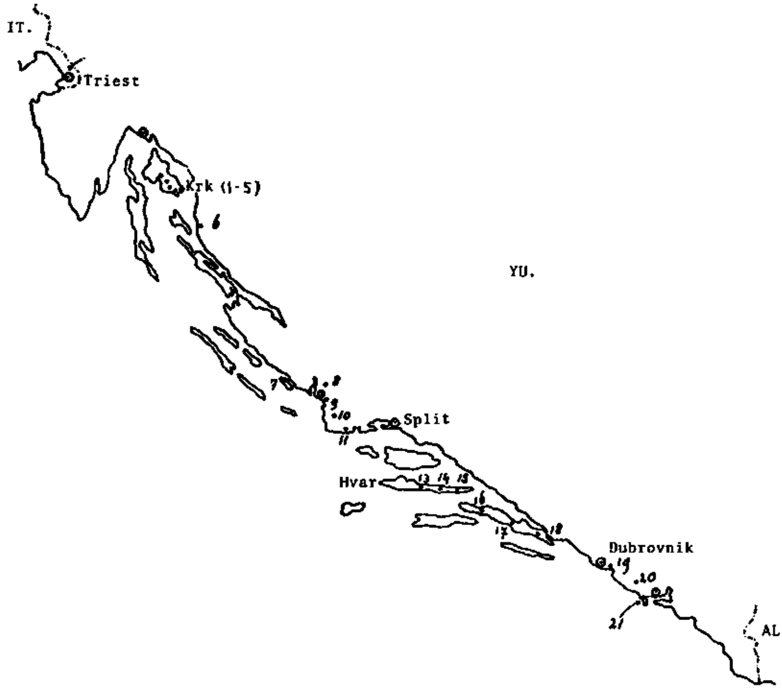

Figure 2. The Dalmatian coast with the approximate location of the 20 sites from which pyrethrum seed was collected. The site numbers in this figure correspond with the Z-numbers in text and tables.

surroundings of this field wild pyrethrum plants occurred abundantly. This situation apparently marks the end of an era of pyrethrum production, where cultivated and wild pyrethrum flowered simultaneously and in close proximity, ensuring mutual gene exchange, a situation which lasted for approximately one hundred years.

Inventory

Seed of each of the 20 populations and of two Kenyan cultivars were sown in the autumn of 1971 and transplanted into replicated yield trials at Molo (2 550 m) and Subukia (2 100 m) in May 1972. At each site, a randomized block design with three replicates was used with plots consisting of two rows of 30 plants each with a spacing of 0.6 m between and 0.3 m within rows. The flowers were picked, dried and weighed every 2 to 3 weeks over a two-year period.

Table 1 shows the yields of dried flowers relative to the Kenyan cultivar K73 and the pyrethrins contents of the dried flowers. The yields and contents of the wild material is much lower than that of the selected Kenyan cultivars. The two Dalmatian populations Z15 and Z5, which were most likely 'cultivated pyrethrum', yielded distinctly better than the wild material. In most populations a substantial to large proportion of the plants remained vegetative, indicating that the temperature requirements for flower initiation (Glover 1955) were not met. This was especially

Table 1. Relative yields of dry flowers and of pyrethrins and pyrethrins contents of two Kenyan cultivars and 20 pyrethrum populations collected in Dalmatia averaged over two sites.

Population		Relative flower yield	Pyrethrins content (%)	Relative yield of Pyrethrins
K73	Kenyan cultivars	100	1.52	100
P4		70	1.49	68
Z15		45	0.97	29
Z5		28	0.93	17
Z21		24	1.04	17
Z 2		25	1.02	17
Z 3		25	0.95	16
Z10		23	0.92	14
Z 1		21	0.98	13
Z13		23	0.82	12
Z14		22	0.81	12
Z 4		21	0.84	12
Z20		18	1.00	12
Z11		14	0.90	8
Z19		12	0.97	8
Z17		15	0.80	8
Z16		16	0.75	8
Z18		13	0.75	6
Z 7		9	0.79	5
Z 9		5	0.78	3
Z 6		4	1.04	3
Z 8		3	0.78	2

Table 2. Yields of dried flowers in g, and pyrethrins contents of six pyrethrum populations at two sites.

Population	Dry flower yields, g		Pyrethrins contents (%)	
	Molo	Subukia	Molo	Subukia
Z13	1390	890	0,81	0,83
Z20	1420	370	0,60	0,93
Z17	1250	230	0,81	0,78
Z11	1210	150	0,91	–
Z18	1260	0	0,76	–
Z 7	940	0	0,80	–

so at the lower and therefore warmer site, Subukia. Some populations remained completely vegetative there, resulting in population × site interactions for flower yields as shown in Table 2.

Among the plants derived from the crosses of Kenyan clones with the introduced pollen the proportion of such vegetative plants was much smaller.

Also for other desirable characters the Dalmatian material, especially the wild populations, was inferior. The flowers were considerably smaller and on shorter flower stalk than K73 and P4. Nevertheless lodging of the flower stalks occurred much more often than in the Kenyan pyrethrum.

Discussion

It is clear, that although pyrethrum is a recent crop, domesticated less than 150 years, with only a short history of purposeful breeding, the distance is large between cultivated pyrethrum, adapted to the Kenyan Highlands, and the wild material.

Introgression of Dalmatian pyrethrum genes into the Kenyan population is therefore a rather time-consuming process, the more difficult since many genotypes hardly flower, if at all, in Kenya. These genotypes cannot exchange genes restricting the available Dalmatian gene pool not inconsiderably. This problem, however, can be circumvented by the introduction of Dalmatian genes in the form of pollen, rather than as seed. The seedlings derived from the pollen collected in Dalmatia and used to fertilize some clones with a high general combining ability in Kenya (Parlevliet & Contant 1970) flowered far more profusely than the seedlings from the Dalmatian populations. At the same time, this approach is a time-saving way of introducing wild genes into the cultivated crop. The disadvantage is of course the impossibility of selecting the wild material before introgression.

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Genetic variation in wild white clover populations in eastern England

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Index words

Trifolium repens, white clover, wild population, genetic variation.

Summary

Populations of wild white clover were collected from a wide range of grassland habitats in eastern England. A number of genotypes of each of 48 populations were established as spaced plants in a field nursery at Cambridge together with two control cultivars, Kent wild white clover and Kersey. Observations were made on several plant characters.

In order to compare the variation found in eastern England with that found in wild white clover populations elsewhere, use was made of the data of Davies & Young (1967) who studied 26 indigenous British populations and a collection of 50 populations from 29 countries throughout the world. The East Anglian collection was much more variable in leaflet length and time of flowering than the British collection of Davies & Young and had a wider range of leaflet lengths than their world collection.

Possible reasons for the variation found in eastern England are examined and the use of these populations for breeding are discussed. The value of assembling germplasm collections of locally adapted material is emphasized.

Introduction

Wild white clover populations were collected from a wide range of grassland habitats in Eastern England. Visits were made to 76 sites such as the parklands of country houses, nature reserves, ancient permanent pastures and archaeological sites, which were thought not to have been ploughed for more than a century. White clover was found at 40 of the sites. The habitats included sparse grassland on dry acid sands, dense turf on chalk, rich grassland managed for hay and pasture on organic peat, silts, loams and poorly drained clays, and the closely-mown lawn of a Cambridge college. Several genotypes were collected from the area in which the parent plant of the commercial variety Kersey white clover was found.

A number of genotypes from each of 48 populations was established as cuttings in the greenhouse together with two control cultivars, Kent wild white clover and

Table 1. Mean values for six characters of the wild populations and the two cultivars.

Population Mean Values	Plant Diameter after 6 months (cm)	Plant Height (cm)	Stolon Diameter (mm)	Terminal Leaflet Length (mm)	Terminal Leaflet Breadth (mm)	Days to first Flower (after Kent W.W.C.)
Maximum	80	24	4.03	35	32	+5
Minimum	41	8	1.92	11	11	−21
Mean	60	16	2.88	25	24	− 4
Kersey	60	26	4.37	44	38	−11
Kent W.W.	44	16	2.43	19	19	0
S.E. $\bar{x}$	6.0	1.09	0.11	1.02	0.98	3.0

Kersey. These were subsequently transplanted as spaced plants 1 m. apart in a field plot at Cambridge where observations were made on several plant characters.

Variation in plant characters

Table 1 gives a summary of the data recorded for six plant characters.

In order to compare the variation found in eastern England with that found in populations of wild white clover elsewhere, use was made of the data of Davies & Young (1967). They studied 26 indigenous populations collected in Britain and a collection of 50 populations from 29 countries throughout the world. In comparing our data with those of Davies & Young, two characters only were chosen as examples, namely terminal leaflet length and the date of first flowering. In order to take into account the environmental variation between the two trials, all results were expressed relative to Kent wild white clover, the control cultivar common to both trials (Table 2).

Table 2. Variation in leaflet length and flowering time relative to Kent wild white clover.

	Leaflet length (% of Kent W.W.C.)	Flowering time (days)
Kent W.W.C.	100	0
Kersey W.C.	225	−11
Range for E. Anglian material	55 -225	−21 to + 5
Range for U.K. material (Davies & Young)	75 -125	− 3 to + 4
Range for world collection (Davies & Young)	80 -220	−30 to +13
S.E. $\bar{x}$ (K.W.W.)	6.1	2.4

It is notable that the East Anglian collection varied much more in both leaflet length and time of flowering than the British collections studied by Davies & Young. It also contained a wider range of leaftet lengths than the world collection of Davies & Young but had a narrower range of flowering times.

Reasons for the variation in eastern England

The range of variation demonstrated may in part be a reflection of the number of genotypes included in the comparison and the range of sites from which collections were made. It is surprising that the range of variation should be so much greater than that recorded for the rest of Britain, although it must be admitted that collecting in Britain has been limited.

A species invading a new area will be subject to selection for adaptation to the environmental conditions pertaining there and if the area is diverse, as is East Anglia, then a wide range of new genotypes may result. In some agricultural areas, a further source of variation, namely gene flow from cultivars, is available. Every attempt was made to ensure that material was collected only from sites on or near which no cultivated varieties of white clover had ever been sown, but it is still possible that gene flow has occurred.

Value of the populations from eastern England for breeding

Whatever the causes of the large variation found in wild white clover in east Anglia, the current collection of local material could well be of considerable use in a breeding program. There is a large variation and all the variants in the collection are presumably well adapted to local conditions, which is an essential property of cultivars to be grown in the region.

There is considerable merit in assembling germplasm collections of locally adapted material. The original Vavilovian centres of diversity provide only a small part of the variation in a species; the situation is dynamic and new secondary centres of diversity are continually arising as crops spread with man and adapt to new environments.

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State, maintenance and use of European genebanks

Chairman: prof. J.G. Hawkes (Birmingham)

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Biosystematic studies of populations in germ plasm collections

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Index words

Germ plasm collection, gene bank, biosystematics, population study, genetic conservation, *Avena* sp., wild oats, *Amaranthus* sp., amaranths, *Helianthus* sp., wild sunflower, *Limnanthes* sp., meadowfoam.

Summary

A wide variety of decisions in designing optimal strategies for genetic conservation depend upon knowledge of genetic variation and biosystematic relationships among the wild, weedy and cultivated forms. Analyses of variability in our present holdings of genetic resources and their data banks in several major crops showed that (1) patterns of variation within and between populations of a species are highly varied; (2) often new collections and population genetic tools for their description will be needed; and (3) more attention needs to be paid to the mapping of gene frequency data. Population studies in wild oats, for example, revealed a worldwide pattern of high polymorphism levels in two hexaploids and a distinct ecotypic pattern in a tetraploid species. Amaranths provided examples of useful applications of numerical taxonomy and allozyme variation in sorting out the crop-weed complexes. Likewise, the model of introgressive hybridization was tested for the widely cited example of California sunflowers. Variation in a new oil crop genus (*Limnanthes*) illustrates the role of population structure in the extent of genetic variation within several related species. A survey of crop gene banks indicates that as yet there are too few geographical rules to guide us in selecting the best collection strategy. However, biosystematic surveys of variation within and between related species are the first essential step toward a planned genetic conservation program.

Introduction

The primary aim of this contribution is to emphasize the central role of biosystematics and population genetics in the scientific knowhow and management of our genetic resources. Many questions are all too familiar in the literature: what, how much and how best to collect, document and conserve from among conceivably a large array of land races or cultivars of economic plants and their potentially useful wild and weedy relatives? The answers have been extremely diverse, ranging from cautious generalizations based on few studies of population variation and modelling of most probable genetic structures (Marshall & Brown 1975), to the combinations of field experience, pragmatism, guesswork and even such truisms as collect-what-you-can-and-urgently-so. A good deal of discussion of land races, ecogeographical patterns of variation in different gene pools, coädaptation, and centres of diversity appears to be based on intuition and unverified assumptions about the history, crop development and evolutionary dynamics (e.g. Frankel 1977; several chapters in Frankel & Hawkes 1975). In his candid reviews of variation patterns, Harlan (1975a, b) pointed out that for the central question in the collector's mind, do gene centres exist, the answer is sometimes yes, sometimes no. In the search for specific genes for disease or pest resistance, he concluded that variation is where you find it. Frankel (1977) and others recognize the need for scientific information in careful planning and execution of collection work, but he also argued that 'elaborate descriptions recommended by some authors are scarcely practical in an inevitably busy collecting season, nor could he see in what manner such detailed information could be put to use ...'.

Not knowing clearly what 'elaborate' and 'detailed' imply here, I suggest that we take a good look at all aspects of genetic resource management. Literature on collections does not often provide much information on the habitat, population size, subdivision, or breeding system so that we know rather too little about the structure and dynamics of populations (Jain 1975). Variation within populations has been only recently studied in crop genera like *Avena, Triticum, Lycopersicon, Oryza* and *Hordeum*, using simply inherited traits and appropriate sampling procedures (e.g. Brown 1978). The so-called 'world collections' of crop germ plasm pose several problems in a population genetic analysis of variation, albeit they represent very useful genetic resources for the plant breeders. Documentation of several large gene banks is now in progress but so far much of the effort is still fragmentary. Availability of adequate and sufficient number of recorded characters in many of the world collection catalogues is still something for the future. Thus, although genetic variation within and between sampled populations is the primary issue in designing optimal collection, utilization or conservation strategies along the scientific principles, we lack the necessary research data on biosystematics and population genetics of variation. However, this is changing rapidly toward a wide recognition of the needs for extensive and good sampling procedures, training in population biology and evolution, provisions for research facilities, computerized data storage and retrieval, and so on. The scope of variation studies has widened from a few taxonomic or Mendelian traits or screening of germplasm for specific resistance and adaptive traits, to a larger array of agronomic, taxonomic, biochemical, and ecophysiological variables. Observations on native habitats provide helpful clues to certain useful genetic resources, and equally important are cytogenetic studies of crossability, gene transfers between related species, or certain variants recovered in the hybridization studies (Rick 1976 & this conference). Protein electrophoresis, gas-liquid chromatography and other assay methods are becoming routine in genetic resource studies. Some of these developments are reviewed next.

Surveys of 'world collections'

Several national or international holdings of germplasm in individual crops have now been listed in catalogues or computerized data banks. We recognized their shortcomings in relation to an ideal collection that should be large, representative,

Table 1. Summary of diversity index analyses in World Collections.

Crop	Number of entries	Number of characters	H′ comparisons	Countries with high H′
barley[1]	17 000	5	characters > regions > countries	Rumania, Hungary, Soviet Union, Ethiopia
safflower[2]	3 000	20	countries > characters > regions	Egypt, Pakistan, Portugal
durum wheat[3]	3 000	5	characters > regions > countries	Ethiopia, Egypt, Portugal
rice[4]	1 900[5]	41	indica > japonica	Indonesia, W. Africa, China

1. Tolbert et al. (to be submitted)
2. Jain & Wu 1977.
3. Jain et al. 1975.
4. Holcomb, et al. 1977.
5. 1900 sampled at random from over 8000 entries.

properly maintained and documented, with authentic land races and a minimum of duplications. The ones we selected for an analysis of geographical patterns of variation had one or more of the following attributes: easy access, large size, record on many traits, interest in races. An International Rice Research Institute catalogue (1970) listed over 8 600 accessions for 41 characters and the *indica-japonica* identity. A total of 1 407 *japonica* and 488 *indica* entries were picked at random from nine countries for our study. The monograph of Ashri (1973, et seq.) on safflower listed 20 characters for more than 3 000 accessions. Data on more than 17 000 barley accessions are maintained on computer tape by the United States Department of Agriculture; only five characters were available for a complete survey of variation among regions and countries. We quickly discovered the incompleteness of such catalogues, since data on screening for resistance to several diseases and a survey of allozyme variation (Kahler 1973) are not yet on that tape. Likewise, data on nearly 3 100 durum wheats were available from our own studies of five characters. In each of these surveys of world collections, individual attributes varied widely in the distribution of polymorphisms such that regions or individual countries could not be easily recognized as the centres of diversity. A pooled diversity index, H′, computed for each accession provided a hierarchical analysis to compare the diversity components due to regions, countries and characters (Table 1). The countries with the high values of H′ do not often correspond to the centres of diversity named in the crop evolution literature (e.g. Harlan 1975a; Zeven & Zhukovsky 1975). Of course, several factors readily account for this result; they include unequal representation of different areas, uncertainty of the history of propagation and records, loss of variation from the original samples, and perhaps the complex nature of both concepts and analyses of diversity centres.

What is the way forward? Our studies are not to be construed as any criticism of the past but simply to reinforce current thoughts on ways of improving our collections of plant materials as well as records. Putting together all the information from gene banks through international collaboration could be an important accomplish-ment of this decade. The materials need to be grown with adequate experimental designs by researchers on individual crops and perhaps scored for a wide spectrum of heritable traits as well as adaptability characteristics. The JIBP volumes (Matsuo 1975), for example, attempted to bring together information on the genetic resources in Japan with emphasis on various analytical tools of multivariate statistics. New conceptual breakthroughs are needed in mapping variation in relation to ecological and agricultural factors.

Some examples of biosystematic research on genetic resources

Taxonomic groupings of races, ecotypes and species are helpful in relation to the utilization of gene transfers among related taxa. About 12 000 samples of maize from Latin America have been studied in terms of races. Goodman & Bird (1977) recently used numerical taxonomy based on 20 ear attributes to classify over 200 races into 14 groups. They noted that Caribbean dents and flints were the best sources of germ plasm for breeders. The genetics of racial divergence and its implications in development of hybrid maize will need further work. Harlan & De Wet (1972) defined sorghum races on the basis of a few discrete traits; however, all combinations of intermediates were also found and therefore, deserve closer study of population genetics. In a series of papers, Rick and his coworkers (Rick et al. 1976; Rick & Fobes 1975) have explored the relationships among and within *Lycopersicon* species. Allozyme variation provided clearcut evidence for the presence of two sibling species in materials from Inter-Andean Peru, previously named as *L. minutum*.

On the other hand, three cultivated and three weedy amaranths were found to have allozyme patterns characterizing the crop and weed groups (Table 2). Very little polymorphism in the existing collections, however, warrants re-examination of this genus by means of new collections (Hauptli & Jain 1978). The role of introgressive hybridization in sunflowers is treated as a classical study of variation as a factor in evolution of crop plants. We recently examined a series of California populations representing the rare endemic *Helianthus exilis* and weedy *H. Bolanderi* in relation to the postulated genetic affinities with the cultivated *H. annuus*. Both morphological and electrophoretic evidence failed to support the introgression hypothesis unequivocally but several descriptors revealed the potential value of the wild relative *H. exilis* as germ plasm (Olivieri & Jain 1977). Many more examples of the recent electrophoretic work on plant systematics were reviewed by Gottlieb (1977) under four major topics: (1) genetic divergence among populations of a species; (2) genetic divergence among related species; (3) role of polyploidy; and (4) specific tests of evolutionary hypotheses. Numerous crop genera will soon provide new information by these means and will most likely stimulate further exploration of wild and weedy relatives of crop plants.

Allozyme variation patterns within species

Brown (1978) reviewed the allozyme variation studies in crop genera with the objective of relating them to the goals and methods of genetic conservation. Table 3 summarizes a few examples in terms of the levels of polymorphism, heter-

Table 2. Allozyme variation in amaranths (Hauptli & Jain, 1978). F = fast, M = medium, S = slow, FS = heterozygotes; a, b, c, d are phenotypes, + = potentially species-specific bands or alleles.

		caudatus BP 352	*cruentus* Rodale	*cruentus* 76-42	*cruentus* CHI-1	*hypochondriacus* P.I.337611	*hypochondriacus.* P.I.288282	*retroflexus* Walnut Grove	*retroflexus* Sutter Buttes	*hybridus* Howard Rd.	*hybridus* Moorpark	*powellii* Tulelake	delta hybrids Thornton Rd.	delta hybrids Acampo Rd.
Adh	F	1.0	1.0	1.0	1.0	1.0	1.0	–	–	0.09	–	–	1.0	1.0
	S	–	–	–	–	–	–	0.90	1.0	0.91	1.0	0.60	–	–
	FS	–	–	–	–	–	–	–	–	–	–	0.07	–	–
	Null	–	–	–	–	–	–	0.10	–	–	–	0.33	–	–
Lap	F	1.0	0.82	1.0	1.0	1.0	1.0	–	–	1.0	1.0	–	1.0	1.0
	S	–	0.18	–	–	–	–	1.0	1.0	–	–	0.65	–	–
	Null	–	–	–	–	–	–	–	–	–	–	0.35+	–	–
Got	a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.40	0.17	1.0	1.0	1.0
	b	–	–	–	–	–	–	–	–	–	0.03+	–	–	–
	c	–	–	–	–	–	–	–	–	0.05+	0.14+	–	–	–
	d	–	–	–	–	–	–	–	–	0.55+	0.66+	–	–	–
Acph	F	–	0.35	–	–	–	–	–	–	–	–	–	–	–
	M	–	0.63	1.0	1.0	1.0	1.0	1.0	0.97	1.0	1.0	1.0	1.0	1.0
	S	1.0+	–	–	–	–	–	–	–	–	–	–	–	–
	Null	–	0.02	–	–	–	–	–	0.03	–	–	–	–	–
Est-1	F	–	–	–	–	–	–	–	–	0.89+	1.0+	–	–	–
	M	–	–	–	–	–	–	–	–	–	–	–	–	–
	S	1.0	1.0	1.0	0.97	1.0	0.97	1.0	1.0	0.03	–	0.78	0.86	1.0
	Others (incl. heterozygotes)	–	–	–	–	–	0.03	–	–	0.08	–	0.22	0.14	–
Est-2	F	–	–	–	–	–	–	–	–	–	1.0	0.78	–	–
	M	–	–	–	–	–	–	0.76+	1.0+	–	–	–	–	–
	S	1.0	0.92	1.0	0.23	1.0	1.0	–	–	–	–	–	1.0	1.0
	Others (incl. heterozygotes)	–	0.08	–	0.77	–	–	0.24	–	1.0	–	0.22	–	–
Number of plants		40	35	30	25	30	50	30	35	40	30	20	50	30

Table 3. Summary data on allozyme variation (adapted from Brown 1978).

Species	Number of populations	Number of loci	Proportion of loci polymorphic (%)	Number of alleles per polymorphic locus	Mean hetero-zygosity	Genetic identity
Avena barbata[1]	9 (region I)	5	0.00	–	0.00	1.00
„	7 (region II)	5	1.00	2.0	0.03	0.54
Lycopersicon[2]						
cheesmanii	54	14	0.57	2.6	–	–
parviflorum	8	14	0.14	2.0	0	–
chmielewski	8	14	0.50	3.5	–	–
Amaranthus[3]						
crop spp.	6	6	0.50	2.3	–	0.83
weedy spp.	7	6	0.83	3.4	–	0.57
Clarkia[4]						
biloba	3	8	0.62	3.6	–	0.92
lingulata	2	8	0.62	3.3	0.07	0.90
rubicunda	4	11	0.64	2.0	0.11	–

1. Clegg & Allard 1972.
2. Rick et al. 1976.
3. Hauptli & Jain 1978.
4. Gottlieb 1977.

Table 4. Summary of allelic diversity patterns (adapted from Brown 1978).

Species and material sampled	Number of loci	Observations	Reference
Avena barbata			
95 populations	4 morphol. loci	Polymorphism within a small region; monomorphism over a large region	Jain 1975; Rai unpubl.
16 populations	5 allozyme loci	as above	Clegg & Allard 1972
Hordeum spontaneum			
28 populations	28 allozyme loci	29% of alleles widespread and common; 16% of alleles rare and localised	Brown et al. 1978
Lycopersicon pimpinellifolium			
11 populations	11 allozyme loci	28% of alleles widespread and common; 36% of alleles rare and localised	Rick et al. 1977
Lycopersicon cheesmanii			
13 populations	8 allozyme loci	21% of alleles widespread and common; none rare	Rick & Fobes 1975

ozygosis and the genetic similarity of conspecific populations. These data merely suggest the use of allozyme loci in describing the patterns of genetic variation, which in turn can be used in defining optimal genetic conservation strategies for individual crop genera. Field observations and various sources of biosystematic information will hopefully aid in understanding the variation pattern and in predicting the status of unexplored genetic resources as well.

Brown (1978) further developed his analysis of allozyme data in terms of scoring different alleles as widespread or localise, and common or rare. Table 4 gives a part of his summary for three of the crop genera. Different species clearly differ in the presence of localised polymorphism and of rare alleles. Brown (1978) concluded that 'the most efficient sampling, storage and evaluation strategy is to concentrate on the variable populations'. However, it is clear from this survey that population genetic studies on the first round of germ plasm collections are essential in deciding on a strategy for subsequent explorations (Jain 1975). That different environments, or isolates and peripheral populations should be sampled has been well recognized; we are essentially arguing about the best ways of collecting representative alleles, all known alleles, or visibly different population samples, with concomitant population studies on variation in our collections.

Our detailed studies in two species of *Avena* have been discussed elsewhere (Jain 1975; Brown 1978). We have initiated similar work in *Limnanthes*, a new candidate for domestication as an industrial oil crop. Populations of 16 different taxa have been sampled over a series of years and described for several sets of characteristics. *Limnanthes* populations occur in island-like wet microhabitats and therefore evolutionary studies seem to offer unique opportunities for developing a well recorded set of germ plasm resources, observing extinction under natural conditions, and testing various ways of sampling and efficient documentation of variation between and within species. Population structure and especially breeding system variables are now being studied. A preliminary survey of allozyme variation suggests that the proportion of polymorphic loci is correlated to number of sites a taxon occupies and of outbreeding (Jain unpubl.; Brown & Jain, in press). New crops like *Limnanthes* could serve as useful test organisms for studies on management of genetic resources.

In this brief survey I have not covered all biochemical discoveries of useful protein variants, nor genes for disease resistance in germ plasm. Mapping of a few loci of direct interest to a breeder is perhaps best illustrated in Qualset's (1975) study of Ethiopian barleys. Even such gene mapping has not been attempted in all collections (e.g. K. Rawal's collections of Nigerian cowpeas and van der Maesen's chickpea collections). Variability within original population samples should be considered in all future collections. Serious efforts must be made to develop information on the concept of land race, and field data on population dynamics should at least cursorily be gathered. Population biologists are getting more and more interested in applied problems, and crop genetic resources should definitely rank very high in their future research priorities.

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The Gatersleben gene bank

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Index Words

Gene bank, technique of maintenance, evaluation, utilization.

Summary

The Gatersleben germplasm collection of the Central Institute for Genetics and Cultivated Plant Research (Academy of Sciences of GDR) comprises at present 43 884 different cultivars, landraces, primitive or wild forms of the cultivated plants and their relatives almost exclusively from temperate regions; among them are more than 22 000 cereals, nearly 8 000 leguminous plants and 6 700 vegetables. This collection is the result of an extensive seed exchange and of many special collecting trips.

Every year part of the collection, approximately 13 000 strains, is cultivated on small plots in the field to obtain the necessary amount of seeds. Techniques have been developed to handle the material in the field guaranteeing the original state and to check the identity by comparing the cultivated material with reference specimens from herbarium, spike, fruit and seed collections which may serve also as material for taxonomic studies.

Two years ago a building for long term storage of seeds was constructed. Its four store rooms can keep a maximum of 100 000 strains. Till now more than 17 000 varieties have been entered into two cool chambers. After the storage of the whole collection in its present size has been completed, the material will be divided into a long-term stored or base collection and into a working or active collection stored under normal room conditions.

Our next step is to prepare a data-bank, which should in the end comprise all data about origin, taxonomic determination, field observations and screening results of the most important parts of the Gatersleben collection. For this purpose a data storage and retrieval system, the so-called INDEX-system, developed in the Academy of Sciences will be used.

The Gatersleben gene bank maintains a very close contact with plant breeders. Its staff members are also co-workers in different associations of breeders, each of which deals with the breeding problems of one crop. Thus an evaluation of the gene resources of our collection in response to the actual need of plant breeding can easily be realized. In order to provide the breeders with already identified material intensive screening experiments have been made in cooperation with other institutions of the country or other laboratories of our institute. During the last years these experiments were for the greater part a screening of disease resistance among the cereal, vegetable and legume collections and the study of protein content and quality of cereals and some leguminous crops. Valuable strains discovered through these screenings have been incorporated in recent breeding work and some of them are already to be found in the pedigrees of breeding stocks and recently released cultivars.

processing. At present, an average of 13 000 accessions are grown in more than 0.1 km^2 annually.

Populations of land races are divided into two parts. If little seed is available, they are first increased. One part is maintained as a population, the other is split into its components on the basis of morphological characters. In this way, the elements of a population can be preserved. If certain components are represented only by few seeds, there is a risk that they be lost. The preservation of physiological characters in a population of land races is still unsolved and will be unsolved until adequate methods for rapid determination are available.

Collections of reference specimens

To ensure the purity of accessions and for various aspects of utilization of collections, archives are indispensable. The archives include: (a) specimens as records of morphological and anatomical characteristics; (b) notes of observations during the growing season, illustrations and publications about cultivars, their features and pedigrees as aids in checking identity.

For accessions with a sufficient number of plants at the first sowing, 2 flowering plants for the herbarium and 10-15 randomly cut ripe spikes or panicles for the inflorescence collection are taken from the plot. It is preferable to take seed for the seed collection from the original sample. Samples of inflorescences or seeds with particularly variable characters should be taken from more than one growing season, so as to demonstrate their full variation, e.g. gradation of glume or seed-coat colour.

Discrepancies that sometimes appear in the collection can be solved largely with the reference specimens. These are more explicit than a verbal description. Therefore collections of reference specimens are not only useful but essential.

Seed storage

Until recently, seed was stored in rooms at atmospheric humidity and accessions were grown generally at intervals of 5 or 6 years. In 1976, a cold store for seed was built. This consists of four storage rooms, each of 170 m^3. It is intended to store about 100 000 entries in this cold storage.

The temperature in three of these storage rooms is regulated between $-5\ ^\circ C$ and $+5\ ^\circ C$, while the fourth chamber is maintained at temperatures between -15 and $-20\ ^\circ C$ and is used for experiments and for extremely long-term storage. At present, there are some difficulties in regulating the relative humidity to around 30% as planned.

The seed is dried to a certain content of moisture, i.e. cereal seeds to 9–7%, and stored on glass jars. They are not hermetically sealed because of the need for fairly frequent sampling. Silica gel with an indicator for humidity is added to each jar: (a) to dehydrate seed gradually (cereals to 7–5% moisture), (b) to indicate changes in humidity within the glasses. By addition of silica gel, an equilibrium is achieved between air moisture in the glasses (relative humidity about 10–15%) and content of moisture in seed (about 5%).

The number of seed stored depends on the species, the difficulty of production and seed size, but, on average, about 10 000 seed of each accession should be stored. Samples of autogamous cereals, i.e. barleys, oats and wheats are stored in a single glass jar and contain 7 000–10 000 seed (0,3–0,5 kg), the average amount of seed obtained from the normal (2½ m^2) plots.

Germination tests of stored seeds are planned for five-year intervals. Annual tests are undertaken on selected stored samples of different kinds to monitor changes in germination capacity. A small number of seed of each collection is grown in the year after storage to check: (a) whether any confusion has occurred, i.e. to confirm that plants are identical with original ones from which seeds were taken for storage; (b) whether there has been admixture within the sample. Mixed or confused samples are discarded.

When all entries maintained in 1975 are stored in the cold store, the collection will be divided into long-term collection (foundation collection) and a working collection.

The working collection will include samples of accessions either frequently required by plant breeders and research institutions or utilized in special investigations, e.g. to evaluate their characteristics for special breeding programs or for a more detailed classification. This collection will be stored under normal room conditions. It is expected that there will be a continous exchange of samples between both collections. At the end of 1977, 17 000 entries were transferred to cold storage.

Evaluation

Evaluation is focussed at present on disease resistance and biochemical characters and is undertaken in cooperation with a number of specialized institutions in the German Democratic Republic. The results of these investigations are regularly published in the journal Kulturpflanze. The seed for all screenings comes from the gene bank at Gatersleben. For example, in 1977, cooperators were supplied with 8 000 seed samples.

Evaluation of disease resistance includes fungal, bacterial and viral diseases.

The search for disease-resistant barleys and wheats in the collection was begun more than 25 years ago by I. Nover at the University of Halle. Material from these two collections were tested for reaction to races of mildew, stripe and leaf rust and to loose smut. As a result of these extensive studies, lines with disease resistance have been incorporated into breeding programs and are to be found in the pedigrees of breeding stocks and recent cultivars. In addition, the sets of differentials for mildew, stripe and leaf rust have been supplemented by lines from the collection.

In the vegetable and legume collections, extensive screenings began in the 1970s in cooperation with the Institute of Phytopathology at Aschersleben to find strains with resistance to economically important diseases. In 1977, the search field beans resistant to mosaic and to root diseases was completed for the time being. Within a collection of 600 field beans, resistance towards five mosaic diseases (bean yellow, pea enation, alfalfa and broad bean true mosaic and broad bean wilt) were found in some cultivars. Within a pea collection of more than 1 200 accessions, several cultivars and lines of local populations showed resistance towards two viruses (pea enation and bean yellow mosaic) and five fungal diseases (*Phoma medicaginis* var.

pinodella, Mycosphaerella pinodes, Fusarium oxysporum f. *pisi, Rhizoctonia solani, Pythium ultimum* and *P. debaryanum*) partly in combination.

The variation in content of protein and lysine in 10 000 wheats and 6 000 barleys, and of protein and methionine/cysteine in 600 field and broad beans was screened in tests between 1970 and 1976. Types selected both for high content of protein and of the amino acids are now undergoing further tests or have been incorporated into breeding programs. In 1 000 accessions of oats and 1 400 of peas, the first round of protein and lysine analysis is now complete. In all these crops, the already known range was confirmed but no accessions were found with exceptional values.

Several years ago, the physiologists and biochemists of the institute began studies on the physiological basis of plant productivity, especially in barley and wheat, and on protein metabolism in legumes. In both of these investigations, material of the collections is widely involved.

Besides these evaluations directed to plant breeding, the feasibility of applying methods and principles of numerical taxonomy in infraspecific classification of cultivated plants is being examined for a collection of Iranian wheats (more than 1 200 strains) collected by H. Kuckuck in the early 1950s. Preliminary results show that: (a) numerical taxonomy can be used as a method for a more detailed classification of cultivated plants below the species level if sufficient detailed data are available; (b) recording of detailed data is expensive and time consuming. Consequently its application cannot as yet be used as a routine taxonomic procedure.

Preparations and first steps are under way for joint computerized recording and exchange for collections in the Socialist countries. For the barley, wheat and cabbage collection at Gatersleben, 'identification data' (such as botanical determinations, designations, origins) and data derived from field observations and evaluations are now transferred onto magnetic tapes and stored in a small computer (KRS-4201) using INDEX system.

The staff of the gene bank has close contact with plant breeders. They cooperate in breeder collectives, in which breeding programs are extensively discussed and finally jointly performed with a broad exchange of breeding material between the stations. New cultivars are released now only by these breeder collectives.

Staff members of the gene bank are included in the breeder collectives. They get acquainted in this way with the problems, and can offer advice and material from the gene bank for the breeding programs. In the same way, breeders have influence on the evaluation of the material. This close cooperation between the breeders and the staff of the gene bank is of mutual benefit and contributes to an effective use of the gene bank.

This brief account should give some idea of the work at Gatersleben, of the material collected and maintained in the gene bank, of its evaluation and of relations with plant breeders who are the 'initial consumers' of all of our activities in broadening the genetic base of crops.

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Gene bank activities in the Federal Republic of Germany

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Index words

Braunschweig gene bank, collections, agricultural plants, horticultural plants, forest trees, micro-organisms.

Summary

The gene bank activities of the Institute of Crop Science and Seed Research started in 1971, when the significance of maintaining plant genetic resources for plant breeding work became more and more evident.

Today the accessions number nearly 25 000, with cereals (wheat, barley, rye) ranking first. New emphasis is given to potatoes (Dutch-German cooperation), beets (acting as main conservation centre on behalf of IBPGR) and grain legumes. Other crop varieties are also maintained but preferably on a more selective basis, e.g. vegetables.

From the beginning documentation and information were given a major role. By developing of a thesaurus (L. Seidewitz) as a basic coordinating language for documentation, the principles of which are now agreed on over the whole world, a foundation has been laid for the exchange of information using the natural languages.

The conservation of collected germ plasm (seeds) is done following the internationally agreed standards for long-term seed storage, i.e. using a moisture content of 6% ± 1%, storage temperature of −10 °C (for orthodox seeds) without controlling RH but air-tight cans.

Utilization of the germ plasm from the working collection is being prepared by evaluation on a broad scale. Quality characteristics as well as physiological studies related to yield and metabolism are carried out in the institute's laboratories, especially for primitive forms and wild species. On a cooperative basis evaluation work is done by specialized institutes at home and abroad for resistance against pests and diseases. Data received from these investigations are published at irregular intervals in the 'Genbank-Informationsdienst (GID)' for a wide distribution.

Besides these activities at Braunschweig-Völkenrode two more programmes within the Federal Republic of Germany are to be mentioned under gene bank aspects:

- The German Collection of Microorganisms (Central Office at Göttingen) in respect to the symbiosis of Rhizobium species and legumenous crop plants. More effective utilization of grain legumes in future will comprise the symbiont also.
- The collection of wild forms, ecotypes etc. of forest trees at the Federal Research Centre of Forestry near Hamburg, which of course is maintaining the forest plant germplasm following about the same principles as we use for crop plants.

Introduction

The title of my paper implies that there is more than one institution in the Federal Republic of Germany with gene bank activities.

First, there is the gene bank of the Institute of Crop Science and Seed Research of the Federal Centre of Agriculture at Braunschweig-Völkenrode (FAL), which covers field crops; secondly we have the German Collection of Micro-organisms (DMS), centred at Göttingen and handling cultures of bacteria and fungi; and thirdly, there is the seed collection of forest trees at the Institute for Forestry Genetics and Breeding of the Federal Research Centre of Forestry and Forest Products in Hamburg.

I will concentrate on out work at Braunschweig-Völkenrode. The initial intention was to have an information service for plant breeders about plant genetic resources only. But soon it turned out to be of greater value to start a gene bank, including collection, conservation, documentation, evaluation and utilization. That was in 1971, but even today documentation and information work headed by Mr L. Seidewitz occupies a central place.

Due to the situation in our own and some neighboring countries, the cereals are numerically most important. But in future our efforts will concentrate on three other main groups of crops:

- the grain legumes of the temperate climate (especially *Lupinus* and *Vicia*),
- potatoes,
- *Beta* roots.

This is not to the exclusion of other species, but certainly they will be considered more selectively.

For potatoes, there is a special scheme. Based on an official agreement the Netherlands (represented by the Foundation of Plant Breeding, Wageningen) and the Federal Republic of Germany (represented by the Institute of Crop Science and Seed Research, Braunschweig-Völkenrode) a 'German-Netherlands Potato Department of the gene bank in the Agricultural Research Centre at Braunschweig' was established in 1974, the primary concerns of which are the wild species and primitive forms of the tuberous *Solanum* species.

For *Beta* roots, we act as a main conservation centre as a result of a proposal of the International Board for Plant Genetic Resources. The material basis for this was primarily the collection of *Beta* wild species and primitive forms comprehending all sections, i.e. Vulgares, Corollinae, Nanae and Patellares, formerly held at the Max-Planck-Institute of Plant Genetics at Ladenburg. Other smaller collections could be added in the meantime.

The germ plasm collection is maintained in the storage chambers, and has the structure shown in Table 1.

Some additional comments are necessary. The total 2 200 potato accessions consists of 82 wild species and 4 species of primitive forms. There are also 230 samples of wild species and 2 600 samples of primitive forms derived from the Birmingham–Wageningen Expedition-1974 now under multiplication at the Internation Potato Center in Peru (CIP).

For wheat and barley, some 7 000 duplicates collected in Ethiopa and maintained at the Plant Genetic Resources Centre at Addis Ababa (an institution estab-

Table 1. Nature of the germplasm collection at Braunschweig-Völkenrode. 1978-07-01.

Crop	Number of accessions[1]	
Wheat	11 000	
Barley	3 500	
Rye	2 000	
Oat	500	
Cereals		17 000
Grain legumes	1 200	
Clover and related species	250	
Grasses	700	
Forage crops		2 150
Potatoes	2 200	
Beets (*Beta* spp.)	600	
Root and tuber crops		2 800
Other species	500	500
Total		22 450

1. Rounded numbers

lished and supported within the frame of technical aid of the Germany Ministry of Technical Cooperation) should be added.

Limits of gene bank

A germplasm collection like ours which later will comprise some 50 000 accessions of the various crops and therefore is to be considered as medium-sized reguises a well differentiated composition of the genetic material. To achieve this complex task, we are now initiating studies on the genetic diversity taking into consideration the evolution of the crops, their improvement by plant breeding and their pedigrees.

Many of the older and the newly collected accessions are small in number. Therefore a comprehensive multiplication had to be done every year. The most crucial crop plants in this respect are the wild species of potatoes, because they need the controlled environment of a climatized greenhouse for successful growth, flowering and seed setting. Moreover careful hand-pollination is necessary. For some species, even more sophisticated techniques have to be used. Taking into account some parameters of population genetics, the following conclusions were drawn for the seed multiplication practice:

- Number of female and male individuals in the crosses should be equal
- Per accession, not less than 20 plants should be used
- Two crossing schemes should be used:

- crossing in pairs 1 × 2, 3 × 4, ...

- pollinating all plants of the accession with pollen mixture of all plants (a procedure we followed this year exclusively)

– Equal distribution of seed lots.

By this practice, move than 300 accessions could be multiplied sufficiently. To avoid duplicate multiplications, an agreement is being made between CIP in Peru, the United States Potato Collection at Sturgeon Bay and us, that each of the three stations will take responsibility for a certain part of the world collection of wild species.

Seed is conserved in modern cold-storage facilities following the international standards, i.e. a seed moisture content of 6% ± 1%, storage temperature –10 °C for orthodox seeds using air-tight containers. Storage capacity is about 250 m^3; accessions are divided into a base collection and a working collection; number of seed ranges from 5 000 to 10 000, depending on seed size.

Evaluation

Utilization of the germplasm from the working collection is being made by evaluation on a broad scale. Besides a rough taxonomic classification, if necessary, and morphological traits, quality characteristics and physiological ones related to yield and metabolism are investigated in the Institute's laboratories, especially for the primitive forms and wild species. These, for instance, include screening for protein quality and quantity, glyco-alkaloids, 5-alkylresorcinols, and development and improvement of the respective screening methods, or investigations on yield structure of grain legumes and yield physiology of the potato.

Evaluation is on a cooperative basis especially for resistance to pests and diseases. In all, there have been eight different institutes involved in these activities. Taking resistance screening of potato species as an example, we now have some 1 500 results referring to *Phytophthora*, cancer, *Fusarium*, *Erwinia* and nematodes from investigations in the Netherlands and our country.

The results are published for breeders information by means of the 'Genbank-Informationsdienst', copies of which are now being circulated. From our experience, this way seems to be the most appropriate to distribute evaluation results. Hitherto the 'Genbank-Informationsdienst', which is published at irregular intervals, is available only in the German language, but publication of an English version is under consideration.

Data bank

The value of the germplasm depends closely on available information, which is processed, stored and retrieved by data banks. Core of our data bank is a database processor for on-line management and retrieval of data running on a Siemens computer. This system called FIDAS (developed by Gesellschaft für Mathematik und Datenverarbeitung, Bonn) is flexible, especially in its file editor and report generator, a fact that meets our requirements. The building up of files follows the convention of this processor; for a passing-on of data different procedures in the file editor can be used in pre-determined data subsets. Further experience with a transfer of data between data bases in different countries should provide a basis for international data exchange.

Search or retrieval terms, in other words descriptors, and their descriptor states are in German, but Seidewitz' efforts are directed towards a computer technically based synonym linkage to be able to address the data set by equivalent descriptors of different natural languages. In this connection also the efforts to achieve internationally agreed descriptors are to be seen in order to transfer foreign data to another data base without problems.

As a means of communication with a data base as well as for a definition of individual user-profiles the edition of a 'Thesaurus for the international standardization of gene bank documentation' has been started. This at the same time forms a basis for a common generally understandable as well as compatible communication language. Because of the rapid development of international cooperation in the field of plant germplasm conservation, editions in Spanish, French and Russian will be provided besides those that already exist in German–English and English–German.

Now published in the English–German version are Part I for cereals (including maize, rice and sorghum), Part II for forage crops (including lawn grasses), Part III for root and tuber crops, Part IV for vegetable, oil and fibre crop plants, and Part V A comprising a selection of common and scientific terms about plant pests and diseases.

These efforts meanwhile got a conclusive response within the international community. The Information Sciences/Genetic Resources Unit at Boulder, Colo United States, and various Crop Advisory Committees, both established by the International Board for Plant Genetic Resources, are using the principles for their respective work. At the Institute of Crop Science and Seed Research, a Seminar on 'Technical Aspects of Information Management and Means of Communication in Plant Genetic Resources, for Utilization of Genetic Material in Plant Breeding' will be organized in November of this year to demonstrate and discuss this subject.

Since referral services are available from computing equipment, it is advisable that terminological links be established between this kind of data documentation related to gene bank and literature documentation. Any terminological harmonization of this nature would contribute to an easier tracing of genetically valuable plant material to which reference happens to be made by literature only.

Other gene bank activities

The main function of the German Collection of Micro-organisms (DMS) is to collect, maintain and supply authentic cultures of bacteria and fungi important for biotechnology, education as well as for applied or general sciences. At present DMS consists of the central collection and four subcollections at different places, each responsible for specific groups of micro-organisms. A second edition of the Catalogue of Strains was published in 1977.

Among the various species, which run to a total of about 3 500 specified accessions, those species are of special interest to us which belong to the group of nitrogen-fixing bacteria, the Rhizobia. Within the Eucarpia Gene Bank Committee, the symbionts were given special consideration during the discussion of grain legumes, because a more effective utilization of these crop plants would comprise the symbiont also. Certainly a closer linkage between crop germplasm conservators or users, and collectors of micro-organisms would be extremely useful.

The third activity to be mentioned here is in forestry: collecting and maintaining ecotypes, wild forms of forest trees and related species, as performed at the Federal Research Centre of Forestry, is as essential as for crop plants, because the problems we are confronted with are the same for forestry improvement, in some countries even greater.

Several thousand accessions have been collected, concentrating on those species which are of special interest to northwest European forestry production. In general, the handling of seed follows about the same procedures as for crops. A special feature is that among those species the number of socalled recalcitrant species is significantly larger than for the crop plants in the same ecological zone. Therefore attempts are being made to overcome the barriers to long-term storage.

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Genebank activities in Italy

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Index words

Genebank activities, germplasm laboratory, collecting expeditions, Italy, Mediterranean region, Ethiopia.

Summary

An account is presented of the activities of the genebank at Bari.

The importance as well as the problems of Italian and Mediterranean crop genetic resources were presented during a conference of our section of Eucarpia at Izmir in 1972 (Scarascia Mugnozza & Porceddu 1973). There a survey was given of the Germplasm Laboratory at Bari along with the aims and goals it proposed to achieve. The present report specifically deals with the activities of the Laboratory over the past six years.

The Laboratory, which was initially housed in the Institute of Agronomy at the University of Bari, is now situated in three new buildings on the University campus. The offices and laboratories for research and seed processing, dehumidification, container closure and germination tests are situated in the main building.

The seed dehumidification is performed in two rooms of 3.6 × 2.4 × 3 m, where the relative humidity of the air can be reduced to 15%, while the temperature does not exceed 30 °C. The humidity control is regulated by a lithium chloride system which is continuously regenerated, the temperature being controlled by a normal refrigeration unit. The dehumidification of the seeds is accomplished over a 45-day period and reduces the original water content of about 12% to approximately 6%.

The seeds are preserved in watertight containers: tin cans of 1 liter and 250 cl, respectively and plastic bags with aluminum foil of 1 liter and 100 cl, respectively.

The germination tests are conducted in accordance with International rules for seed testing. Seed is stored in 4 cold rooms of 6 × 3.35 × 3 m situated in a second building; 2 rooms operate at −15 and are used for long term conservation of base collections, while the other two operate at 0 °C and 30% relative humidity and are for the preservation of active collections. Growth chambers and rooms for seed cleaning are located in the third building. These growth chambers and a greenhouse with 10 small rooms, independently controlled, enable the study of material which requires particular environmental conditions.

The Laboratory is staffed by the National Research Council: 9 researchers, 13 technicians, 3 administrators and 5 scholarship holders are presently employed.

Collaboration with institutes of plant breeding, agronomy and field crops and plant pathology of universities and C.N.R. laboratories ensures specific competency and provides environmental conditions suitable for the material under cultivation and study.

The activities of the Laboratory include:

- exploration and collection;
- multiplication and evaluation of the material;
- seed physiology;
- storage of seeds and documentation on the collections.

Exploration was done mainly in the Mediterranean basin and, on a smaller scale, in Ethiopia.

The activity in the Mediterranean basin brought forth a series of 24 missions to places in Italy, especially in the south and on the islands, in order to collect old varieties of wheat and ecotypes of vegetables and fodder plant. Students of the M.Sc. Course on 'Exploration and utilisation of Plant Genetic Resources' at Birmingham participated in some of these missions.

The missions in the Mediterranean outside Italy were directed to North-Africa (Algeria, Tunisia) and Greece for the collection of cereals and grain legumes, and to Spain for the collection of maize and wheat. Many of these activities were organized and conducted in collaboration with FAO and IBPGR.

The Laboratory also organized two excursions to Ethiopia to collect tetraploid wheats.

The collected material is multiplied, classified and evaluated in the course of two successive years and inter plant competition is avoided. Part of the harvested seeds is then used for long-term preservation and part forms the active collections. Plants are studied for their disease resistance in the field, their height, flowering time and maturation, while seeds are analyzed for technological and qualitative parameters, e.g. protein content. Pea lines resistant to nematodes are one of the results obtained during these studies.

Table 1. Collections stored at Germplasm Laboratory, Bari (Italy).

Species	Acc. No.
Triticum	26.500
Pisum	4.090
Vicia spp.	2.210
Vicia faba	1.250
Hordeum	956
Zea	327
Avena	114
Vegetables	523
Grasses and clovers	965

In addition, these materials are subjected to a series of studies to ascertain the genetic structure of populations, relationships among materials belonging to the same species but evolved in different areas, material belonging to different species but evolved in the same area, etc.

Also, the duration of seed viability is being studied. This is conducted on diverse crop species as wheat, pea, tomato and sunflower. The data obtained from the evaluation and classification work are stored in the University of Bari computer by means of a terminal located at the Laboratory, using the EXIR system. Print-outs for wheat and peas have already been published. Others are in preparation. The number of accessions being conserved is reported in Table 1. Part of them are destined for long term conservation and the others for exchange and distribution. The available space is still considerable, but additional storage rooms have already been planned. Following an invitation from IBPGR and subsequent acceptance by the National Research Council, the Laboratory has, in fact, become one of the four centres (Fort Collins, Leningrad, Kyoto) for the preservation of base wheat collections and related species. For this reason it is thought that in the near future a great number of seed samples will arrive in Bari and exhaust the available space.

As for the international cooperation, it should be mentioned that a network for evaluation of genetic resources of durum wheat has also been organized as part of the research network on durum wheat set up by the European Office of FAO.

During 1977–78 approximately 500 accessions have been studied in 10 localities over six Mediterranean countries (Spain, Portugal, Algeria, Italy, Greece and Cyprus).

This was a first attempt which, I hope, will lead to the setting up of a Mediterranean network for genetic resources. Further to the international collaboration, Dr. Perrino from our staff is presently acting as a scientific advisory to the Gene Bank which is being built in Tehran (Iran). This can enrich the Laboratory through new contacts and experiences, in addition to opening up the possibility of a much easier exchange of material.

The collaboration which has been set up between the Laboratory and I.C.A.R.D.A. is extremely interesting. The cooperation involves genetic resources of cereals, grain legumes and forage crops and forsees the possibility of evaluating our collections in areas very different from those that can be found in Italy.

In the national field, I like to mention that we are trying to organize a network for the preservation of the fruit tree germplasm, with the cooperation of 20 Italian institutions. The work was started in 1976 as a part of a practical breeding programme on vineyards, lemons, almonds, etc..

Collection fields were set up and evaluation was begun. It is hoped that this could constitute the beginning of an interest of scientists in an area of genetic resources which is endangered by erosion, for the field crops have not yet had the attention they merit.

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Describing, evaluating and utilizing a germplasm collection of Phaseolus vulgaris beans

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Index words

Phaseolus vulgaris, common bean, evolution under domestication, collection, databank, evaluation, use in breeding programmes.

Summary

The paper begins with a brief account of the origin and evolution of *Phaseolus vulgaris* L. The changes that have occurred under domestication and as a result of the dispersal of the species from the centre of diversity in Latin America to many countries of the old and new worlds, are outlined.

The Cambridge germplasm collection of 5 000 accessions is then described. The facilities available for seed storage and the methods employed for maintenance and description of the genotypes are reviewed. 28 descriptor characters are recorded for each accession and the data are fed into the Executive Information Retrieval Computer Program (EXIR). An example of the use of this program for information retrieval is presented.

The possible use of two multivariate statistical techniques for the evaluation of the data from the collection is then assessed.

Finally, the utilization of the germplasm collection in two breeding programmes designed to develop *Phaseolus vulgaris* as a pulse crop for Britain is briefly described.

Introduction

The Cambridge germplasm collection of common bean, *Phaseolus vulgaris* L., totals about 5 000 accessions. The major sources of the collection are shown in

Table 1. Major sources and numbers of bean accessions in the Cambridge Collection

North America	190	United Kingdom	60
Mexico	250	Spain and Portugal	400
Colombia	270	Other European	190
Brazil	140	Turkey	880
Other S. American	200	Iran	430
Uganda	390	Afghanistan	70
South Africa	80	India	80

Table 1. The species has its centre of diversity in Central and South America and from there it has been taken to many parts of the Old and New Worlds.

Ph. vulgaris is an ideal model for the study of the evolution of crop plants; wild populations are still available, primitive cultivars are found in the centres of diversity, local varieties in the African tropics and a range of more evolved cultivars in Europe and North America.

The dispersal of these beans is fairly well documented (Evans, 1976). With their spread from the tropical Americas to temperate regions, there has been genetic adaptation to the new environments. Since the genus is native to the tropics, many cultivars from that region are photoperiod-sensitive and respond to short days. There are also photoperiod-neutral types and all the temperate cultivars have been selected for photoperiod-neutrality or tolerance of long days. There has been a modification of the growth habit to give five main groups of races (Evans & Davis, 1978). In Europe and North America selection for the bush habit has allowed the crop to be harvested mechanically. Furthermore seed numbers per pod have changed where up to 9 seeds are found in wild forms but rarely more than 5 in most bush cultivars. Pod structure has also been altered in cultivars, with a reduction in dehiscence and in fibre content.

Maintenance of the germplasm collection

Seed of the Cambridge germplasm collection is stored in screw-top polythene jars in cold-rooms; 'long term' storage at –5 °C and 'short term' storage at 2 °C. New introductions are grown in aphid-proof cages to guard against the field introduction of any new viruses. The description of each line is based on spaced plants grown in the field nursery.

Table 2. The 28 descriptor characters used in describing the bean collection.

1. The Cambridge accession number
2. Any other designation
3. Name of cultivar
4. Origin or earliest recorded source
5. Season in which accession is grown

Seed and seedling characters

6. Seed weight (mg.)
7. Seed shape – longitudinal – scale 1-5
8. Seed shape – transverse – scale 1-5
9. Seed coat pattern, 0 to 7
10. Seed coat, major colour
11. Seed coat, minor colour
12. Seedling pigmentation

Plant characters

13. Plant habit
14. Branching pattern
15. Number of nodes per main stem
16. Height of plant (cm.)

Leaf and flower characters

17. Leaf shade
18. Leaf size (scale 1-5)
19. Flower, standard colour
20. Flower, wing colour

Physiological characters

21. Time from sowing to first flowering (d)
22. Time to maturity (d)
23. Position of pods

Pod characters

24. Number of pods per plant
25. Pod length (mm.)
26. Pod curvature, scale 0-5
27. Pod fibre, scale 0-9
28. Number of seeds per pod

Table 3. Example of the use of the Executive Information Retrieval Computer Program.

```
Print: (Cam. no., days to maturity, seed wt.,
node number, pods per plant, seeds per pod) for all with
habit, 5

and node number, from 2 to 10 and no. of days to flowering, from 10 to 60
and pods per plant, from 40 to 250 and pod texture, from 5 to 9
and seeds per pod, from 5.0 to 15.0

no. of items in query response = 6
no. of items in the data bank = 3929
percentage of response/total data bank = 0.15

2358   123   164 mg    9   50   5.2
2788   109   567 mg    7   46   5.2
2789   106   341 mg   10   62   5.7
2856   133   201 mg    6   52   5.2
2918   102   210 mg    7   52   6.9
5353   120   482 mg    7   49   5.9
```

Describing the collection

Table 2 shows the 28 data elements in the collection. These are mainly higher heritable characters, which are not greatly influenced by the environmental conditions.

The data collected are fed into the Executive Information Retrieval Computer Program (EXIR) produced by the Taximetrics Laboratory of the University of Colorado, Colorado, U.S. Table 3 gives an example of the use of this program for information retrieval.

Evaluation of the collection

Attempts are being made to evaluate the collection by the use of multivariate statistical techniques. If *p* quantitative variables are observed on each accession, then the complete plot of the data would be in a *p*-dimensional space. Many multivariate techniques such as principal component analysis and canonical variates analysis attempt to compress the data into fewer than *p* dimensions so that examination for structural patterns is eased. If most of the information can be compressed into two dimensions, then the data can be convientently presented in a two dimensional plot. To illustrate the use of these techniques, 8 quantitative variates were taken from 658 accessions originating from 20 countries. The eight variates considered for the exercise were; days to maturity, individual seed weight, growth habit score, number of nodes per main stem, plant height, days to first flowering, length of pod, number of seeds per pod.

By considering the 658 accessions as a single population the principal components analysis derives linear functions that contain the maximum information. The first two principal components were plotted but it was found that there was no obvious classification of the accessions into distinct sub-populations.

The canonical variate analysis was therefore used. By taking account of the

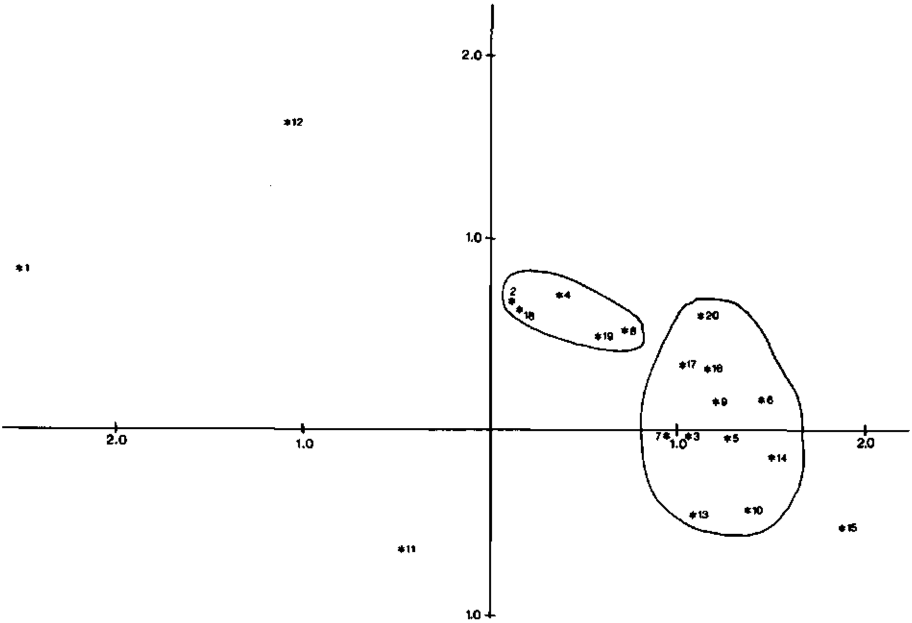

Fig. 1. Plot of first two Canonical Variates (country mean values). 1 Afghanistan, 2 Argentina, 3 Brazil, 4 Chile, 5 Colombia, 6 Costa Rica, 7 El Salvador, 8 Ethiopia, 9 Guatemala, 10 Honduras, 11 Iran, 12 Malawi, 13 Mexico, 14 Nicaragua, 15 Paraguay, 16 Peru, 17 Syria, 18 Turkey, 19 United States, 20 Venezuela.

national grouping of the lines, this technique derives the linear functions that maximizes the separation of the groups. The device thus compresses the differences between countries into as few dimensions as possible. The first two canonical variates accounted for 73% of the variation between countries and the mean values for each country are plotted in Fig. 1.

This separation between countries can of course be considered on a univariate basis, taking each of the eight variates in turn and performing a 'one-way' F test of variance (the between country mean square/the within country mean square). The F ratios for the eight variates were as follows:

days to maturity	7.0	height of plant	21.6
seed weight	4.6	days to first flower	11.9
growth habit score	9.4	length of pod	9.5
number of nodes per main stem	10.1	number of seeds per pod	3.0

By far the greatest F ratio and consequently the greatest separation between countries was for height of plant and the smallest was for number of seeds per pod. The first canonical variate was associated with height, so that the position of the country along the X axis of Fig. 1 was heavily dependent on the average height of the plants.

The countries Afghanistan, Malawi and Iran are clearly separated from the others; two clusters can be identified, one including Argentina, Chile, Turkey, Ethiopia and the United States and the other cluster including most of the Latin American countries. Perhaps because only one accession was available from Paraguay, it did not seem to fall into the cluster for the Latin American countries.

Utilization of the germplasm collection in plant breeding

The germplasm collection has provided an opportunity of describing the variation within the species *Phaseolus vulgaris*. Two breeding programs are in progress at Cambridge, one with the large-seeded food beans and the second with the small-seeded navy beans used for processing. Both breeding programmes are concerned with the development for Britain of beans as pulse crops, i.e. for seed production rather than for production of green pods.

Breeding programs for large-seeded bean

The collection has allowed the selection of promising parental material for the development of high yielding, high protein varieties of bush beans. From the collection 400 lines were initially utilized (Hamblin 1973) in an attempt to isolate the potentially high yielding types of bush habit. 64 of these were then selected and these were reduced to 25 varieties on the basis of yield, protein content, and yield and protein content together. Within these 25 varieties, 4 types of crosses were made:
high yield × high yield; high protein × high protein; high yield × high protein; high rank × high rank (i.e. parents above average for both yield and protein content).

With crosses of high yield × high yield and high protein × high protein methods are being used to select recurrently for high yield and high protein both between and within these two populations (Evans & Gridley 1978) with the aim of improving breeding methods for simultaneous selection for high yield and high protein in a crop plant.

The ultimate aim of the breeding program for large-seeded beans is to develop cultivars suitable for direct human consumption as dry beans, also cultivars suitable for producing textured proteins in meat substitutes and for animal feed. In the last case, however, toxicity would have to be overcome by breeding or, as in soya bean, by a heat treatment.

Breeding program for small-seeded, navy beans

The program for navy beans aims to develop small white-seeded cultivars adapted to the British climate. The 7 existing cultivars were produced in Michigan, US and are not adapted to the British climate. Furthermore they have a very narrow genetic base (Anderson, Adams & Whitford 1968).

The germplasm collection was used to identify other genotypes, which could be used as parents in breeding. The parents included types from Central America, South America, Turkey and northern Europe. (Evans & Davis 1978). A large number of advanced generation lines were grown in the field in 1977 and selections were made of those lines, which were early flowering, early maturing, tall and high yielding. Of these 15 lines proved to be superior to the Michigan cultivar, Seafarer, in the four characters and equivalent to Seafarer in canning quality.

Conclusions

The germplasm collection has proved useful in broadening the genetic base of material used in two breeding programs for Britain. It has been particularly valuable in providing genotypes suitable for breeding a new seed crop for a new environment.

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The Wageningen collections

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Index words

Collections at Wageningen, plant breeding institutes, gene bank.

Summary

A review is given of the working collections of the three Wageningen plant breeding institutes (IvP, IVT and SVP). Both the contents and the status of these collections are discussed and some examples of active introductions are given. The preparation of a national Dutch gene bank is announced.

Introduction

To explain why the collections of gene material are spread all over Wageningen, it is necessary to know the organization of plant breeding research in the Netherlands. This research comes under the Ministry of Agriculture and Fisheries, whose tasks are delegated to directorates, two of which concern us here, namely the Directorate for Agricultural Research and the Directorate for Agricultural Education.

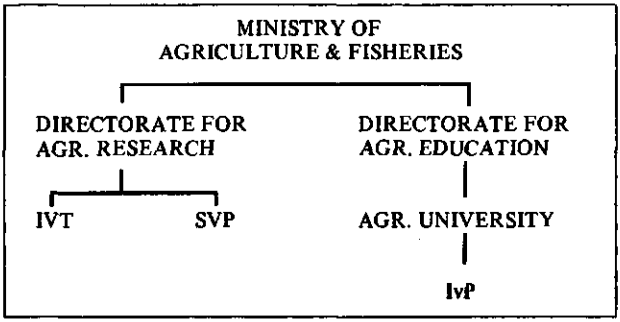

Fig. 1. Organization of plant breeding research in the Netherlands.

The Directorate for Agricultural Research supervises agricultural research in the 33 research institutes. Two of those institutes deal with plant breeding:
plant breeding:

- The Institute for Horticultural Plant Breeding (IVT)
- The Foundation for Agricultural Plant Breeding (SVP).

The Directorate for Agricultural Education is responsible for the Agricultural University, one of whose departments is the Institute for Plant Breeding (IvP). The main difference between the research of the IvP and the other two institutes is that IvP is oriented to breeding problems in general, whereas IVT and SVP are limited to several horticultural and agricultural crops, respectively. This is why there are different points of contact between the institutes, also for collection management. None of the three institutes, however, has the appointed task of building up or maintaining collections of germplasm. Every available collection serves primarily for current plant breeding research.

Composition of the collections

Table 1 shows both the size and the location of the most important collections at the three breeding institutes at Wageningen.

The genetic wealth of these collections depends on their composition rather than on the mere number of accessions. A major part of most collections consists of cultivars and breeding lines, whose genetic variation is very narrow. But even between cultivars and breeding lines, variation is limited, because they are normally intended for practical breeding purposes and these tend to differ as little as possible from one another for most characters: only a few phenotypes are acceptable for modern horticulture and agriculture and selection is against deviating types.

If more variation is needed for plant breeding than can be found in such closely related populations, the collections are supplemented with less related materials.

Table 1. Number of accessions and site of the main Wageningen collections. Numbers are rounded.

	Number of accessions		
Crop	IVP	IVT	SVP
Barley			4 000
Bean *(Phaseolus)*	100	900	
Beet			1 350
Cucumber		1 800	
Flax			1 000
Lettuce		1 500	
Pepper *(Capsicum)*		1 200	
Potato	3 000		
Tomato		3 000	
Wheat	2 800		3 500

Such collections of wild and primitive material are likely to contain much more genetic variation. The total amount of variation that can be used for plant breeding is in the first place crop-specific. How much of the existing variation is represented in a certain collection depends mainly on the introduction policy of the plant breeder concerned. What proportion of available variation is to be found within accessions depends on a number of factors, such as the breeding system, the system of collecting and preserving the material, and the manpower and facilities.

Since they are special-purpose collections, the Wageningen collections are primarily built up in the easiest and cheapest way possible. They consist largely of commercially available cultivars, special breeding populations developed by the institute itself or supplied by investigators or private breeders and of wild and cultivated material asked for at genebanks, botanic gardens and research institutes.

The Dutch collection of old cultivars, land races and primitive or wild material began mainly because of a shortage of certain characteristics. Usually the characteristics wanted in breeding modern cultivars are disease resistances, which are poorly represented in the material already available.

The most important crop in which active introduction is done is undoubtedly the potato. To find resistance to late blight and the golden nematode, three joint expeditions of IvP and SVP were organized to its main centre of origin Peru, Bolivia and northern Argentina. In the first two expeditions, only cultivated material was collected; in the last (in 1974) over 200 samples of wild populations were collected as well. Altogether, these expeditions resulted in over 3 000 acquisitions. They are used as follows: after increasing the material once at IvP, it is sent to the German-Dutch Gene Bank in Braunschweig for maintenance. The IvP works on the accessibility of the material by bridging crosses and SVP takes care of the breeding work.

The 1976 expedition of SVP to Pakistan was not in the first place induced by a lack of genetic variation, but by a chance partnership with a mountaineering club. The expedition covered the north of Pakistan, one of the gene centres for wheat. The emphasis was on spring wheat (320 accessions) and spring barley (195 accessions), but also some maize, grasses and pulses were collected. Most of the samples were obtained from farmers or collected in the field.

A third example of active collection is to be found in the lettuce collection of IVT. In 1976, over 200 samples of wild relatives of our cultivated lettuce were collected in Israel. This material contains interesting resistances to *Bremia lactucae*.

Other crops of which material was collected to save it from genetic erosion is bean *(Phaseolus)*:

- IvP collected 110 Dutch land races
- Department of Plant Taxonomy and Plant Geography of the Agricultural University collected land races in Ethiopia
- Pakistan expedition of SVP also yielded bean samples. Most of this material was or will be added to the IVT bean collection.

Also the beet collection of SVP contains 160 accessions of wild *Beta maritima* collected by staff of this institute in north-western France.

Management of the collections

The examples I have given represent the largest and best collections in Wageningen. For most of the lesser crops, the situation is different however. The primary use of the collections for current plant breeding research brings a large risk of genetic erosion, which is the main reason why most of the collections cannot be regarded as small genebanks. The collections are used for immediate benefits: for a breeding or research program, the germplasm is screened for some useful characters and for its crossability with modern varieties. Because no Dutch institute has the task of preserving the material that cannot be used or that has no further use, little priority can be claimed for preservation. The most usual techniques of plant breeders to deal with this problem are:

- maintaining a small, selected number of accessions instead of the whole collection
- maintaining as one group several accessions that look alike.

A second implication of the low priority the maintenance of germplasm has had in Dutch agricultural research is that even for the preservation of selected accessions manpower and equipment is minimal. So normally a very limited number of plants is used for maintenance, in violation of population genetics. Moreover, the amount of seed harvested is normally insufficient for exchange, and its quality is suboptimal as a result of inadequate drying and storage facilities. Further, the populations are increased only when the seed quality is so low that it is really necessary and the standards applied for this criterion by plant breeders and seed technologists are not always the same. Sometimes accessions are lost this way.

Such a low priority brings a toll for evaluation research, seed administration and germination tests on collection materials. Dutch breeders made themselves highly dependent on cooperation with foreign colleagues for an essential phase of their work, namely the provision of base material.

Establishment of the Dutch genebank

Fortunately, there is a growing awareness of the necessity of proper collection management and the plant breeding institutes took the initiative to improve the current situation. Since 1976, there has been an active working group in Wageningen preparing the establishment of a Dutch gene bank. This working group consists of representatives of the Directorate for Agricultural Research, the three plant breeding institutes IvP, IVT and SVP, the Government Seed Testing Station and the Government Institute for the Research on Varieties of Cultivated Plants (RIVRO). The aim is to create a gene bank for that material threatened by genetic erosion within our borders, and to improve the quality of the collections available in the institutes at this moment. In order to ensure the necessary continuity of the genebank work, the working group considers that the Dutch genebank should be run independently of the plant breeding institutes. The tasks of the genebank should be laid down by the law.

I think that our concept of the gene bank does not differ essentially from that abroad: both maintenance and evaluation are considered to belong to its tasks. The four points I have mentioned – the choice of material, the quality of maintenance, quality and amount of seed, and the accessibility of the collections – are the points that need improvement before most of our collections can be called a gene bank. First equipment for seed growing, seed drying and seed storage have to be obtained, so that collection will be followed by careful increase of the material. Only if one can rely on the preservation of the populations of a collection is it useful to describe and evaluate them extensively.

The simplicity of this concept is striking. Even the equipment needed for major improvement is simple and well known. So if the working group can overcome the financial and administrative obstacles, we should soon be able to maintain land races and other cultivars that fall into disuse, to keep for the future those parts of the working collections that lose direct significance for plant breeding and to change our introduction policy into an exchange policy.

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Collections of species and ancient cultivars of tulip (Tulipa L.) in the Netherlands

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Index words

Tulipa, tulip, collections, origin, species, cultivars, breeding.

Summary

The tulip industry will always require new flower colours and forms, while ever higher priorities are established for such characteristics as good bulb production, a short juvenile period, ability to be forced, good keeping quality and disease resistance.

In breeding, crosses with wild species or old cultivars could help to achieve these objectives. For this reason a collection of about 90 species has been built up at the Institute for Horticultural Plant Breeding. Besides, about 1 000 old cultivars are maintained in the 'Hortus Bulborum' at Limmen. Origin and properties of this material are being studied.

In the Middle Asian centres of diversity, the Pamir-Alay and Tien-Shan mountain systems, about 60 species occur at altitudes ranging from 400 to 4 000 m, e.g. *T. kaufmanniana*, which occupies a zone from about 400–2 000 m in the western Tien Shan. This explains the great variability within certain species and their adaptability when migrating to south-west Asia, the Mediterranean and eastern Europe.

The genus has been divided into the sections *Eriostemones* and *Leiostemones*. The *Eriostemones* are thought to have separated at an early stage of the evolution of the genus. They can grow in regions with a limited water supply and unfavourable soil types. The present-day cultivars are practically all *Leiostemones*, derived from a cultigen which was introduced from Turkey in the 16th Century and which is referred to as *Tulipa gesneriana* L.

A number of species from Middle Asia were introduced into western Europe around 1900. One of these, *T. fosteriana*, became the parent of the Darwin hybrids.

Only during recent decades has more information become available from population studies. It is evident that species segregation is continuing in our times. The wild species and old cultivars exhibit a greater variation in the most important characters than do the present-day commercial cultivars. They may therefore be a source of parental material in breeding for resistance to *Fusarium oxysporum* and tulip breaking virus, and for increased bulb production and early forcing.

Introduction

For centuries, tulip breeding has been a favourite and very profitable pastime of Dutch bulb growers, as is demonstrated by the bewildering number and range of registered cultivars. Further progress in breeding and selection is essential for the bulb industry. A need will undoubtedly arise for new colours and forms while ever higher demands will be made on properties that affect the economics of tulip growing, like bulb production (number and weight), duration of the juvenile period, ability to be forced (lower energy requirement), keeping quality and resistance to pathogens like *Fusarium oxysporum* and tulip breaking virus.

For improvements in this direction, wild species may be used considerably in breeding. Also crosses with old cultivars could be of advantage.

So the Institute for Horticultural Plant Breeding started to collect wild species in 1964. Moreover, a collection of about 1 000 old cultivars is being maintained in the 50-year-old 'Hortus Bulborum', a garden of the Netherlands Society for Breeding of Ornamentals ('Neversie') at Limmen. Information obtained about the origin and properties of the material is useful in our breeding work.

Origin and some characters of the genus Tulipa

In the Middle Asian centres of diversity, the Pamir-Alay and Tien Shan mountain systems, the genus *Tulipa* grows at altitudes ranging from 400–4 000 m, predominantly between 800–2 000 m. In those regions about 60 species occur. Several species grow at a range of altitudes, as does e.g. *T. kaufmanniana* in the range 400–2 000 m. This wide range with the fluctuating conditions of a montane climate explains the great variability of these species and their ability to adapt to diverse environmental conditions (Wood 1971) when they migrated from these centres to south-west Asia, the Mediterranean region and parts of eastern Europe.

The tulip collection at IVT includes about 90 species from more than 250 locations. In total there are probably about 100 well defined species for which nearly 300 names have been published. The collection of IVT has been built up and is being evaluated to provide material for our breeding program.

Boissier (1882) subdivided the genus into the two sections *Eriostemones* and *Leiostemones* and his classification has been maintained by later botanists, although some of them changed the rank of those taxa to subgenus. The *Eriostemones* represent a natural group recognizable by its morphological characters and karyotypes. It is thought that they have separated in the early stages of the evolution of the genus (Bochanceva 1962). They are in general dwarf-growing narrow-leaved species with filaments at their bases clothed with short hairs. They have more uniform chromosomes than the *Leiostemones* and have primitive characters, like monocolpate pollen (Romanov 1959; Walker 1974), as well as secondary ones in their inflorescences and embryo-sacs (Cave 1951; Romanov 1944). They have colonized habitats in the more extreme situations of limited water supply and unfavourable soil types (salt, gypsum). Vvedenskiĭ in his treatment of the genus in 'Flora of the USSR, Vol. 4' (1935) mentions 65 tulip species for the Soviet Union, and Bochanceva (1962) cites another 19 species which have since been described. She also describes the karyotypes of 50 wild species on the basis of an investigation of 10 individuals of each population. Hall (1940) dealt with the genus for south-west Asia, the Mediterranean region and eastern Europe and also included the cultivated Middle Asian species, being well aware of his limitations in the latter case. Exploration of the Middle Asian centres began in the last quarter of the 19th Century. E. Regel (1873-1887), at that time Director of the Imperial Botanical Gardens at

St. Petersburg, was the first botanist to describe tulips from these areas. Some of the species were introduced into western Europe around the beginning of the century; six of them were described and named by J.M.C. Hoog in the Netherlands. One species, *T. fosteriana*, became the male progenitor of our present-day Darwin hybrids. The sites where these early introductions were collected are not exactly known. Clonal selections by growers, and maintainance as small clones in botanical gardens did not permit population studies and has resulted in many taxonomic synonyms. Only in recent decades has more information, mainly from Soviet sources, become available about the areas where *Tulipa* is a component of the vegetation cover in spring. Silina (1969) investigated the intraspecific variability of *T. kaufmanniana* in the western Tien Shan over a period of 10 years and observed that vegetative propagation in nature was virtually absent and that the populations maintained themselves by seed. Abramova (1964) surveyed the genus for Turkmenistan. Kupcov (1975) studying 51 populations of *T. schrenkii* of the Ukranian steppes, the Trans-Volga region and northern Kazakhstan, made a statistical analysis of colour distribution. He could not establish any influence of soil type or the prevailing climate on colour distribution. We could cite many others. Russian authors and British botanists exploring Iran believe that species segregation is continuing in our times (Taliev 1930; Hall 1940; Bochanceva 1962; Bykov 1972). The wide variability in the more than 40 populations collected in Iran and Afghanistan and now being studied at the IVT supports this.

Practically all present-day cultivars are *Leiostemones*. They do not constitute a natural group and are derived from a cultigen introduced from Turkey in the 16th Century. Attempts to cross *Eriostemones* with *Leiostemones* species have never been successful.

Use of species and ancient cultivars

For future breeding, crosses with wild species or old cultivars could be of advantage. Of the wild species, the early flowering ones with resistance to *Fusarium oxysporum* or tulip breaking virus are the most interesting. At IVT some species, among them certain clones of *Tulipa fosteriana* and *T. greigii*, have been found to have a high degree of resistance to *Fusarium oxysporum*. Systematic screening for resistance to the notorious tulip breaking virus will be started this year, because one species, *T. fosteriana*, has already been found which is not, or only slightly, affected by tulip breaking virus. A negative property of this species is the poor keeping quality of the flowers. It may therefore be necessary to cross it with commercial cultivars of *T. gesneriana* of better keeping quality. In seed populations of *T. hungarica*, *T. rhodopea* and *T. vvedenskyi* we found a small proportion of individuals that already flowered in the third year after sowing, while first flowering is normally delayed until 4 to 6 years from sowing. Perhaps this property could be exploited in breeding for a shorter juvenile period and perhaps the duration of the juvenile period is correlated with bulb production and forcing ability.

Not only wild species but also old cultivars from the 'Hortus Bulborum' could be used in breeding. There is strong evidence that some old cultivars are resistant to *Fusarium oxysporum*. As it was recently confirmed that resistance to *Fusarium* is controlled by additive gene action, the detection and use of a range of resistant parents in breeding should greatly improve resistance in progenies. More old cultivars and populations of species will be tested at IVT for resistance both to *Fusarium oxysporum* and tulip breaking virus.

Old cultivars can also be used to increase bulb production and ability to be forced. Because breeders in the past have made use of few parent clones, some inbreeding is likely. The unformity noted by earlier breeders in populations from crosses among Darwin tulips suggests the same. The Mendel and Triumph tulips have shown that plants with good hybrid vigour can be obtained by crossing tulips of different origin.

By tracing the parentage of tulips that respond favourably to early forcing, we have found that this earliness has generally been derived from certain early flowering species (e.g. *T. schrenkii* from the Crimea) and old cultivars. Use of such species and old cultivars as parents may further reduce the cold requirement and forcing period. Still unknown is whether an extreme shortening of the glasshouse period can be achieved without simultaneously shortening vase life. It is in any case strongly recommended to use parents of good keeping quality. Because tetraploids generally show a better lasting quality than diploids, tetraploidy was introduced artificially in cultivars and species. For this, Zeilinga, Schouten and Kroon (IVT) used nitrous oxide (N_2O) on early flowering species such as *T. fosteriana, T. kaufmanniana* and *T. greigii*. This resulted in tetraploids that are being crossed with tetraploids of *T. gesneriana*, also obtained by treatment with nitrous oxide. In the course of time, these crosses may yield offspring combining suitability for early forcing with a long vase life.

Results of systematic crosses between older and more recent cultivars in a diallel program at IVT have shown that one of the older cultivars ('Golden Harvest' registered in 1928) gave a good bulb production in all its combinations. Another old cultivar 'Duc de Berlin' (1860) generally transmits earliness to its offspring.

These examples show how the collections of wild species and ancient cultivars can be used to provide us and others with the right parents for breeding.

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Study of genetic resources of crops in Czechoslovakia

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Index words

Gene bank, genetic resources in Czechoslovakia, field crops, vegetables, fruit crops, ornamentals.

Summary

In the CSSR, planned research on genetic resources started in 1951, when 6 000 cultivars from various places collected since the beginning of the Century were concentrated under the supervision of the Institute of Genetics and Plant Breeding of the Research Institutes of Crop Production, Prague-Ruzyně. At present, the collection is distributed over 14 research institutes according to group of crops; it now comprises 40 274 cultivars; some of the items are double in various institutes so that the total number reaches 45 548 cultivars of field crops, vegetables, fruit crops and ornamentals. Of that total, 3 000 cultivars are of Czechoslovak origin. About 5 000 cultivars are introduced every year into the collection and the same number is distributed to the foreign countries. Research is directed to use in breeding and to direct introduction into practice. Unification of methods of evaluation, computerized output of information within the countries of COMECON and construction of a gene-bank is being planned.

Introduction

In Czechoslovakia, genetic resources of crops have been investigate for many years. The present program has continued the successful activity of the State Research Agricultural Institutes in Prague and Brno that even in the 1920s had systematically collected and evaluated domestic and foreign cultivars. Collections of some crops have been thus maintained since that time, as for instance grapevines since 1903, wheats and barleys since 1910, potatoes since 1922, and most other crops since the 1930s.

A large-scale investigation was started in since 1951 when agricultural research institutes were reorganized. The initial collection of 6 000 cultivars in 1951 was expanded to 18 600, 35 636, and 40 274 cultivars in the years 1960, 1970 and 1977, respectively.

At present, maintenance of the Czechoslovak collection of genetic resources is dispersed, being recorded in fourteen Research Institutes, sometimes in Breeding or Research Stations that are affiliated to them. Eleven of these institutes are special-

Table 1. Survey on institutes, species in the collection, and number of accessions (31 december, 1977)

Institute	Crops	Number of
Institute of Genetics and Plant Breading, Research Institutes of Plant Production, Prague – Ruzyně	wheat, winter barley, rye, oats, poppy, vegetables, grapevine	7 529
Research Institutes of Plant Production, Piešťany	wheat, barley, Triticale, soya, beans, lentil, clover, lucesne	2 506
Department of Variety Testing, Central Institute for Control and Testing in Agriculture, Prague	field crops, vegetables, fruits, grapevine	1 497
Research and Breeding Institute of Cereals, Kroměříž	cereals	7 779
Research and Breeding Institute of Maize, Trnava	maize	1 306
Research and Breeding Institute of Technical Crops and Legumes Šumperk	legumes, technical plants, oil plants	3 458
Research and Breeding Institute of Beets, Semčice	beets	149
Research and Breeding Institute of Potatoe, Havlíčkův Brod	potato	620
Research and Breeding Institute of Fodder Plants, Troubsko u Brna	clover, grasses, wild fodder plants	1 784
Research and Breeding Institute of Hops, Žatec	hops	139
Research and Breeding Institute of Vegetables, Olomouc	vegetables, aromatic plants	10 166
Research Institute of Viticulture and Vines, Bratislava	grape vines	1 400
Research and Breeding Institute of Fruits, Holovousy	fruits	2 132
Research and Breeding Institute of Ornamental Plants, Průhonice	flowers, ornamental plants	6 230
Total		45 695

ized in individual groups of crops (Table 1). Their program includes also exploiting of plant resources in practical breeding.

Investigations have been coordinated by the Institute of Genetics and Plant Breeding of Research Institutes of Plant Production, Prague-Ruzyně. There is also a

Table 2. Survey on Czechoslovak Collection of Cultivars (31 December, 1977).

Group of crops	Number of samples
Cereals	10 070
Maize	1 306
Legumes	2 624
Oil plants	408
Technical plants	1 695
Beets	149
Potatoes	620
Fodder plants and grasses	2 504
Hops	139
Vegetables	10 390
Aromatic plants	36
Grape vine	1 471
Fruits	2 132
Ornamental plants	6 230
Collection of the Department of Variety Testing of the Central Institute for Control and Testing in Agriculture	500
Total	40 274

Centre of Introduction, which organizes international exchange of seed samples. For instance in 1977, the Centre imported 3 085 cultivars (inclusive of 5 090 cultivars imported by other institutes) and exported 3 824 cultivars (inclusive of 4 969 cultivars exported by other institutes). Exchange has been encouraged by Indexes seminum which are published every third year. Each of them lists about 12 000–15 000 cultivars of the major species. Import is partly by buying seed samples from breeding firms of the whole world. Coordination of investigations includes also technical supervision of the research. Thus uniform registration (data bank) and the plan for a central conditioned store (gene bank) are being elaborated. In cooperation with other institutes, the Institute of Genetics and Plant Breeding supplies 8 000 cultivars every year, inclusive of the necessary documents for immediate use in breeding.

The total of 40 274 collected cultivars, by crop groups, is listed in Table 2. The Table does not include cultivars that have been separately recorded in several institutes, which would bring the total to 45 548. The collection includes about 3 000 samples of old Czech regional cultivars and both old and current Czech cultivars.

Evaluation

Immediate agricultural exploitation, i.e. selection of cultivars for testing at Stations for State Variety Testing, or eventual registration of cultivars is Czechoslovakia, has been performed by the Department of Variety Testing of the Central Institute for Control and Testing in Agriculture. Foreign cultivars can be also tested in Czechoslovak State Variety Tests under contract with the breeding firm abroad, a certain tax being levied. If the cultivar manifests a higher quality in agricultural trait, it can be registred in Czechoslovakia or for regular, (i.e. yearly) import, or for occasional import. For instance in 1976, 27 new domestic and 20 foreign cultivars were registered for occasional import. In 1977, 12 domestic, 7 foreign cultivars for regular import, and 23 cultivars for occasional import were registered with field crops, vegetables, fruits and grapevines. As usual from Western countries, seed is imported for licensed propagation. Use of foreign cultivars is very important, even in countries like Czechoslovakia where breeding with main crops has been successful.

Registration

The system of registration and evaluation of collections in specialized research institutes, sited most favourably in climate for the particular crop, proved completely practical. It allowed extension of studies on the collection. Cultivars are now evaluated in field trials covering about 60 ha every year. Detailed descriptions have been made since 1951, which are valuable for breeding, and 380 detailed reports have been completed which are exploited by teams of breeders. This activity has still reflected in the quality of present breeding material and new cultivars bred in Czechoslovakia.

Present and future development

At present, work is directed to programming the activity of the future gene bank, namely in following two directions:

- establishing a data bank comprising information on genetic resources
- elaborating the plan for a long-term store for samples.

The data bank is being planned in a close cooperation between countries of COMECON, and also with advice from FAO.

The work includes the following five items:

- registration of resources gathered by expeditions, the program of which was agreed with the AUICP, Leningrad
- registration of resources imported into Czechoslovakia, the national program has been computer used since 1975
- registration in the card-index, i.e. essential information for registering the cultivars in the collection
- description of the cultivar according to the international classification system. (Classification systems for 21 crops have been elaborated in Czechoslovakia and 12 classification systems have already been unified within the countries of COMECON).
- registration of storage and distribution in the gene bank.

Description of cultivar, and of storage and distribution are computerized in a model of a selected set of wheat cultivars. Having been verified, the model will be employed also for other species and cultivars in their terms. The last point will be executed later.

The system for long-term storage of seed samples profits from experience of various countries, mainly the AUICP Leningrad, and also from local experimental data with the collection of vegetable crops stored at temperatures below zero. The project expects that 8 boxes, each of 50 m^3, will be built up, the total capacity of them being 120 000 samples. Each sample is to be stored under two conditions: at −18 °C, and at 2 °C and controlled relative humidity of 20–30%.

The system of storage should ensure preservation of genetic resources for future generations and supply the breeders and farmers with seed samples as well as ample information from the computerized data bank.

Conclusion

The paper gives a short information on registration and exploiting the collection of crop resources in the ČSSR. By developing a many year cooperation with main research institutes of COMECON, headed by the AUICP Leningrad, our research workers contribute to the all world effort to preserve and use the gene pool of crops. Exchange of crop resources concern important research institutes in all the world. Recently, cooperation is being developed with FAO, EUCARPIA, and Gene Bank Committee.

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Wild forms of Trifolium resupinatum collected in Asia Minor succeed in Central Europe

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Index words

Trifolium resupinatum, evaluation of collection.

Summary

In 1969, I collected seeds of wild forms of *Trifolium resupinatum* in the western part of Anatolia with the assistance of Dr W. Hertzsch, who was at that time specialist in forage crops at the Izmir FAO Centre in Turkey. Also in 1969, W. Hertzsch sent me two further samples collected in eastern Anatolia near Lake Van. All seeds came from areas that had never been cultivated. The clover plants grew on the border of roads and fields and sometimes in non-arable habitats occassionally used for cutting or grazing.

Although the samples were not isolated during the first years, they did not lose their special characters. First of all they differed in growing type. Some plants started developmment as a rosette and later branches crept on the ground; other plants developed erect stems immediately. Other differences between the origins were flower size, flower colour and number of leaves. All forms flowered freely; all had small seeds with thousand-seed weight of about 0.5 g, whereas the cultivated forms from Iran had a thousand-seed weight of 1.5 g. The leaves were smaller and the stems finer than those of the cultivated forms.

In field trials selected strains of that material yielded only one productive cut, whereas most of the cultivated forms from Iran showed 3-4 times a regrowth after cutting during vegetative development. After these experiments the selected material of Anatolian wild forms was tested as a second crop. When sown on 16 July 1974, all 18 strains under test yielded 1.5 or 2 times as much as an Irani provenance and as cv. Maral from Portugal. In 1975, 45 strains that were tested, gave similar results. When sown on 23 July after harvesting winter barley, the best strains yielded more than 6.0 kg/m^2 of fresh matter and 0.60-0.70 kg/m^2 of dry matter. But stands sown 4 weeks later did not develop well. In the very dry year of 1976, all catch crops grew poorly. But in 1977, extended field trials with Persian clover selected from wild forms gave good results. The main date of sowing was 2 August. Those strains of which more seed was available were sown too in spring on 2 May and if possible on 16 August. The yield of the strains was again compared with that

Table 1. Trials with strains from Anatolian provenances of Persian clover and relation to average of a cultivated provenance of Iran and cultivar Maral.
n = number of strains tested

Date of sowing	n	Proportion of strains classed against standards (%)		
		better	same	poorer
1977-05-02	104	17	74	9
1977-08-02	126	76	18	6
1977-08-16	83	46	52	2

of an Irani provenance and cv. Maral, used as standards in Table 1.
Only one cut was evaluated of the spring seed. The trial sown on 2 August was cut on 31 October. Whereas the average yield of the standards was 3.05 kg/m^2 for fresh matter and 0.33 kg/m^2 for dry matter, the wild forms yielded more than 6.0 kg/m^2 for fresh matter and 0.60 kg/m^2 for dry matter. The stands of the last sown trial also covered the soil excellently but unfortunately we could not gather the crop before the clover plants were knocked by frost. Therefore evaluation of the third sowing in Table 1 was based on an estimate on 11 November before the cold nights.

The strains developed from Anatolian wild forms were more suitable for growins a second crop than the commercial provenances.

German sugar-beet farmers now prefer Persian clover as a catch crop after early ripening cereals to crucifers, because that way they avoid propagation of the sugar-beet nematode *Heterodera schachtii*. I think the selected material of Anatolian wild forms offer a better and safer yielding catch crop, when sown by 15 September.

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Aegean clover (Trifolium meneghinianum Clem.): a legume for intercropping

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Index words

Trifolium meneghinanum, Aegean clover, forage crop, intercropping, hydrocyanin content, leaf mark.

Summary

A rapidly growing strain with high suitability for greenmanuring was bred by selection from material collected in Turkey. Sown after the harvest of early cereals at mid-July it produces *more than 40 t/ha green matter till autumn. It could be of special importance for cultivation in* sugar beet areas because this clover is no host plant of the beet-nematode.

In 1970 the Institute for Plant Breeding of our station received a seed sample of *Trifolium meneghinanum*, a clover collected by Hertzsch in 1969 in Turkey. This material was described by Hertzsch et al. (1974). According to their first investigations, it is an annual rapidly growing clover with a high content of protein. Most of plants do not regrow after cutting. If there is regrowth, its yield is only a quarter of the first cut. In greenhouse trials in 1973, ten plants were found with good regrowth after cutting. After pollination by means of bumble-bees in the greenhouse, sufficient seed was produced to lay out a performance test of the progeny (Morgner 1973). The yield of the strain bred by this simple method exceeded the yield of the initial material by 30%. Nearly all plants showed good regrowth after cutting.

The initial population consists of both plants with and without leaf marks. The new strain has no leaf marks. Cross-experiments showed that leaf-marking is inherited by only one gene. Another bred strain has only plants with leaf marks but its yield is too low.

Though it contains hydrocyanic acid, cattle like Aegean clover. But we have deferred our original intention to use it as a forage crop. It is not clear yet whether there are other components responsible for its bitter taste. Single plants show quantitative differences in hydrocyanin content but plants free of this component were not found. We hope to find appropriate initial material among other provenances of this clover.

To promote soil fertility in crop farming, there is an increasing need for green manure. This rapidly growing clover could find such an application. In areas where sugar-beet are grown, legumes are especially suitable for this purpose because they are no hosts of the beet nematode (*Heterodera schachtii*). Trials to undersow Aegean clover in cereals were not successful (Hertzsch et al. 1974) because, in contrast to red clover, the growth of the clover after harvest of the cereals was poor. In other trials, when the new strain of Aegean clover was sown after harvest of early cereals, results were promising. After sowing at mid-July 1973, we harvested ten weeks later 48 t/ha fresh matter or 4.8 t/ha dry matter. Including roots and stubble, the dry matter yield was 6.5 t/ha. In 1977 when the clover was sown at the beginning of August, we obtained the same yield. For comparison, Persian clover yielded only 31 t/ha (origin Iran) and 26 t/ha (cultivar 'Maral') respectively of fresh matter. When sown at mid-August the new strain reached a height of about 20 cm.

The ability of Aegean clover to grow and to cover the soil surface rapidly and its low requirements for nutrients – 20 kg//ha N was applied in all cases –, indicate the suitability of this species as green manure. Our breeding work so far with Aegean clover has demonstrated that collected material of a 'new' species can be improved rapidly by simple breeding methods. This agrees with our experience in breeding with Persian clover.

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Establishment of a gene bank for crop plants in Israel

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Index words

Data bank, dynamic preservation, indigenous species, land races, onion, safflower, wheat, EXIR II, Israel.

Summary

The establishment and activities of the Israelian gene bank have been discussed.

The need for the establishment of a gene bank in Israel comes in response to the realization that there is a need for Israel to collect and maintain its rich heritage of germplasm and to join the world wide efforts for the preservation of valuable genetic resources, their evaluation, documentation and utilization. Small countries like Israel, with limited financial resources and many other priorities, have to identify those areas in which they can make a unique contribution to germplasm conservation and use. A committee was appointed by the Agricultural Research Organization and the National Council for Research and Development with the task of establishing a gene bank in Israel. It is composed of breeders, pathologists and geneticists from the Ministry of Agriculture, academic institutions and private seed companies. The committee had the task of developing the organizational system, identifying those areas in which maximum effort should be placed and cooperation with the existing genetic resource facilities both in Israel and in other countries. The decisions of the committee were geared to finding the golden path between unrealistic ideals on the one hand, and insufficient effort to preserve genetic resources on the other.

One of the first decisions of the committee was that the Israel Gene Bank for Agricultural Crops would adopt a decentralized participatory approach. Further, it was decided that the bank's functions would encompass plant introduction, seed storage, genetic resources evaluation and information gathering, analysis, dissemination and exchange. These efforts would be proportionate to the total plant breeding effort in Israel and geared to assist it. Whenever possible, the breeders would maintain their material, evaluate the collections (with financial assistance from the Bank) and supply the records to the Bank where these data would be

Table 1. Ten Crop Committees of Israel Gene Bank for Crop Plants

Winter Cereals
Summer grains
Industrial crops
Medicinal and spice plants
Solanaceous vegetables
Other vegetables
Pulses
Forage and pasture plants
Ornamentals
Cucurbits.

transcribed into machine-readable form.

The gene bank would guide these efforts by the following:

- Long-term storage in the Bank's own facilities.
- Air and increase know-how in improving the existing storage facilities of individual programs.
- The establishment of a central data services using the Exir system developed by IS/GR center in Boulder, Colorado, USA.
- Developing an exchange network with the worldwide system of genetic resources.

The second major step of the establishing committee was to appoint 10 committees (Table 1) to determine the needs and priorities of genetic resource collection, maintenance, information and use in the major crops of Israel (excluding perennial fruit-trees, citrus and similar plants, which will be dealt with at a later date). One of the benefits of having these committees was to increase awareness of the genetic resources program by involving most of the leading breeders in this cooperative effort.

The role of these committees in suggesting strategies for data collection, elimination of duplication, and determining priorities for collection, and increase of material is an essential ingredient for the success of this form of decentralized bank.

The major conclusions of the crop committees are as follows:

- There is an urgent need to enhance the plant introduction activities.
- Primitive varieties and land races should be collected immediately.
- Collections in indigenous species related to the cultivated crops should be made on a selective basis.
- Computarised data banks for each crop and its wild relatives should be established.

The collection of primitive cultivars is one of the areas where Israel can make a contribution to the world effort and high priority has been assigned to this task which includes the following:

- Collection and organization of information from the collections made in the past.
- Intensive collection in those areas where primitive varieties of cereals, legumes and pulses are still grown with efforts being made to reach those areas where collecting has not been undertaken.
- Cooperation with scientists from other countries.

The rich diversity of indigenous species in Israel, which are either the established direct progenitors of cultivated plants or species closely related to the cultivated crops, prompted the establishment of a special committee. This committee consisting of experts in research and use of wild species in breeding was charged with determining a policy for the conservation of the rich resources of germplasm, this being one of the areas in which the greatest contribution can be made. The following was decided:

- An effort be made to collect information about the wild relatives of cultivated plants that grow in Israel. This information is to be obtained from previous studies as well as new studies where data is not yet available about the geographic and ecological distribution, population, size and variation.
- To collect samples of species that have limited populations or distributions and where there is a distinct danger of their elimination. The preservation would include seed storage as well as inclusion where possible in new or existing botanical gardens.
- The development of techniques of dynamic preservation (Browning 1974; Dinoor 1976) for those species that are common and not in any immediate danger of elimination. In order to ensure their continued survival, a watch system would be developed to maintain surveillance of future developments such as urban sprawl, road networks, and agricultural encroachment which might endanger a particular ecological niche of any of these species. The well structured and developed system of Nature Reserves of the Nature Reserves Authority and the well organized and influential volunteer Society for Nature Preservation affords Israel an interesting opportunity of developing and testing the concept of dynamic preservation of genetic resources.

The EXIR II programme has already been successfully integrated and was used in preparing the register of the materials stored now at the control seed storage facilities. The individual crop committees have been asked to survey this listing for the following:

- to eliminate duplications
- to select lines for long-term storage
- to transfer to larger collections in gene banks abroad.
- to fix priorities for increasing the material

The data on collection of cultivated safflower (*C. tinctorius*) assembled by Ashri (1975) was successfully integrated into the EXIR program and is ready for world-wide use.

The Gene Bank Committee decided to proceed this year mainly with two crops from which much experience will be gained: wheat and onion. Crop committees composed of breeders were appointed for each crop in order to integrate the breeding lines, cultivars and wild species into the EXIR system, plan evaluations and collections. These two committees face different problems; the onion committee has already taken active steps:

- Preparation of a thesaurus for integration of their breeding lines and other materials, which includes morphological descriptions and states, physiological behaviour and reactions to several important diseases.
- Collection of data on the nature and distribution of indigenous *Allium* species and development of recommendations for collecting and preserving these species.
- Determining of priorities for the increase and storage of existing collections.

- The establishment of a common library of literature on genetic resources and their use in onion.
- Contacts with existing collections of wild and cultivated material of onion in other parts of the world.

One of the main objectives of a gene bank is to stimulate interest in the maintenance and use of germplasm as a tool in breeding, combating the danger of vulnerability resulting from a narrow genetic base and providing information about new and unusual crops. To move in this direction, a successful one-day symposium was organized by the Israel Gene Bank and over 100 breeders, researchers and agronomists attended. The interest generated should help stimulate future meetings on topics of genetic resources and initiate supporting research, which is an integral part of improving germplasm preservation. Israel's contribution as part of the worldwide efforts will focus on:

- Collection and maintenance of land races.
- Collection and dynamic preservation of indigenous species which are related to cultivated crops.
- Development of the research potential in the area of genetic resources.
- Information systems evaluation and analysis.

Despite its size and limited resources a substantial contribution is possible.

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Native Turkish forage legumes and grasses in agriculture

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Index words

Forage legumes, grasses, *Lolium perenne*, perennial ryegrass, *Trifolium pratense*, tetraploid red clover, *Vicia sativa*, vetch, Turkey.

Summary

The native collections of perennial ryegrass, tetraploid red clover and vetch have been discussed.

To increase agricultural production, better-yielding cultivars of forage crops have to be found. In the Turkish breeding program for forage crops, native material that occurs in different regions of Turkey has to be observed.

Native perennial ryegrass (Lolium perenne L.)

In our breeding program for perennial ryegrass, native perennial ryegrass material from different regions of Turkey has been collected. In one region near the Province of Burdur, the material was collected from a native pasture with a crust of white salt on the surface of the soil. The soil type was a heavy wet clay. In another region in the south-east of Turkey, I have collected native perennial ryegrass from the Province of Urfa; the maximum temperature in this area is 46.5 °C. with a minimum of −12.4 °C. The average annual rainfall in this area is 473 mm., mostly in the fall and winter. The altitude of the area from which material was collected was 547 m above sea level. The plants collected had a range of growth habit. Two plants were prostrate and three plants were upright. After clonal propagation, we transplanted the plants to fields at different substations in the country. This material survived at Ankara without irrigation. Seed setting was normal. The clones produced bigger spikes than the originally collected material.

In general, the material has a wide range of adaptability to different climatic conditions. There are quite large differences in growth habit among clones collected from the same vicinity.

Native tetraploid red clover (Trifolium pratense L.) (2n = 28)

Red clover cultivation in Turkey is becoming more important as the demand for forage increases. But, except for introductions from abroad, there are no improved cultivars available. To obtain more productive forage material for Turkey, we have collected native red clover from the east of the country and have selected from it a native tetraploid plant. Tetraploid red clovers were produced by colchicine treatment in many countries. But there are no reports in the literature of native cultivated tetraploid red clovers. Seed setting of the material has been studied. We have randomly collected 100 heads from our trial plots. We have counted the number of the flowers and determined seed set, which averaged 49.51% at Ankara with irrigation.

Vetch (Vicia sativa L.)

Vetch is an important forage legume in Turkey. In our collection, we have found two distinct types of material for seed-coat color and winter hardiness. So far, we have selected one strain with black seed-coat and another with yellow seed-coat. The black-seeded vetch does not have hard seeds and appears to be more suitable for winter cultivation in the coastal cotton-growing areas. The yellow-seeded vetch is more suitable for fall sowing in the transitional areas because of its tolerance to cold.

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Gene bank activities at the International Potato Center

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Index words

Solanum sp., potato, wild potato, gene bank.

Summary

A short review is presented about the activities of the International Potato Center at Lima.

One of the main goals of the International Potato Center (CIP) is the preservation of the genetic resources of the potato. As CIP is a member of a network of international centres sponsored by the Consultative Group on International Agricultural Research, it is also concerned with the utilization of these genetic resources to increase potato productivity in developing countries.

The indigenous reservoir of potato genetic variability is known to be threatened by extinction in its centre of diversity. It is also recognized that the danger of genetic erosion is more serious in the native Andean cultivars than amongst its wild relatives because of their rapid replacement by locally bred cultivars. Despite the numerous collections made during the 50 years before the creation of CIP, a large proportion of the cultivated material collected had been lost and consequently there was not an adequate sample of the cultivated potato species in existing potato gene banks.

Accordingly, during the past five years, CIP efforts conducted under the leadership of Professor C. Ochoa, have been concentrated on the collection of native cultivars. The strategic location of CIP in Peru, where the greatest genetic diversity of the tuber-bearing *Solanum* species exists, has facilitated the organization of systematic collecting expeditions to countries from Guatemala southwards to Argentina. It is now considered that most of the existing cultivated germplasm of the Andes is available within the 11 000 accessions assembled in the CIP potato gene bank. Consequently, efforts have now been shifted to the exploration and collection of wild potato species. First priority has been the collection of those wild species not available in the living state in existing potato gene banks around the world.

The cultivated germ plasm is currently being maintained by annual asexual propagation. All the non-sterile entries are also maintained as botanical seed. In addition, culture techniques in vitro have been developed and are being tested as a means of long-term storage and for the future distribution of genotypes.

Detailed taxonomic studies conducted in the cultivated collection have shown the presence of many duplicates, which constitute different samples of the same morphotypes or cultivars. They were most likely collected under different vernacular names or at different localities in their geographic area of distribution. Furthermore, it is worth noting that they are not only morphologically identical but also have the same electrophoretic pattern and similar reaction to some pests or diseases. In such cases, the best clone is chosen to represent the group and the rest are converted into botanical seed and are no longer maintained vegetatively.

The collection is being evaluated for several pests and diseases, environmental parameters and agronomic characteristics. Morphological descriptors have been recorded to maintain the identity of the stocks. In addition, acquisition of data will be greatly facilitated by using a recently developed list of standard descriptors for the cultivated potato.

All available data from these evaluations as well as from the collection localities, native names and taxonomic identifications of the entire cultivated collection have been stored in a computerized data-management system. Thus, all the documentary information related to each entry in the gene bank will be readily retrievable.

In terms of international collaboration for the conservation of potato genetic resources, CIP has given high priority to the development of a cooperative system between gene banks to maintain, regenerate, distribute, and exchange botanical seed of the wild potato species.

There is no need to stress that there is considerable duplication of effort in the maintenance of the same accessions in several gene banks. However, intensive collections and exploration are still needed to obtain a more representative sampling of the genetic diversity present in each tuber-bearing *Solanum* species, so that its potentialities can be fully exploited. Consequently, if large numbers of new accessions are to be obtained by collecting expeditions, the present problems of maintenance and the future problems of rejuvenation of stocks will be increased to a point where their conservation in a single potato gene bank may not be feasible.

The proposal for such cooperation between gene banks has received endorsement, in principle, by the United States Potato Collection at Sturgeon Bay, Wisconsin and the German-Dutch Potato Collection at Brunswick, West Germany. Therefore, it appears that the necessary conditions are present to begin an effective international collaboration for preserving the genetic resources of the potato.

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Plant genetic resources in Poland

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Index words

Poland, genetic resources, gene bank, collection, evolution.

Summary

Based upon recent estimation, the whole genetic stock of the crop plants has about 50 000 accessions which are maintained at various collections in Poland. This includes 8 000 accessions of wheat, 3 500 of barley, 1 600 of oat, over 6 000 of grasses, 3 000 of leguminous plants and over 1 000 of potato. The largest collection (15 000 accessions, mainly cereals) is maintained at the Central Plant Collection Department at Radzików. The Plant Breeding and Acclimatization Division in Bydgoszcz takes care of genetic stock of grasses (8 000 accessions).

Activities

The Central Plant Collection Department is responsible for conservation of germplasm of wheat, barley, oat, rye, and some pulses in Poland. The activities are: exploration, storage, evaluation, rejuvenation and multiplication as well as documentation and distribution of the material collected.

Exploration

Every year an expedition is organized for collecting chiefly old cultivated forms of cereals and legumes, but samples of wild stands are also collected. A joint exploration with DDR (Gatersleben staff) was held in 1976, covering the Karpathian mountain region. A similar expedition took place last year and the next one is planned for August and September this year.

Evaluation

Every new accession is subjected to 3 years field and laboratory evaluation in order to obtain reliable results. Evaluated are several morphological and agronomical characteristics. The results are distributed among the breeders. For evaluation and documentation of wheat stock, the minimum descriptors list suggested by FAO was adhered to.

Storage, rejuvenation and multiplication

Lack of controlled storage conditions entails frequent rejuvenation of the seed samples (3–4 years). In order to improve storage conditions, and thus prolong seed viability and eliminate frequent rejuvenation, silica gel will be added to every hermetic glass container. When the amount of seeds of any accession decreases to the minimum designed for the species, then additional multiplication in the field is performed.

Documentation

This year, for the first time, a computerised storage and retrieval system has been applied for passport data documentation in rye, oat and, partly, barley collections. The agronomical and morphological data will be added to this in the near future.

Prospectives

Based upon the decision reached by the Ministry of Agriculture in cooperation with collaborating Ministries and the Secretary of The Polish Academy of Science, the Central Plant Collection Department will change its name into Plant Genetic Resources Department and will take responsibility for the plant genetic conservation programme in Poland. The programme is to cover all major crop plants in this country. For this, 22 active collections will be established in 16 stations responsible for a certain crop or crops. The base collections will be stored in Radzików in a climatized building. Unfortunately, this store will be constructed only in 5 years time and till then the responsible stations will have to provide their own storage facilities. Efforts will be made to ensure proper conditions for active collection storage as well. The Plant Genetic Resources Department will set up files covering the whole of the genetic resources in Poland. It is hoped for this to introduce an information system for storage and retrieval (e.g. EXIR) together with new computer equipment.

Contribution of wild species and primitive forms to the performance of modern cultivars

Chairmen: prof. Ch.M. Rick (Davis) and dr S. Blixt (Landskrona)

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Potential improvement of tomatoes by controlled introgression of genes from wild species[1]

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Index words

Lycopersicon sp., tomatoes, wild tomato, collection, germ plasm, introgression, evaluation.

Summary

The Germplasm resources available for improvement of the tomato include, in addition to a wide array of cultivated types, the wild var. *cerasiforme* of the cultivated species, *Lycopersicon esculentum*, and eight other, exclusively wild species. The number of available accessions of such wild taxa is large and may even exceed 1 000 items, which are maintained in several living collections. Depending upon the nature of their respective mating systems, substantial genetic variation can be detected within and between living accessions of the wild species. All such items hybridize with *L. esculentum* with varying degrees of difficulty. A massive source of wild germplasm is therefore available for the breeding of improved cultivars.

The worth of this material can be evaluated in several ways. Observations made in the native habitat can yield clues as to the existence of potentially valuable traits. Careful evaluation of accessions in test plantings often reveals the presence of other useful characteristics. Also available for exploitation are various kinds of 'novel variations' encountered in later generations derived from interspecific hybridization. Fullest utilization of the wild germplasm is therefore realized by hybridization with *L. esculentum* followed by systematic backcrossing to that species accompanied by generations of pedigree testing. Various examples will be presented to illustrate that tomato breeding has already benefitted and will continue to benefit from the application of these methods.

Genetic resources

The tomato (*Lycopersicon esculentum* Mill.) is an excellent example of a crop plant that has been extensively improved by controlled introgression of genes from primitive cultivars, wild species, and other forms of exotic germplasm. It is doubtful whether so many stress problems have been solved or are being solved genetically in any other cultigen. In the list of diseases that have been controlled by deriving

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resistance from such sources, at least ten examples could be cited for the cultivated tomato.

Success owes to many factors, not least, the availability of a wide array of primitive cultivars, of accessions of the wild form (var. *cerasiforme*) of the cultivated species, and of eight other, exclusively wild species. My presentation is based entirely on the last group. The nine species (including var. *cerasiforme*) are diverse in their adaptations, breeding systems, patterns of variability, and attributes for tomato improvement. Another factor that accounts for their exploitation is the fact that they can all be hybridized with *L. esculentum*, albeit with varying degrees of difficulty. The situation ranges from crosses that can be made with the ease of intraspecific hybridizations to those that require embryo culture on a large scale for success (Rick 1978).

All of these species have been used to varying extents as sources of germplasm for tomato improvement. Their pertinent features are presented as follows.

1. *L. esculentum* var. *cerasiforme* has spread since the conquest of the New World as a weed throughout the tropics and sub-tropics. Completely congruous with the cultivated forms, and derived forms fully fertile and viable. Fruit red, cherry-size, and generally of high quality. Grows in a wide variety of habitats, though generally mesophytic. The races found in the wet tropics possess natural resistance to leaf-attacking fungi and to other problems associated with high watertables, saturated humidity, and high temperatures. Accessions are rather uniform genetically and not greatly diversified outside the native region in Ecuador and Peru.

Accessions are rather uniform genetically and not greatly diversified outside the native region in Ecuador and Peru.

2. *L. pimpinellifolium* (Jusl.) Mill. Appropriately named the currant tomato. Crosses with *L. esculentum* as easily as var. *cerasiforme*. Morphologically a diminutive tomato in all respects. Mesophytic but often found in very dry situations. Source of much important disease resistance and such quality characteristics as high content of vitamins and soluble solids, low pH, and strong color. Highly self-pollinated and homozygous at the extremities of the range, but subject to much outcrossing and genetic variation in the north coastal districts of Peru (Rick et al. 1977).

3. *L. cheesmanii* Riley, endemic to the Galapagos Islands. Fruits 1 cm, yellow or orange, owing to the presence of the *B* gene. In a wide range of habitats, often xeric. Crosses freely with *L. esculentum*. Almost exclusively self-pollinating and colonies usually approximate pure-line status, yet considerable genetic variation found between accessions (Rick & Fobes 1975). Evolving in a void of parasitic disease and insects, generally susceptible to pests.

4 & 5. The sibling species *L. chmielewskii* and *L. parviflorum* described and named by Rick et al. (1976). Generally similar in slender diminutive character, mesic habitats in the intercordilleran valleys of the Peruvian Andes. Fruits tiny, whitish-green at maturity, never synthesizing colored carotenoids. Source of high soluble solids and intensifier of fruit color. *L. parviflorum* highly self-pollinated and extremely uniform genetically. *L. chmielewskii* facultatively outcrossed and more variable.

6. *L. hirsutum* Humb. & Bonpl., a robust rank-growing green-fruited species from mesic to moist situations in western Ecuador and Peru. Possibly associated with its glandular nature and rank odor, resistant to many insect pests. Found at

higher altitudes than any other tomato species. High altitude forms are more resistant to chilling temperatures according to unpublished research of E. Vallejos and J. Lyons of the University of California, Davis. Unilateral incongruity permits crosses with *L. esculentum* only with the latter as female parent. Self-incompatible and highly variable in central part of range, self-compatible and much more uniform in the north and south limits of the range (Rick et al. 1979).

7. *Solanum pennellii* Corr., another green-fruited species that belongs to the same intercongruous group, the *esculentum* complex. Endemic to highly xeric situations in western Peru. Like *L. hirsutum*, crosses unilaterally with *L. esculentum*. Except for one accession at the southern extremity of its range, self-incompatible and highly variable.

8. *L. peruvianum* (L.) Mill. and the closely related *L. chilense* constitute the other major subgeneric group. Hybridizes with *L. esculentum* but only with embryo culture and with much effort to find embryos large enough to culture. The incongruity barrier can also be circumvented by use of such special aids as interspecific chimeras (Günther 1964) and selected inbred *peruvianum* lines (Hogenboom 1972). Unquestionably the most variable, consequently the least exploited, tomato species. In every tested criterion of genetic variability, is heterogeneous both within and between accessions. Source of various pest resistances and potentially the richest source of vitamin C.

9. *L. chilense* Dun. More restricted in range (southern Peru and northern Chile) and less variable than *L. peruvianum*. Barrier to crossing with *L. esculentum* less severe than that of *L. peruvianum*, hence better source of the mutual characters. Tends to be taller, less tractable to culture and more drought-resistant than *L. peruvianum*.

Another favorable attribute of the tomato species is their availability. Thanks to various agencies and to the efforts of many workers, numerous accessions have been acquired and maintained in living collections. Thus, the Tomato Genetics Stock Center at the University of California at Davis maintains about 500 accessions of these species. And possibly a comparable number in the aggregate is kept by the North Central Plant Introduction Station of the USDA at Ames, Iowa and other governmental agencies. In general, most of the biotypes and geographic races are represented in these collections, but certain gaps are known. Effective maintenance requires large-scale matings inter se to retain the variation of the self-incompatible and the highly outcrossed self-compatible items. To be comprehensive, evaluation of such items should be done on a large scale. The less or seldom outcrossed species are simpler to maintain, but a wide representation of races and biotypes is essential (Rick 1976).

Evaluation

The value of wild species as sources of useful germplasm can be evaluated in several ways. One source of information that is frequently neglected is observations made by collectors in the native habitat. It is commonly accepted that, as a species evolves in a given series of habitats, it will acquire genes that endow it with adaptation to those habitats. Thus genes conferring resistance to certain stress situations are presumably present in wild populations growing successfully in the presence of such stresses. Consequently a study of autecology can yield clues as to the existence of valuable inherited traits. The discovery of such genotypes is well exemplified in the tomato species (Rick 1973). Observations on a series of such examples are reviewed briefly as follows.

The cultivated tomato is remarkably sensitive to saline conditions. Yet, certain species, particularly certain biotypes within species, are decidedly more tolerant. A dramatic example is found in maritime biotypes of the Galapagos species *L. cheesmanii.* While collecting along a beach on the western side of Isabela Island in January 1970, we encountered several populations that were thriving there despite their remarkable exposure to salt spray and high salt content of the soil. They were situated scarcely 2 m above, and 5 m total distance from, the high tide line and were associated there with recognized halophytes.

Since collection, these accessions have been tested extensively by E. Epstein and his colleagues at the University of California, Davis. Rush & Epstein (1976) discovered, for example, that such accessions could be grown in hydroponic culture which was gradually adjusted to full-strength sea-water. In contrast, cultivars of *L. esculentum* collapse before the concentration reaches half. Their research further demonstrated that the salinity tolerance of the *cheesemanii* accession is vested in the ability of the cells to cope with high sodium levels, not in any mechanism to restrict sodium uptake or translocation. Preliminary investigations indicate that the genetic determination of the tolerance is complex, but that selection for high tolerance might be effective.

Another dramatic stress resistance was encountered in the semi-xerophytic *Solanum pennellii.* The ability of this species to survive the arid conditions of its habitat is based on the water retention of its foliage (Yu 1972). Selection experiments to date have successfully maintained this character through several backcross generations. It remains for future research to assess the potential value of this character for reducing water requirement of tomatoes.

Another character sensed by a study of autecology is the high content of soluble solids in fruits of *L. chmielewskii.* In collecting this species, we were impressed by the lack of ripe fruit on the plants despite their large size, abundant flowering, and good fruit-set. The lack of ripe fruit suggested animal predation, which in turn suggested attractiveness of the fruits. On analysing ripe fruit at Davis, we obtained refractometer readings of 10–11%, more than twice the level in standard cultivars. A backcross selection experiment was successful in raising the content of soluble solids from roughly 5% to 7% in lines that produced a normal crop of large red fruits (Rick 1974). Although the derived lines suffer various defects and would therefore not qualify as suitable cultivars, they provide breeders with raw material that is considerably more advanced than the wild species. Other investigators have further ascertained that the hybrids between these lines and other *esculentum* parents have higher contents of soluble solids, thereby pointing the way for exploitation of the derived lines as parents of improved F_1 hybrid cultivars.

The most widely practised method of assaying wild material is by observations of the accessions in test cultures. The method has become so widely practised in breeding for such characters as disease and insect resistance that it scarcely needs elaboration here. Jointless pedicel (*j–2*) derived from *L. cheesmanii* and now used in tomato breeding programs as a device to prevent excessive fruit shattering in mechanical harvesting and to eliminate undesirable pedicel stubs is an example of a character assayed in this fashion (Rick 1967).

'Novel variation'

A third category of evaluation is one that I have labelled 'novel variation', referring to characteristics that are not normally observed in the wild species, either in its native habitat or in cultures. Novel variants usually require hybridization and analysis of segregating progenies for their detection. The literature is replete with examples of the four modes of origin known for such unpredictable variants. These categories are: 1) genic interaction; 2) plasmatic interaction; 3) parental latent variation; 4) mutation.

1. Genic interaction is demonstrated most clearly by a monogenic trait from the wild parent gaining expression when introgressed into a milieu of the cultivated parent genotype. A well known example of this kind of interaction is that of the *B* gene universally present in *L. hirsutum* and other green-fruited species. Although *B* codes for the synthesis of β-carotene in the ripe fruit, it has no detectable effect in the green-fruited species because they do not synthesize colored carotenoids. But when bred into red-fruited *L. esculentum*, *B* exerts a dramatic effect, a 1:1 segregation of red to orange being detected in the first backcross generation (Lincoln & Porter 1950). The *B* gene thus derived has been incorporated into several cultivars, for example, Caro-Red (Tomes & Quackenbush 1958). A similar example is the discovery of a fruit pigment intensifier *Ip* from *L. chmielewskii*, which is expressed after transfer to an *esculentum* background (Rick 1974). Basically, transgressive variation also belongs to this category, since it depends upon interaction of sets of quantitative genes of the two parental lines. It is exemplified in Yu's (1972) research on the water-loss character derived from *S. pennellii*, as well as in many other investigations of other cultivated plants, for instance, the drastic improvement in yields measured in progenies of interspecific hybrids of *Avena* (Frey 1976).
2. Plasmatic interaction is seldom encountered in the use of interspecific hybrids in tomatoes. Almost invariably, *L. esculentum* is used as the female parent in such crosses, so that the derived products have *esculentum* cytoplasm and most of the *esculentum* genome in their make-up. But if reciprocal hybrids are studied in which the wild genome is combined with *esculentum* cytoplasm, novel characters tend to appear. An example is the extreme reduction of androecium that takes place progressively when the genome of *S. pennellii* is backcrossed to *esculentum* cytoplasm (Andersen 1963). Although such a combination would be of no direct use to the plant breeder, it might be possible to manipulate the materials so that the *esculentum* genome is combined with wild cytoplasm as accomplished in *esculentum-peruvianum* hybrids by Hogenboom (1976).
3. Latent variation refers to recessive genes of the self-incompatible species that are seldom expressed in the accessions because they are maintained by cross-pollination on a large scale to retain sufficient vigor and maximum genetic variation. Of this category we have encountered numerous examples of serendipitous characters, including several genes for male sterility derived from *L. chilense* (Rick & Smith, 1953), and *L. peruvianum* (my unpublished work).
4. Mutation rates are frequently observed to be high in interspecific hybrids. Variants of this origin appear sporadically and in fewer backcross sublines than

those of any other category. Our research has turned up numerous examples (Rick 1966; 1968). The rates of mutation thus induced are not comparable to those resulting from application of such efficient mutagens as EMS, but the spectrum of mutations might conceivably be different.

The moral implicit in this experience is that in systematic backcrossing it behoves the experimenter to alternate occasional selfed generations with backcrosses, a procedure that will permit the expression of novel variants of all enumerated categories.

Cell and tissue cultures

My discussion has been concerned with more or less traditional methods, but the treatment would not be complete without mentioning the prospects for cell and tissue cultures. The possible applications of these techniques have been discussed by Carlson (1975), Melchers (1977), and many others. Although recent progress in this field has been disappointingly slow, intensive study of culture requirements and the application of new techniques might expand the opportunity for cell hybridization and thereby permit the matings of species and differential elimination of wild chromosomes heretofore impossible by normal methods. It would be a tremendous boon, for example, to have access to the frost hardiness of *Solanum lycopersicoides*, the alkali tolerance of *S. rickii*, and the woody perennial nature and pest resistance of *S. ochranthum*. It remains for future research to develop these opportunities. An intriguing new development is the production by cell fusion of tomato-potato hybrids (Melchers et al. 1978).

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Use of Agropyron species for wheat improvement

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Index words

Agropyron, Elytrigia, partial amphiploid, addition line, substitution line, homoeologous pairing, transfer derivatives.

Summary

After a discussion dealing with the patterns of phylogenetical differentiation of the genera *Triticum* and *Agropyron* within tribe *Triticeae*, the hybrids obtained between the two genera are listed. Subsequently, the methods are reviewed which made it possible to obtain progenies.

The sterile F_1 show no or nearly no pairing at meiosis between parental chromosomes. Backcrosses to common wheat varieties opened up the way to create stable addition types with either one genome (partial amphiploids), or one chromosome (addition lines) added to the full complement of the wheat. From this type of material in which no more wheat/*Agropyron* recombinations occur, it is possible to have a better knowledge of the genetic behaviour of the characters that we wish to transfer from *Agropyron* into wheat. Also the evidence of the activity of gene *Ph* (on chromosome 5B of wheat) has led to the use of methods that induce an exchange between homoeologous segments of *Agropyron* and wheat.

Introduction

Agropyron species have long attracted the attention of wheat breeders for the same reasons as rye or *Aegilops*, because some species display agronomically interesting features, especially resistances (winter cold, salt, drought, pests, diseases ...). So, since the beginning of this century, people have tried to use *Agropyron* to increase the genetic variation in wheats, or to create new species (for reviews see Cauderon 1958; 1966a).

In the 1920's and 1930's, breeding programs were undertaken, particularly in the Soviet Union, United States, Canada and Germany, to obtain perennial wheats adapted to steppe-like zones with continental climate. The hope to create a new species that would produce green forage and grain raised much enthusiasm and the first hybrids were as topical to the scientists, technicians and reporters as *Triticale* is now. Perennial wheats were obtained; they generally were complete or partial amphiploids with 70 or 56 chromosomes. Meanwhile they did not fulfil all the hopes. Nevertheless some of the amphiploids have been backcrossed with wheat;

with appropriate methods, it has been possible to transfer useful characters of *Agropyron* into wheat, mainly disease resistances (for reviews cf. Cauderon 1966a; Knott & Dvorak 1976). Only a few *Agropyron* gametes were involved in those programs. We have tapped little of the resources of that large genus, which has a key position within tribe *Triticeae*; there are many species of *Agropyron* each with a large natural genetic variation (Cauderon 1966b). Furthermore, major advances have been made in the methods of obtaining and using hybrids between distant species (Sears 1970; 1972).

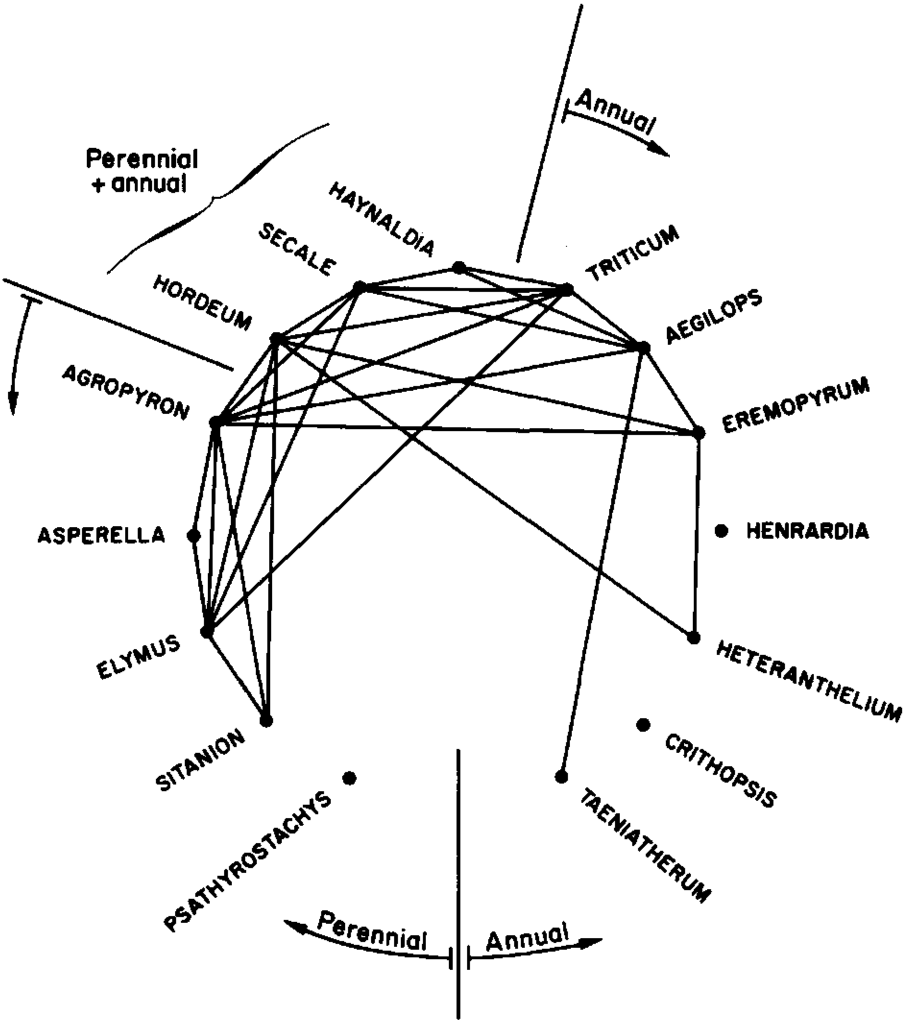

Figure 1. Genetic relationships within the tribe *Triticeae* in terms of successful intergeneric hybrids (after Sakamoto 1973).

Table 1. Subdivision of the genus *Triticum* (after Mac Key 1975)

Monococca Flaksb. ($2n = 14$)	Dicoccoidea Flaksb. ($2n = 28$)	Speltoidea Flaksb. ($2n = 42$)
T. Monococcum (L.) MK (A Genome) ssp. *boeoticum* (Boiss.) MK var. *aegilopoides* (Bal. ex Korn.) MK var. *thaoudar* (Reut.) Perc. ssp. *monococcum*	*T. timopheevi* (Zhuk. MK (AG Genomes) ssp. *araraticum* (Jakubz.) MK ssp. *timopheevi*	*T. zhukovskyi* Men. et al Er (AAG Genomes)
T. urartu Tum. (A Genome, modified)	*T. turgidum (L.) Thell. (AB Genomes)* ssp. *dicoccoides* (Korn.) Thell. ssp. *dicoccum* (Schrank.) Thell. ssp. *paleocolchicum* (Men.) MK ssp. *turgidum* conv. *turgidum* conv. *durum* (Desf.) MK conv. *turanicum* (Jakub.) MK conv. *polonicum* (L.) MK ssp. *carthlicum* (Nevski) MK	*T. aestivum (L.) Thell. (ABD Genomes)* ssp. *spelta* (L.) Thell. ssp. *macha* (Dex. et Men.) MK ssp. *vulgare* (Vill.) MK ssp. *compactum* (Host) MK ssp. *sphaerococcum* (Perc.) MK

An understanding of the cytotaxonomic relationships, cytogenetic structure and evolutionary history of the two genera is a prerequisite.

Long included in the Linnaean genus *Triticum* because of morphological affinities, the two groups are today classified as two distinct genera within tribe *Triticeae* Dum., a vast taxon differentiated in the Pleistocene, with well defined limits, but with a generic status difficult to establish: the absence of genetic barriers between groups has favored the development of a complex reticulate system in which polyploidy has played an important role. Taxonomical problems are still under debate. There are many inconsistencies in the views held. The situation as shown in Fig. 1 was summarized by Sakomoto (1973): *Triticum* and *Agropyron* are supposed to derive from the same ancestor, probably diploid perennial and allogamous with basic chromosome number, x, of 7. By different evolutionary trends, with a major step of phylogenetic differentiation at the diploid level, then by polyploidy, both genera have colonized now nearly the same regions with Artic-temperate or Mediterranean climate.

The genus *Triticum L.* has been studied extensively; it comprises only annual species. In this whole taxon, without clear discontinuities, a situation preserved by alloploidy and autogamy, Mac Key (1975) defined six species on the basis of morphological, genetical and cytogenetical data (Table 1). This polyploid complex supposedly developed around a wheat species with the A genome (*T. monococcum*) (L) M.K.; already an annual and autogamous, the latter has highly impressed on the whole group the characteristics that have facilitated its domestication: wide ecological adaptation, suitability for fertile land and cold tolerance. By crosses with other diploid species of the tribe *Triticeae*, the incorporation of B and G genomes (from other wheats or *Aegilops* species) and later of the D genome (from *Ae. squarrosa*) would have added the other valuable characters: improvement in heat tolerance and chlorophyll content, combination of good vegetative growth with high seed production, better tolerance to soil acidity, and a protein quality more balanced for man. So arose the present situation with two species for each of these levels of ploidy:

T.monococcum (L.) MK and *T. urartu* Tum.,
$2n = 14$, with only the A genome
T. turgidum (L.) Thell and *T. timopheevi* (Zhuk.) MK,
$2n = 28$, with respectively AB or AG genomes
T. aestivum (L) Thell and *T. zhukovskyi* Men. et Er.,
$2n = 42$ with respectively ABD or AAG genomes

The genus *Triticum* has long been considered as a model alloploid series. It is known that the genomes are closely related (homoeologous) (Sears 1966). The inability of the homoeologues to pair with each other is due to the presence of a gene *Ph* on chromosome 5B which prevents homoeologous pairing, reinforcing preferential pairing (Okamoto 1957; Riley & Chapman 1958)..

The genus *Agropyron* has been less studied and is poorly understood. It presents serious difficulties of taxonomy and nomenclature; there is still disagreement about the number and rank of the taxa to be included in it. The species readily crossed with wheat have been successively classified in the *Triticum, Agropyron, Elymus* or

Elytrigia genera. They are now joined together, under the name *Elytrigia* Desvaux, as a section in the genus *Agropyron*, or as a genus. This group includes only perennial species with caespitose and rhizomatous types. Native to western Asia, they are now distributed over the Arctic-temperate regions of the whole world and also over the Mediterranean Basin. A complex endemism in each area has been established. Perenniality and allogamy have been maintained and have played an important role in evolution. Both of these features may have helped to maintain structural heterozygosity within genomes and could have allowed the setting of a segmental alloploidy. The latter assumption, blurring the genetic barriers, has eased natural hybridization and favored the creation of polyploid complexes with several levels of ploidy. A new adaptability associated with efficient systems of vegetative propagation thus insured geographical extension and allowed permanent diversification. The floras of the Near East (Nevski 1934; Bor 1970; Tzelev 1976) list many (40 to 60), probably too many species (as shown by the recent studies of Dewey 1978a,b). But what we know about that group is limited, and our information about relationships within *Elytrigia* concerns only few species and samples. Probably only few diploid progenitors have contributed to the creation of the species investigated: among them *A. elongatum* (Host) P.B. (E genome), *A. junceum* (L.) P.B. (J genome structurally close to E) and *A. spicatum* (Pursh) Scribn. et Smith (S genome, classed today under *Roegneria*, another section of the *Agropyron* and related to *Hordeum*). Present knowledge does not allow precise formulation of the genomes for each species. Anyway, the original genomes have been modified. As shown on Table 2, we may recognize two polyploid complexes (Heneen 1972; Heneen & Runemark 1972), and four aggregate species. For the best known taxa, we have symbolized their genomic structure as indicated by cytogenetic analysis of species and interspecific hybrids (Cauderon 1958, 1966b; Breton & Cauderon 1978). At present, only the E, J and S genomes can be indentified. As far as it is

Table 2. Genome analysis in the *Agropyron-Elytrigia* group.

Name	2*n*	Genome(s)				Remarks
Polyploid complexes						
A. elongatum (Host) P.B.	14	E				E close to J
	28	E6	E7			
	70	?				
A. junceum (L.) P.B.	14	J				J close to E
	28	J 1	J 2			
	42	J 1	J 2	E3		
	56	?				
Aggregate species						
A. intermedium (Host) P.B.	42	E1	E2	N1		
A. littorale (Host) Dum.	42	N2	N3	Y1		
A. repens (L.) P.B.	42	R1	R2	S 1		S in *A. spicatum* (Roegneria group)
A. campestre G.G.	56	K1	K2	E4	E5	

known, only preferential pairing is working as a diploidizing system in the polyploid types.

F_1 Hybrids obtained between the two genera

The relationships between wheat and *Agropyron* are not so direct as between wheat and *Aegilops*, and as far as I know, natural hybrids have been never mentioned between them; meanwhile the two genera have still some affinities and six species or subspecies of *Agropyron* have been successfully crossed with wheats (Figure 2):

- *A. elongatum* (2*x*) with *T. turgidum*, *T. timopheevi* and *T. aestivum* (Jenkins & Mochizuki 1957; Jenkins 1958),
- *A. elongatum* (4*x*) with *T. aestivum* (El Ghawas & Khabil 1973),
- *A. elongatum* (10*x*) with *T. turgidum*, *T. timopheevi*, *T. aestivum* (review cf. in Cauderon 1958),
- *A. junceum* (4*x*) with *T. turgidum* (Ostergren 1940),
- *A. campestre* (8*x*) with *T. turgidum* and *T. timopheevi* (Cauderon 1958),
- *A. intermedium* (6*x*) with *T. turgidum*, *T. timopheevi*, *T. aestivum* and also with induced autotetraploids of *T. monococcum* (cf. in Cauderon 1958).

The results of crosses implying *A. junceum* 6*x*, *A. littorale*, *A. repens* were negative or doubtful; *A. junceum* 2*x* and 8*x*, and also *T. urartu* and *T. zhukovskyi*

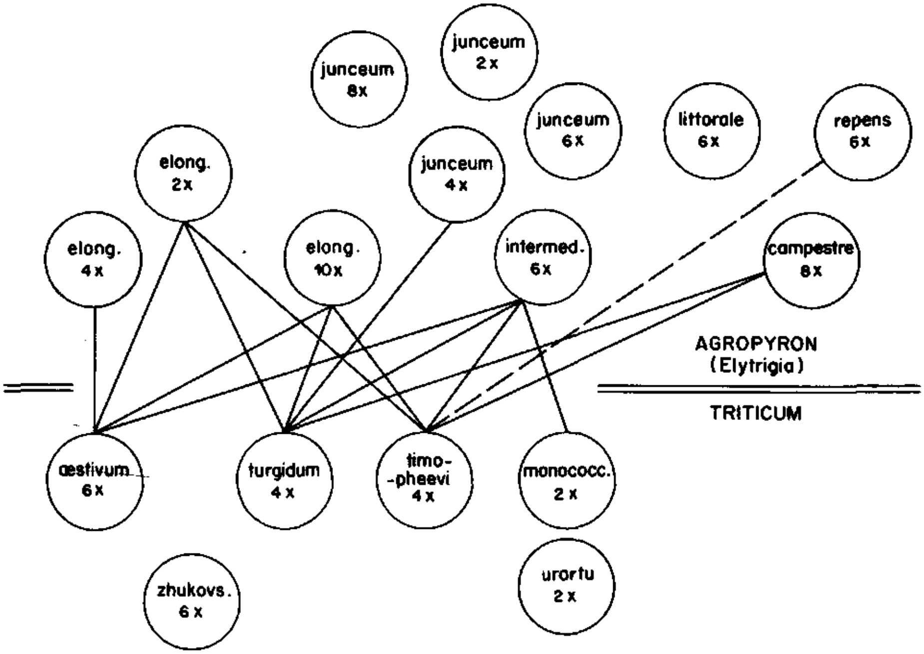

Figure 2. Successful (___) and doubtful (- - -) crosses of *Agropyron* spp. with *Triticum* spp.

have been never used. Finally, we could not find any information about the use of the other species mentioned in the floras of the Near East.

Ways to obtain progenies

With a few exceptions, the F_1's were vigorous but sterile. A study of their meiosis revealed little or no homology, as there were only a few, if any pairings between wheat and *Agropyron* chromosomes. However, most of the hybrids gave progenies if treated with colchicine, or if backcrossed with wheat. In fact only the hybrids obtained with *A. junceum* and *A. campestre* did not produce any progenies.

Amphiploids

The complete amphiploids involving hybrids between wheats and the decaploid *A. elongatum* have never been obtained. Those with F_1 between *T. aestivum* and *A. intermedium*, were not very fertile (Cauderon 1958). On the other hand amphiploids have been readily obtained from F_1:

- between the diploid *A. elongatum* and *T. turgidum, T. timopheevi* or *T. aestivum* (Jenkins 1958; Jenkins & Mochizuki 1957),
- between *A. intermedium* and *T. turgidum* or *T. timopheevi* (Cauderon 1958).

Only the last ones have been selected for forage, but, as yet, they have not attracted attention of wheat breeders.

All those amphiploids might be reservoirs of genes to improve wheats or Triticale.

Back-crosses

Backcrossing the F_1 or the amphiploids to common wheat, with selection for useful *Agropyron* characters, nearly always proved to lead to intermediates with extra genomes or entire chromosomes from *Agropyron*. At this step, as in the F_1, there is practically no pairing between chromosomes of the two genera.

Depending on the number of backcrosses, and on the genetic behaviour of the characters under selection, different addition types have been isolated in the progenies of hybrids involving *A. intermedium* or the decaploid *A. elongatum* and common wheats.

The first, partial amphiploids, are octaploid ($2n = 56$) and possess one more genome besides the chromosome complement of common wheat. In spite of their agronomic value they retain many undesirable characters of *Agropyron* and a poor yield (Cauderon 1966a, et al. 1973) so it is doubtful whether they have the same potential as *Triticale*.

Partial amphiploids may readily be obtained. They are automatically selected for tolerance to homozygosis and for a good balance of the *Agropyron* chromosomes with the wheat genomes. This is in fact a good step to test *Agropyron* as a source of germplasm for wheat breeding: it is difficult to judge *Agropyron* spp. themselves as annual crops!

The second are *alien addition lines* in which one pair of *Agropyron* chromosomes has been added to the chromosome complement of common wheat. They have been obtained mostly after backcrossing the partial amphiploid to wheat and selection for resistance to disease (review in Dvorak & Knott 1974). They had little impact as a crop, because they were nearly always less fertile or less stable than wheat. However, they are a prerequisite for an understanding of the genetic behaviour of *Agropyron* characters in the wheat background. It is the reason why programs have been initiated in order to produce complete sets of alien addition lines. Some of them have been isolated from the partial amphiploids *T. aestivum* × *A. intermedium* $2n = 56$ (Cauderon et al. 1973; Sinigovets 1976; Zeller, unpub.) and also from the amphiploids *T. aestivum* × *A. elongatum* $2x$, $2n = 56$ (Dvorak & Knott 1974), *T. turgidum* × *A. elongatum* $2x$, $2n = 42$ (Mochizuki, 1962).

Occasionally, *balanced substitution* lines in which a pair of *Agropyron* chromosomes had replaced a wheat pair, have also been obtained (Tables 3 and 4). They have arisen from hybrids between wheat and *A. elongatum* $10x$ or *A. intermedium* backcrossed to the wheat parent; in the progenies, selection have been made for a

Table 3. *Agropyron elongatum* 10x / *Triticum* homoeologous relationships, from substitutions and transfers.

Substitutions			Transfer derivatives, from translocation or recombination and related references
A.elong. 10x chromosome substituted	*T. aestivum* chromosome(s) replaced	Markers of the *Agropyron* chromosomes and related references	
1 Ag el	1 A, 1 D	– Stem rust resist. (Townley - Smith 1965)	unknown
3 Ag el^1	3 D	– Leaf rust resist. (Bakshi; & Schlehuber 1958) – G.O. Transaminase activity (Hart et al. 1975)	Yes: 1. spontaneous (Schlehuber; & Sebesta 1959), gave cv. "Agent" (Smith et al. 1968) 2. removing 5B chrom. (Sears 1972, 73)
4 Ag el 5 Ag el 6 Ag el	4 D 5 D 6 D	– Streak mosaic resist., triple substitution (Larson & Atkinson 1970, 1972)	unknown
6 Ag el	6 D 6 A, 6 D	– Stem rust (Knott 1964) – Stem rust (Jonhson 1966)	Yes: after irradiation (Knott 1961) gave cv. "Eagle", "Kite" (Knott & Dvorak 1976)
7 Ag el$^2_1$	7 D	– Leaf rust (Quinn & Driscoll 1967) – Yellow flour (Knott et al. 1976)	Yes: 1. after irradiation (Sharna & Knott 1966) gave cv. "Agatha" (Knott & Dvorak 1976) 2. removing 5B chrom. (Sears 1972, 73)
7 Ag el$_2$	7 D	– Stem rust (Knott et – Yellow flour al 1977)	unknown

1. Substitution line 'TAP 67'
2. Substitution line 'Argus'

Table 4. *Agropyron intermedium / Triticum aestivum* homoeologous relationships, from substitutions and transfers.

Substitutions			Transfer derivatives from translocation or recombination and related references
A. interm. chromosome substituted	*T. aestivum* chromosome replaced	Markers of the *Agropyron* chromosomes and related references	
3 Agi	3 A	– Leaf rust resist. – Hairy leaves (Cauderon 1966; The & Baker 1970)	– In progress: cross with *Ae. spelt.* (Chueca & Cauderon 1977)
4 Agi	4 B	– Streak mosaic resist. (Wong et al. 1974)	– In progress: using ph mutant of Chinese Spring (Wang, et al. 1977)
7 Agi	7 A	– Leaf and stripe rust resistance (Wienhues 1973)	– Yes: spontaneous and after irradiation (Wienhues 1973)
7 Agi	7 D	– Stem rust (Cauderon 1966; The & Baker 1970) – Peroxydasic activity (Cauderon et al. 1978)	– In progress: cross with *Ae. spelt.* (Cauderon & Ryan 1974)

simple character and for general vigor. Such lines are generally stable and fertile, and some of them have been used as a source of resistance to rusts, for instance 'Agrus' (Caldwell et al. 1956) and 'TAP 67' (Bakshi & Schlehuber 1959) (Table 3) both resistant to leaf rust. But they are valuable only if the *Agropyron* chromosome can compensate the unbalance provoked by the loss of the wheat chromosome. This implies an homoeology between the two chromosomes. To get such a substitution line systematically by using the monosomic deficient for the wheat homoeologue is a rather tremendous step (Cauderon 1973). Moreover, they often bear unfavorable genes difficult to eliminate because the chromosome is the 'unit' of transfer. To reduce this 'unit', transfer by irradiation has been tried. It consists in an induction of translocations between wheat and *Agropyron* chromosomes after breakage of the two chromosomes by ionizing radiations. Theoretically, this method does not imply any homoeology between exchanged segments. Different procedures have been successfully applied to partial amphiploids, addition or substitution lines (for reviews see in Cauderon 1966a, 1973). Though this material offers an advantage, it also raises problems such as bad transmission through the pollen, linkage with unfavorable genes (for instance yellow flour) and poor yield. Obviously, a segment of the alien chromosome cannot successfully replace a random segment of a wheat chromosome. In practice, satisfactory transfer involves also substitution of the alien segment for a homoeologous wheat segment. Such transfers are more frequent as expected because of their competitive advantage and they can be obtained in experiments of modest size. So leaf rust resistance has been transferred to common wheat from *A. elongatum* 10*x* (Sharma & Knott 1966), *A. intermedium* (Wienhues

1973), and stem rust from *A. elongatum* 10x (Knott 1961). This material has been successfully used in wheat breeding programs (Knott & Dvorak 1976).

The necessity of using special procedures to reduce the *Agropyron* contribution is due to a lack of pairing and crossing-over between *Agropyron* and wheat chromosomes, but it was demonstrated that this situation depended on two series of effects:

– the homology between wheat and *Agropyron* chromosomes is weak but there is some homoeology as demonstrated in several cases by the production of the specific substitution lines (Table 3 and 4) and recently by Dvorak & Sosulski (1974) for the E genome of *A. elongatum* 2x,

– The *Ph* gene, on 5B chromosome, largely responsible for the suppression of homoeologous pairing in hexaploid wheat, is efficient also in suppressing pairing between wheat and *Agropyron* chromosomes (Johnson & Kimber 1967; Sears 1972; Dvorak & Knott 1972; Cauderon & Ryan 1974; Chueca & Cauderon 1977), (Table 3 and 4).

Several methods have been proposed to suppress this effect in order to open the way for exchange of genes between wheat and relatives. The first is removal of the chromosome 5B, by a series of crosses implying monosomic 5B and some other aneuploids (Sears 1973). The second is utilization of *Ae. speltoides* to neutralize the effect of the *Ph* gene (Riley et al. 1968). Both methods have proved efficient in the case of wheat/*Agropyron* derivatives. The first was successfully used by Sears (1973), for the transfer of the leaf rust resistance of *A. elongatum* 10x to wheat by the way of 'Argus' and 'TAP 67' lines. In cooperation with Ryan and Chueca (Table 4), we are using the second one for the transfer of leaf and stem rust resistance of *A. intermedium* to wheat.

A third method seems now possible thanks to wheat mutants of 'Chinese spring' carrying the recessive *Ph* allele, or a deletion of *Ph* locus (Sears 1977). Using such mutants in a cross with a substitution line (Table 4) Wang et al. (1977) induced pairing between chromosome 4 Agi of *A. intermedium* and its wheat homoeologue 4B.

Conclusion

Several methods, rather complicated but efficient, have been developed and allow us to transfer genes from *Agropyron* to wheat; and simpler methods will certainly appear in the near future. The hexaploid derivatives obtained so far by cytogeneticists through homoeologous pairing or irradiation are results and proofs of the usefulness of this methodological work. These derivatives probably do not represent any *important* improvement of the assortiment of wheat cultivars, probably because of the genes chosen for transfer (mainly rust resistances). We specially need now better information from the physiologists, agronomists and pathologists about the interesting characters present in the genus *Agropyron*, so poorly explored: the priority for wheat improvement is the evaluation of the genes of *Agropyron* to be transferred which must be estimated in the genetic background of wheat, in amphiploids, addition or substitution lines.

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The potato gene-pool, and benefits deriving from its supplementation

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Index words

Solanum tuberosum, potato, wild *Solanum* species, gene-pool, Neo-Tuberosum.

Summary

Potatoes are autotetraploid clones, modern varieties being bred by crossing and a few varieties can represent a large gene-pool. However, due to common ancestries, the gene-pool is not as large nor the varieties as heterozygous as they could be. Inadequacy is shown by the constant efforts to add disease-resistance genes from wild species etc. There is evidence that maximum yield requires four distinct alleles per locus, and any supplementation of the gene-pool, except with undesirable dominant alleles, may thus be beneficial.

Very many specific characters are required in a successful variety and breeding to secure their presence, by back-crossing to existing varieties, is not conducive to maximum heterozygosity. In autotetraploids, maximum heterozygosity is not attained in a first cross between unrelated inbreds and would require two or more generations of crossing of unrelated parents. No varieties have emerged from breeding aimed at maximising heterozygosity, though some success has been achieved incidentally to breeding aimed at combining specific characters from various sources.

Initial potato introductions to Europe were few. In the 18th century the number of varieties multiplied due to seedlings being raised to replace virus-diseased parents; selection among these improved adaptation to our climate and further introductions then appeared inferior. These seedlings were from natural pollination which mainly involves selfing, and varieties probably became very inbred. Breeding by crossing commenced in the mid 19th century and many varieties were grown, though all traced to the few early introductions or the few subsequent successful ones. Most breeding was with local stocks and local gene-pools were somewhat distinct.

State-supported breeding, employing scientists able to distinguish between diseases and recognize resistances to them, commenced in the early 20th century. This led to interchange of mainly virus-resistant parents between countries, and the development of successful varieties combining different local gene-pools. It also facilitated long-term breeding, e.g. from wild species and primitive cultivars; earlier breeding consisted of crossing between named varieties, numbered breeding-lines being virtually unknown.

Explorations this century have revealed the vast variation in Latin American primitive cultivars and wild species. Use of introductions with desirable properties has frequently led to other desirable properties being introduced incidentally. It seems that the gene-pool is so limited that almost any supplementation might confer benefits additional to those for which it is used.

The Neo-Tuberosum and related diploid projects are aimed at enabling varieties to be selected from initial or early generations of crossing with commercials, enabling breeding for

characters which would not justify back-crossing programmes, increasing the likelihood of incidental introduction of other useful characters, and maximising the likelihood of yield heterosis.

Introduction

There is a paradox about the gene-pool of our cultivated potatoes. They are vegetatively propagated autotetraploids, and most modern varieties are selected from the F_1 generation of crosses. Consequently there may, in theory, be as many as four different alleles per locus in each cultivar and a few cultivars could represent a very large gene-pool. Through inter-relationships between varieties and between their parents and ancestors, the actual gene-pool is limited, and the varieties are not as heterozygous as, in theory, they could be. Even so, the gene-pool may be substantially larger than could be represented by a similar number of cultivars of a diploid inbreeding seed-propagated species.

However, the gene-pool still restricts breeding. On the one hand, its inadequacy is illustrated by the constant efforts to add disease resistances from wild species and primitive cultivars to it. On the other hand, Mendoza & Haynes (1974) have shown that published data fits an overdominance model for yield heterosis in the potato. On this basis there will be a requirement for four distinct alleles at many loci for maximum performance. To provide such a level of heterozygosity would require skilled manipulation of a large gene-pool. Thus any additions to the gene-pool, other than of clearly undesirable dominant alleles, are likely to be intrinsically beneficial.

There is also a paradox about breeding procedures. A great many characters must be considered in assessing a cultivar, perhaps as many as forty or fifty, though with varying degrees of importance. A wide range of fungal, viral and bacterial diseases can attack the potato and, while immunities are frequently both unnecessary and unavailable, excessive susceptibilities to any of these diseases will reduce the value of a cultivar, sometimes to zero. A cultivar must be of suitable maturity and the tubers of suitable size, shape and colour; a large number of probably dominant characters adversely affecting shape or colour must be absent. Then there are characters affecting the cooking qualities of the tuber and its general acceptability to the housewife, or the processer, and so on. While in these respects a 'perfect' cultivar may be an unattainable ideal, and, as Simmonds (1969) has pointed out, a realistic objective must be a not-very-imperfect cultivar rather than a perfect one, attainment of any level of success must almost inevitably involve building on previous successes.

The easiest way to produce a new cultivar is to cross together existing commercial cultivars. Useful recombinations of characters, but not major advances, can be made in this way. The traditional way to introduce new characters from wild species or primitive cultivars involves back-crossing to commercial-type material, generally through three or more generations. The genomes of the resulting cultivars derive substantially from the existing commercial gene-pool, with at most 12% of new material, the increase in the level of heterozygosity consequently being limited. Where one requires adequate levels of disease resistance or of other characters of the type controlled either by multiple loci, or by numbers of resistance factors at one or two loci, inter-crossing of close relatives may be needed, with further reduction in heterozygosity.

Thus, in breeding, the requirements for particular characters run counter to the requirement for high heterozygosity to maximise yields.

Another feature of autotetraploids may be briefly mentioned. If a locus in a parent is homozygous, all its gametes will be homozygous for that allele. Consequently, irrespective of the genotype of another parent with which it is crossed, all the progeny will be at least duplex for that allele; they cannot have four different alleles at that locus. Part of the inbred status of a parent is transmitted to its outcrossed progeny in that way. In fact, leaving aside an additional minor factor known as 'double-reduction', the inbred status of a genotype obtained by crossing two unrelated genotypes is a third of the average inbred status of the two parents, and is not zero as in diploids. Therefore, achieving high heterozygosity may require two or more generations of crossing of unrelated parents.

As far as I know, no cultivars have yet emerged from breeding aimed specifically at maximising heterozygosity. Such success as has been achieved has been incidental to breeding aimed at combining specific characters from various sources. The Neo-Tuberosum work (contrib. 1.5, this book) has the increasing of heterozygosity and yield potentials as a major aim.

History of potato breeding

I will review, briefly, the history of potato breeding with reference to the nature of the gene-pool.

We know little of the earliest introductions but believe them to have been few and mainly casual. Most probably derived from food supplies assembled at Cartagena in Colombia for the voyage of sailing ships to Spain. A British writer (Rye) in 1730 knew only five types of potatoes, and his descriptions leave no doubt that he was well acquainted with them.

Following the appearance of virus diseases ('the curl'), farmers found that they could 'rejuvenate' the diseased stocks by raising seedlings. The viruses did not pass through the seed. The raising of seedlings, undoubtedly accompanied by selection, led to the emergence of a range of cultivars termed 'endless' by another British writer (Marshall) in 1785. These derived mainly if not entirely from the few preceeding cultivars. The gene-pool was being sorted, desirable characters concentrated and undesirable ones eliminated, but little if any variation was being added.

This breeding, induced by virus epidemics, improved the adaptation of the potato to our climate and our requirements. In consequence, subsequent introductions generally appeared inferior. Modern breeding concepts had not emerged, and the prime requirement in an introduction was that it should itself be an acceptable commercial cultivar. This remained true until the early years of this century. Thus when the next major problem emerged, the blight epidemics of the mid Nineteenth Century, and at least one pioneer sought new breeding material from South America, Goodrich of New York State, he discarded most of his introductions because of their unsatisfactory climatic adaptation; they were too late maturing (Goodrich 1863). The exception was his 'Rough Purple Chili' which is in the ancestries of many cultivars grown today.

Thus far, the progenies raised were from natural pollination and the great majority of seedlings would be selfed derivatives of their parents. Few ancient cultivars have survived into modern times but Salaman in 1926 mentioned that one, 'Myatt's Ashleaf', bred true for most observable characters, and this suggests that it was highly inbred. The approach to homozygosity on inbreeding autotetraploids is much slower than in diploids, one generation of selfing of a diploid having an effect equivalent to $3\frac{1}{2}$ generations in an autotetraploid, and the levels of heterozygosity in the cultivars would be reduced only slowly. However, a tendency for several cultivars to be derived from the same inbred progenies, as observed in the Neo-Tuberosum work, may have seriously affected the gene-pool.

Breeding by crossing commenced after the blight epidemics, though for a long time selection from open-pollinated progenies continued alongside this. While breeding had previously been by farmers using locally available cultivars as parents, individuals specializing in breeding as a commercial venture emerged and some of these looked further afield for parents. Nevertheless, most breeding was with local stocks and the gene-pools of different countries or regions remained somewhat distinct. Early this century, North American cultivars were mostly pollen sterile and introductions from Europe were made to contribute both fertility and disease resistances (Hougas & Ross 1956). Though susceptible to most diseases, they seemed invariably to have the *Nc* gene for resistance to virus C while only a proportion of British and European cultivars had this gene (Dykstra 1936; 1939; Cockerham 1943). A colleague tells me that the *Nb* gene was more frequent in continental European than in British cultivars while the reverse applied to the *Nx* Gene, and some evidence for this can be extracted from the data tabulated by Cockerham (1943).

In the early years of this century, many cultivars were grown. In Britain, there were about 500 distinct ones of which about 90 were in fairly widespread cultivation (Salaman 1926). But these would all trace back to the few initial introductions and to the few subsequent successful ones, to a very limited gene-pool which had been eroded selectively to meet our requirements and as a result of disease epidemics and, incidentally to such selection, unselectively due to non-random gene-associations deriving from inbreeding.

Government-supported breeding subsequently emerged and, at least in Britain and the United States, this has largely supplanted private breeding. Only a handful of the pre-existing cultivars have been incorporated into the few resultant major programmes. Whether further substantial erosion of the pre-existing gene-pool has resulted from this is uncertain; it depends on the adequacy of those few parents to represent the gene-pool and, as they mostly derived from crossing, most of the available genes may have been present in them.

The major Government-supported programmes employed scientists who were able to distinguish between diseases and recognize and utilise resistances to them. An immediate effect was an exchange between countries of parents having specific characters of value; a bringing together of the various local gene-pools. An early success in the United States was the cultivar Katahdin which had a British, a Polish, and two American grandparents. It has resistance to leaf-roll virus and to virus Y and is good in other respects; in 1955 about a third of the certified seed produced in the United States was of this cultivar (Hougas & Ross 1956). It has been extensively used in subsequent breeding. The Scottish Plant Breeding Station (SPBS) released 'Craigs Defiance' in 1938; this combined virus resistances from a British and a European parent and, although it is no longer in cultivation, it has been extensively used in subsequent breeding and is in the ancestry of many SPBS varieties. Perhaps the greatest success in British breeding has been 'Pentland Crown', released by SPBS in 1958. This also was bred for virus resistance and has 'Katahdin' in its ancestry together with a British, a European and an Australian cultivar and a Chilean importation to the United States. In addition to its resistance to leafroll-virus and virus Y, it is our highest-yielding cultivar, is resistant to scab, and is more resistant than most older cultivar to tuber blight. The yield may stem from its highly diverse ancestry while its fortuitous scab and tuber blight resistances are of unknown origin, bonuses deriving from the use of material from elsewhere.

Another aspect of Government-supported breeding has been the provision of the security, as well as the know-how, which has allowed long-term breeding through several generations. Previous breeding almost invariably used named cultivars as parents; numbered breeding lines were virtually unknown. Breeding from wild species or primitive cultivars is essentially long-term, and requires a stable environment for the breeders. Before Government-supported breeding, the only long-term effort known to me was that by Dr. John H. Wilson of St. Andrews University, who died in 1920 leaving a fifth-generation breeding line which was subsequently used at the SPBS. His position in the University presumably provided the necessary environment.

I have indicated that the gene-pool of our cultivated potatoes early this century was limited and was subdivided into various local gene-pools, which were subsequently combined, to some extent, in virus-resistance breeding with benefits extending beyond virus resistance. There is still some separation due to regional preferences, e.g. for white or for yellow flesh. Other developments this century have been the recognition of the immense store of untapped variation remaining in the primitive cultivars of South America, and the discovery of the very large number of wild species, many of which have been found to have disease resistances of value, and some of which have still not been collected as living plants so that their properties remain unknown.

Use of wild species and primitive forms

Rather than listing wild species and primitive forms used in breeding for particular purposes, I propose to discuss the unexpected benefits that have often resulted from their use. I mentioned a Chilean accession in the ancestry of 'Pentland Crown'. This, called 'Villaroella' or 'Villaroela' (the spelling varies in publications), was used in virus-resistance breeding in the United States before 1921, apparently for resistance to virus X. It was some ten years later that immunity as distinct from resistance to X was observed in its derivative, seedling 41956; hypersensitive resistance was available from other sources but this was the first recognition of immunity (Stevenson 1956). Subsequently the cultivar Saco was bred from 41956 × 96–56 and proved to be immune to virus S ('latent mosaic') (Akeley et al. 1955), this being the first finding of such immunity. The inheritance of this immunity is complex; neither 41956 nor 96–56 is immune, yet some of the selfed progeny of

'Saco' are susceptible (Bagnall & Young 1960). It seems probable that 'Villaroella' may have borne a gene which contributes to S immunity when combined with some other gene. This appears to be a bonus derived from the use of 'Villaroella'. Neither X nor S immunity has been inherited by 'Pentland Crown', but one wonders whether or to what extent the other attributes of 'Crown', mentioned previously, may trace to 'Villaroella'.

An eelworm-resistant primitive cultivar, 'CPC 1673', happened to carry an X-immunity gene apparently identical with that in 'Villaroella', and one of our eelworm-resistant cultivars, 'Pentland Meteor', has by chance inherited it; again a bonus.

Solanum demissum and its relatives have been extensively used for its *R* genes for immunity to blight. This breeding could be viewed as a failure because races of blight able to infect varieties bearing any of the *R* genes, individually or in combination, ultimately appeared. But it then proved that some *demissum* derivatives had generalized blight resistance, falling short of immunity (which in fact was hypersensitivity) but enabling the plants to survive blight attack without excessive damage. This different resistance mechanism, which is now being used in breeding, constitutes a bonus deriving from the use of *demissum*. Additionally, cultivars and breeding lines derived from *demissum* were often high-yielding and had good shapes and other properties and were by no means to be discarded when their blight immunities broke down; whether these aspects were to be attributed to *demissum* or whether they arose from the broad range of other parents used in such breeding, and the selective skills of the workers involved, remains uncertain. Also, high protein contents and various virus resistances have been found in *demissum* derivatives, bonuses accruing from the use of its blight hypersensitivities.

Other primitive cultivars and wild species are in use in breeding in Britain or elsewhere for reasons such as virus or eelworm, frost or insect resistance, but either have not yet given rise to commercial cultivars or have done so elsewhere, any bonuses accruing from the use of its blight hypersensitivities.

But the general picture that emerges is that the potato gene-pool is so limited that any supplementation might prove to be of value in ways other than those for which it is chosen for use, this in spite of backcrossing procedures in which the final cultivars contain relatively little of the genomes of the introduced parents.

Use of Neo-Tuberosum

One objective of the Neo-Tuberosum work is to eliminate the need for backcrossing, giving cultivars more rapidly, and allowing a greater part of the introduced genome to be present in them. One advantage will be that Neo-Tuberosum parents can be introduced to breeding programmes by reason of properties, perhaps resistances to relatively minor diseases, which would not justify lengthy backcrossing programmes. Another will be the greater likelihood of 'bonuses' of the type discussed above. A third, and in my view an especially important one, is the likelihood of high yields due to heterosis.

There are already indications of 'bonuses' from the use of Neo-Tuberosum in that higher frequencies of scab and of gangrene resistance have been found in progenies of Neo-Tuberosum derivation than in the general through-put of our cultivar breeding programme, this although these properties were not known to be present in the parents concerned. These particular parents were some of the first used and were chosen rather casually; parents used subsequently have been assessed more extensively but their progenies have not yet reached the stage of disease-resistance assessment. Whether any of the derivatives of the first batch have stronger resistances than were previously available is not yet known.

Heterosis for yield

Evidence of yield heterosis was found in pilot work by myself (Glendinning 1969) and by various others (John Innes Res. Inst. 1966; Plaisted 1972; Plaisted & Cubillos 1973; Tarn & Tai 1973) but it has not so far been confirmed in our cultivar breeding programme, where only the first batch of progenies, referred to above, has reached a stage of detailed assessment. This is rather disappointing, and further experiments are in hand or planned. One reason could be that the parents concerned might have been inbred, and might have transmitted part of their inbred status to their progenies. Some subsequently used parents were derived from manual intercrossing within Neo-Tuberosum, and their progenies may prove superior.

Diploid breeding programme

Our diploid breeding programme is also aimed at securing high heterosis in the ultimate tetraploid cultivars. Diploids are being developed to near-commercial standards so that little if any further breeding will be required after crossing with tetraploid cultivars; it should thus be possible to select cultivars with possibly half their genomes of diploid origin. Further, the diploid contribution will be in the form of an unreduced pollen-grain containing almost the whole genotype of the diploid parent and thus itself highly heterotic (Sudheer 1977). This work is as yet at an experimental stage, no actual inputs to the cultivar breeding programme having been made.

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Some genes of importance for the evolution of the pea in cultivation (and a short presentation of the Weibullsholm-P.G.A. pea collection)

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Index words

Pisum sp., pea, wild pea, genes, evolution, Weibullsholm pea collection.

Summary

Presently 295 known and symbolized genes are preserved in the pea collection at Weibullsholm Plant Breeding Institute. Out of these, *dpo*, *a*, *le*, *p*, *v* and *r*, have been particularly important in the transformation of the wild peas into modern cultivars. Several others, e.g., *n*, *m*, *i*, *l*, *z*, seem to be present in a large part of modern varieties.

As far as could be found out, these have all originated as spontaneous mutants. The oldest and most basic of all is probably *dpo*, *Dpo* to-day being found only in some wild forms. The recessive *a*-mutant seems to have originated around 1200–1300, *le*, *r* and *v* around 1500–1600, the rest probably after 1800.

Out of 10 analysed disease resistance genes, 3 are dominant, originating from wild or very primitive material, 7 are recessive mutants fairly recently isolated.

Most of these mutants seem to have been isolated from material in cultivation. The contribution from wild and primitive material, with the possible exception of disease resistance, seems to be restricted; perhaps this is only to be expected, the pea as a cultivated plant still being very little different from some of the wild forms.

Finally, the information system of the pea genebank, utilizing a Wang 2 200, is presented.

Introduction

Several taxa have been described in the genus *Pisum* and a differing number given specific rank, such as *P. arvense* L., *P. sativum* L., *P. abyssinicum* Braun, *P. fulvum* Sibth. & Sm., *P. aucheri* Jaubert & Spach., *P. elatius* Stev., *P. formosum* Alefeld, *P. humile* Boiss. & Noe, *P. jomardi* Schrank, *P. transcaucasicum* (Gov.) Stankov (Lamprecht 1974). All these can, with the exception of *P. formosum*, be crossed and the gene exchange is completely free. *P. formosum* is regarded as belonging to a separate genus, *Alophotropis*. Extreme forms, such as on the one hand *P. fulvum* and on the other *P. elatius*, do differ in chromosome structure and therefore F_1 can be highly sterile, sometimes up to 95%. Similar sterility can, however, also be found in crosses, for instance among *P. sativum* forms, provided

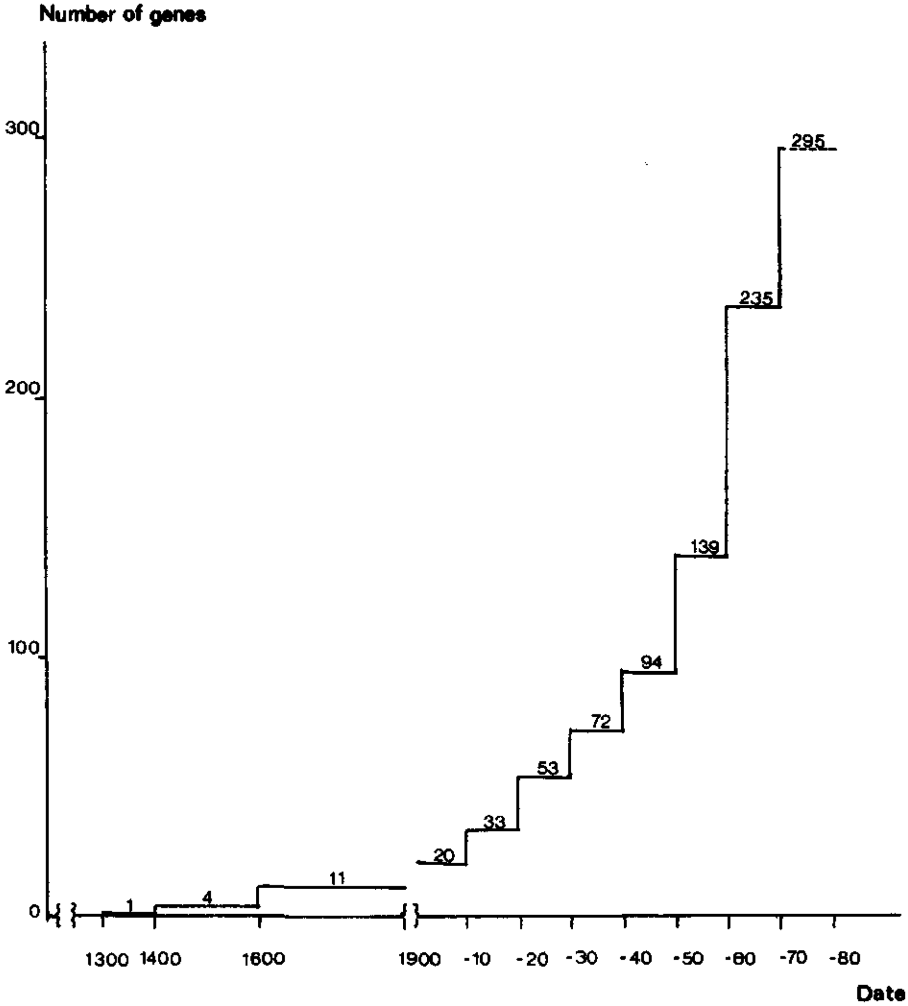


Figure 1. Number of known genes in *Pisum* during different intervals of history.

the right different translocation types are chosen. The F_2 and later generations always have fully restored fertility in combination with all genes after selection of the different homogeneous translocation lines, making allowance for the known difficulties connected with genes closely linked to the breakage-points.

The genus *Pisum* is therefore here regarded as monospecific and the genes can be discussed as freely transferable over the whole range of forms (Blixt 1972).

Genes in wild and cultivated peas

Wild peas still exist and some of these differ very little from some of the field peas still grown and cultivated. Despite this, there are certain difficulties in defining precisely the wild type of *Pisum*. A main obstacle is that the different forms, or taxa, that may perhaps be called ecotypes, carry different recessive alleles. All forms except *P. fulvum* seem, for instance, to be recessive for *Cit*, *Cm* and *Umb*. The gene *Ser* seems to be present only as recessive in all *P. sativum* and all *P. elatius*. For practical purposes, the Weibullsholm gene bank works presently with a 'standard type' (App. 1), which is close to a European *P. arvense* or *P. elatius*.

To become aware of the existence of a gene obviously at least two alleles must exist simultaneously. Fig. 1 shows the growth of our knowledge of pea genetics over a timespan (data from the latest gene list, Blixt 1977). Numbers are conservative estimates, only such genes being counted as are presently represented by type-lines in the collection. The date of 'discovery' of a gene has been set at the date of publication, when such a date was known. Besides these genes, which have been analysed in some detail, approximately twice as many, at least, are isolated in lines, waiting to be analysed and symbolized. And I would like to emphasize that the diagram, at least after 1960, does not reflect the availability of genetic variation as much as the availability of funds for genetic work.

As shown by archeological finds, man used the pea about 5 000 years ago (Schiemann 1932). To follow, in terms of genes, the evolution from a wild to a cultivated plant is, of course, limited to present genetic knowledge. Also, the domestication of the pea seems, for different reasons, to have been superficial. Below are listed a number of genes that have participated in domestication, the order of importance being, of course, as judged by me.

Dpo. Demonstrated by Marx only in 1971 (Marx 1971), this gene is probably by far the most important for domestication of the pea. The dominant allele, found as yet exclusively in wild peas, e.g. in *P. fulvum*, and some *P. arvense* lines, cause the pod to dehisce explosively, twisting the pod-halves and throwing the seeds out. Recessive *dpo* pods do open, when also dominant in *P* and *V*, but the seeds usually remain in the pod unless mechanically disturbed.

This mutant may perhaps be analogous in function and importance with, for instance, the non-brittle spike of the hexaploid wheat or the cob of maize. When it was first used by man is not known, but it certainly dates back very far. Archeological finds of pods might here be of interest, the twisted shape of the opened *Dpo* pod being characteristic.

A. All wild peas are dominant *A*, i.e. have anthocyanin. Recessive *a* inhibits anthocyanin production in the whole plant and with the disappearance of anthocyanin from the seed-coat most of that bitter pea taste disappears too.

The *a* mutant was first mentioned, it seems, by Petrus de Crescentii in 1306 (Crescentii 1458). For such a drastic and easy mutant to discover, changing flower colour from red to white, and with such a distinct change of taste, the actual discovery was perhaps not much earlier. Anyway, in the days of Linnaeus it must have been firmly established, as Linnaeus named the pea with the white flowers first and called it *P. sativum*. It is unfortunate, however, that this was taken as a basis for naming the species and the wild plant was reduced to a subspecies. Mendel, using $A - a$ as one of his character pairs, showed that white-flowered *P. sativum* and red-flowered *P. arvense* (Blixt 1975) could be crossed without any sterility in F_1. To-day, all modern pea varieties for human consumption are *a*, and for the sake of quality, also fodder-peas, traditionally *A*, should be replaced by *a* varieties, because of their lower content of tannins and other anti-nutritional substances.

Le. The first mention of the short, zig-zag stem seems to be around 1500 (Bock 1539). The recessive *le* was used by Mendel and transforms the pea plant from a long rather indeterminate climber to a short more determinate plant, at the cost, however, of a reduction in yield.

It seems that the *dpo* mutant soon became homozygous in the entire cultivated population of peas, even in primitive cultivars. Recessive *a* seems also to have gained ground quite rapidly, at least in Europe. The *le* mutant, on the other hand, was long used in the garden and only recently gained interest as a field crop, despite its many weaknesses. With the introduction of 'industrial methods' in agriculture, cultivars and cultivation methods that reduced yields to a fraction of the maximum could be economically favorable.

Besides *le*, about 15 genes with minor effect on internode length and number are known (e.g. *coe, cotr, cona, cot, cry, la, mie, mier, mine, miu, prae*). The ffect and interaction of these is poorly known. Some seem to be used by breeders presently producing very short plants or short plants without using *le*.

An aspect still insufficiently known in its genetic details, but of major importance for the utility of dry peas for human consumption, is cooking properties. Part of this is associated with the hardness of seeds and all wild forms and many primitive or old cultivars have a varying fraction of hard seeds, again partly a pleiotropic effect of *A*.

Some genes associated mainly with quality should be mentioned.

P, *V* and *N*. The character determined by *p* or *v*, partly or entirely unparchmented pods, seems to have been known at least since 1537 (Ruel 1537). The recessive *v* mutant might well have been the first discovered, as it is one of the commonest spontaneous mutants in peas, both ways, dominant to recessive and vice versa. Mendel used one of them, either *v* or *p* (Novitsky & Blixt 1977). The *n* mutant in combination with *p v* gives delicious edible-podded forms, and was discovered about 1900 (Denaiffe 1906).

R and *Rb*. The *r* mutant seems to have arisen at about the same period as *le* and *v*, around 1500. At least, it was also mentioned by Bock (1539). Because of the sweet taste and tenderness, it gave the pea considerable importance as a vegetable, particularly in modern times for canning and freezing. Another gene, with very similar effect, the *rb* mutant, was only recently discovered (Kooistra 1962). The most relevant part of the story here is that probably all lines carrying *rb* can be traced back, it seems, to the cultivar Alsweet, thus indicating one and the same mutation as origin. Though, of course, impossible to prove, the same probably holds true for *a*, *le* and *r*, so that all cultivars and forms existing today that carry those alleles originate from a few mutants.

M. Another gene of some importance for taste, *M*, is, unlike most other bitter-tasting seed-coat color genes not totally inhibited by recessivity in *a*. Most wild forms of pea and primitive cultivars are *M*, all modern cultivars for human consumption *m*. One may ask why *M*, *Fs*, *U* and many other dominants producing these bitter tasting substances are still as common as they are in fodder peas and most primitive cultivars also used by man. The answer is probably that most of these substances (and for some it has been experimentally verified) give resistance or tolerance to certain fungal diseases.

Man's taste is largely governed by his eyes and this has been and still is no mean problem. The local preferences for seed-color in beans in Brazil is but one example. In peas, the distribution of certain alleles is governed by similar preferences.

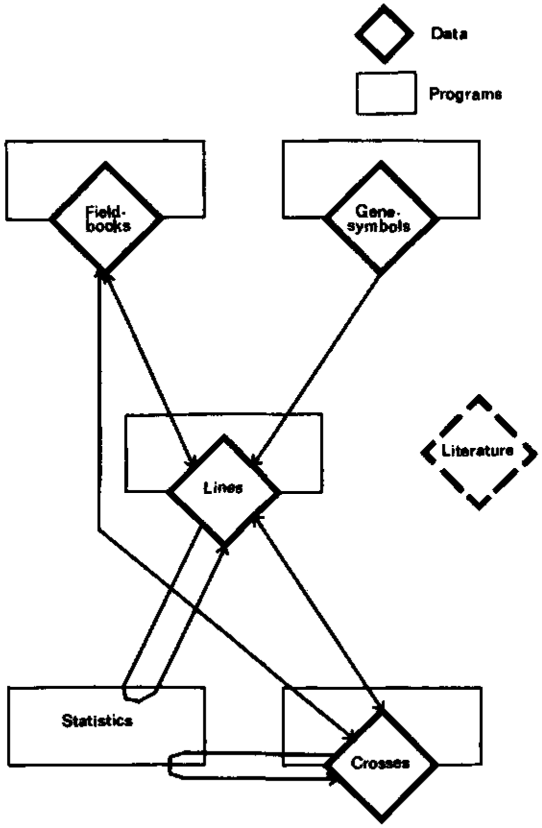
I. One pair of the alleles for seed-color is *I*, yellow cotyledons, and *i*, green, known at least since Mendel. The fashion in Scandinavia is nowadays for cooking peas to be yellow and green peas, used for instance in Belgium and the Netherlands, are not used.

L and *Z*. The recent mutants *l* (Tschermak 1912) and *z* (Kajanus 1923), both giving round smooth seed, *z* also a clear transparent color, are rather essential in a Scandinavian yellow dry pea, but seem of little consequence elsewhere in Europe. Genes for disease resistance in peas are insufficiently analysed. Recessivity and the seemingly limited distribution indicate fairly recent mutation of seven genes: *er-1*, *er-2*, mildew resistance (Harland 1948; Heringa et al. 1969); *lr*, pea leaf-roll virus resistance (Drijfhout 1968); *mo*, common pea mosaic virus resistance (Yen & Fry 1956); *rpv-1*, *rpv-2*, resistance to *Peronospora viciae* (Matthews & Dow 1969); *sbm*, resistance to seed-borne mosaic virus (Hagedorn & Gritton 1971). Dominance and primitive origin apply for three genes: *En*, resistance to pea enation mosaic virus (Schroeder & Barton 1958); *Fnw*, resistance to near-wilt (Snyder & Walker 1935); *Fw*, *Fusarium* wilt resistance (Wade 1929).

Finally, two quite drastic characters should be mentioned: fasciation, genes *fa*, *fas*, the Mummy pea, which has raised interest for centuries (Bauhin 1620) but still remains a promise; and *af*, the semileafless (Kujala 1953), that is now being introduced in a wide enough genetic background to receive a meaningful test.

In conclusion: Though the pea is old as a cultivated plant, it has diverged little from wild ancestors, which still exist. With the exception of *dpo*, the major genes distinguishing domesticated pea were all discovered after AD 1000, usually originating, it seems, as unique events (exception: *v*). The contribution from primitive cultivars or wild forms seems to be quite limited, also for disease resistance, where three dominants have been found in such material among 10 known genes. This, however, should perhaps not be interpreted to mean that wild forms are of no interest in pea breeding; but certainly that the genetic basis of the pea has not been much narrowed by intensive plant breeding.

Figure 2. The *Pisum* gene data bank.



The pea collection

The pea collection at Weibullsholm seems actually to have been initiated around 1915 by Birger Kajanus when he found and isolated the *z* mutant. About 1930, Herbert Lamprecht actually initiated a gene bank, concentrating on preserving genes rather than genotypes.

At present, the same general policy is continued. Part of the material is of spontaneous origin, part is induced mutants. Most important is probably the collection of type-lines, i.e., lines typifying the described and published gene symbols.

Lines are classed into three groups, pure lines, collections and cultivars, and into two classes with or without lethal or sterile genes, and the different groups and classes are treated differently in practical handling.

The standard observation comprises observation of roughly 200 individual genes and about 45 characters expressed quantitatively.

The information is stored and handled on a Wang 2 200 minicomputer (Blixt 1976), comprising a central unit, a cathode-ray tube screen with key board and magnetic cassette station, two printers and one disk unit for one fixed and one removable 2.5 Mb disk.

As extra input channels two field-portable memories are used (Frequensor Terminal 450).

The information is divided into different separate partly communicating databanks (Fig. 2). The 'Lines' stores data on the individual lines or accessions of the collection; the 'Gene Symbols' the information pertaining the genes; the 'Crosses' information on purpose and experimental details on out own crosses; 'Linkage data' information on genetic results (segregation) on our own crosses and crosses published by others; 'Field-books', for instance sowing-lists, field-books; 'Literature' is a collection of reprints on *Pisum*.

The information is available partly as general lists on lines and gene symbols, partly as particular answers to specific questions, for instance whether there are lines answering certain descriptions, or statistical parameters of the material, such as correlations between characters, like protein content and yield.

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Appendix 1. Standard type of *Pisum*. Valid genes.

Dominants

Amp-1, Asc, Astr, Br, Bra, Ca, Cgf, CHI-24, Cit, Cm, Cv, Dpo, E, Ed, Ef, En, F, Fnw, Fs, Fw, Gty, Him, Hr, I, Inci, Int, Kp, Kpa, Lap, M, Np, Obs, Pl, Pu, Pur, Rf, Ser, Sym-1, Tra, Umb, Vl, X, Y.

Recessives

a, ac, acu, ad, ade, af, age, alb, alt, am, ang, ar, as-1, as-2, as-3, as-4, as-5, as-6, as-7, as-8, aure, au, auv, b, beg, bif, bip, bips, brac, brev, bt, bulf, cal, calf, cat, cc, ce, ceo, ch-1, ch-2, ch-3, ch-4, chi-1, chi-2, chi-3, chi-4, chi-5, chi-6, chi-7,chi-8, chi-9, chi-10, chi-11, chi-12, chi-13, chi-14, chi-15, chi-16, chi-17, chi-18, chi-19, chi-20, chi-21, chi-22, chi-23, chi-25, chi-26, chi-27, chi-28, chi-29, chi-30, chi-31, chrw, cht, cist, co, coch, coe, coh, com, con, cona, cont, cor, cot, cotr, cov, cp-1, cp-2, cr, creep, cri, crif, cris, crpt, cry, curl, curt, cvit, d, def, dem, den, dgl, di, dim, disp, dn, dp, ds-1, ds-2, ds-3, ds-4, dt, elo, em-1, em-2, ep-1, ep-2, er-1, er-2, exal, fa, fas, fe, ff, fil, fl, fla, fn, fna, fo, fob, foe, fol, fom, fov, fr, fru, gfc, gl, gla, gp, gri, h, ho, hyt, ib, iba, inc, ins, k, l, la, lac, laf, lat, lath, lc, ld, le, leflo, let, lf, lm, lo, lob, lr, lt, lum, mex, mie, mier, mifo, min, mine, mis, miu, miv, mo, mp, ms-1, ms-2, ms-3, ms-4, n, na, nap, nlb, no, nod-1, nod-2, nol, nr, o, obo, och, oh, oli, olv, op, p, pa, paf, pafl, pal, par, pat, pe, ph, pla, pn, pr, pra, prae, pre, pro, psp, pt, py, qua, r, rag, ram, rb, re, red, rfe, rms, rpv-1, rpv-2, ru, rup, rups, s, sa-1, sa-2, sa-3, sal, sat, sb, sbm, sc, serp, sg-1, sg-2, sifl, sil, sin, siv, sn, sob, sre, sru, srub, st, ster, sti, stia, stim, stp, stpr, str, sub, sul, sup, sym-2, sym-3, t, tac, td, te, ten, teu, tl, trp, twp, u, un, uni, up, v, vac, ve, vim, wa, was, wb, wel, wex, wil, win, wlo, wp, wsp, xa-1, xa-2, xa-3, xa-4, xa-5, xat, yg, yp, z.

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Tripsacum introgression and agronomic fitness in maize (Zea mays L.)

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Index words

Tripsacum, Zea, maize, teosinte, intergeneric gene exchange.

Summary

Maize (*Zea mays* L.) crosses with species of *Tripsacum* when the parents are not isolated by gametophytic barriers. In hybrids that combine 10 chromosomes from *Zea mays* and 18 from *Tripsacum dactyloides* (L.) L., little gene exchange between the *Zea* and *Tripsacum* genomes is obvious in backcross derivatives. Hybrids that combine 10 *Zea* and 36 *Tripsacum* chromosomes are often characterized by pairing between as many as four different *Zea* chromosomes and four independent *Tripsacum* chromosomes. Backcross derivatives of these hybrids are cytogenetically complex. Highly tripsacoid maize with $2n = 20$ *Zea* chromosomes can usually be recovered within eight generations when the hybrids are backcrossed with the maize parent. Tripsacoid-recovered maize is partially sterile. Full fertility can usually be restored by crossing between lines, and tripsacoidness can at the same time be maintained by selection. Some selected lines breed true to type while others segregate for degree of fertility and tripsacoid traits. The agronomic potential of various tripsacoid traits are being investigated. Genetically dominant resistance to six common maize diseases (anthracnose, fusarium stalk rot, northern and southern corn leaf blight, rust, and Stewart's bacterial blight) was identified in tripsacoid recovered maize. This resistance was successfully transferred to commercial maize inbreds. Other potentially useful tripsacoid traits that were transferred to maize through *Tripsacum* introgression are gametophytic apomixis, weak perennialism, a tillering habit, an increase in inflorescence-bearing culm nodes, and an increase in number of fully developed ears at each node.

Introduction

The closest wild relative of maize (*Zea mays* L.) is subspecies *mexicana* (Schrad.) Iltis (teosinte). Maize hybridizes with teosinte wherever they grow together across Central America. Mexican farmers sometimes encourage such introgression to improve agronomic fitness of the crop (Lumholtz 1902; Wilkes 1970, 1977). Comparative morphology indicates that introgression with teosinte played a major role in evolution among races of Mexican maize (Wellhausen et al. 1952). Introgression of maize with species of the distantly related *Tripsacum* can be induced experimentally (de Wet & Harlan 1974; Harlan & de Wet 1977). However maize and *Trip-*

sacum are reproductively isolated in nature. Yet it has been postulated that *Zea-Tripsacum* introgression contributed to racial evolution of South American maize (Roberts et al. 1957; Mangelsdorf 1961, 1968).

Tripsacum is a small, but morphologically variable genus with a wide tolerance of soil condition. It is resistant to most common maize diseases and insect pests of maize, and has an almost continuous distribution from latitude 42 ° N to 42 ° S. Sporadic attempts have been made with some success to exploit *Tripsacum* germplasm in maize improvement. Reeves & Bockholt (1964) succeeded in increasing vigor of a maize inbred through introgression from diploid *T. dactyloides* (L.) L. ($2n = 36$). Simone & Hooker (1976) transferred resistance to northern corn leaf blight from *T. floridanum* Porter ex Vasey ($2n = 36$) to corn belt maize. Petrov et al. (1971) introduced gametophytic apomixis into maize from tetraploid *T. dactyloides* ($2n = 72$), and Bergquist (1977) identified resistance to six diseases in tripsacoid maize derived from introgression with tetraploid *T. dactyloides*. The enormous gene pool of *Tripsacum*, however, remains largely unexplored. This is primarily due to the difficulties in transferring genes of *Tripsacum* to *Zea*. These difficulties have now largely been overcome. The choice of *Tripsacum* parent is critical to successful hybridization, and cytological events occurring in the hybrids and early backcross derivatives determine the success or failure to recover tripsacoid maize (de Wet & Harlan 1974). In this paper, the cytogenetics of maize - *Tripsacum* introgression, the morphology of tripsacoid maize, and the potential significance of *Tripsacum* germplasm in maize improvement are discussed.

Cytogenetics of maize - Tripsacum introgression

Maize ($2n = 20$) crosses with both diploid ($2n = 36$) and tetraploid ($2n = 72$) taxa of *Tripsacum*. *Zea* and *Tripsacum*, however, are reproductively isolated by gametophytic and sporophytic barriers, and less than 1% of *Tripsacum* collections studied will cross with maize. When maize is used as the female parent, endosperm is poorly developed in hybrid caryopses and special care is required for hybrid seedlings to survive. Once established, however, they are usually vigorous, perennial and resemble the *Tripsacum* parent in vegetative morphology. Endosperm development is essentially normal in reciprocal crosses, and vigorous hybrid seedlings are usually produced. Hybrids with *Tripsacum* as the female parent have as yet been backcrossed with maize for only four generations, and diploid individuals with 20 *Zea* chromosomes in *Tripsacum* cytoplasm have yet to be recovered. Hybrids discussed in this paper all involve maize as the female parent and either diploid or tetraploid *T. dactyloides* as the pollen parent. Hybrids were successively backcrossed with maize for eight or more generations until tripsacoid offspring with 20 maize chromosomes were recovered.

Hybrids between diploid ($2n = 20$) *Zea mays* (Zm) and diploid ($2n = 36$) *Tripsacum dactyloides* (Td) combine 10Zm + 18Td chromosomes. Intergenome pairing in these hybrids is minimal (Chaganti 1965; Newell & de Wet 1973). They are male-sterile, but the cytologically unreduced female gamete can function sexually. When these hybrids are pollinated by maize, their offspring regularly have 20Zm + 18Td chromosomes. Plants with 38 chromosomes remain male sterile but frequently produce offspring when pollinated by maize. Second generation backcross derivatives combine 20Zm + 1–18Td chromosomes, and one or two further backcrosses commonly produce some fully fertile individuals with 20 maize chromosomes. Recovered maize rarely exhibits tripsacoid traits (Mangelsdorf & Reeves 1939). But, Simone & Hooker (1976) succeeded in transferring resistance to northern corn leaf blight from *T. floridanum* ($2n = 36$) into corn belt maize.

Hybrids between maize and tetraploid *T. dactyloides* ($2n = 72$) are characterized by 10Zm + 36Td chromosomes. These hybrids are usually vigorous but often completely sterile. Some hybrids, however, are partially female fertile, and produce offspring when pollinated by maize.

Tetraploid *T. dactyloides* are of two basic cytological types. The one race is characterized by essentially normal chromosomal behavior during meiosis of microsporogenesis. These plants reproduce sexually, and hybrids between this race and maize are characterized by autosyndetic chromosome pairing during meiotic prophase. The 36 *Tripsacum* chromosomes regularly form 18 bivalents, and the 10 *Zea* chromosomes remain as univalents or rarely pair among themselves (Harlan et al. 1970). These hybrids are frequently sterile, and when female fertile, essentially pure maize is recovered within five backcross generations using maize as the pollen parent. The other race of *T. dactyloides* ($2n = 72$) behaves cytogenetically as an autotetraploid. Tetravalents are commonly produced during meiosis of microsporogenesis (Newell & de Wet 1974), and individuals reproduce as facultative gametophytic apomicts (de Wet et al. 1973).

Hybrids between maize as the female parent and facultative apomictic tetraploid *T. dactyloides* combine 10Zm + 36Td chromosomes, and are male-sterile but partially female fertile. Female gametophytes can function sexually or asexually. These hybrids are characterized during meiotic prophase of microsporogenesis by autosyndetic bivalent formation among the *Tripsacum* chromosomes, while the *Zea* chromosomes usually remain unpaired. Occasionally, however, as many as four *Zea* chromosomes pair with individual *Tripsacum* chromosomes or enter into trivalent associations with *Tripsacum* bivalents. The chromosomes involved are consistently 2, 4, 7 and 9 of maize, and probably those *Tripsacum* chromosomes identified by Galinat (1973) to have loci in common with them. These hybrids commonly reproduce by means of gametophytic apomixis. But, sexually reproduced offspring with 10Zm + 36Td or 20Zm + 36Td chromosomes are also produced. Maize chromosomes are often eliminated during the first meiotic division of macrosporogenesis, and failure of cytokinesis during the second meiotic division produces female gametophytes with 36 *Tripsacum* chromosomes (de Wet et al. 1970; de Wet et al. 1973). Similarly, offspring with 46 chromosomes can be obtained in later backcross generations. However, judging by the appearance of maizoid traits in these backcross derivatives, the *Tripsacum* genome becomes successively more contaminated with *Zea* genetic material with each additional maize backcross. In plants with 10Zm + 36Td chromosomes, in which the *Tripsacum* genome is contaminated with *Zea* genetic material intergenome pairing becomes the rule rather than the exception, and an array of chromosome numbers are common in their offspring (Table 1). Individuals with 20 maize chromosomes can usually be recovered from any of these genome combinatios after three or more additional backcrosses. The most interesting pathway to recovered maize involves selected plants with 20Zm + 36Td chromosomes in which the *Tripsacum* genome is con-

Table 1. Offspring of hybrids with 10 *Zea* + 36 *Tripsacum* chromosomes when pollinated with maize.

Generation	Number of parents	Number of offspring with chromosome number					
		46	48	50	52	54	56
F_1		84					
BC_1	2	23					2
BC_2	1	12				2	2
BC_3	2	45		1	1	1	2
BC_4	9	31	1	2		2	3
BC_5	19	253	1	1	5	7	12

Table 2. Selected derivatives of a BC_3 individual with 20Zm + 36Td chromosomes in which the *Tripsacum* (Td) genome is contaminated with *Zea* (Zm) genetic material, when backcrossed with maize.

Genome Constitution			
BC_4	BC_5	BC_6	BC_7
10Zm + 18Td	20Zm + 18Td	20Zm + 1-4Td	20Zm
20Zm + 18Td	20Zm + 1-4Td	20Zm	20Zm
20Zm + 36Td	20Zm + 18Td	20Zm + 1-4Td	20Zm
30Zm + 36Td	20Zm + 18Td	20Zm + 1-4Td	20Zm
34Zm + 36Td	20Zm + 18Td	20Zm + 1-4Td	20Zm

taminated with maize genetic material, and half the *Zea* genome is contaminated with *Tripsacum* genetic material. These backcross derivatives do not behave cytogenetically as allotetraploids. One to four trivalents, or an occasional tetravalent involving *Zea* and *Tripsacum* chromosomes are formed during meiotic prophase (Engle et al. 1974). They produce offspring with various combinations of maize and *Tripsacum* chromosomes (Table 2). Maize recovered from these derivatives exhibits various degree of *Tripsacum* introgression.

Morphology of tripsacoid maize

Tripsacoid maize recovered after *Tripsacum* introgression is partially sterile. Fertility can usually be restored through hybridization between tripsacoid lines, and tripsacoid traits can be maintained through selection. Fertile tripsacoid selections breed true to type or segregate for tripsacoidness as well as fertility. They vary extensively in degree of tripsacoidness (Stalker et al. 1977a, b).

Highly tripsacoid lines with 20 maize chromosomes often resemble early derivatives of maize-teosinte hybridization in inflorescence and vegetative morphology. These plants tiller, their inflorescence branches are often long, and the branched inflorescences are frequently bisexual. Female spikelets are commonly paired and arranged below the paired male spikelets on a common rachis. Ears are in four or eight rows, with yoked opposite pairs, or yoked pairs fused into whorls of eight (de Wet et al. 1978). Yoked pairs sometimes disarticulate at maturity. Glume and rachis tissues are strongly indurated, and the cupules on the female section of the inflorescence are variable in size and shape. Highly tripsacoid selections are now being studied genetically to determine whether genes introduced from *Tripsacum* are homologous with, or different from teosinte genes known to produce similar characteristics in maize.

Moderately tripsacoid populations are typically maize-like in general ear morphology, but variable in detailed inflorescence structure. The number of culm nodes bearing inflorescences is increased from one or two to as many as eight, and each inflorescence often produces more than two well developed ears. Ears range from 6 to 20 cm in length, are variously shaped, and range from 8 to 14 in number of rows. Glumes are usually more indurated than in the maize parent, rachis induration is increased, and the cupules are generally deeper than in pure maize. Plants often tiller and some are weak perennials.

Derivatives of maize - *Tripsacum* introgression are tripsacoid in exactly the same traits as Mexican races of maize are teosintoid and South American races of maize are 'tripsacoid' (de Wet et al. 1978): This would suggest that *Tripsacum* introgression could have played a role in racial evolution of South American maize as suggested by Roberts et al. (1957). Evidence of natural introgression between modern races of maize and *Tripsacum*, however, is absent. The only possible natural hybrid between *Tripsacum* and maize is *T. andersonii* Gray. This species seems to combine 54 *Tripsacum* and 10 *Zea* Chromosomes in its genome constitution (de Wet et al. 1976). This species is highly sterile sexually, and backcross derivatives are absent in nature. It seems more likely that South American races of maize are teosintoid rather than tripsacoid. They were probably derived from teosintoid Mesoamerican races of maize (de Wet et al. 1978). Indeed, evidence is accumulating to show that teosinte is wild maize (Beadle 1972). Races of maize that do not show the characteristic induration of glume and rachis tissues are not teosintoid because of intermediate expressions of alleles at the tunicate locus. With the effects of this gene removed, all races of maize are teosintoid in these respects.

Tripsacoid traits and maize improvement

Little is known about the potential agronomic usefulness of *Tripsacum* germplasm that can be transferred to maize. In general, introgression with *Tripsacum* reduces yield and agronomic suitability of standard maize inbreds. Vegetative vigor is sometimes improved but changes in inflorescence morphology are generally undesirable. Individual *Tripsacum* genes or gene complexes, however, may prove useful in maize improvement. The most successful *Tripsacum* traits so far transferred into maize breeding material are resistance to various common maize diseases. Simone & Hooker (1976) of the University of Illinois extracted resistance to northern corn leaf blight from *T. floridanum*. Bergquist (1977) of Pfister Hybrid Corn Company identified resistance to anthracnose, fusarium stalk rot, northern corn leaf blight, southern corn leaf blight, common rust and Stewart's bacterial blight in highly tripsacoid lines derived by us from introgression with tetraploid *T. dactyloides*. Resistance in these tripsacoid lines proved to be genetically dominant when crossed with susceptible inbreds, and the genes controlling resistance have been transferred to commercial maize inbreds. However, it remains to be seen whether other undersirable *Tripsacum* traits transferred with these genes for resistance can be eliminated from the breeding material. Some success in this direction has been achieved by both groups.

Resistance to rootworm damage and to waterlogged soil is also present in selected tripsacoid lines. Furthermore, tripsacoid lines can perhaps be used to change the architecture of the plant. Number of ear-bearing nodes, as well as number of ears produced at a node can be increased through *Tripsacum* introgression as can strengthening material of stems and cobs. Male sterility is common in some tripsacoid lines, but the genetics of this trait need to be studied before new sources of genetic male sterility can be made available for commercial production of hybrid seed maize. Gametophytic apomixis is readily transferred to maize from tetraploid *Tripsacum* (Petrov et al. 1971). However, the desirability of apomixis in an annual crop such as corn belt maize is doubtful.

This study was originally designed to study the cytogenetics of intergeneric gene exchange between *Zea* and *Tripsacum*. This process is genetically complex, but by selecting desirable pathways the maize genome can be loaded with *Tripsacum* genetic material. The second phase of the project, which has just been started, is to transfer selected tripsacoid traits into standard inbred lines of maize. These tripsacoid lines will be made available to breeders for use in maize improvement. The usefulness of *Tripsacum* germplasm is impossible to predict. It is not known whether traits similar to those introduced from *Tripsacum* cannot more successfully be extracted from teosinte or exotic races of maize.

Maize-breeding projects have so far been very successful in increasing agronomic suitability in an ever-changing agricultural environment without having to look for traits not present in domesticated maize. However, corn belt maize is derived from a narrow genetic base and any mechanism to increase the gene pool of *Zea mays* is desirable. Concern has repeatedly been expressed over the genetic vulnerability of corn belt maize. A narrow gene pool allows for immediate fitness and high yield under ideal conditions. Such populations, unfortunately are vulnerable to fluctuations in the environment. This was demonstrated in 1970 when an attack by a particularly virulent race of southern corn leaf blight reduced maize yields in the Midwest by some 15%. This epidemic was due largely to the susceptibility of T-cytoplasm, which is used to induce male sterility in commercial inbreds, to this race of *Helminthosporium maydis*. A ready solution to this problem was at hand by simply returning to normal cytoplasm. The next epidemic, however, may not be as easy to control. A broadening and diversification of the genetic base of maize is imperative for continuous maize improvement.

The gene pool of *Zea mays* is large. Primitive races are still widely grown, and teosinte, the wild ancestor of maize is still extant. It also appears fully feasible to broaden the genetic base of maize through introgression with *Tripsacum*. To what extent *Tripsacum* germplasm will affect agronomic suitability of maize is not yet known. It is known that hybrid vigor and disease resistance can be transferred to selected inbreds from tripsacoid recovered maize. If useful *Tripsacum* genes can be transferred to commercial inbreds of maize without adversely affecting agronomic performance, valuable breeding lines can be generated. Preliminary studies suggest that gene transfer from *Tripsacum* to maize has promise in breeding for disease resistance.

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Wild species for the breeding of grasses

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Index words

Grass breeding, natural resources, hybridization, *Dactylis glomerata, Dactylis marina, Lolium perenne, Lolium multiflorum, Festuca arundinacea, Festuca pratensis, Festuca rubra, Holcus lanatus, Phleum pratense, Poa chaixii, Poa longifolia, Poa pratensis.*

Summary

In old grasslands on fertile soils and under good management, grass populations evolved that were well adapted, productive and palatable. Expanding cities and new agricultural techniques threaten these gene reservoirs.

For their breeding programs, research institutes and commercial breeders collected indigenous grasses. A range of specialized cultivars were developed. Only a part of the germplasm collected has been conserved in clonal nurseries and in cultivars.

Newer cultivars are mostly genetically narrower than older ones, but too narrow a base for cultivars has never been much problem in cross-fertilizing grasses. Nevertheless, many attempts to broaden the genetic basis of grass species have been made to obtain new combinations of desirable characteristics. Within the species, both crossing between genotypes of different climatic regions and intercrossing of pasture and hay types have been carried out, for instance in *Phleum pratense*. A more radical approach is interspecific (*Dactylis* or *Lolium*) and even intergeneric hybridization (*Lolium* with *Festuca*). These distant crosses often have given disappointing results because the hybrids were not sufficiently fertile and cytologically unstable. Backcrossing the hybrids to the parental species seems an attractive method of introducing new variability into grass species. Apomictic *Poa pratensis* crosses with *P. longifolia* and *P. chaixii* can be used in breeding to introduce a completely sexual stage in the breeding scheme. The crosses are also used for breeding *P. pratensis* with a glabrous lemma.

Natural resources and breeding

The differences between grass cultivars and ecotypes of old grasslands and other habitats are small. This has not only to do with the short breeding history of grasses as compared to other crops but also and especially with the high value of some ecotypes. Grasses were used by man long before they were domesticated (Frankel 1977). They were used in natural unfertilized places but also in old grasslands that were properly fertilized and well managed. In such conditions populations evolved that were persistent and highly adapted to agricultural practice.

In old grasslands, the farmer possessed suitable plants of the perennial grasses before the breeder and the seed trade. Before home-bred cultivars became available, resowing of grassland was risky. The commercial seeds, often imported, were mostly harvested from stemmy plants, which were more suitable for seed production under arable conditions than for leafy persistent swards.

The more the difference between plants from commercial seeds and plants from old pastures was realized, the more the need was felt to explore indigenous sources. It was not sufficient for a reliable seed supply to gather seeds in nature or to harvest seed from old pastures, both for direct commercial use. Such was only achieved by collecting plants in pastures and subsequent selection of the best plants from the populations as parents for synthetic varieties. By various breeding procedures, a range of special cultivars was developed: for hay and for pasture, for sports fields and for lawns. Understandably the emphasis has been on collection and breeding of species of generally accepted value. However some species that are neglected at present or are appreciated elsewhere in the world would be put to use. For instance in the Netherlands, *Holcus lanatus* is common but not very much appreciated. According to recent British data (Haggar 1976), *H. lanatus* is more productive than *Lolium perenne* when nitrogen application is low. Therefore, if fertilizer policy were to change, *H. lanatus* might be reappreciated. In New Zealand, *H. lanatus* is being used because its wide adaptibility (Jacques et al. 1974). This example of *H. lanatus* may indicate that our natural resources have not been fully explored.

The collection work of research institutes can mostly be found out without too much difficulty, but collection work by commercial breeders is poorly documentated. The question arises how much of the collected germplasm has been preserved. Populations that did not satisfy the breeder's standard and selections that dit not achieve cultivar status have probably been lost. Therefore, only a part of the collected germplasm will still be available in clonal nurseries and in cultivars. Although breeders' practices are diverse, most modern cultivars are based on fewer clones than older ones. A possible production of hybrid grass seed by using male sterility (Wit 1974b) could further narrow the genetic basis of cultivars.

Such a los of genetic variation will not be insurmountable as long as the sources remain available. As yet, the grass breeder in western Europe has had a lot of germplasm in his surroundings. However, the area under old grasslands is decreasing. Growing cities, construction of new roads, and new agricultural techniques for tillage and resowing grasslands all threaten these natural and semi-natural gene reservoirs.

Novoselova (1977) believes that the conservation of germplasm for many high-quality species and cultivars of forage plants is just as important as for other crops. In the Netherlands gene banks seem to be more urgently needed for other crops. Nevertheless more vigilance to preservation of grass germplasm is certainly necessary.

Wide crosses

Although modern cultivars may be narrower based than older ones, too narrow-based cultivars have never been much of a problem in cross-fertilizing grasses. This does not mean that no attempts are made to broaden the genetic basis of grass crops. There is more than I can review briefly.

In many species like *Phleum* pratense and *Lolium perenne* rather distinct early and late flowering types occur, usually called hay and pasture types. In a collection of *Phleum pratense* ($2n = 42$) populations from the Netherlands, the plants fell rather sharply into two groups: a late-flowering well-tillered type which was taken for indigenous and a more erect early-flowering type that was probably introduced as seed but had maintained itself for several years (van Dijk 1961). These plants were intercrossed, yielding an F_2 consisting mainly of intermediate types. Many F_3-progenies were rather uniform for heading date and phenotype, presumably because of their hexaploidy. Such crossing of distinct types can be applied both for indigenous and for foreign sources. Breese & Davies (1976) mention the crossing of British *Lolium* and *Dactylis* to Mediterranean introductions to combine summer growth of the first with autumn and spring production of the latter. Such attempts to extend the growing season show promise, especially for regions with a mild winter. Most wide crosses within the species offer little breeding problem, but sterile F_1 hybrids have been reported (Jauhar 1974).

Interspecific crosses

A more radical approach is interspecific or even intergeneric crosses. The number of attempts that have been made is indicated by Knoblock's checklist of crosses in the Gramineae (1968).

The genus *Dactylis* comprises both tetraploid and diploid species, of which *D. glomerata* ($2n = 28$) is widely used. Many species are interfertile (Borrill 1961). Crossing of tetraploid with diploid gives triploid and tetraploid hybrids. Backcrossing to the tetraploid parents results in a mainly tetraploid offspring, also from triploid hybrids. In this way, genes can be transferred from diploids to *D. glomerata* (Jones & Borrill 1962).

Of the tetraploids, the cross *D. glomerata* × *D. marina* has been made to combine the high production of *D. glomerata* with the better digestibility of the latter (Borrill et al. 1973). One of the characteristics of *D. marina*, leaves without silicified teeth, was also found after inbreeding *D. glomerata* (van Dijk 1964). To obtain this leaf characteristic, both inbreeding and interspecific crossings can be used.

In the breeding of *Lolium perenne*, a range of interspecific crosses is known. *Lolium multiflorum* is closely related to *L. perenne* and fertile hybrids are easily obtained. Well known is the New Zealand short-rotation ryegrass derived from *L. perenne* × *L. multiflorum*. The aim of hybridization programs is to combine the rapid and high production of *L. multiflorum* with the greater persistence and better winterhardiness of *L. perenne*. However, many breeders are disappointed because the hybrids are not genetically stable. Three ways have been used to achieve more stable products:

- crossing at tetraploid level (Breese et al. 1975).
- production of F_1 hybrids from male sterile *L. perenne* and normal *L. multiflorum*, practised by one Dutch breeder (Joordens 1977).
- backcrossing the hybrids to *L. perenne*, as done in New Zealand and in the Netherlands by the Foundation for Agricultural Plant Breeding.

Fertility is usually no problem in the hybrids and some people even doubt if the parents should be taken as separate species.

Intergeneric crosses

Lolium multiflorum ($2n = 14$) and *L. perenne* ($2n = 14$) are to some extent crossable with *Festuca pratensis* ($2n = 14$) and *F. arundinacea* ($2n = 42$), in fact hybrids are found in nature (Wit 1974a). In hybridization programs, tetraploid versions of the diploid parents are used too; the hybrids can be doubled by colchicine treatment or meiotically (Dijkstra 1975). Hybrids showing the high quality and ease of establishment of *Lolium* and the summer production, winterhardiness and rust resistance of *Festuca* could be useful. According to Lewis (1974), hybridization programs are in progress in eight countries. In crosses between *Lolium* and *F. pratensis*, most viable seeds are obtained from *Lolium* $2x \times$ *F. pratensis* $4x$ (Wit 1974a). Nitzsche (1974) mentiones promising diploid hybrids. The following schemes of genomes present some options for making hybrids. No distinction is made between *L. perenne* and *L. multiflorum*.

a) *Lolium* (L_2) × *Festuca pratensis* (Fp_2)
$L_2 \times Fp_4 \rightarrow LFp_2 \rightarrow$ colchicine $\rightarrow L_2Fp_4$
$L_2Fp_4 \times L_2 \rightarrow L_2Fp_2$
$L_2Fp_2 \times L_2 \rightarrow L_2Fp \rightarrow$ colchicine $\rightarrow L_4Fp_2$
$L_2Fp \times L_2 \rightarrow L_2^+$ introgression L_2^+

b) *Lolium* (L_2) × *F. arundinacea* (Fa_6)
$L_2 \times Fa_6 \rightarrow$ colchicine $\rightarrow Lm_2Fa_6$
$LFa_3 \times (L_4 + L_2) \rightarrow L_4^+ + L_2^+$(introgression)
$L_2Fa_6 \times (L_4 + L_2) \rightarrow L_4^+ + L_2^+$ (introgression)

The main problems are cytological instability and lack of fertility in the newly formed hybrids. G.J. Speckmann and H. Dijkstra of SVP tell me they have analysed a population of newly formed Lm_2Fa_6 ($2n = 56$). Chromosome numbers, $2n$, in the fourth generation showed a range 45–55, 22% being 45. Similar phenomena were observed in *Phalaris*. McWilliam (1974) obtained a hybrid population by *Phalaris tuberosa* ($2n = 28$) × *Phalaris arundinacea* ($2n = 42$) $\rightarrow 2n = 35 +$ colchicine $\rightarrow 2n = 70$. After eight generations of the selection, the average chromosome number was 56–58.

Ahloowalia (1974) assumed that cytological instability in *Festulolium* occurred especially beyond the tetraploid level. Thomas (1974) expected more genomic stability by using B chromosomes.

The cytological instability of the hybrids has lead to more emphasis on introgression. In many programs, diploid and tetraploid *Lolium* plants with some *Festuca* characteristics are under investigation. Some show a good seed set but are male-sterile. Such male sterility can be an useful tool in grass breeding (Wit 1974b).

In spite of the problems in fertility and stability some hybrid cultivars are under test. 'Hazel', a Dutch octoploid variety from *Lolium* and *F. arundinacea*, is accepted for the French List of Varieties. Buckner (1974) bred 'Kenhy', a *F. arundinacea* with some characteristics of *Lolium*.

Recently, J. Dijkstra (SVP) crossed *L. perenne* with *Festuca rubra* and obtained Lp_2Fr_6 and Lp_2Fr_4 plants that were completely female-sterile and highly male-sterile.

Apomixis and interspecific crosses

Another genus that allows many interspecific crosses is *Poa* (Clausen 1961). For brevity, I present only data from my own breeding program for *P. pratensis*. *P. pratensis* ranges in somatic number 36–150 and is a facultative apomict. Interspecific crosses are used to introduce a sexual stage in breeding and to introgress for some characteristics (van Dijk 1974). The cross *P. longifolia* ($2n = 42$) a cross-fertilizing perennial from near the Black Sea in the Soviet Union, and *P. pratensis* produced hybrids that were sexual and sufficiently fertile. In F_2 a wide range of types was found. By direct selection and by crossing to *P. pratensis*, plants could be selected that were rather close to *P. pratensis*, some of them highly apomictic. Glabrousness of the lemma could be introduced in *Poa pratensis* from *Poa longifolia*. A glabrous lemma facilitates seed threshing and cleaning because hairiness causes sticking together of the fresh seeds.

Also the reciprocal cross is realized by using a sexual genotype of *P. pratensis*. The chromosome numbers of the F_1 hybrids could not easily be derived from those of the parents (contrast Almgård 1966). How parental chromosomes are distributed to the offspring is difficult to investigate in these highly polyploid species. It needs a lot of painstaking chromosome counts. This lack of knowledge does not prevent use in breeding.

I also used the diploid cross-fertilizing *P. chaixii*, clearly different from *P. pratensis*. It is rare in the Netherlands. Two types of crossing behaviour were found.

- *P. chaixii* ($2n = 14$) × *P. pratensis* ($2n = 84$) → F_1 $2n = 35$, little yellowish plants, presumably polyhaploids. Some F_2 plants looked identical as the F_1 (formed apomictically?) but other types were formed too, as normal green plants, phenotypically *P. pratensis* and mostly highly apomictic. The latter were useful for breeding. F_1 $2n = 35 \rightarrow 2n = (35, 42, 65)$ yellowish and $2n = (65–70)$ green. In the next generation, yellowish plants segregated in the same way.
- With other plants, different results were obtained: *P. chaixii* ($2n = 14$) × *P. pratensis* ($2n = 84$) → $5F_1$ plants $2n = (42, 66, 68, 70, 76)$. All F_1 plants were normal green; the hybrid $2n = 42$ more or less intermediate in phenotype; the others very close to *P. pratensis*. The F_2 progenies segregated but the F_2 plants closely resembled *P. pratensis*; only the characteristics glabrous lemmas or a somewhat lighter colour in some plants harked back to *P. chaixii*. The hybrid $2n = 42$ gave some intermediate F_2 plants. It was surprising how strongly the phenotype of *P. chaixii* was surpressed. In the *P. chaixii* hybrids, apomixis was easier recovered than in *P. longifolia* hybrids.

In crossing *Poa* species, there is no certainty that the best combination of species was chosen. Novoselova (1977) mentioned more than hundred *Poa* species growing in the Soviet Union; Hair (1968) mentioned 35 in New Zealand, most of them endemic. Therefore, the chance for better combinations is high!

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Breeding barriers between Brassica campestris L. and Raphanus sativus L.

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Index words

Intergeneric hybridization, *Brassica campestris, Raphanus sativus*, crossability, matromorphy, embryo culture.

Summary

The causes of poor crossability between *Brassica campestris* L. (♀) and *Raphanus sativus* L. (♂) have been studied. In all crosses strong incompatibility reactions at the stigma as well as disturbance of the orientation of pollen tube growth within the ovary occurred.

At three days after pollination in most pistils some pollen tubes (mostly even more than 10) had entered the ovary, but the number of ovules/ovary with a pollen tube in the micropyle was much lower. Development and growth of ovules and embryos occurred to a certain extent, but were variable.

In three types of cross in vitro culture of embryos gave rise to viable plants. Most were matromorphs; hybrids were obtained from the crosses at the tetraploid level. It is suggested, that selection in *B. campestris* for genotypes with a good crossability with *Raphanus* will be worthwhile.

Introduction

Intergeneric hybridization between *Brassica campestris* L. and *Raphanus sativus* L. could have interesting agronomic potential, both for direct use of the amphidiploid and for the transfer of desired characteristics of *Raphanus* to *B. campestris* and *B. napus*. Several authors (for review see Yarnell 1956) reported the production of hybrids between the two species, but crossability was poor in both directions.

This paper reports the results of a study on the causes of poor crossability between *B. campestris* and *R. sativus*. Of both species diploid as well as tetraploid forms were used and the former species was always the female parent.

Material and methods

The following forms of *B. campestris* were used: ssp. *chinensis* (4*x*), ssp. *perviridis* (4*x*), turnip-rape (2*x*) and turnip (2*x*) and of *R. sativus* a diploid variety of fodder radish ('Siletta') and its autotetraploid ('Palet').

All plants were grown in a greenhouse at temperatures between 15 en 20 °C. About 14 flower buds per inflorescence were emasculated. Two days after emasculation, when several of these buds had opened, all pistils were pollinated by brushing mixtures of pollen of 30 plants onto the stigmas. Three days after pollination, 2 pistils per inflorescence, of which at the time of pollination one was still at the bud stage and the other at the flower stage, were fixed and studied by ultraviolet microscopy (Johnson 1971). Between 22 and 30 days after pollination, the developed ovules and embryos were isolated and cultured in vitro by the method of Harberd (1969).

Results and discussion

In all crosses incompatibility reactions occurred on the stigma. Usually, many pollen grains had germinated, but the pollen tubes were short and strongly fluorescent through callose formation. In many stigma papillae, callose deposits were observed. In most pistils, however, some pollen tubes penetrated the stigma.

No barrier seemed to exist in the style and in nearly all pistils one or more pollen tubes entered the ovary. After pollinating *B. campestris* ssp. *chinensis* (4*x*), ssp. *perviridis* (4*x*) and *B. campestris* (2*x*) with 'Palet', 54, 89 and 70% of the ovaries contained 10 or more pollen tubes; and after pollination with 'Siletta' the proportions were 50, 78 and 52%, respectively. Bud pollination had no clear positive effect on the penetration of pollen tubes into the style.

The number of ovules per ovary ranged from 21 to 30, depending on the form of *B. campestris*. The average numbers of ovules per ovary with a pollen tube in the micropyle were low: for the pollinations with 'Palet' on *B. campestris* ssp. *chinensis* (4*x*), ssp. *perviridis* (4*x*) and *B. campestris* (2*x*), 0.6, 1.1 and 1.3, and for the pollinations with 'Siletta' 1.4, 0.8 and 1.6, respectively. Therefore, the number of ovules that might have been fertilized was much lower than would be expected from the number of pollen tubes in the ovaries.

The orientation of the pollen tubes in the ovary indicated that the ovules failed to attract the pollen tubes. Berger (1968) reported a comparable barrier for interspecific crosses in *Arabidopsis*.

Although for the six types of cross, the average proportion of ovules with a pollen tube in the micropyle ranged from 2.8 to 6.8%, only 1.3 to 3.8% of the ovules showed visible development 22–30 days after pollination and only 0.9 to 2.4% of ovules contained an embryo. In the crosses with *B. campestris* (4*x*), the isolated ovules were mostly small and milky looking and sometimes even brown, whereas in the crosses with *B. campestris* (2*x*), they were mostly well developed. For the crosses 4*x* × 4*x*, 4*x* × 2*x* and 2*x* × 2*x* the isolated embryos were between the stages globular and mature and for 2*x* × 4*x* between the stages torpedo and mature. In vitro culture of the embryos of the various types of cross yielded 5, 0, 3 and 19 plants, which represented 4, 0, 10 and 30% of the cultured embryos, respectively. Nearly all plants were matromorphs, only 3 hybrids were obtained from the crosses at the tetraploid level. The size and the appearance of the isolated ovules and embryos suggested, however, that at least a great majority of the embryos of the 4*x* × 4*x* and 4*x* × 2*x* crosses and also a part of the embryos of the 2*x* × 2*x* crosses were of hybrid origin.

A large variation was observed between *Brassica* plants for pollen tube penetration into the micropyle, for ovules that showed post-fertilization development and for embryo development. Selection for these characteristics in *B. campestris* may improve crossability with *Raphanus*.

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The use of Secale vavilovii in rye breeding

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Index words

Secale vavilovii, wild rye, tetraploid stocks, evaluation.

Summary

The use of the wild rye species, *Secale vavilovii* has been discussed.

The annual wild species *Secale vavilovii* Grossh. is distinguished by a number of interesting characteristics. Some of them are detrimental, i.e. the fragile rachis, the tiny seeds, the very weak straw and the high susceptibility to all *Puccinia* pathotypes and to powdery mildew. On the other hand, *S. vavilovii* has some very useful characters, of which its high self-fertility is most important. The genome of *S. vavilovii* is different from that of the cultivated rye (*S. cereale*) with regard to at least two structural rearrangements (Kranz 1973) as well as to the amount and distribution of heterochromatin (Bennett et al. 1977).

Two samples of *S. vavilovii* had been collected by Kuckuck (1973) in Iran. He succeeded in crossing this *S. vavilovii* material as male parent to *S. cereale* cv. 'Heines Hellkorn' and was able to select self-fertile 'cultivated' stocks after seven inbreeding generations. The material shows almost no detectable inbreeding depression. Therefore, at the Eucarpia Conference in Poland, Kuckuck (1974) concluded, that *S. cereale* × *S. vavilovii (c/v)* crosses can efficiently be used in broadening the genetic variation of rye and facilitating the differentiation and fixation of genetic stocks.

This paper reports on the progress made in the breeding of fertile tetraploid stocks of rye. From diploid *c/v*-stocks tetraploid progenies have been derived which have been backcrossed to cultivated tetraploid varieties/-stocks (Kuckuck und Peters 1977). In the progenies, types with improved seed setting have been selected. In many cases not only the percentage seed set but also the number of seeds per head is higher than in the control variety Tero. A comparison of the frequency distributions for seed set of the best family (F IV) with 'Tero' reveals that their seed set is not different after open pollination (Fig. 1). After bagging, however,

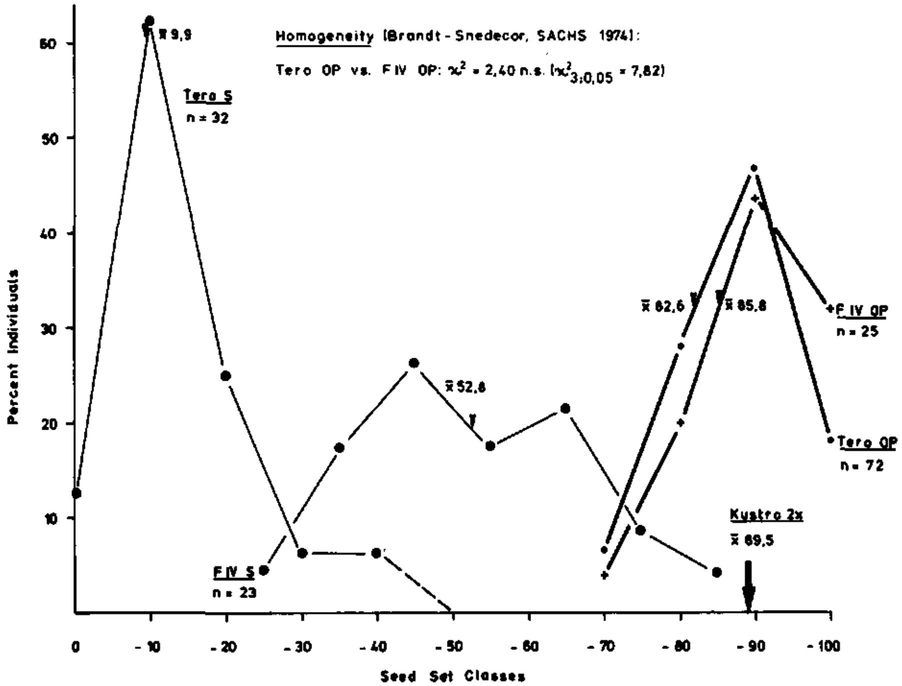

Figure 1. , Fertility of the tetraploid 'Family IV' (F IV) derived from a cross ((*S. cereale* X *S. vavilovii*) X *S. cereale*) as compared to the tetraploid cultivar 'Tero' after open pollination (OP) and selfing (S) at Grünbach, FRG, in 1977.

'Tero' is highly sterile, whereas 'F IV' is self-fertile (Fig. 1), so that in the latter the differentiation and fixation of genotypes can easily be acquired.

What are the reasons for the higher seed set of 'Family IV'? From Fig. 2 is evident that the increase of the number of kernels per spike (fertility) with increasing number of florets per spike (criterion of plant vigour) is stronger in 'F IV' than in 'Tero' as revealed by the respective regression lines, which are significantly different. As a consequence the percentage seed set of 'Tero' is negatively related to its plant vigour (no. florets), whereas the seed set of 'F IV' increases with increasing number of florets. Since the seed set of 'Family IV' depends directly on its plant vigour it is obviously less influenced by other factors like cytological anomalies and is therefore more stable than the fertility of 'Tero'. This is also supported by cytological data.

From Fig. 2 it is also evident that the number of florets per spike as well as the number of seeds per spike are generally higher in 'F IV' than in 'Tero', which means that the former has a higher yield potential per spike. The total kernel yield of some of our stocks is comparable to that of 'Tero', others are significantly poorer in yield (Table 1). This is obviously due to other yield components like tillering ability etc. which need further improvement.

Another positive result is the successful reduction of plant height as indicated

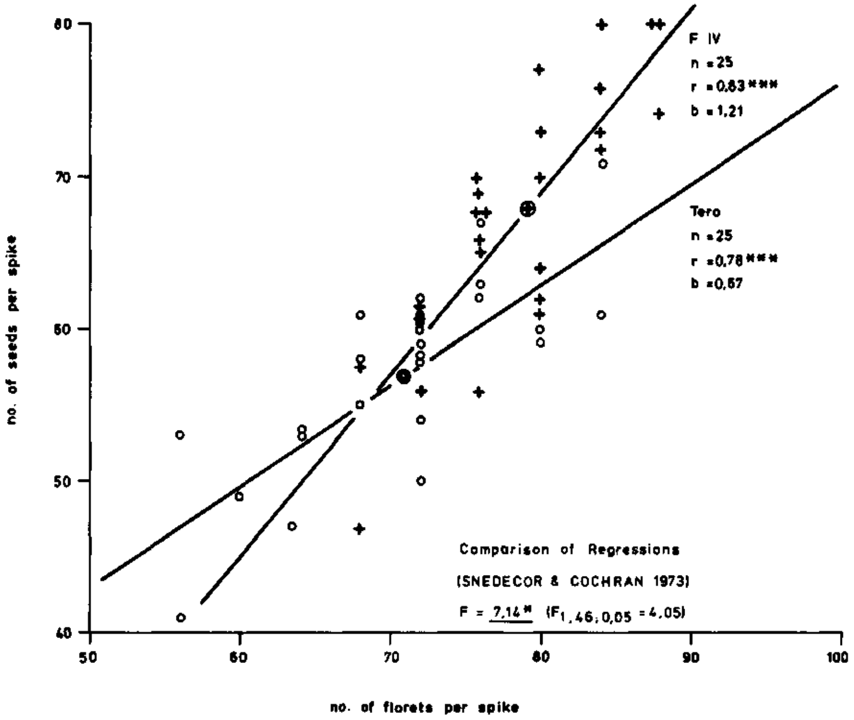

Figure 2. Relationship between the number of florets per spike (vigour) and the number of seeds per spike (fertility) of 'Family IV' (F IV) and 'Tero' after open pollination at Grünbach, FRG, in 1977.

Table 1. Plant height and yield performance of four tetraploid stocks of rye as compared to the commercial tetraploid cultivar 'Tero' in two years, 1976 and 1977, at Grünbach/Bavaria. Plant height of the diploid cv. 'Kustro' (1977) = 127,7 ± 1,2 cm.

Material	Plant height		Kernel yield rel. 'Tero'	
[(*S. cereale* x *S. vavilovii*) x *S. cereale*]	1976 $\overline{x} \pm SE^1$	1977 $\overline{x} \pm SE$	1976 (%)	1977 (%)
F IV	140.7 ± 2.3	135.2 ± 1.4	71.8**	83.9*
F VIII	139.1 ± 2.1	128.0 ± 1.7	99.0	103.5
B III	151.9 ± 1.9	125.9 ± 1.8	100.0	94.3
S I	128.3 ± 1.7	124.2 ± 1.5	67.0**	83.3*
cv. 'Tero'	161.1 ± 1.7	143.0 ± 1.6	100.0	100.0

1. $\overline{x} \pm SE$ = mean value ± standard error
*, ** significantly lower than 'Tero' at P = 0,05 and P = 0,01, respectively

in Table 1, where all of the stocks are clearly shorter than 'Tero'. Plant height (lodging resistance) is a main objective in rye breeding. We have in progress some *c/v*-material which is satisfactorily short and also fertile and vigorous. However, improving lodging resistance and kernel yield at the tetraploid as well as the diploid level needs intensive future efforts.

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Use of wild emmer (Triticum dicoccoides, AABB) in the breeding of common wheat (T. aestivum, AABBDD)

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Index words

Wild emmer, common wheat, *Triticum dicoccoides, Triticum aestivum*, protein content, stripe rust resistance.

Summary

Wild emmer may be used in wheat breeding as a source for high content of protein, leaf sheath pubescence and resistance to stripe rust. Crosses between *Triticum diccoides* and *T. tauschii* (DD) were unsuccessful. Crosses between *T. dicoccoides* and *T. aestivum* yielded hybrid seed. Differences in crossability occurred when *T. aestivum* was used as the female parent: 'Chinese Spring' gave better results than other varieties.

Only part of the hybrid seed germinated. The hybrids were pentaploid, and seed-set after pollination with common wheat was better than after selfing. Since the first backcross, repeated selfing and/or more backcrosses have given rise to fertile plants with 42 chromosomes. Preliminary experiments showed that the desired characteristics of *T. dicoccoides* were retained.

Introduction

Recently, Israeli and Russian workers (Avivi 1977; Brezhnev 1977; Gerechter-Amitai & Grama 1977; Migushova & Pokrovskaja 1976; Shestakova & Migushova 1976) reported the possible value of wild emmer (*Triticum dicoccoides*, AABB) as a source for high content of protein in the breeding of tetraploid and hexaploid wheat (*T. durum*, AABB, $2n = 28$ and *T. aestivum*, AABBDD, $2n = 42$). Earlier, Gerechter-Amitai & Stubbs (1970) and Grama & Gerechter-Amitai (1974) had shown already that several lines of wild emmer were highly resistant to stripe rust of wheat. In 1972, a study on the use of wild emmer in the breeding of common wheat was started at SVP.

Material

The material consisted of line G25 of wild emmer (originally from Israel), nine varieties of common wheat (eight of winter wheat and 'Chinese Spring'), and one accession of *T. tauschii* (DD). Line G25 has strong resistance to stripe rust (Gerechter-Amitai & Stubbs 1970) and dense pubescence on the leaf sheath. It also has a high content of protein and a thousand-grain weight nearly as high as of common wheat (Gerechter-Amitai & Grama 1977), as was confirmed in the present study.

Results

Reciprocal crosses between wild emmer and *T. tauschii* were unsuccessful. The reciprocal crosses between winter wheat and wild emmer differed. With winter wheat as female parent, only 0.1 seeds were formed per spike, and the reciprocal cross yielded 1.2 seeds per spike. In both crosses the germinability of the seed was about 50% and about half of the seedlings were viable. However, when 'Chinese Spring' was used as female parent, 1.8 seeds were formed per spike; they germinated well and gave rise to viable hybrids. 'Chinese Spring' differs from the other varieties in carrying the recessive alleles of the crossability genes Kr_1 and Kr_2, making this variety readily crossable with rye. So these genes may also affect the crossability between common wheat and wild emmer. Furthermore, there appeared to be a relation between germinability of the seed and viability of the seedlings, but there is no reason yet to relate the latter phenomena to the crossability as such.

The hybrids were pentaploid and highly sterile. After selfing, only one of the hybrids that had common wheat as female parent produced two seeds. Selfing of the hybrids of the reciprocal cross was successful on half the plants, the average seed-set being 7. In this way 61 plants were obtained. Their chromosome number ranged from 28 to 40, with a mean of about 32 and a peak (23%) at 28. A third of these plants were not viable, and after selfing of the remaining individuals only a fifth produced seed.

Pollinating the pentaploid hybrids with common wheat (mostly winter wheat) yielded 3–4 seeds per spike on nearly all plants. The chromosome number in the first backcross progeny was between 35 and 41, with a mean of nearly 38. About 15% of the plants were not viable, and after selfing about 40% were sterile. There was a general tendency for fertile plants with higher chromosome number to produce more seed. The plants that originated from hybrids with common wheat ('Chinese Spring') as the female parent had a mean chromosome number which was nearly one higher than the mean of the other plants, and the proportion of fertile plants was higher too.

Since the first backcross, the fourth generation of selfing or of a combination of further backcrossing and selfing has been reached. In general, the number of chromosomes increased by 1 per generation. The most advanced material has 42 chromosomes and reasonable fertility. Preliminary experiments indicated that lines are available, in which the thousand-grain weight equals that of common wheat, and the content of protein is considerably higher; but the yielding capacity is not yet known. Also the resistance to stripe rust and the pubescence of the leaf sheath were retained in some of the material. A good basis has been established for further breeding work.

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Biosystematic investigations in the genus Hordeum

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Index words

Hordeum, wild barley, cultivated barley, taxonomic monograph, introgression, interspecific hybridization.

Summary

To evaluate ways of incorporating genetic material from wild species of *Hordeum* into the cultivated varieties, it has been necessary to make a taxonomical study of the genus as a whole. Live material has proved rather poor, and new collections have to be established. The genus contains some 25 species occurring in most of the temperate regions. Both diploid, tetraploid, and hexaploid levels were found, even within the same species. Crosses are being made between the different species. By embryo culture, it was possible to obtain a high proportion of interspecific hybrids in very high frequencies.

Introduction

The wild relatives of cultivated plants have been a subject of increased interest. A closer study of wild relatives and ancestors is often necessary to produce a meaningful picture of how cultivated species and varieties have evolved.

There is a growing realization among plant breeders that to counteract a detrimental impoverishment of genetic resources in cultivated plants, we have to preserve primitive varieties of these species, and gene banks have been established to cope with this task. However, there are motives also to examine more distantly related wild species as possible donors of genetic material to cultivated plants. In the last decade a number of techniques have been developed or improved which raise the prospect of distant crossings. By means of embryo culture, we can now, for instance in the genus *Hordeum*, obtain hybrids between rather distantly related species, hybrids that could not have been produced by conventional methods. The chromosome-banding technique has also allowed study of differences and similarities of chromosomes in much more detail than before.

In many genera that contain important cultivated plants, taxonomic knowledge is surprisingly poor. Any biosystematic study of such a group of plants must go hand in hand with a taxonomic revision establishing the morphological characteristics, natural distribution and correct nomenclature of the taxa involved.

Taxonomic monograph

In Denmark there is a long tradition for barley research, both practical and theoretical. Since 1976, the Danish Natural Science Research Council have sponsored a project for worldwide study of the genus *Hordeum*. The aim of the project is primarily to produce cytogenetic and biosystematic data that should improve understanding of the evolutionary relationships in the genus. It soon turned out, however, that the taxonomy and nomenclature of *Hordeum* needed urgent revision. Parallel with the experimental studies, we have therefore spent much time studying herbarium material and literature with the intent of producing a worldwide taxonomic monograph.

Our first problem was to establish a sufficiently large and representative collection of living plants. We were careful to use only material of known wild origin, preferably seed collected directly in nature. Samples from gene banks were often disappointing, as they frequently lacked information on origin or were incorrectly identified. Our main sources of material were thus our own field trips, a network of contacts with collecting botanists in many countries, and seed catalogues from botanic gardens, which often have a section with spontaneous material. So far we have collected in Iran, Turkey, and various parts of the Mediterranean; in the coming winter, we expect to join a Danish expedition to southern South America and thus hope to obtain living material of several species that have so far been poorly represented. Seed from several recently collected herbarium specimens has been induced to germinate. The establishment of a satisfactory collection of living plants is a time-consuming task, and we would still welcome seed samples that colleagues may be able to send from anywhere in the world, but particularly from central Asia and the Americas.

The genus *Hordeum* contains about 25 species; most of them are perennial, although some of the most widespread species are annual. There is wide variation in reproductive patterns, from species that are obligate cross-fertilizers with incompatibility systems like *H. bulbosum* and *H. violaceum* to species which are almost completely self-fertilized like *H. murinum* and *H. vulgare*. The chromosome numbers vary from the diploid level ($2n = 14$) up to at least the hexaploid level ($2n = 42$). Infraspecific polyploid series occur in some species, e.g. *H. murinum* and *H. violaceum*.

There is far from universal agreement about generic limits. The small genera *Hordelymus, Taeniatherum* and *Psathyrostachys* are sometimes included.

Three main centres of distribution occur, one in central Asia, one in the Mediterranean area and the Near East, and one in South America. A few species are native to North America and one to South Africa. Some species have become naturalized as weeds almost all over the world, e.g. *H. murinum* and *H. jubatum*.

Interspecific hybridization

In our crossing program, we try to include all possible combinations between species, also the reciprocal ones, but we concentrate on crosses between species on the same ploidy level as well as on crosses with *H. vulgare*. Hybrids are raised by embryo culture since development of endosperm is often poor in hybrid seeds. So far more than 200 F_1 families have been obtained, representing different interspecific crosses.

Plants were obtained in more than half the attempted crosses, and we believe that this proportion can be raised by further improving the technique. Most of these plants were hybrids, although haploids occurred as well. The cross *H. vulgare* × *H. bulbosum* had long been known to produce haploid *H. vulgare* plants by elimination of the *H. bulbosum* chromosomes at an early stage of the embryoric development. We were thus interested to learn whether such chromosome elimination would occur also in other combinations.

Hybrids were obtained also in crossings between rather distantly related species, and were often easier to produce when ploidy was high, e.g. *H. vulgare* (2*x*) × *H. turkestanicum* (6*x*). *H. vulgare* functioned best when used as a male parent. Frequently the hybrids were not strictly intermediate in morphological characters, but were more like one of the parents. Apparently this was not a consequence of elimination or other chromosome irregularities; in some cases we, demonstrated, by giemsa banding, the presence of both sets of parental chromosomes.

Haploids were also obtained, but never as regularly as in the previously described *H. vulgare* × *H. bulbosum* crossing. In some cases, we undoubtedly lost the haploids, since all embryos received standard treatment and the weaker ones were not treated separately. In many cases, the embryos germinated but died after a short time; we assume that many of these were haploids. In some combinations, both hybrids and haploids were found in the progeny from a single spike. The problem will be further investigated by differential treatment of embryos.

Chromosome doubling has been carried out in some of the sterile hybrids to re-establish fertility. We will examine these plants in F_2 to see whether they reproduce normally or eliminate chromosomes.

Crosses were also attempted between *Hordeum* species and the closely related genera *Hordelymus*, *Taeniatherum* and *Psathyrostachys*. Hybrids have so far been obtained in several crosses with *Hordelymus*.

Aim of project

The aim of the project is thus:

- To produce a taxonomic monograph of the genus *Hordeum* and to stabilize the nomenclature in order to enable workers on wild barley species to communicate without misunderstandings.
- To obtain information, by means of crossing experiments and cytogenetic studies, on the evolutionary relationships within the genus. Such information may provide a basis for attempts to transfer valuable genetic characteristics (such as disease resistance or improved protein composition) from wild species to cultivated barley.

All the material that we have brought together will be maintained in such a way as to be available for other research workers on request.

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Solanum sucrense Hawkes – a clarification

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Index words

Solanum sucrense, origin, weed potato, potato breeding.

Summary

A study to elucidate the taxonomic position and evolutionary relationships of the Bolivian tetraploid weed potato, *Solanum sucrense* Hawkes was carried out in Birmingham. Two hypotheses for an allopolyploid origin were tested. It is suggested that the *S. sucrense* gene pool is mainly formed by hybrids from the Andean cultivated potato *S. tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes and the southern Bolivian wild species *S. oplocense* Hawkes. The significance of the results in relation to the future use of *S. sucrense* in breeding programmes is discussed.

Two hypotheses for an allopolyploid origin were tested: first, that *S. sucrense* was derived from crosses between the tetraploid Andean cultivated potato *Solanum tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes with the tetraploid cytotype of the Bolivian wild species *Solanum oplocense* Hawkes.
or, secondly, that *S. sucrense* was a hybrid of *S. oplocense* with the diploid Bolivian/Peruvian weed potato *Solanum sparsipilum* (Bitt.) Juz. et Buk., the diploid contributing an unreduced gamete.

The species are easily distinguishable on morphological characteristics. The characters of *S. sucrense*, more often that not, fall between those of *S. tuberosum* ssp. *andigena* and *S. oplocense*, while the characters of *S. sparsipilum* do not show affinity to any one of the other species.

Results

Essential to testing both hypotheses is the production of 'artificial' *S. sucrense* (*S. tuberosum* ssp. *andigena* × *S. oplocense* or *S. sparsipilum* × *S. oplocense*). The success of these crosses is high if *S. oplocense* is used as the male parent, but the reciprocal cross is difficult to obtain. The two 'artificial' hybrid groups closely match the variation found in *S. sucrense*. Individuals from both hybrid groups are recognizable as *S. sucrense*. However, there are certain characters such as rotate corolla, broad stigma, leaf pigmentation) that occur within *S. sucrense* but not within the *S. sparsipilum* × *S. oplocense* hybrids.

The crossability of *S. sucrense* and of the hybrids is interesting. The hybrid *S. tuberosum* ssp. *andigena* × *S. oplocense* is as self-fertile and cross-fertile as *S. sucrense*, whereas the *S. sparsipilum* × *S. oplocense* hybrid is poorly self-fertile and all attempts to cross-pollinate are abortive.

On the basis of these and other evidence, I suggest that the hybrids *S. tuberosum* ssp. *andigena* × *S. oplocense* form the basis of the *S. sucrense* genepool. Subsequent backcrosses to both parents and inter-population crosses provide the array of morphological complexes common in central and southern Bolivia.

The hybrids *S. sparsipilum* × *S. oplocense* may *contribute* to the variation of *S. sucrense* in the limited areas where the putative parents are sympatric. However, the low crossability of the hybrid labels it as an evolutionary dead end, the survival depending upon a stable niche or chance mutations of selective value in a changing environment.

Individuals of *S. sucrense* are variable and yet are still recognizable as a group. I recommend, therefore, that the specific status of *S. sucrense* be maintained, albeit as the hybridogenic taxon *Solanum* × *sucrense*.

Early interest in these wild species was concentrated on the search for resistance to the potato cyst-nematode, *Globodera* (formerly *Heterodera*) *rostochiensis*. Mai & Peterson (1952) in the United States planned to use *S. sucrense* in a breeding program for resistance to the golden nematode. Later in Europe, Rothacker et al. (1966) found resistance to pathotype A of *G. rostochiensis* in *S. tuberosum* ssp. *andigena, S. sucrense, S. oplocense* and *S. sparsipilum*. Ross (1966) found similar resistance in *Solanum camarguense* Card., now synonymous with *S. oplocense*. Further work by Ross (1969) showed accessions of *S. oplocense* to be resistant to all known German trophotypes and hybrids of *S. tuberosum* ssp. *tuberosum* × *S. oplocense* to be resistant to 80% of these German trophotypes. In the same year, Ross & Huijsman (1969) tested *S. oplocense* and found accessions with resistance to pathotype A and 11 races of Pathotype B of *G. rostochiensis*.

In two cases, good crossability with cultivars of *S. tuberosum* ssp. *tuberosum* is recorded viz. with *S. sucrense* (Mai & Peterson 1952) and with *S. oplocense* (Ross 1969).

The search for resistance to potato cyst-nematodes was expanded when Stone (1973) formally recognized a second species, *Globodera pallida*. Kort et al. (1977) clarified a complex situation by designing alphanumeric codes for cyst-nematode pathotypes to replace various European synonyms, 5 for *G. rostochiensis* and 3 for *G. pallida*. They listed known tuberous species resistant to these pathotypes, noting that only the diploid species *Solanum vernei* Bitt. et Wittm. and *Solanum multidissectum* Hawkes were resistant to *G. pallida* pathotypes.

Recently, work by Dr C.A. Huijsman (personal communication) at the Foundation for Plant Breeding, Wageningen has revealed resistance to Pa2 and Pa3 (Dutch D and Dutch/British E) races of *G. pallida* in accessions of *S. oplocense* and *S. sucrense* and one accession of *S. tuberosum* ssp. *andigena* from the Lecherias area of Potosi, Bolivia. Central and southern Bolivia should therefore be considered a valuable source area for potato cyst-nematode resistance. In view of the obviously close evolutionary relationships of the two resistant wild species with *S. tuberosum* ssp. *andigena* in this region of Bolivia the clarification of the taxonomy of this tetraploid complex offers promise for breeding against cyst-nematode.

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Wild species and primitive cultivars as ancestors of potato varieties

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Index words

Tuberous *solanum* species, utilization, potato breeding, *Solanum tuberosum*, primitive cultivars.

Summary

The first introgression of foreign genes into the ancestry of European potato cultivars of today took place during 1890–1921. It was the intention to combine valuable properties, preferably resistance to late blight of *S. demissum* via *S.* X *edinense*, with varietal characters. Besides that: the higher backcross generations were partly outstanding in vigour and yield. This experience favored in the following decades the use of primitive cultivars and wild species and led to performing expeditions in the gene-center by many European countries. – Genes of at least 7 different sources of *S. tuberosum* spp. *andigena* and 2 of *S. phureja* are present in modern cultivars. Among the wild species *S. demissum* attributed genes most frequently. Today 49% of the cultivars of the five European countries possess genes from *S. demissum*. Other wild species used in modern breeding are *S. acaule, S. spegazzinii S. stoloniferum, S. vallis-mexici* and *S. vernei*. Some cultivars are based on several different primitive cultivars and wild species together, in the extreme on *S. andigena* and 4 wild species. Neo-*tuberosum*-breeding, as the breeding of hybrids arisen out of very many *andigenas* by panmixie and natural selection under long-day conditions in consecutive cycles is an excellent method to utilize *andigena*-populations and so to augment the genetic diversity of the potato even more.

Introduction

The broadening of the genetic base of the potato went through three stages. The first is characterized by hybridization of occasional acquisitions of wild species, mainly *S. demissum*, with cultivars in the 19th Century. The hybridizations were made partly out of botanical interest but partly also with the intention of combating late blight epidemics. They left no traces in the modern assortment of potato.

A second stage of hybrization started about 1890 in several countries by a number of workers being interested mainly in the late blight resistance of *S. demissum*. Only the endeavours of five of them were fruitful in so far as their hybrids became ancestors of many cultivars of today. A. Findlay in Scotland started his work probably in 1890[1], Salaman with *S.* × *edinense* probably in 1906, Wilson with *S. demissum* in 1908, Knappe with *S. demissum* in 1909 and Broili with Chilean *S. tuberosum* and *S.* × *edinense* in 1921[2].

The researchers at first did not believe, as they used to say, that the hybrids with wild species could give rise to cultivated varieties, but those who continued with backcrossing soon became aware that seedling lines appeared among the backcross progenies coming very close to cultivated varieties in yield and other properties. Resistance to late blight and to 'degeneration', later known as virus diseases, was also observed among these offspring.

The third stage began in 1926 when under the direction of Vavilov, Bukasov and his colleagues started expeditions to the gene-centre for systematic collection of wild species and primitive cultivars. From that time on the genetic base of the potato has changed slowly but in its effect dramatically. The Russian expeditions were followed by those of Germany, the United Kingdom and the United States. Today most species of the section Tuberozium have been brought to Europe where they are maintained in germ-plasm banks.

Evaluation and screening started also systematically preferently in order to find resistance to late blight, potato leaf roll virus, potato virus X and Y and Colorado beetle (for literature on these matters see Hawkes this volume, and Ross 1958a, b). By 1952, it was recognized that the introduction of a foreign gene-pool (*S. demissum*) had increased yield (Toxopeus 1952). This, as would be expected, appeared not in the F_1 but in later backcross generations. This experience, confirmed by Black and K.O. Müller, spread among potato breeders, changed their attitude towards the utilization of 'wild material' and caused them to breed intensively with wild breeding parents.

The material made available to breeders from Institutes with notes on their specific properties was accompanied by special instructions on mass selection methods among the seedlings. The susceptibles were to be discarded and only the resistant progeny preserved. After some years, that recommendation was not implemented. The favorable influence of the foreign gene-pool, for instance on vigour and yield, became apparent. The susceptibles too turned out to be valuable breeding material. So breeders preserved good susceptibles and ran them through the normal selection work of successive years, thus giving them too a chance to become a cultivar. Today nearly half of the cultivars with wild species in their ancestry lack specific genes or gene combinations originally present in the wild ancestor and formerly believed to be the only important ones.

Utilization of wild species and primitive cultivars in modern potato breeding

Table 1 shows the wild species and primitive cultivars utilized in breeding of modern cultivars of five European countries. Different accessions of *S. demissum*

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[1] We do not know for sure which was the 'wild potato' A. Findlay used in the parentage of Majestic. Because of the relatively widespread use of *S.* × *edinense* in Scotland in those years, we assume that Findlay used the same.

[2] The information that *S. commersonii, S. fendleri* and *S. maglia* were used in breeding of varieties is considered doubtful and omitted here.

Table 1. Wild species and primitive cultivars in the ancestry of modern cultivars (Hogen Esch & Zingstra 1972; Holden 1977; Möller 1962; Netherlands 1978; Ross 1958a, b, 1978, my own enquiries; Siebeneick 1948; Soviet Union 1977; Stegemann & Loeschcke 1976)

Country code	1	Date of variety list	2	Cultivars with additional introductions in their parentage										
				adg	phu	dem	chil+ adg+ dem	adg+ dem	adg+ dem+ phu	adg+ dem+ acl	adg+ dem+ vrn	adg+ dem+ sto	adg+ dem+ acl+ sto	adg+ dem+ acl+ sto+ spe
DE	118	1978	16	19	1	18	–	43	–	5	4[3]	4[4]	4[5]	5
DD	21	1976	1	–	–	–	9[6]	11	–	–	–	–	–	–
NL	81	1978	41	6	1	2	–	23	–	–	7	1[7]	–	–
SU	71	1977	35	6	–	18	2	9	–	–	–	1[8]	–	–
GB	56	1976	27	–	1	4	2	13	10[9]	–	–	–	–	–

adg = *S. tuberosum* ssp. *andigena*; acl = *S. acaule*; chil = Chilean *S. tuberosum* ssp *tuberosum*; spe = *S. spegazzinii*; sto = *S. stoloniferum*; vrn = *S. vernei*

1. total number of cultivars of known origin; 2. number of cultivars based on early introductions of *S. tuberosum* ssp. *andigena* and ssp. *tuberosum* only; 3. one cultivar with vrn only; 4. one cultivar with spe instead of sto; 5. one cultivar with chil in addition; 6. one cultivar with chil only; 7. cultivar with sto only; 8. cultivar with *S.* × *vallis-mexici* only; 9. two cultivars with phu and dem only.

and *S. andigena* are represented most. It follows Chilean *S. tuberosum, S. phureja, S. stoloniferum, S. vernei, S. spegazzinii* and *S. acaule*. Many cultivars possess genes form more than one foreign gene-pool. This was arranged in order to combine several major gene inherited properties in one cultivar. Evidently this composition could be achieved without disadvantage and was often even favorable. At the extreme, genes of four wild species are combined in the cultivars Cordia, Esta, Franzi Heidrun and Wega, partly with extreme resistance to potato virus Y, resistance to the pathotypes Ro 1–5 of *Globodera rostochiensis* and over average resistance to potato leaf roll virus.

– Chilean *S. tuberosum* ssp. *tuberosum*. Two introductions are known. Villareola, an acquisition from the Chilote Indians of the Island of Chiloe, immune to potato virus X, found entrance into United States cultivars and thence through the line 41956 into Scottish cultivars. Another primitive cultivar of the Chilote Indians was utilized by Broili and K.O. Müller as a parent and gave rise to the hybrid 9089, which is the ancestor of many East German and Soviet cultivars because of its resistance to potato virus Y and to races of *Synchytrium endobioticum* (Frandsen 1958).

– *S. tuberosum* ssp. *andigena*. Probably the first introductions were different accessions of the hybrid species *S.* × *edinense*, a hybrid between *S. andigena* and *S. demissum* occuring in Mexican fields. The fate of this material is described under *S. demissum*. Several *andigena* lines went into the pedigree of many Soviet cultivars like Imandra. *Andigena* lines like 44.685/1 from Baur's and Schick's expeditions occur as ancestors of many Continental cultivars. They occur nearly exclusively in combination with *S. demissum* (see below) as breeding lines of the work of Max-Planck-Institut, for instance 44.335/130 a.o.

Very beneficial in general and widely utilized was the introduction of the *andigena*-lines CPC 1673 and 1960. They carry genes for resistance to the pathotypes Ro1 and Ro4 of *Globodera rostochiensis* (H_1, Toxopeus & Huijsman 1953) and the first a gene for immunity potato virus X (Rxadg, Wiersema 1961). Other cultivars, based onto the line P.I. 258907 hypersensitive to potato virus S (gene Ns, Baerecke 1967), are being developed in West-Germany and other ones, based on an *andigena* line with monomer inherited extreme resistance to potato virus Y (Munoz et al. 1975) in the International Potato Center, Lima, and in the United States.

– *S. phureja*. Two accessions appear in the ancestry of Scottish cultivars and through the cv. Dr. McIntosh entered one West-German and one Dutch cultivar. Other introductions of the International Potato Center proved to be resistant to *Pseudomonas solanacearum* and went into the breeding work of Rowe & Sequeira (1972).

– *S. demissum*. This species together with *S. andigena* as hybrid species *S.* × *edinense* is the oldest donor of genetic material after the early introductions up to 1850. The species occurs in the pedigree of cultivars of nearly every country.

The main value of this species was its resistance to late blight. Major genes as well as minor genes determine the resistance. After the outbreak of various races of *Phytophthora infestans* in 1936, the major gene resistance became nearly worthless, but it retained the race-independent field resistance based on minor genes (Frandsen 1958). Another important characteristic is resistance to potato leaf roll virus showing up in later backcross generations (Müller 1951). These properties would perhaps be insufficient to explain the popularity of these hybrids as breeding parents by the potato breeders. Heterosis in vigour and yield may have played its part too.

Findlay's hybrids through Majestic, Doon Star and Herald as parents found entrance in the range of cultivars in Great Britain and the Netherlands. Wilson's crosses occur in the pedigree of Scottish cultivars and Knappe's in two Russian cultivars. Black's hybrids are the base of many cultivars of Scotland and many other countries. Salaman's hybrids have gone into the breeding work of Howard at the Plant Breeding Institute at Cambridge.

Those *edinense* introductions sent from the United States Department of Agriculture to the Biologische Bundesanstalt, Berlin, achieved the widest distribution. Broili and after him K.O. Müller bred this 'Ef'-material, producing the 'W-races'. Famous as breeding parents are Aquila, Apta, Capella, Maritta and Schwalbe. They were utilized abundantly as parents in both Germanies, Scotland, Soviet Union, and, via Kennebec, in the United States and Canada (Akeley et al. 1948).

Breeding work with several provenances of *S. demissum* at Max-Planck-Institut resulted in many cultivars, such as Cosima and Rheinhort and many breeding lines, of which the line 19268 gave rise to 10 Dutch cultivars, among them Jaerla and Marijke.

– *S. vernei* from several expeditions went into the pedigree of 11 modern cultivars of the Netherlands and West-Germany because of its resistance to some pathotypes of *Globodera pallida* (Ross 1967, 1972, Huijsman 1959, Kort et al. 1972). Here too favorable properties like high starch content turned up unexpectedly during breeding with this species.

– *S. stoloniferum* accessions were sent from Leningrad to the Max-Planck-Institut where an extreme resistance to potato viruses A and Y was found (gene Ry, Ross 1958 c,d, 1961) as well as field resistance to late blight (Rudorf & Schaper 1951) in some of them. In West-Germany it gave rise to 10 cultivers, 6 with extreme resistance to potato virus Y (Cordia, Esta, Fanal, Franzi, Heidrun, Wega). Another accession of the Commonwealth Potato Collection by the work of Dodds gave rise to backcross hybrids, out of which the Dutch cultivar Corine originated. The Scottish breeding work of Cockerham (1970) and Davidson (1978) is near to producing cultivars too.

– *S. spegazzinii* EBS 510, collected by H. Brücher, was introduced into breeding because of its resistance to the pathotypes Ro 1, 2, 3, 4, 5 of *Globodera rostochiensis* (gene Fb, Ross 1962). The backcross hybrids lateron revealed additional advantages like resistance to scab and *Fusarium coeruleum*.

– *S. acaule*, collected by the expeditions of Baur and Schick, was hybridized when screening detected its extreme resistance to potato virus X, controlled by the dominant gene Rxacl (Ross 1954; Cockerham 1970). The backcross hybrids later showed a considerable resistance to potato leaf roll virus.

Altogether there have been yet more cultivars originating from the 5 species mentioned than enumerated in Table 1, but with continual changes in varietal lists they have been replaced by better ones.

Discussion and conclusions

Experience with introduction of additional gene-pools into *S. tuberosum* suggests the following conclusions. It was possible to transfer any property so far tested of a wild species into a cultivar. Beyond the planned transfer of distinctive major genes, there is a heterotic effect on yield and a more conspicuous expression of many characters. This general benefit, explicable by modern theories of heterosis, must be attributed to the increase in heterozygosity, which allows more diverse interactions within and between loci. The benefit appears after 5–8 backcrosses, is not restricted to *S. demissum* and is even true for species remotely related to the series *Tuberosa* as those of the series *Acaulia* and *Longipedicellata*. Besides the 5 species in Table 1, I found the same with *S. megistacrolobum* *S. oplocense* and *S. sparsipilum* (Ross unpubl.). There is no unfavorable trait known that would prohibit the utilization of a wild species hithertoo envisaged for introduction into potato breeding. Even the very high glykoalkaloid content often found in the tubers of wild species could be reduced to normal concentrations after some backcrosses (Ross et al. 1978).

The ease with which genes of different species can be combined in a fertile cultivar not distinguishable from pure *tuberosum* by its habit indicates a rather free exchange of chromosomal material at least among *S. tuberosum* and the 5 or 8 wild species. There is no unthriftyness in the backcross offspring. Cytological investigations do not suggest a close homology of the diploid and the tetraploid genomes of wild species and cultivars. Bivalents are formed in the tetraploid wild species *S. acaule, S. demissum* and *S. stoloniferum* and are often observed in their hybrids (Howard 1960, 1970; Ramanna 1977; Swaminathan & Howard 1953). These bivalents could, however, be at least partly allosyndetically paired. My data (1961) observing the segregation of the gene Ry in *stoloniferum–tuberosum* hybrids favor allosyndesis.

The varietal lists of the last few years reflect the situation in actual European breeding 12–15 years before. It takes that long to breed the valuable character of a wild species into a commercial cultivar. This work, except the last steps, was taken over in the Federal Republic of Germany by the Biologische Bundesanstalt (till about 1934), the Bayerische Landessaatzuchtanstalt, now Bayerische Landesanstalt für Bodenkultur und Pflanzenbau and the Max-Planck-Institut für Züchtungsforschung (since 1928). These institutions have concentrated in potato breeding on adding valuable characters from wild species and primitive cultivars to the 'cultivated' genetic background. The best offspring, usually one or two backcross generations removed from cultivar registration were handed over to potato breeders and Institutes in Germany as well as to those of other countries very freely. The present situation in the Federal Republic of Germany is illustrated by the fact that at least 90% of the more than one million seedlings produced in a year have wild species or primitive cultivars in their ancestry. The situation may be similar in other European countries.

Nevertheless there is unanimity that the potato needs more improvement. The genetic treasure of more than 60 wild species crossable easily with *S. tuberosum* and more than 600 different primitive cultivars should be evaluated and utilized. This must be achieved in a right and economic way. Nobody wants to introduce wild species or primitive cultivars just to broaden the genetic base. Breeders and Institutions will hardly take up such a costly and time consuming work (5–8 generations of backcross and selection work during this period and thereafter) unless there is a specially wanted property at the end. Such transfer of a particular property is expected to be accompanied by the transfer of other genetic material offering further scope for heterozygosity and allelic as well as non-allelic interactions.

Screening and maintenance of single primitive varieties carrying important genes in order to cross them with advanced cultivars or breeding lines was the usual way to utilize this material in the past and certainly will be one way in the future. But the primitive cultivars deserve special consideration. In contrast to the wild species, the primitive cultivars have most of the 'cultivated' genes in common with advanced cultivars. So, many generations of backcrossing are superfluous. The bulk of old South American cultivars can be considered as one great gene-pool. The Neo-*tuberosum* work of Glendinning (see his paper for other literature; Plaisted 1972; Plaisted et al. 1975) has shown that breeding parents outstanding in vigour and yield can result from selection by natural environmental factors only after a few generations. This process can be enhanced by selective pollination and selection among offspring.

Tapping of the genetic resource hidden in South American cultivars has only just commenced. Undoubtedly broadening of the genetic base by wild species and primitive cultivars will lead to further improvement, which the potato, still one of the world leading crops, needs urgently.

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Transfer of quantitative characters from wild and primitive forms

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Transfer of quantitative characters from wild and primitive forms

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Summary

Distant hybridization is one of the key factors in plant evolution. The existence of numerous allopolyploid species within cultivated plants is sufficient evidence of its creative role. Introgressive hybridization though more difficult to detect undoubtedly was of similar importance for crop improvement throughout the centuries. But alloploidly is an evolutionary dead end and plant breeding continuously widens the distance between primitive and cultivated forms. There is no lack of techniques today for interspecific transfer of valuable genetic entities either on the gene, the chromosome, or the genome level. We may look forward in near future to have even more effective methods of experimental management of gene recombination, chromosome translocation, and genome composition. In recent plant breeding programs qualitative characters of wild species, e.g., disease resistance or content of protein and specific amino acids, have been efficiently used already now. But are the prospects for interspecific transfer of quantitative characters equally good, such as for grain yield or any of its fundamental components, e.g., harvest index, photosynthetic rate, or substrate translocation? The larger the distance of productivity to bridge the less is such transfer practical. The desired traits must be fitted into a highly balanced system of metabolic and genetic interactions and selection is difficult because of this complexity. There is easy support of directed introgression for qualitative characters by backcrossing. But if the polygenes to be transferred are scattered over several chromosomes or segments, it will be impossible to separate them from the association of undesired 'wild' gene components without break-down of the complex quantitative characteristic. Fortunately, there are instances of evidence for spatial assemblage of genes for related functions. In these cases, 'indirect' selection for part components will greatly facilitate transfer methods and there is any reason to expect ready progress in this direction by straight exploitation of isozyme patterns or other biochemical markers. The question, however, remains whether the top productive varieties of the main crops of today can still take advantage of the quantitative diversity present in the natural resources or wether they already passed the 'point of no return' at which further addition of foreign variation does nothing but spoil the achieved high level of performance.

Genetic characters are defined to be quantitative or qualitative with reference to the number and expressivity of genes that control them.

Qualitative characters, as they have been dealt with in Section 4 of this Conference, are those dependent on a single locus or a few genes only. Though multiple alleles or genetic polymorphisms became increasingly evident, as investigations became more sophisticated, genetic variation remained relatively small because of the few loci involved in qualitative characters. In addition, typically mono- or oligogenic phenotypes are clearly expressed in Mendelian populations. Thus selection by natural conditions or by normal plant breeding is effective for these characters and severely diminishes undesirable alleles. The classical illustration of characters that can be alarmingly exposed to gene erosion is disease resistance. Here, primary selection is even for virulence within the pathogenic fungus, which can be especially severe because of high multiplication rates. In many such instances, the need to broaden the genetic base of crops has been well demonstrated.

Quantitative traits, on the other hand, are controlled by the interaction of a large number of genes, called polygenes, which condition a large variability of crop plants in these characters. Each gene, however, exerts only minor phenotypic effects and consequently natural or directed selection is weak in result. Even with severe selection progress is sometimes difficult to measure and biometric methods are required for analysis in all cases. Such polygenes are believed to control characters with continuous variation, such as grain yield of cereals.

Polygenes are presumably often closely linked. Such chromosomal clusters have been named 'linkats' by Demarly, and are described in more detail in his paper. Their evolutionary origin is easily explicable by gene duplication via unequal crossing-over or other mutational means sometimes followed by mutation and selection among the unemployed copies. All the same, these neighboring loci kept their functional relationship; they usually still interact in controlling the same quantitative character or similar biochemical pathways. Other hypotheses refer to their weak phenotypic expressivity and try to locate polygene complexes within heterochromatic blocks or Giemsa bands. All these assumptions are far from being verified. The question of the genetic structure of quantitative characters is, however, crucial for their management in breeding programs.

The answer to the question of how to transfer such quantitative characters from wild and primitive forms to highbred cultivars depends on the object in view. In this paper, I consider especially, for instance, European winter wheats or barleys with a maximum yield capacity of 8 to 10 t/ha (0.8-1 kg . m^{-2}). The complete genetic make-up of the present days cultivars has originated from wild and primitive ancestors. Modern plant breeding in Europe started 100 years ago from a wide range of primitive forms and land races from the various corners of Russia, Germany, France, or England. From that base, the development of improved cultivars was mainly by continuous combination of the best available and rejection of the unwanted types. This led people to state that the genetic base for further selection got increasingly narrow. This is undoubtedly true. There is no theoretical objection, except for the conclusion that this procedure must be immediately dangerous. On the contrary, only by gathering the more productive genes from the pool and discarding the negative alleles was the present productivity reached. The art of plant breeding is to maximize the genetic exploitation and its efficiency is determined (1) by the available means to reveal genetic divergence and (2) by the realized genetic selection intensities. Admittedly, progress is not automatic and selection within pure lines in efficient. But with adequate methods, breeders can still be increasingly successful.

The *mechanism of continuous improvement of quantitative characters* can best be demonstrated by the famous selection trial on maize that John Hopkins started in 1896 at the Illinois Experiment Station. The continuous stepless increase in oil or protein content of grain indicates that improvement is not by transfer of complete polygenic complexes but rather by permanent recombination and reshuffling of single weaker subunits against the more productive ones. Intensifying introgression requires additional new subunits that fit better into the given complex loci. But we lack realistic estimates of the total number and variability of the polygenes controlling yield or similar quantitative traits of agronomic performance. Even though Glendinning in his paper pointed out that European potato breeding was originally based on not more than about 5 different introductions, this does not necessarily mean disastrous genetic poverty for quantitative genes. For instance, from only 10 alleles (5 genes) for a given trait, many millions of genotypes can be derived. Genetic progress is still linear within most of the present crops and with available breeding methods. There is still enormous variation that breeding can exploit by intelligent recombination and selection or even by selfing as in an open-pollinated population of rye. There is little reason at present to use wild and primitive forms to improve quantitative characters in cereal crops!

If, however, for any reason transfer of genetic entities from primitive into high-bred cultivars seems desirable, there is no lack of techniques today for such transfer, even between species. Many of them are listed in the table of contents of the last Eucarpia Congress in Madrid (Sanchez-Monge & Garcia-Olmedo 1977). They were broadly discussed during this meeting:

- Interspecific hybridization
  - Selection in segregant populations
  - Backcrossing
  - Genetic bridges

- Artificial allopolyploidy
  - Direct utilization
    - Amphidiploids
    - Partial amphidiploids
    - Somatic fusion of protoplasts
  - Indirect utilization
    - Genetic bridges
    - Genome construction
    - Genome extraction

- Chromosome manipulation
  - Additions and substitutions
  - Translocation lines
  - Recombination systems

- Molecular manipulation
  - Protoplasts: DNA uptake
  - Plasmids: DNA recombinant molecules

- Cytoplasmic manipulation
  - Male sterility

But little use has been made of these techniques in practical breeding for improving yield or adaptation of modern cultivars. Evidently, there has been no urgency as the conventional approach is still effective. But it is also true that transfer of polygenes is much more difficult than that, for instance, of single resistance genes because of the problems of selecting for quantitative traits. This is even so if we disregard cytological complications that often create sterility or upset the pattern of recombination in remote crosses. Even within the limits of complete fertility and genetic exchange, there are severe restrictions of quantitative gene transfer.

Let me now confine myself to these intraspecific fertile crosses. An essential for continuous progress in breeding is the choice of appropriate parents for promising cross combinations. Effective parents must have an optimum genetic distance to ensure further success of selection. It may well be that progress by crossing within modern cultivars will ultimately or even soon slow down if nothing new is added to the combinations. But the correct way of overcoming this limitation is not crossing with primitive forms. Too distant crosses lead to segregant generations with unbalanced poor phenotypes, even without chromosomal instability. The problem is to find the maximum useful genetic variance and there is usually little of that in wild and primitive forms. This has been demonstrated by Escuro et al. (1963) in oat crosses (Table 1). For all the investigated quantities, genetic variance was increased in F_2 where unrelated parents were chosen. This should give better selection gain in F_3 as could be calculated. But the values for the selected F_3 plants, which are the sum of the F_3 means + the gain by selection, were not much different or even less in such F_3 progenies of crosses between unrelated parents because of their lower population means.

The same situation may change somewhat with breeding methods. Hybrid breeding additionally utilizes the immediate heterotic effects resulting from crosses. These may be more dependent on genetic diversity and the optimum genetic distance may well be larger in hybrid cultivars than in self-fertilized cultivars. But there is an optimum distance too for high combining ability of inbred lines and wild types are in general far beyond.

Several techniques may surmount the difficulty of low population means after distant crosses:

– The breeder may increase the size of the treated progenies, hoping to intensify selection by the higher number of the available segregants. This procedure, of

Table 1. Crosses between differently related oat cultivars. (Escuro et al. 1963; after Schnell 1974). I = related parents; II = unrelated parents. For each pair the higher value is italicized.

Quantity		Mean from 3 crosses				Genetic variance in $\overline{F}_2$	Progress from F_2 selection $= G_s$	Selected $F_3 = F_3 + G_s$
		$\overline{P}$	$\overline{F}_1$	$\overline{F}_2$	$\overline{F}_3$			
Panicles per plant	I	*9.7*	*10.1*	*9.5*	*9.3*	*1.1*	*0.1*	*9.4*
	II	9.1	9.6	9.1	8.6	*1.9*	*0.5*	9.1
Mass (g) of 50 grains	I	*1.45*	*1.54*	*1.46*	1.45	0.008	0.04	1.49
	II	1.45	1.52	*1.46*	1.48	*0.016*	*0.09*	*1.57*
Grain yield per plant (g)	I	*12.8*	17.3	12.8	*12.0*	2.9	-0.5	11.5
	II	12.6	*18.0*	13.1	10.4	*3.0*	*1.4*	*11.8*

course, increases nursery area, labor and costs, which is evidently one reason why plant breeders avoid crosses with exotics as much as possible.

– Backcrosses may be made to the adapted parent. Table 2 shows data of this type from soya crosses between adapted and exotic cultivars. Backcrossing definitely increased the chance of obtaining useful lines after selection. The problem, however, was the large probability of losing the minor effects of the desired polygenic units from the exotic parent. The success of the backcross procedure depends on effective selection for characters of the non-recurrent parent. The method is suited for transfer of phenotypically expressive characters, like resistance, for which large batches can be screened. To recover more of the yield polygenes from the exotic parents, Schnell suggested not to backcross in the manner of a three-way cross but to re-use for backcrossing the same adapted cultivar A_1. This inbreeds A characters, whereas A × E combinations gain by heterosis. By such means, the expression of polygenes from the exotic form is intensified and selection favors exotic additions.

– Another way to keep quantitative complex loci within the selected group is to use marker genes. Frey & Browning (1971) found that by introducing a resistance gene from *Avena sterilis* into oats even under conditions free from infection, yield performance of the isoline was definitely increased. Similar experience was mentioned by Hänsel (1964) who transferred mildew resistance into barley with parents from Lyallpur. Apparently, under favourable conditions useful polygenes can sometimes be recovered from wild types, even beyond the optimum distance of genetic relationship.

There has, however, been little research on directed marking of polygenic complexes for plant breeding. Animal breeders have been much more successful in determining biochemical characters, especially isozymes, and analysing their linkage to performance, for instance to protein or fat content of milk, growth rate and body weight (Geldermann 1976). The necessary chemical methods are easier in

Table 2. Crosses between differently related soya cultivars. (Thorne & Fehr 1970; after Schnell 1974). E = exotic cultivars; A = adapted cultivars. For each pair the higher value is italicized.

Quantity	Parents		Lines in F_6			Lines among 10% best
	type	mean	cross	mean	genotyp. variance	
Protein content (%)	E	46.6	(A_1xE)	43.9	*1.66*	24
	A_1	41.3	$(A_1xE)xA_2$	*44.3*	1.62	*36*
	A_2	44.5				
Oil content (%)	E	18.6	(A_1xE)	20.2	0.49	17.5
	A_1	21.7	$(A_1xE)xA_2$	*20.4*	*0.56*	*42.5*
	A_2	20.6				
Yield (g/m^2)	E	530	(A_1xE)	607	1.85	13
	A_1	667	$(A_1xE)xA_2$	*635*	*3.40*	*47*
	A_2	679				

animals than in plants; blood sera or antibodies against proteins are more readily available. But there is no reason not to do the same in plants. At the best, biochemical markers can be part of the polygene complex of interest. Many biochemical data are available, e.g. from wheat monosomics indicating that certain chromosome segments carry multigene complexes for particular quantitative traits. These should be traced not only in wild forms but also in those with the optimum genetic distance from modern high-yielding cultivars.

According to adaptation or performance, the breeder is then interested to transfer such complete set of polygenes or to use only part of it for substitutive improvement. Complete transfer will be requested if the donor type offers the character at an immediately useful level. If so, break-up of the composite by recombination should be avoided. For instance, crossing of high-yielding rapeseed cultivars newly resynthesized from the diploid ancestors with the commonly cultivated varieties gave much better offspring than crosses within the group of the adapted cultivars. Such complete transfer of polygenic segments may also explain the occurrence of superior progenitors in other crop species. Cryptic structural differentiation favors this development of balanced polygene segments.

On the other hand, utilization of valuable genes from more primitive forms though still within the useful distance is generally greatly impeded by too little crossover to uncouple the wanted units from any undesired ones. Insertion of intercross generations (Hänsel 1964) or diallele selective matings by the method of Jensen (1970) may help to loosen the linkage. Sophisticated genic control of meiotic processes in wheat could also facilitate introgression from wild into cultivated species.

Summarizing, four debating points on the subject may be formulated:

– The continuous increase of genetic performance in plant breeding programs directed to the improvement of quantitative traits is the best proof for an enormous genetic diversity still existing in crop plants and available for exploitation by traditional plant breeding procedures. Domestication is known to create explosions of phenotypic variability in animals and plants.

– Transfer of quantitative genes will rarely be reasonable beyond the limits of complete fertility. Problems of sterility barriers or low chromosomal pairing abundant in the relevant literature are insignificant in this context.

– Even within this range of fertile hybridization further progress needs an optimum genetic distance between parental types for crosses, because of the needs of a good functional balance. Wild and primitive forms are usually too remote and are therefore generally useless for the improvement of quantitative traits such as high yield in wheats.

– The optimum genetic distance is different for breeding of selfing or hybrid cultivars. Improvement of quantitative traits within this optimum follows different pathways: transfer of complete polygene segments may be useful if these express high productivity, for instance if they belong to rather closely related types. The lower the immediate value, the more the need for efficient recombination to break up linkages between the desired and undesired polygenes. Research on ways for a better genetic control of recombination is much needed.

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The concept of linkat

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Index words

Linkat, gene cluster, coadaptation of genes, semi-stable functional units, evolution, domestication.

Summary

The most recent analyses suggest that genetic structures in higher plants may be characterized as follows:

– In the course of evolution ancestor genes gave rise to numerous duplicates. These duplicates remained tightly linked or in some cases moved to other loci. They may too present allelic series at the same locus. They evolve independently and show more or less important differences between them.

– Dominance interactions produced new phenotypes and epistatic interactions determined hierarchical regulations. Therefore the genome of eucaryotes contains a high rate of more or less silent duplicates.

– After transcription of gene units themselves, during the maturation of nuclear RNA, excisions would take place followed by specific splicing. It would appear expressed sequences or exons and silent sequences or introns.

If we add to these characteristics that:

– Crossing over is not a randomised event but is under genic and epigenic control. Therefore its intensity in a chromosomal sector can be determined by natural selection. Due to numerous rearrangements on the chromosomes, natural selection retained adapted clusters of genes.

– Natural or induced mutations are under genotypic control too. Analyses in *E. coli* and Bacteriophage suggest that in eucaryotes there must exist mutator and antimutator genes.

The linkat is presented as:

1. A set of loci which aggregated in a same chromosomal sector during species differentiation. These clusters show strong epistasy and generally represent coadapted functions.

2. Each of the loci are constituted by a series duplicated transcriptional units. Their expression has some flexibility caused either by hierarchical repression or derepression between slightly differentiated duplicates or by rearrangements of introns to exons after DNA transcription, which breaks the dogma 'one gene one polypeptide chain'.

3. On these chromosomal segments the allelic arrangements which possess the highest adaptive value have been stabilized by genic and epigenic factors lowering the rate of recombination between them. Therefore they are inherited as a semistable block.

4. In some case it could be postulated that these arrangements contain inside them antimutator factors which give a longer perennity to the clusters.

Therefore linkats appear to be semi-stable functional units, the expression of which having some flexibility following environmental correlations and genetic background. This concept is included in a genetic system which minimizes genetic load. Its consequences for plant domestication and breeding will be developed (inbreeding, introgression, recurrent selection, source-population).

Introduction

During the process of selection, which led from primitive forms to domesticated cultigens, not only were many genes lost but also many genetic structures were modified.

It is the intention of this analysis of linkat to discuss the impact of domestication on the structural organization of genes.

Structural organization in natural equilibrium

Effects of coding genes

An allele situated in a locus of chromosome may act on the expressed phenotype in various ways:

– By its own value, which is the mean effect in morphogenesis or metabolism of its coded protein. The additive value of the gene is generally derived from this effect.

– By the interaction of this allele with its homologous allele. This interaction known as dominance can be partial or total dominance or overdominance ($ab > aa > bb$) which gives superiority to heterozygosity per se.

– By numerous interactions of the allele with other related loci. These effects can act along the one chromosome: cis-epistasis, or between loci of other chromosomes: trans-epistasis (either they are homologous or not).

Evolution did not select genes

During evolution the frequency of a genetic code increased not by itself but by the multiplication rate of the plants possessing it. The adaptive value of an allele can be defined only by taking account of the whole phenotypic effect of this allele on the plant. Therefore in some cases, it may be the only interactions which have been selected.

Now we have to analyse how polymorphism appeared in the various effects of a gene.

– Diversification of the proper value of a gene. A high proportion of structural genes show allelic polymorphism (in many cases alleles differ only by one amino acid). In other words, the evolution of additive value of genes operated through mutations. No canalizing effect of these mutations has been detected. Yet it seems that the older variants were progressively eliminated as more sophisticated ones appeared. In a chromosomal sector with five loci *a, b, c, d* and *e*, we may imagine the following allelic polyphormism:

$$\begin{array}{ccccc} a_1 & b_1 & c_1 & d_1 & e_1 \\ a_2 & b_2 & c_2 & d_2 & e_2 \\ : & : & : & : & : \\ : & : & : & : & : \\ a_l & b_m & c_n & d_p & e_q \end{array}$$

Every plant has its own arrangement for instance: $a_2\ b_3\ c_1\ d_4\ e_2$.

– Diversification of dominance interactions. When there are n different allelic forms in a locus, $\frac{1}{2}\, n\, (n + 1)$ dominance effects can be observed (homozygotic effects being included).

It might be admitted that mutations in the code arise at random. It might be admitted too that, for small populations, fixation of a new mutant is mainly at random. But the future of a mutation is strongly conditioned by the change in interactions between the existing allele and the new mutant. If strong overdominance appears, the plants that possess the new allele will have a larger progeny. As a consequence, all mechanisms will be improved that reinforce this privileged heterosis (such as floral morphology or incompatibility alleles). If these steps are successfull the species may turn to autopolyploidy; this will considerably increase the number of dominance interactions.

– Diversification of epistasy. Duplication of genes is another means to ensure flexibility between related codes in one cell. Irregular crossing over is generally at the origin of duplicates. It has been shown that duplicates, similar at the beginning, will diverge during evolution (for instance genes for ribosomal ribonucleic acid, genes coding for histones). The process of gene duplication, as first step of gene adaptation, seems to provide material for establishing new enzymic chains. These may often include structurally related proteins with similar or complementary or different functions (Horowitz 1965, Zuckerkandl 1975). Gene duplication is probably much easier to achieve than the improvement of new functions. Therefore *structural adaptation could arise from duplication followed by mutations.* Among these duplicates, a hierarchical control mechanism is selected. Some master switches of higher rank will control the activity of the others. Two types of regulatory mutations may be considered: first the mutant is the regulatory gene itself affecting the rate of production of the enzymic pool of regulated sequences; second, mutational event changes the order of genes on chromosomes bringing new relationships between genes and migration to other loci. In these new situations, most of the duplicates had to become neutral or unuseful codes alongside the primary genes. This suggests, as experimentally demonstrated by cultures in vitro, that almost any wanted chemical reaction or utilization of substrate can be induced from regulatory modifications of already existing genetic information. This process must be of major importance for domestication and differentiation of species.

– Transcriptional units. A new source of genetic flexibility comes from recent concepts of gene transcription in higher organisms. From the new analysis of eukaryotic coding sequences of DNA, we know that the parts translated into amino acid sequence are not continuous but are interrupted by regions which will be lost during maturation of the messenger. Then the cistron unit, thought to correspond to one polypeptide chain, must be replaced by the notion of transcription unit localized in a segment of DNA that can correspond to several polypeptide chains. Gilbert (1978) uses the term introns (for intragenic regions) for the silent DNA alternating with expressed parts: the exons. The possibility of rearrangements or substitutions from introns to exons – probably under environmental or epigenic control – suggests that introns are either remnants of ancient functions or reserves for future improvement or differentiation.

Crossing-over does not happen at random

It is now trivial to recall that crossing-over is under definite genetic control. The most outstanding experiments of Chinnici (1971) on *Drosophila*, of Poisson (1970) on cotton, of Sears (1969), Okamoto (1963), and Riley (1963) on wheat, suggest that the hypothesis of Pandey (1972) is valuable for most of plants.

Since the 'relations de lien' (Gallais 1970, 1977) between two loci do not occur at random, we are inclined to think that the situation of loci in chromosomes is strongly conditioned by selective values of their trans and cis epistasis. This topological organization of loci influences chromosomal numbers and distribution of chromosomal arms (evolution from mediocentric to telocentric chromosomes must strongly change genic epistasy) giving rise to clusters of genes, recombination of which could be prevented by local control of crossing-over.

Genetic control on the rate of mutation

Some time ago, it was thought that mutations occurred at random. Yet breeders stated that various genotypes did not react similarly to induced mutagenesis. For spontaneous mutations, it has been shown that lines with high instability can be achieved by selection.

If we discuss what is known about the loci that control mutations in *Escherichia coli* and other organisms like T_4 bacteriophage, we may speculate about the evolution of mutation rates in populations of higher plants. Research by Cox (1976) shows that mutator genes are situated in numerous loci. They control various types of mutations. Antimutator genes have been demonstrated too. If a new mutator allele appears in a population, advantageous, deleterious or neutral mutants will soon arise. With the more or less unpredictable environment, selection pressure gives rise to a balance between mutator and antimutator genes. Therefore in most situations the evolutionary process operates to fix lower mutations rates, in other words, weak mutator alleles or strong antimutator alleles specifically linked to favorable genes.

The linkat

Definition

From previous theoretical and experimental considerations, it may be conceived that there is a chromosomal organization for most quantitative or agronomic traits – which I named linkat – characterized as follows:

– During phylogenesis, rearrangement procedures, such as inversions, translocations and deletions, may associate close to an allele other alleles coding for other functions related into an adaptive system (floral morphology, incompatibility mechanisms).

– Each unit of such a cluster comprises several duplicates modified to some degree. There is a flexibility in the expression of each unit, due either to a transitory hierarchical balance between the duplicates (some being repressed and neutral, other being expressed) or to post-transcriptional rearrangements between introns and exons.

– These adaptively coordinate units have been associated during evolution to

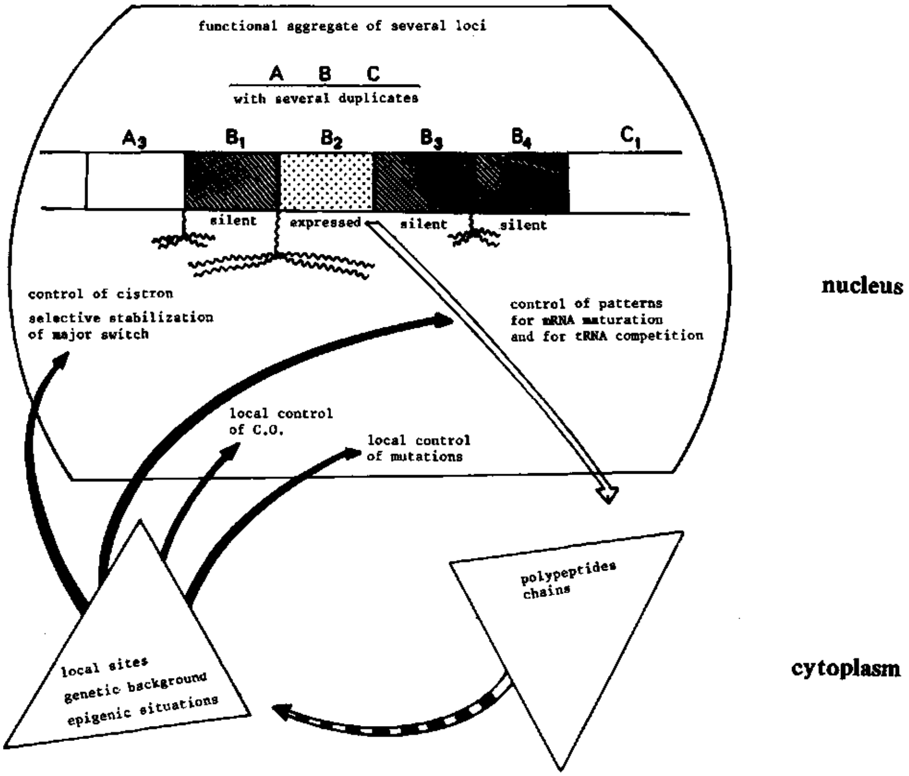

Fig. 1. The linkat organization and its controlling elements.

hereditary factors that locally weaken crossing-over. Specific genes can control it, (Pandey 1972), but the whole genetic structure plays its part too. This low cross-over rate ensure preservation of linked alleles, selected either naturally, or by man, for their strong cis or trans epistatic interactions. Chromosomal arms might contain several linkats (which may superpose in superlinkats), disconnected by sectors where cross-over rates are very high. In this sense, the distribution of a genome in a given number of chromosomes has an important meaning as well as linked sequence on both sides of the centromere.

– A number of these segments probably contain an internal control of sensitivity to spontaneous or induced mutations. This control might be achieved by a balance between mutator and antimutator factors.

Therefore a linkat appears as a multi-transcriptional linked unit, with a strong internal regulation for its hereditary stability and with a sophisticated mechanism providing its phenotypic flexibility (Fig. 1).

Facts about linkats

Most research done in molecular genetics concerns microorganisms or animals. When picturing protein evolution since various evolutionary line separated, it is stated that related gene duplicates often remain adjacent for a long time, as for instance genes for Haemoglobin β, δ and ν, though the gene for α -haemoglobin moved to another locus. In a species of *Chironomus*, Plagens (1971) found 15 structurally distinct globin duplicates. During embryogenesis of mouse, a significative cluster of *t* genes controls the sequential steps of differentiation. Many other examples could be quoted for *Drosophila* (Mukai 1967). In higher plants such sequences were often regarded as pleiotropy. But finer analysis showed that *S* locus of incompatibility is a cluster of genes. For some genera like *Primula*, morphological characteristics of floral morphology are included in such a block. Dwarf genes, restorer and modifier genes, teosinte complex, speltoid mutation are other examples of adaptive complex. Other illustrations could be found in caryogram comparisons during species differentiation, where block rearrangements are significant too.

Moreover numerous models and simulated analysis demonstrate that in the following conditions – genic polymorphism with marked epistatic effects between loci, after several generations of outbreeding, in a situation of selection – only a few blocks of allele arrangements will survive and, if simultaneously crossing-over is restricted to this segment, will give rise to linkats (e.g. Franklin & Lewontin 1970).

Meaning of linkats in evolution

The necessary polymorphism for a population to survive is as large as the variety of environmental conditions. Genetic load, which represents genotypic variation around a natural ideotype, is a hereditary reserve of polymorphism. In the course of coadaptation in an ecosystem, genetic load is adjusted to a minimum, which allows adaptation of a population to fluctuating environment. The principle of such an adjustment is called load resonance (Demarly 1976, 1977). Natural selection gives advantage to every mechanism that increases load efficiency as a source of polymorphism. In this sense, ploidy level, rate of allogamy and heterosis are elements of a system economizing load at population level; equally dominance interactions, epistasis and linkats are parts of an economizing system at individual level. One will notice the triple aspect of the adaptive flexibility of linkat: ability of various proteins from one set of data, modulation of the strength of alleles clusters by crossing-over control and adjustment of the ratio mutator/antimutator genes.

Instability of linkats

Tensions network

The creode[1], according to which a plant develops during vegetative growth, as well as during reproduction, controls tensions (or correlations, or flux exchanges, or signals) at every level: morphology and functions of tissues, positioning and stratification of cells and regulation of genetic expression such as code transcription, unfolding of meiosis, control of recombinations and selection of mutations). The network of these tensions that determines the stability of structure and expression for a linkat, is called linkat tension. Normal environmental and epigenic conditions maintain the established linkats. Each deep disturbance of these networks can lead to a modified expression, or a modified structure of genetic information.

Linkats during domestication

Domestication of a species will simultaneously change natural pressure on required load for adaptation in the ecosystem (such as better control of physical factors of competition), decrease the reproducing effectives and in some species, inbreeding. It can be established experimentally that transition from outbreeding to inbreeding modifies the expression of quantitative genetic factors or linkats.

Moreover, it has been shown in *Petunia* (Bergounioux 1978) that correlation network does not follows the same programme in heterozygous and in homozygous individuals. The change of breeding system seems to induce a change in linkat tension. Therefore these modifications of quantitative heredity could be explained by two non-exclusive mechanisms: either there are other exons that give rise to different proteins, or hierarchical regulations have changed between silent and expressed duplicates.

Linkats in in vitro cultures

It follows from previous remarks that if internal correlations of the plant be broken down by cell or tissue cultures, organizational tension network will disappear. During last 10 years, the experiments conducted in vitro showed linkat relaxation either at vegetative stage (somatic variants, Sibi 1976) or at meiosis in chro-

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[1] A creode is a representation (e.g. by a trajectory in phase space) of a temporal succession of states of a system characterized by the property that the system, if constrained to move slightly away from the creode, will return to it (Waddington 1957).

mosomal pairing (Tran 1978) or at gametophytic level (variant from androgenesis, André-Truong 1977, Odjo 1978, Picard 1976). Though at the moment we do not know the exact mechanisms involved, the breeders interest will lie on transmissibility (mainly maternal) of this effects. It might suggest that, for some aspects, in vitro culture can provide a short cut for evolution.

Consequences for plant improvement

The consequences of the linkat as a cluster of alleles semi-stable in its heredity, which possess a flexibility for its expression, are important in plant domestication and in plant breeding. The most outstanding are as follows:

- Quantitative analysis derived from inbred progeny will not regularly reflect genetic inheritance of outbreeding species because of rearrangements of linkats tensions.
- Then major interest of inbreeding seems to become a tool for new rearrangements of cis-epistatic effects. Perhaps will it induce intra-linkat recombinations.
- When introgression is used to introduce quantitative or complex adaptive traits, a change in natural breeding system, or relaxed linkats by the use of in vitro cultures could considerably increase the efficiency of hybridization.
- A wider use of amorphous stage of in vitro culture before regeneration of plants could afford new sources of polymorphism or greater efficiency in mutagenesis.

In recurrent selection cycles, breeders can better introduce a selfing before panmixia: it will raise the rate of intra-linkat recombinations.

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Biosystematics and evolution of species barriers

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Principles of evolution and cereal domestication

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Breeding, cereals, domestication, evolution.

Summary

Plant domestication is evolution in man-made habitats. Weeds and domesticates are so closely associated with man that they can no longer compete succesfully with their wild relatives for natural habitats. They are adapted to habitats that are continuously being disturbed by man. Weeds are spontaneous in disturbed habitats. Domesticated cereals rely on man for seed dispersal. Wild races of domesticated cereal species are aggressive natural colonizers. They are preadapted to cultivated fields, and numerous wild cereals probably failed to become domesticated because of a lack of natural colonizing ability. Domestication is a process not an event. Domestication of cereals was initiated when seeds of harvested wild grass were sown in habitats specially prepared for them by man. Domestication continues as long as seeds harvested from a planted population are again sown. Harvesting or sowing cannot independently lead to domestication. But changes in selection pressures leading to domestication are automatic under a regime of harvesting and sowing in successive generations. Evolution is rapid under domestication. Smal samples of seed are sown, and the sown populations rapidly become essentially uniform under the influence of selection and genetic drift. Isolation of cultivated populations by man, habitat adaptation, occasional recombination between cultivated populations, conscious selection by man, and rare introgression of cultivated populations with wild relatives contribute to racial evolution in cereals. Traditional races of cereals have a unity of form and usually also of habitat. However, they vary in form and respond favorably to changes in natural pressures. Modern plant breeding favors genetic uniformity and immediate fitness. Care must be taken that sufficient variation is preserved in domesticated cereals to insure future progressive evolution under experimental breeding.

Introduction

Variation is essential for progressive evolution. Plant domestication is evolution in habitats by man disturbed. Two kinds of organisms, weeds and domesticates, evolved in man-made habitats. Both weeds and domesticates have become so dependent on man that they can no longer compete succesfully with their wild relatives for natural habitats. Natural selection for adaption to disturbed habitats by man gave rise to weeds (Harlan & de Wet 1965; Baker 1974). Natural selection within an environment created by harvesting and sowing gave rise to cereal domestication (de Wet & Harlan 1975; de Wet 1975). Evolution is rapid under domestication. Cereal cultivation dates back some 10 000 years, and during this relatively short evolutionary timespan domesticated races evolved differences in form that allow for their recognition as distinct species. A better understanding of the principles of evolution in the man-made habitat is needed before the potential of cereal germplasm can be achieved.

Principles of population biology

There are three kinds of evolutionary active populations in nature. There are small phylogenetically related populations that are reproductively isolated from one another, large interbreeding populations, and populations that are reproductively divided into almost isolated subpopulations (Mayr 1954). Different kinds of population structures allow for different responses to changing environments (Grant & Flake 1974; Grant 1977).

Large outbreeding populations are genetically continuous. Adaptive gene combinations at one extreme of the population can spread to the other extreme through gene exchange. In such populations, selection is relatively ineffective on new mutations. Even those mutations with harmful effects can become fixed in the population. New alleles are rapidly swamped by hybridization, and genetic systems that promote homeostatic buffering of the genotype tend to develop. In relatively stable environments, large interbreeding populations are evolutionary successful. Adaptive gene combinations are maintained, and new adaptive norms develop with slowly changing environments. Large populations are at a disadvantage when their environments change rapidly. They cannot sustain rapid evolution. Intense selection removes part of the population, breaking up better adapted colonies into relict populations.

Populations that are partially isolated from one another are better adapted to cope with changing environments than are large interbreeding populations. Individual subpopulations are essentially uniform, each with a specific adaptation. However, the population as a whole is diverse, and this large gene pool is available to most subpopulations through occasional gene exchange. Under conditions of changing selection, some subpopulations will be more successful than others. Successful subpopulations will expand at the expense of less successful neighbors, and expanding ones will come into genetic reach of one another. Recombination takes place, and new bursts of evolutionary activity become possible.

Small genetically isolated populations respond rapidly to changes in selection pressures (Wright 1966). Non-adaptive gene combinations can become fixed by chance if they happen to be part of the isolated gene pool. In relatively stable environments, successful small populations rapidly reach the highest adaptive peak possible with the limited resources for variation. Once this peak is reached, evolutionary progress essentially ceases. In rapidly changing environments, such populations survive in ecological refuges, become extinct, or by chance reach new adaptive peaks where they again become inactive in evolutionary development. Obligate inbreeders under similar adaptive stress behave essentially as do small isolated populations during rapid environmental change. Facultative inbreeders are phylogenetically similar to almost isolated colonies of large outbreeding populations (Baker 1959).

Cereal domestication combines several evolutionary principles of population biology. Artificial sowing selects a fraction of the available genepool of the species for multiplication. Sowing by individual farmers creates series of isolated populations in which different combinations of polymorphic genes become fixed through the actions of drift and selection. Attempts to sow wild or domesticated cereals in new environments frequently fail. This certainly must have been so during the sowing of wild cereals. Species that were not adapted to disturbed habitats are difficult to domesticate. Among several hundred grass species that are regularly harvested as wild cereals, only 34 are known to have ever been succesfully cultivated (App. 1). The wild progenitors of these cereals are all aggressive colinizers of disturbed habitats. Once adapted to cultivation, the size of domesticated populations is determined by the availability of habitats prepared by man. In traditional agriculture, such populations are generally small and adaptation soon reaches equilibrium with the fluctuating environment. The cultivated population therefore rarely reaches optimum adaptive fitness. But, this variation ensures against total failure under extreme environmental fluctuations. A balance is maintained in cultivated populations of traditional type agriculture between immediate fitness and progressive evolution. Variation in local races is further assured by occasional hybridization between populations from adjacent fields, and by farmers occasionally exchanging seed from distantly isolated populations.

Traditional agriculture is an ideal system for progressive evolution of cereals under cultivation. This evolutionary system is rapidly being undermined by modern methods of plant breeding. Breeders strive for immediate fitness for maximum yields. Uniform cultivars are rapidly replacing the established local races even in systems of traditional agriculture. This reduction in the gene pool of cereal species must eventually lead to agronomic disaster. Experience has taught that even minor environmental changes can drastically reduce yield of modern widely used cultivars of cereals.

Dynamics of cereal domestication

Domestication is the result of selection pressures associated with harvesting and sowing (Harlan et al. 1973). Conscious selection by the cultivator is not essential. Domestication is automatic in the man-made habitat. Sowing leads to better adaption in cultivated fields, and harvesting usually leads to loss of natural mechanisms of seed dispersal. However neither occasional sowing nor occasional harvesting can lead to domestication. Selection for man-made habitats becomes effective only when sown generations follow one another in succession. Under a regime of sowing and harvesting that follow one another, pressures for a shift to new adaptive peaks within the cultivated habitat become more demanding with each generation, and the population eventually becomes totally dependent on man for survival.

Sowing of wild cereals is a common practice among some nomads. They sow to increase the size and density of natural populations. This facilitates harvesting and provides better grazing for their herds. Habitat preparation is limited or not practised at all, and the process is rarely repeated within the same gene pool for more than one generation at a time. Except for competition within population, selection pressures are not significantly altered by this kind of sowing. Competition for space is ever present in natural populations, and is particularly strong among natural colonizers from which domesticated cereals were developed.

Harvesting as a process in natural populations merely reduces the number of seeds available from which the next generation will become established. Rarely is more than half the available seeds harvested in populations of wild cereals. The remaining seeds are naturally dispersed. Harvesting samples the population at random, and gene frequencies in such large natural stand of wild cereals remain essentially at equilibrium. Grazing animals and seed-eating birds are at least as effective as man in harvesting. The process of harvesting intensifies rather than reduces natural adaption. It is only when a selected sample of harvested seeds is sown and genetically protected from the wild parental population that domestication is initiated. Burkill (1952) suggests that sowing of small isolated colonies came about when man transported his favorite wild cereals beyond their natural ranges. Sowing became imperative and isolation from the population was complete.

The circumstances that led to plant domestication are not known. Cultivation was probably not initiated to overcome food shortages. It is safer to move to new areas where food is still available even if that means war, than to risk starvation by sowing your remaining food supply and to wait for an uncertain crop to mature. Historically man rather eats what he has in hand than gambles on agriculture in times of scarcity. It is possible that sowing started as a means of increasing food supplies during times of abundance when a sedentary way of life contributed to an increase in human population. It is equally likely that during such times of abundance, sowing was initiated to increase the frequency of particularly desirable traits of wild food-plants.

Inability to dispersel seed greatly facilitates harvesting and is one of the most desirable characteristics in cultivated cereals. American wild rice (*Zizania aquatica* L.) is widely harvested on a commercial scale in the north-central United States and adjacent Canada. Because of efficient natural dispersal of seed, it is estimated that more than 80% of the grain escapes the harvesters. This wild cereal has recently become cultivated after the discovery of a small population in which spikelets tardily disarticulated from mature inflorescences (Hofstrand 1970; Oelke et al. 1973). An increase in harvestable seed from 20 to 50% made the expense of paddy cultivation economical. Other desirable traits may also prompt sowing and cultivation of wild cereals.

Teosinte, the ancestor of maize (*Zea mays* L.) is characterized by caryopses that are covered by an indurate rachis and glumes (Galinat 1971; Beadle 1972; de Wet & Harlan 1972). A single dominant gene, tunicate, reduces this induration and provides some stability to the teosinte rachis at maturity. The oldest known race of maize in the archaeological record is tunicate (Mangelsdorf et al. 1967). It has long papery glumes and soft rachis. Certainly, the discovery of a tunicate teosinte could have led to the cultivation of this grass (Galinat 1977). Such a teosinte has soft glumes and the spikelets are free from the rachis cupules. The indurate rachis and glumes make treshing of teosinte difficult, and the species may first have been harvested in the wild or cultivated as a green vegetable. Immature female inflorescences are sweet and appetitizing when cooked. Its use as a cereal probably dates from the discovery of a tunicate population. This mutation would greatly facilitate harvesting and treshing.

Be that as it may, cultivation of cereals became a way of life in the Near East by 7 000 BC, and possibly around the same time in many other parts of the world. Cereals were independently domesticated in Europe, south Asia, China, West Africa, Central America and South America (App. 1). Tuber domestication may have preceded cereal cultivation in some regions. Domestication of a tuber species is genetically a simpler process than cereal domestication. Tubers are often vegetatively reproduced and the inflorescences are little altered under cultivation, except for partial sterility in some domesticated species. In cereals under domestication, yields depend largely on changes in the architecture of the whole plant. Domesticated cereals differ from their wild ancestors in habitat and gross form.

Domestication and habitat adaptation

Domesticated cereals are adapted to habitats specially created for them by man. Inability to adapt to habitats that are continuously being disturbed probably excluded numerous wild cereals with excellent yield potential from becoming domesticated. Domestication is evolution in man-made habitats. It is a continuous process, as is evolution in natural populations.

The ancestors of domestic cereals are agressive colonizers of naturally disturbed habitats. They are pioneers in the vegetational succession. Colonizers succesfully invade habitats disturbed by man, and become better adapted to permanently disturbed habitats with each generation they remain associated with man. Weeds and domestic crops are artifacts of man's agricutural activities. They evolve independently under different pressures within the man-made habitat. Domestic crops are rarely derived from weeds, but abandoned domestic crops often become weedy (de Wet & Harlan 1975). Weed races of domesticated species commonly evolve through natural selection within the man-made habitat, or originate from crosses between wild and cultivated races of domesticated species.

Ecological boundaries between colonizer, weed, cultivated and domestic races of a species are rarely well defined. Higgs & Jarman (1969, 1972) point out that the wild class of organism merges into the domestic class by a more or less continuous series of stages according to their dependence on man for survival. In general, plants growing outside habitats disturbed by man are wild. Those that invade naturally disturbed habitats are colonizers, and those that are spontaneous in habitats disturbed by man are weeds. Plants that are cared for by man are cultivated or domesticated. Crabgrass (*Digitaria sanguinalis* (L.) Scop.) forms part of the natural grassland of Eurasia where the species is wild or weedy. It was introduced to the New World where it occupies permanently disturbed man-made habitats and is an obligate weed. In south-eastern Europe, the species was at one time widely cultivated and harvested as a wild cereal. In Kashmir and the Caucasus, crabgrass is grown as a domestocated cereal. There it depends on man not only for a habitat, but also for seed dispersal.

Sowing automatically increases intensity of competition for space in the habitat prepared by man. Seed dormancy becomes selectively more and more disadvantageous in cultivated fields. Seedlings that emerge first have the best chance of becoming established. Dormancy is advantageous in wild populations since germination must wait for favorable conditions. Domestic races are sown only when conditions are most favorable, and immediate germination increases competitive ability.

Seedling vigor is selectively advantageous in all colonizers. Once germination starts, the more vigorous seedlings successfully occupy the available space. Energy for seedling development is stored in the endosperm. Domestication therefore automatically selects towards an increase in seed size. Seed size is also a factor in yield, and it is possible that man consciously contributed to selection pressures that produced the relatively large grains characteristic of most cereals.

Habitat adaptation is genetically complex. It is controlled by genes distributed over several chromosomes (Clausen 1959, 1967). Major adaptive shifts are not easily accomplished. Wild races of domesticated species are ready adapted for cultivation. They are colonizers of naturally disturbed habitats. The ranges of most domesticated cereal species are large. This allows extensive ranges for domestic races as well. Cultivated *Sorghum bicolor* (L.) Moench, as an example, is grown in the wet tropics of West Africa, fog belt of the East African mountains, African savanna, and semi-arid regions of Africa and Asia. The species was probably first cultivated in the West African savanna (de Wet & Harlan 1971). Wild races have as wide an adaptation as present-day domesticated races (de Wet et al. 1970).

Phenotype of domesticated cereals

Selection associated with cultivation gave rise to characteristics that distinguish the form of cultivated races of cereals from that of their wild progenitors (Harlan et al. 1973). The major changes are loss of natural seed dispersal mechanisms, and shifts to synchronized tillering and branching, or to apical dominance.

Harvesting and sowing often automatically lead to a loss of natural mechanisms of seed dispersal. The spikelets or florets of many cultivated cereals do not disarticulate at maturity, and sowing becomes obligatory for survival. Selection against disarticulation is automatic under a regime where inflorescences are harvested for later threshing (Wilke et al. 1972). The weedy *Avena abyssinica* Hochst. (Ethiopian oats) has lost its natural ability to disperse seed, although it is neither consciously harvested nor consciously sown (Rajhathy & Thomas 1974; Ladizinsky 1975). It is accidentally sown, harvested and threshed with the barley (*Hordeum vulgare* L.) it accompanies as an obligate weed. Individuals with the least efficient methods of dispersal are selectively harvested. Since the caryopses of barley and Ethiopian oats are difficult to separate, the weed is automatically included in the seed sample for the next crop. A natural shift from seed dispersal by disarticulation to one dependent on harvesting, threshing and sowing by man was automatic. These same kinds of selection would operate when wild cereals were brought into cultivation and a sample of harvested seeds were sown to establish the next generation. Some harvesting methods, however, favor natural seed dispersal. Sauwi millet of north-western Mexico (*Panicum sonorum* Beal) is harvested by uprooting whole plants before the inflorescences are fully matured. These are allowed to dry in the sun before the florets with their grains are shaken from the spikelets. Domestic races of *Panicum sonorum* differ from wild races conspicuously only in habitat preference, and by having larger inflorescences and larger caryopses. These domesticated races readily become established as weeds. They are aggressive colonizers of all disturbed habitats and, although no longer cultivated, are widely distributed along the mountains of western Mexico. Natural selection can decrease or increase ability to seed disperse. In Malawi, a kind of Guinea sorghum is grown for its sweet grains that are eaten raw (de Wet et al. 1972). Inflorescences are harvested before the grains are fully matured, and florets are shaken from the spikelets after they have dried in the sun for a few days. When this sorghum matures in the field, seed dispersal is as efficient as in wild races of *Sorghum bicolor*. However, the florets rather than the spikelets disarticulate. That new seed dispersal mechanisms can become established in domesticated cereals is particularly obvious in Mississippi chicken corn (*S. bicolor*, spp. drumondii). This weed originated from an abandoned grain sorghum. To survive as an escape from cultivation, natural seed dispersal became essential. In wild *S. bicolor*, disarticulation of spikelets is achieved through an abscission layer that forms between the glumes and pedicel. Seed dispersal in chicken corn is due to breaks in the rachis between nodes.

Synchronized tillering or branching on individual plants of cultivated cereals are similarly automatic on selection associated with domestication. Tufted wild grasses produce several main branches, which are often characterized by several secondary branches each of which produces an inflorescence. Maturity of these inflorescences is usually reached over an extended period during the growing season. At least three harvests are required to get a reasonable yield of American rice (*Zizania aquatica*), a species that is commercially collected as a wild cereal. This is not desirable under cultivation. Harvesting is regularly done only once at the end of the growing season. Individuals with most inflorescences mature at the time of harvest will automatically make a proportionally larger contribution to the next crop sown than do plants with fewer inflorescences mature at one time. Selection under domestication favors uniform population as well as uniform maturity of the individual plant. Uniform maturity of the population is achieved by loss of seed dormancy and increase in seedling vigor. Uniform maturity of the individual plant produces two basic phenotypes. Domesticated cereals that retained their tillering habit such as rice (*Oryza sativa* L.) and other small grains, are characterized by synchronous tillering. Tillering is completed early in the growing season and all inflorescences mature at the same time. In other cereals such as *Pennisetum americanum* (L.) Leecke (pearl millet), sorghum, maize and others, a single stem is produced with usually a much enlarged solitary inflorescence. In some races of cereals such as *Panicum miliaceum* L. (proso millet) and many minor cereals, tiller or branch synchronization is not complete. Such races are commonly grown under traditional farming systems, and are frequently harvested several times during the growing season. This precludes the need to store grain during the early part of the harvesting season.

Domestication of cereals often changes the phenotype of the inflorescence so drastically that different cultivated races and their wild progenitors are recognized as different species or even genera. The compact inflorescence of durra or kafir sorghums is very different from the large and open inflorescence of the wild *S. bicolor* (Harlan & de Wet 1971). Similarly, the gaping glumes that expose the mature grain distinguish Guinea sorghums from all other races of *S. bicolor*. These taxa are all interfertile and belong to a single species. Yet, morphologically they represent distinct taxonomic units (Snowden 1936). Even more spectacular are the differences between *Zea mays* subsp. *mexicana* (Schrad.) Iltis (teosinte) and maize

(Mangelsdorf 1974; Wilkes 1977). Although conspecific genetically, teosinte is often treated as belonging to the genus *Euchlaena* (Randolph 1976). The female inflorescence of teosinte is a distichous spike, with solitary fertile spikelets alternately arranged in cupules on an indurate fragile rachis. In maize, the female inflorescence is a polystichous spike with paired fertile female spikelets arranged in shallow cupules along a continuous rachis. Mangelsdorf (1974) argues that maize could not have been derived from teosinte since the traits that distinguish them are phylogenetically more advanced in teosinte. These changes, however, are of the same order as those distinguishing wild and domestic races of other cereals (de Wet 1975). Differences between wild and cultivated races of domesticated species are genetically complex and not all simple alternatives. In maize and teosinte, differences in inflorescence are controlled by at least five major gene complexes (Collins 1919; Langham 1940; Rogers 1950). The change from a distichous spike (teosinte) to a polystichous spike (maize) is associated with loss of natural ability to disperse seed. Alternate spikelets became yoked, and pairs of spikelets became cross-yoked into whorls (de Wet & Harlan 1976). These changes restored fertility of the rudimentary spikelet of each pair at a node, and produced the basic eight-rowed ear characteristic of maize.

Variation in domesticated species

Domesticated species are diverse. Races distinct in form are often alike in habitat. Snowden (1936, 1955) divides *Sorghum bicolor* into 13 wild, 7 weed, and 28 domesticated species. The 28 cultivated taxa are further divided into 156 varieties and 521 forms. These taxa all cross to produce fertile hybrids wherever they are sympatric (de Wet & Shechter 1977). The wild taxa differ in habitat. The weeds originate from hybrids between wild and cultivated taxa, and the domestic races are artefacts of man's agricultural activities. Different kinds of sorghum are grown for different uses, and adjacent villages often grow morphologically different kinds for similar uses. Man selects new mutations and gene combinations that he fancies and multiplies these in isolation. This is true of all cereals. Some 300 different races of maize are commonly recognized.

Domestication is an ongoing process. Small isolated populations are subjected to different selection with each generation the cereal is grown. Furthermore, selected seed samples are transported across the range of cultivation of the species, and previously isolated populations become sympatric by such migrations. Populations as close as adjacent villages or even adjacent fields usually differ genetically, and occasional crosses between populations provide variability for continuous evolution. The age of cereal domestication, selection and isolation by man, and the nomadic nature of man can explain most of the variation characteristic of cereals. Five race complexes are recognized among cultivated sorghums. Equally common are hybrid races that combine characteristics of two or more basic races in almost all possible combinations (Harlan & de Wet 1972). Domestication provides an ideal environment for rapid differentiation between population as well as for continuous evolution.

Hybridization of wild and weed races with cultivated races contributes to racial evolution in cereal species (Harlan 1976). Durra sorghums and wild aethiopicum sorghums show evidence of introgression. Guinea sorghums may have inherited their adaptation to a forest habitat from wild arundinaceum, and kafir sorghums incorporate genetic traits of verticilliflorum from south of the equator (Shechter & de Wet 1975). Archeological evidence shows that the primitive Coxcatlan maize of the Valley of Mexico hybridized with teosinte some 3 000 years ago, and that such introgression contributed to racial differentiation of maize (Mangelsforf et al. 1967). In the Nobogame Valley, Mexican farmers unconsciously encourage introgression from teosinte into maize (Wilkes 1970), and in the Valley of Mexico gene transfer between maize and teosinte is commonly encountered.

Evolutionary basis of plant breeding

Plant breeding is as old as plant domestication. Selection of small samples of seed for sowing, and the environmental variation encountered under cultivation change gene frequencies of planted populations from one generation to the next. Selection remains the basis of plant breeding and, together with production of hybrid seed, made possible the tremendous increases in yield achieved for cereals such as maize, wheat, rice and sorghum during the last few decades.

Plant breeders strive for immediate fitness, and modern farmers strive for an essentially uniform environment in which to grow their crops. This makes possible excellent yields as long as the environment remains stable. Unfortunately for immediate fitness, one does not consider variation in habitat, and even small environmental fluctuations can seriously affect yields. Domestication favors uniform populations. Initial domestication must have involved only a fraction of the total gene pool of the species. Different gene combination, however, become established in different cultivated populations through a combination of drift and selection. The environment of traditional agriculture varies, and genetic variation is automatically maintained within cultivated populations. Yields are relatively low, but essentially stable from year to year except with extreme environmental fluctuations.

Modern cultivars of cereals are seriously depleted of genetic variation. I estimate that 70% of corn belt maize hybrids have two or more of seven 'station lines' in their pedigrees, and the majority of these were derived from two basic breeding lines, 'lancaster' and 'Stiff Stalk Synthetic'. These two gene pools represent recombinations of selections out of cultivars grown in the corn belt some 50 years ago. Modern commercial maize hybrids have high agronomic suitability and the introduction of any alien germplasm severely reduces yield. They are also vulnerable to fluctuations in the environment. Too much or too little rain, competition from weeds, lack of adequate fertilizer, and attacks by insects or other pathogens drastically reduce yield. This was clearly demonstrated for corn belt hybrids in 1970 when an attack by a particularly virulent race of southern corn leaf blight (*Helminthosporum maydis* Nisik et Miy.) reduced yield by some 15%. As far as agricultural catastrophies go, the corn blight disaster was a minor one. It could, however, have been more severe. The famine in Bengal during 1943 was caused primarily by an infection of the rice crop by *Helminthosporium oryzae* de Haan.

Concern has repeatedly been expressed about the genetic vulnerability of corn belt maize and most other commercially grown cereals. The 1970 leaf blight epidemic in maize was largely due to the susceptibility of T-cytoplasm to race T of the pathogen. This cytoplasm confers male sterility to breeding lines. A ready solution, was at hand by simply returning to normal cytoplasm. This, however, also meant a return to the slow and expensive hand detasselling of female lines in hybrid maize seed production. Genetically dominant resistance to *Helminthosporium maydis* has now been introduced into commercial maize inbreds from tetraploid ($2n = 72$) *Tripsacum dactyloides* (L.) L. (Harlan & de Wet 1977).

Genetic variation is essential if progressive improvement of cereals is to be maintained through breeding. A fraction of the total available gene pool of any domesticated species is now being used by plant breeders. Breeding is generally restricted to germplasm within selected races of the cultivated complex of domesticated species (Harlan & de Wet 1971). This gene pool is rapidly being eroded away by replacing traditional local races with experimentally bred lines. A treasure house of variability in wild races of domesticated species is largely being ignored (Harlan 1976). Increasing yield through breeding is evolution under domestication. Progressive evolution is a change in gene frequencies that allow a population to adjust successfully to changing environments. Maintaining and using all the natural variation that characterizes domesticated species must become part of modern plant breeding.

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Appendix 1. Cultivated cereals, their possible areas of first cultivation, their wild ancestors, and their weed relatives as commonly recognized taxonomically.

AVENA, L.

A. abyssinica Hochst. (Ethiopian oats). – Obligate weed of Ethiopia that has lost its natural mechanism to disperse seed; introduced from the Mediterranean; wild progenitor *A. barbata* Pott.; weed relative *A. vaviloviana* (Malz.) Mord..

A. sativa L. (Oats). – Domesticated in the northern Near East; widely grown as a crop; wild progenitor *A. sterilis* L.; weed relative *A. fatua* L..

A. strigosa Schreb. (sand oats). – Domesticated in the southern Mediterranean; cultivated in south-western Europe; wild progenitor *A. hirtula* Lag.; weed relative *A. strigosa.*

BROMUS L.

B. mango Desv. (mango). – Domesticated in the Andes of Central Chile; no longer cultivated as a cereal; wild progenitor *B. mango*; weed relative *B. mango*

BRACHIARIA (TRIN.) GRISEB.

B. deflexa (Schum.) C.E. Hubbard (animal fonio). – Domesticated in the Futa-Jalon of Guinea; collected as a wild cereal across the African savanna; wild progenitor *B. deflexa*; weed relative *B. deflexa.*

COIX L.

C. lacryma-jobi L. (Job's tears). – Domesticated in south Asia; cultivated in Assam, Burma and south-east Asia; wild progenitor *C. lacryma-jobi*; weed relative *C. lacryma-jobi.*

DIGITARIA HEIST. ex FABRIC.

D. cruciata (Nees) A. Camus (kaishan). – Domesticated in the Khasi Hills of Assam; cultivated in India as a minor cereal; wild progenitor *D. cruciata*; weed relative *D cruciata.*

D. exilis (Kippist) Stapf (fonio). – Domesticated in the West African savanna; cultivated in West Africa as a minor cereal; wild progenitor *D. longiflora* (Retz.) Pers.; weed relative *D. exilis.*

D. iburua Stapf (black fonio). – Domesticated in the West African savanna; cultivated in West Africa; wild progenitor *D. barbinodis* Henrard; weed relative *D. iburua.*

D. sanguinalis (L.) Scop. (manna). – Domesticated in the Caucasus and Kashmir; collected as a wild cereal in south-eastern Europe; wild progenitor *D. sanguinalis*; weed relative *D. sanguinalis.*

ECHINOCHLOA BEAUV.

E. colona (L.) Link. (shama). – Domesticated in south India; collected as a wild cereal across the warmer parts of Africa and Asia; wild progenitor *E. colona*; weed relative *E. colona.*

E. frumentacea (Roxb.) Link. (Japanese millet.). – Domesticated in the Far East; cultivated across Asia; wild progenitor *E. crus-galli* (L.) Beauv.; weed relative *E. crus-galli.*

ELEUSINE GAERTN.

E. coracana (L.) Gaertn. (finger millet). – Domesticated in Ethiopia and adjacent Uganda; cultivated across the African savanna from Chad to South Africa and in south Asia; wild progenitor *E. africana* Kennedy-O'Byrne; weed relative *E. africana.*

ERAGROSTIS BEAUV.

E. tef (Zucc.) Trotter (tef). – Domesticated in Ethiopia; endemic as a cultivated cereal to Ethiopia; wild progenitor *E. pilosa* (L.) Beauv.; weed relative *E. pilosa.*

HORDEUM L.

H. vulgare L. (barley). – Domesticated in the northern Near East; widely cultivated; wild progenitor *H. spontaneum* C. Koch; weed relative *H. spontaneum.*

ORYZA L.

O. glaberrima Steud. (African rice). – Domesticated in the West African savanna; cultivated in West Africa; wild progenitor *O. barthii* A. Chev.; weed relative *O. stapfii* Roshev..

O. sativa L. (rice). – Domesticated in south Asia; widely cultivated; wild progenitor *O. rufipogon* Griff.; weed relative *O. nivara* Sharma & Shastry.

PANICUM L.

P. miliaceum L. (proso millet). – Domesticated in Europe and China; cultivated in Eurasia; wild progenitor *P. spontaneum* Lyssov.; weed relative *P. spontaneum.*

P. sumatrense Roth. ex Roem. & Schult. (sawan). – Domesticated in south India; cultivated in south Asia; wild progenitor *P. sumatrense*; weed relative *P. sumatrense.*

P. sonorum Beal (sauwi). – Domesticated in the mountains of north-western Mexico; no longer cultivated as a cereal; wild progenitor *P. sonorum*; weed relative *P. sonorum.*

PASPALUM L.

P. scrobiculatum L. (khodo millet). – Domesticated in the Indian plains; collected as a wild cereal across south Asia; wild progenitor *P. orbiculare* Forst.; weed relative *P. orbiculare.*

PENNISETUM RICH.

P. americanum (L.) Leecke (pearl millet). – Domesticated in the African Sahel; cultivated across the African savanna and semi-arid Asia; wild progenitor *P. violaceum* (Lam.) L. Rich.; weed relative *P. stenostachyum* (Klotzsch) Stapf & C.E. Hubbard.

PHALARIS L.

P. canariensis L. (canary grass). – Domesticated in south-western Europe; cultivated as a feed for caged birds; wild progenitor *P. canariensis*; weed relative *P. canariensis*.

SECALE L.

S. cereale L. (Rey). – Domesticated in the Near East; widely cultivated; wild progenitor *S. ancestrale* Zhuk.; weed relative *S. ancestrale*.

SETARIA BEAUV.

S. geniculata (Lam.) Beauv. (brittle grass). – Domesticated in the Valley of Mexico; cultivated during prehistoric times in central and north-eastern Mexico, but no longer grown as a cereal; wild progenitor *S. geniculata*; weed relative *S. geniculata*.

S. glauca (L.) Beauv. (korali). – Domesticated in Central India; cultivated in India as a Kharif crop; wild progenitor *S. glauca* weed relative *S. glauca*.

S. italica (L.) Beauv. (foxtail millet). – Domesticated in southern Europe and southern China; cultivated across Eurasia; wild progenitor *S. viridis* (L.) Beauv.; weed relative *S. viridis*.

SORGHUM MOENCH

S. bicolor (L.) Moench (sorghum). – Domesticated in the West African savanna; cultivated across Africa and Eurasia; wild progenitor *S. verticilliflorum* (Steud.) Stapf; weed relative *S. drummondii* (Steud.) Millsp. & Chase.

TRITICUML.

T. monococcum L. (einkorn). – Domesticated in the Near East; once widely cultivated in Eurasia, now a relic crop in the Balkans, Turkey and south-western Europe; wild progenitor *T. boeoticum* Bois.; weed relative *T. boeoticum*.

T. turgidum L. (emmer). – Domesticated in the Near East; widely cultivated; wild progenitor *T. dicoccoides* Korn.; weed relative *T. dicoccoides*.

T. timopheevi (Zhuk.) Zhuk. (timopheev wheat). – Domesticated in western Georgia, Soviet Union; endemic as cereal in Georgia; wild progenitor *T. armenianum* (Jakubz.) Mukushina; weed relative *T. timopheevi*.

T. aestivum L. (bread wheat). – Domesticated of the Near East; widely cultivated; strictly a cultivated species.

ZEA L.

Z. mays L. (maize). – Domesticated in Central America; widely cultivated; wild progenitor *Z. mexicana* (Schrad.) Kuntze; weed relative *Z. mexicana*.

ZIZANIA L.

Z. aquatica L. (American rice). – Recently domesticated crop of north-central United States; collected as a wild cereal in southern Canada and the adjacent United States west to the Dakotas; wild progenitor *Z. aquatica*; weed relative *Z. aquatica*.

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Evolution of interspecific barriers in Lycopersicon[1]

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Index words

Lycopersicon, Lycopersicon peruvianum, evolution, interspecific barrier, intraspecific barrier, insect pollen vector, isolation.

Summary

Species of *Lycopersicon* have evolved without changes in chromosome number and with minimal variation in chromosome structure. Interspecific and intraspecific barriers have therefore evolved by genic differentiation. Examples are known of presyngamic barriers to hybridization, embryonic breakdown, hybrid sterility, reduced viability, lowered recombination rates, and other postsyngamic restrictions to introgression. Despite close sympatry between intercrossable species in certain regions, evidence has been found of introgression in only one pair of species. This situation suggests that ethological barriers based on behavior of insect pollen vectors may also effect isolation.

Analysis of the nature of sterility barriers within *L. peruvianum* indicates that their genetic determination is complex and that the nature of postsyngamic developmental breakdown can differ reciprocally. According to the known facts, certain interspecific barriers have evolved by processes that were secondary to geographic isolation.

Considerations of the evolution of *Lycopersicon* species are somewhat simplified by the facts that they are rather closely related (hence probably monophyletic) and that, save for rare exceptions of autotetraploidy, the chromosomes of all species are alike in number and the karyotypes are nearly identical in morphology. The F_1 interspecific hybrids show complete pairing, and, except for occasional structural changes, meiosis is normal. Differentiation between species and the barriers between and within species have therefore arisen almost entirely by gene substitutions.

Barriers to interbreeding fall into two main categories: presyngamic and postsyngamic. Many instances of the former are known, the most prevalent type being an obstruction to pollen-tube growth in the style preventing fertilization and fruit development. Although superficially resembling the self-incompatibility reaction

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found in *L. chilense, hirsutum* and *peruvianum* and *Solanum pennellii*, it differs in several respects and therefore should be distinguished from it. Recognizing this difference, Hogenboom (1973) proposed the name incongruity for this kind of barrier.

Another class of presyngamic barrier that may function in the isolation of tomato species is ethological blockage based on behavior of insect pollen vectors. No direct evidence of this category was obtained, but it is suggested by certain observations. Oligolectic behavior was suggested, for example, in the pollination of *L. pimpinellifolium* in a test plot in Peru (Rick et al. 1978). There a single species of the solitary bee *Exomalopsis* accounted for at least 95% of the pollination, and nearly all of the pollen carried by it was *Lycopersicon*. Other evidence accrues from the fact that natural introgression is known for only one combination despite sympatry and cohabitation of intercrossable species in certain areas.

An additional class of presyngamic barrier is geographic isolation, of which the most extreme type is found in *L. cheesmanii*, which is restricted to the Galápagos archipelago. It can be readily crossed reciprocally with *L. esculentum*, *L. pimpinellifolium*, and other members of the *esculentum* complex. F_1 hybrids and subsequent generations are fully fertile in the sense that gamete abortion does not exceed that of the parents, but many segregants are unfruitful for other reasons. Poor fruit-set as well as weak growth of the majority of segregants when grown under field conditions at Davis reveal disharmonious interaction between genes of the parental species. Thus, despite the ease of hybridization and high fertility of the hybrids, geographic isolation may be reinforced by the aforementioned classes of postsyngamic obstacles. It is not known whether the same problems are exhibited in other environments, but it is entirely possible that the genetic disharmony is general and might serve as an effective barrier to gene exchange in the native environments.

Postsyngamic barriers take many forms and are subject to various intensities. The most prevalent types are embryonic breakdown after hybridization and hybrid sterility. The former characterizes the reaction when *L. cheesmanii, chmielewskii, esculentum, parviflorum*, and *pimpinellifolium* are pollinated by *L. chilense* and *peruvianum*. In those crosses, embryo development is blocked at very early stages with *peruvianum*, but variably later with *chilense* (Rick & Lamm 1955). The problem can be circumvented, albeit with great effort, by excising embryos and planting them in sterile culture media. After such embryo culture, the young plant can be transferred to soil, where it grows normally.

The reciprocal of these crosses generally fails to set fruit as a result of the incongruity reaction, hence it qualifies as a presyngamic type. This barrier has been circumvented ingeniously by Hogenboom (1972), who found that in certain selected self-fertile inbred lines of *L. peruvianum* the barrier was relaxed sufficiently to permit these lines to be used successfully as female parents. Lack of this reaction in some self-fertile lines demonstrates that the interspecific barrier is not determined solely by self-incompatibility. Another solution to the problem was formulated by Günther (1964) by synthesizing an *esculentum-peruvianum* chimera whose stylar reaction was like that of *esculentum* but whose sporogenous tissue (hence L_2) is *peruvianum*. This very useful combination has benefitted many workers by providing them with hybrids with desired *esculentum* lines.

Another form of postsyngamic obstruction is restricted recombination. Even though an interspecific hybrid might be sufficiently fertile to yield progeny and though chromosomes of the wild parent might be successfully backcrossed into the milieu of the cultivated parent, introgression will not be realized to the fullest extent if recombination is restricted. The best opportunity for testing this phenomenon was afforded by *esculentum-pennellii* hybrids (Rick 1969, 1971). By using *esculentum* stocks with multiple recessive markers on seven chromosomes, it was possible to introgress the intact *pennellii* homologues or homologous segments through five backcrosses and to compare segregation ratios and recombination frequencies with those of *esculentum* controls. Monogenic ratios were frequently distorted, favoring alleles of the recurrent parent. Recombination frequencies in the introgressed chromosomes were reduced in every tested chromosome to as low as 14% of controls. The reduction was greatest nearest the centromere and diminished distally to values not differing significantly from those of controls. Frequencies continued to diminish consistently from BC_1 to BC_3 and BC_5. In some instances reciprocal crosses differed significantly. Differential elimination of recombinant gametes and zygotic products could account for at least part of the reduction. According to certain evidence, the modifications of segregation and recombination are governed independently.

These observed modifications of segregation and recombination could have effects on population structure in the wild and in experimental cultures. Tighter linkages tend to preserve blocks of wild-parent genes intact, particularly in the vicinity of the centromere. The effect would obstruct the efforts of plant breeders to transfer single desirable genes and eliminate the remainder of the generally undesirable wild genes. This phenomenon probably accounts for the difficulties experienced by J.C. Gilbert of the University of Hawaii and others (personal communications) in breeding nematode-resistant lines with acceptable fruit type in the course of backcrossing the *Mi* gene from its *peruvianum* parent and possibly also for the apparently tightly linked loci controlling resistance reported by Sidhu & Webster (1975).

The logical material for investigating the developmental and evolutionary nature of postsyngamic blocks is found within, rather than between, species. An opportunity for such a study was provided by biotypes of the highly polymorphic *L. peruvianum* (Rick 1963). Amongst the many forms of this species, var. *humifusum* C.H. Mull., which is native to the north-east limits of the species, does not hybridize with the great majority of other *peruvianum* races. After such crosses, fruits set readily but breakdown in embryo development prevents the formation of any mature, viable seeds. The barrier is effective reciprocally, but the two crosses differ in development.

Exceptions to this behavior are found in the successful crosses between var. *humifusum* and certain accessions which are found in the region between the former and the remainder of the species. These biotypes are intermediate morphologically and genetically in the sense that they constitute a crossability bridge. The nature of the barrier can therefore be analysed genetically by hybridizing var. *humifusum* with the intermediate types and testing the consequent F_1 and segregating generations against typical *peruvianum*. Similarly, the progeny of intermediate × typical can be tested against *humifusum*. The results indicate that genetic control of the reactions is complex, but they do not permit an estimate of the number of responsible genes.

In all tests of the ability of these materials to cross, it is safe to generalize that the closer the biotypes are geographically, the greater the probability of hybridization. If the barrier arose by natural selection to prevent interbreeding of certain biotypes like *humifusum*, it would be expected to be strongest between nearby races. These observations and deductions, together with more recent studies on accessions from the margins of the *peruvianum* range, are therefore compatible with the hypothesis that the barriers arose, not as a primary isolating event, but secondarily after geographic isolation. Clearly other instances of intraspecific barriers should be investigated in order to ascertain how general the *humifusum* example might be for patterns of establishment of isolating mechanisms in tomato species.

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Patterns of variation of chromosal types in Lycopersicon and Solanum

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Index words

Solanum, Lypersicon, pachytene morphology, structural differences, heterochromatin.

Summary

The genus *Solanum* has existed for several millions of years, but the chromosome morphology of tuber-bearing *Solanums* are remarkably similar in many respects to those of the chromosomes of the related generas like *Lycopersicon* and *Neolycopersicon*. This similarity in chromosome morphology does not mean that there is no variation. A comparison of some of the homeologous chromosomes of *Lycopersicon, Neolycopersicon* and *Solanum* revealed certain differences in clearly recognisable, and constant heterochromatic blocks. Such differences can be observed in intergeneric, interspecific and intraspecific hybrids. The changes in heterochromatin appear to be unaccompanied by any major chromosomal structural changes. Since heterochromatin is genetically intert, the variation in heterochromatin might be of little evolutionary significance. Nevertheless, the heterochromatic blocks in some of the chromosomes may serve as reliable cytological markers to characterise and identify genomes in *Solanums*.

Introduction

According to a reasonable estimate the genus *Solanum* has existed for about 100 million years (Hawkes & Smith 1965). During this long period of evolution, the karyotypes of tuber-bearing *Solanums* have changed very little, if any, from the karyotypes of the related genera such as *Lycopersicon* and *Neolycopersicon*. The similarities between the pachytene chromosomes of *Lycopersicon* and *Solanum* was first discovered by Gottschalk (1954). This fact was further confirmed by a more direct comparison of homoeologous chromosomes at pachytene stage in the hybrids between *L. esculentum* × *S. lycopersicoides* (Menzel 1962), and *L. esculentum* × *S. pennellii* (Khush & Rick 1963). In these hybrids the chromosomes were also found to be completely synapsed.

The morphology of pachytene chromosomes of tomato and potato are in fact so similar, that the key for identification of the dihaploid-*S tuberosum* is nearly the same as that of tomato (Yeh & Peloquin 1965). However, in spite of such similarities between the chromosomes of different genera, there are also reports that indicate considerable differences between tuber-bearing *Solanum* species (Fielder & Shreiter 1959; Haynes 1964; Lam & Erickson 1971; Marks 1969). But these differences are mostly in chromosome lengths and arm ratios, which are highly variable at pachytene stage and therefore may not represent real differences between the karyotypes of different species.

During the past few years, I have examined the pachytene chromosomes of tomato as well as several wild and cultivated diploid species of potato (Ramanna & Prakken 1967; Ramanna & Wagenvoort 1977) and interspecific *Solanum* hybrids (Hermsen & Ramanna 1969; Ramanna & Hermsen 1971; Ramanna & Abdalla 1970; Ramanna & Hermsen 1976). On the basis of these studies, published and unpublished, this paper compares the karyotypes of *L. esculentum* and *Solanum* species and describes some of the variation in chromosome morphology between genera, between species and within species.

Material and methods

To reconstruct the standard karyotype of tomato, three cultivars of *L. esculentum*, viz. Moneymaker, Glory and San Marzono were used. Besides, two genetic stocks L-41 and LS-1 were used. The general karyotype of *Solanum* species was reconstructed after examining the pachytene chromosomes of three central American species, viz. *S. bulbocastanum, S. verrucosum* and *S. pinnatisectum*; four South American species, *S. phureja, S. chacoense* and *S. vernei* and *S. cansense*, and several di-aneuhaploids of *S. tuberosum* var. Gineke. The interspecific hybrids of *Solanum* that have been analysed are described elsewhere (Ramanna & Hermsen 1971, 1976 and 1977; Ramanna & Abdalla 1970).

The hybrid material of *L. esculentum* × *S. pinnellii* was obtained from Ir O.M.B. de Ponti (I.V.T., Wageningen).

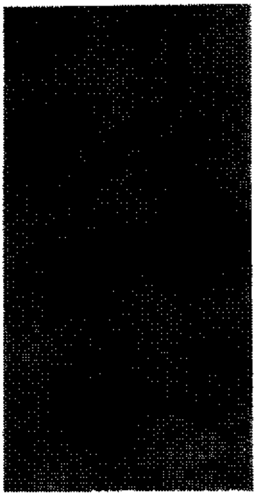
All the crosses between tomato cultivars and genetic stocks were made in the field in the summers of 1972, 1973 and 1974. The genetic stock *yv* was obtained from Prof. C.M. Rick (Davis, California).

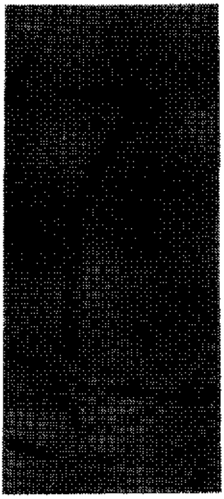
Cytological techniques for *Lycopersicon* and *Solanum* have been described previously (Ramanna & Prakken 1967; Ramanna & Wagenvoort 1977).

Results

Similarities and differences between the pachytene chromosome morphologies of Lycopersicon and Solanum

The species of both genera have a basic chromosome number of 12. The standard system of numbering of pachytene chromosomes of tomato is that described by Barton (1950), and the same system has been adapted for the dihaploid *S. tuberosum* (Yeh & Peloquin 1965). A comparison of chromosomes of the two genera, based on Barton's system of numbering, indicates that there are 9 asymmetric chromosomes with sub-median constriction (chromosomes, 1, 2, 3, 4, 6, 7, 8, 9 and 10) and 3 symmetric chromosomes with median constriction (5, 11, and 12). Morphologically the euchromatic (achromatic) and heterochromatic (chromatic) parts also show a striking correspondence between *Lycopersicon* and *Solanum*,


Money maker (mm)


L41


F_1 mm x L41

Figure 1. Standard chromosome 6 of tomato. Note the two heterochromatic blocks on the long arm.
Figure 2. Variant chromosome 6. Note only one heterochromatic block on the long arm of chromosome 6.
Figure 3. Heterozygous condition of the standard and the variant types.

which are commonly found also in *Neolycopersicon*, several wild diploid *Solanum* species, and several dihaploids and their derivatives of *S. tuberosum*.

There are a few major differences between the two genera in chromosomes 6 and 11. In *Lycopersicon* and *Neolycopersicon*, the asymmetric chromosome 6 is rather characteristic in having two blocks of heterochromatin on the long arm. (Fig. 1). Equivalents of this chromosome have never been found in *Solanum*, so far as examined. Also, in *Lycopersicon*, the long arm of chromosome 11 has a distinct gap in the heterochromatic part. Equivalents of this chromosome have not been recorded in *Solanum*. Except for these two major characteristic differences, the chromosomes of the two genera are remarkably similar in appearance.

From the morphological comparison alone one may not be able to detect small structural differences that might exist between the chromosomes of different genera. However, clear structural differences in the heterochromatic parts of pachytene bivalents have been recorded in intergeneric hybrids (Menzel 1962; Khush & Rick 1963).

Variation of chromosomes within and between species

Although the general features of the chromosomes of most of the diploid tuber-bearing *Solanum* species studied were alike, small differences between chromosomes of different species were clearly demonstrated. Such a difference has been reported for chromosome 11 of *S. pinnatisectum* and *S. bulbocastanum* (Ramanna & Hermsen 1976). Considerable variation for one and the same chromosome in different cultivars can be demonstrated as of the nucleolar chromosome (chromosome 2) of tomato where the length of the sat-region varied enormously between cultivars (Gottschalk 1954). So the chromosome variation observed between species may often not be of much significance for characterization of a species.

As in the nucleolar chromosome of tomato, variation can occur in other chromosomes in tomato too. Chromosome 6 (Fig. 1) has two blocks of heterochromatin on the long arm in the standard morphological type. This can be observed invariably in most of the cultivars. However, I found a rare variant of chromosome 6 (Fig. 2) in a genetic stock of *L. esculentum*. It had only one block of heterochromatin on the long arm. The nature of this variation will be described later.

As in tomato, variation in heterochromatic parts can also be observed within a species of *Solanum*. In *S. bulbocastanum* I have found three distinct types of chromosomes 11. These differ in the blocks of heterochromatin in the distal parts.

Nature of variation in heterochromatin

The differences in the heterochromatic parts in the chromosomes has been known for sometime (Gottschalk & Peters 1954; Menzel 1962; Khush & Rick 1963), but the nature of such differences has not been investigated. Differences in heterochromatin may represent structural differences in chromosomes such as transpositions, duplications or deletions, alternatively such differences result from tandem repeats of heterochromatic chromomeres; if so, the differences may not involve any structural alterations. To test between the alternatives, crosses were made between types with the standard chromosome 6 (cv. Moneymaker) and with the variant (L41). In this cross, the heterozygous condition for the heterochromatic block on the long arm of chromosome 6 was clearly recognizable in the F_1 (Fig. 3). When F_1 was backcrossed to the variant type (L41), a typical back-cross ratio (39 heterozygous for block as in Fig. 3, and 34 variant type as in Fig. 2) was observed. So the variant has a normal female transmission rate and, therefore might not have suffered any deletion or major structural change. This view was supported by crossing the variant with the genetic stock *yv*. The recessive gene *yv* has been localized by deletion mapping, around the distal heterochromatic block of the long arm of chromosome 6 (Khush & Rick, 1968). The F_1 of this cross was normal and did not express the recessive phenotype of *yv*. If there were a deletion, or normal allele YV were absent in the corresponding locus, the recessive phenotype would be expressed in F_1 because of hemizygous condition. Since the F_1 was normal, the variant had not suffered any structural alteration of the chromosome. So it could perhaps be an ancestral type; the standard chromosome might have arisen by tandem duplication of a heterochromatic chromosome in the distal part of the long arm.

Significance of variation in heterochromatin in Solanaceous species

It is well established that heterochromatin is genetically inert, or nearly so. Since Mendelian genes are absent in these regions, the alterations in heterochromatin might be of limited importance in speciation. However, extensive studies on the maize pachytene chromosomes have indicated that heterochromatic chromosome knobs are of considerable genetic significance (Galinat 1977). The chromosome knobs can restrict crossing over, and may favour 'block inheritance' of genes. Indirectly the knobs in maize may serve the same function of the small structural differences of chromosomes – which are known to suppress crossing over.

It is interesting to note that a disproportionately large number of marker genes in tomato are localized in the junctions between the heterochromatic and euchromatic portions in most of the chromosomes (Khush & Rick 1968). These junctions are also the ones that show considerable, but evasive, variation in the genome.

In spite of the fact that heterochromatin may not be of great evolutionary significance, it deserves attention for another reason. When properly recorded, heterochromatic blocks may be useful cytological markers that could be helpful in identification of chromosomes, and even of genomes.

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Significance of certain chromosomes of rye (Secale cereale L.) for the wheat improvement

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Index words

Wheat-rye chromosome substitution, wheat-rye chromosome translocation, disease resistance, breeding for grain yield.

Summary

Evidence is presented to show the significance of 1B/1R wheat–rye chromosome substitution and 6B/6R translocation lines for the improvement of both resistance to diseases and grain yield in wheat. In crosses of wheat × triticale substitution of 1B by the rye chromosome 1R is genetically and agronomically favoured.

Introduction

The cultivated rye is closely related to wheat (*Triticum aestivum* L. em. Thell.); five of the seven pairs of its chromosomes are known to compensate well for their missing homeologues in wheat. Individual rye chromosomes, however, vary greatly in their effects both on fertility and stability when present as disomic substitutions in wheat (Gupta 1971). Recent evidence (Gustafson & Bennett 1976) from triticale, which possesses complete sets of wheat and rye genomes, indicated that the rye chromosomes 1R and 6R were the most stable and co-adopted with wheat genomes; hence they may be of value in improvement of wheat.

The present paper considers the possible significance of the rye chromosomes 1R and 6R for improvement of grain yield and resistance to diseases in wheat.

Materials and methods

The cross Hope/Timstein//3*Heine-7/3/Riebesel 47–51/2*Heine-7/4/Cleo that involved the wheat–rye 1B/1R substitution line Riebesel 47/51 (Zeller 1973) was made in 1959 by Mr C.L. Zeeman at Cebeco Plant Breeding Station, then situated at Hoofddorp, with the aim of producing a high-yielding, diseasefree cultivar for commercial use. Three advanced (F_8) lines, selected by the pedigree method, that were equally resistant to the prevalent races of yellow and brown rusts and mildew, were yield-tested in the Netherlands during the years 1968–1971. The yield trials were conducted with normal seed rates in randomized complete block design with 10 m^2 plots, 2–4 replications at 1–3 locations, depending upon the stage of the trials. The lines were classified for the presence or absence of the rye chromosome 1R from Riebesel 47/51 on the basis of chromosome checks in root tips, and or scores in the United Kingdom of mildew and yellow rust.

Results and discussion

Among the three advanced lines in the main trials from the cross involving Riebesel 47/51, one line Cebeco 148 (cv. Clement) was found to possess resistance to yellow and brown rusts, and mildew, on the rye chromosome 1R, derived from Riebesel 47/51. The other two lines, Cebeco 144 and 150 did not derive their resistance to these diseases from the rye chromosome. All the three lines, however, were equally resistant in the Netherlands to the prevalent races of the wheat pathogens. Two lines, Cebeco 144 and 150, yielded, over 8 location-year tests during 1968–1971, 103 and 111, 2% respectively, of the standard cultivars Manella, Caribo and Lely, whereas Cebeco 148 (Clement) yielded 121% of the standards. All the three lines were similar for days to heading and plant heights and were in general agronomically acceptable. Data are still somewhat limited. The results, however, suggest that thee rye chromosome 1R can be exploited for breeding high-yielding cultivars of wheat. Support for this conclusion comes from the frequently high-yielding wheat cultivars bred in Europe using 1B/1R spontaneous chromosome substitution lines of wheat (Mettin et al. 1973; Zeller 1973).

However, only a few substitution lines have been used. New 1B/1R chromosome substitution lines have to be produced in order to diversify the source of resistance to the diseases. A cross between the winter wheat line Cebeco 180 and 'Armadillo' hexaploid triticale was made in this study. Selections among back-cross progenies for agronomic characters and for resistance to wheat mildew resulted in a normal plant with rye chromosome 1R from triticale substituted for chromosome 1B. Introduction of the rye chromosome 1R into wheat was compatible with agronomic desirability in wheat and supported the conclusion of Gustafson & Bennett (1976), in that, when both natural and artificial selection coincided in favouring a particular substitution, it was quickly fixed in the breeding process. Many genetic events might work together to fix the 1R chromosome in wheat–rye crosses. According to Scoles & Kaltsikes (1974), in the presence of wheat–rye genomes, as it occurs in triticale, aneuploids for the chromosome 1B are frequently produced. Deficiency, especially for 1B chromosome, is transmitted through male gametes in high frequencies, apparently compensated by the presence of 1R chromosome from rye. The chromosome 1B^s of wheat carries a gene inhibiting pairing of rye chromosomes (Scoles & Kaltsikes 1974), absence of which may be expected to lead to greater stability of rye chromosome(s) in wheat. Compared with the other rye chromosomes, chromosome 1R causes the least meiotic disturbance in wheat (Riley 1960).

The wheat line 1222, homozygous for 6B/6R wheat–rye translocation (obtained from Dr N.A. Tuleen, Texas A & M University, College Station, Texas, U.S.A.), was found under Dutch conditions to be resistant to mildew. Initial observations indicated normal fertility and normal transmission of the rye segment through the male gametes in this line in contrast to observations by Riley (1960) with 6B/6R wheat–rye disomic substitution lines, which had reduced pollen fertility. Initial crosses of this line with normal wheat lines in this study appeared promising.

Disease resistance

The chromosomes 1R and 6R from rye are especially attractive to wheat breeders in that they carry genes for resistance to the wheat pathogens yellow, brown and stem rusts, and mildew (Mettin et al. 1973; Zeller 1973). The nature of the genes, in durability and stability to a new virulent race of the wheat pathogens, may not be completely different from that found in *Triticum* spp. Rye, however, offers some additional advantages. The resistance genes on a rye chromosome normally behave as linked genes in wheat. Incorporation of the gene resistant to a particular disease from rye would thus allow simultaneous introduction of gene(s) for resistance to the other diseases on the same chromosome. The linked disease-resistance gene can be used as a marker gene to facilitate combining of different major genes into one line in order to provide better protection against the new virulent races of the wheat pathogens.

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Genetic methods of overcoming breeding barriers

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Exploitation of incongruity, a new tool for hybrid seed production

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Index words

Incongruity, matching genic systems, regulation of fertilization, hybrid seed production, introgression.

Summary

Incongruity, besides being treated as a cause of problems in interspecific crosses, can also be exploited for the regulation of fertilization.

Models are shown to demonstrate how barrier genes and corresponding penetration genes can be transferred from one species to another and how these genes may be used in hybrid seed production. Advantages of the system and difficulties to be expected in its exploitation are mentioned and results of research on *Lycopersicon* accessions are discussed.

Introduction

The intimate relationship between pistil and pollen was earlier described in evolutionary terms as an increasingly specialized interaction, as two chains of accurately coordinated processes, based on matching genic systems in the partners and resulting from co-evolution of pistil and pollen (Hogenboom 1973; 1975). Incongruity in this relationship, resulting from evolutionary divergence of populations, was defined as non-functioning through a lack of genetic information in one partner about some relevant characteristic of the other. The relationship was then said to be incomplete. Research produced interesting results and allowed reinterpretation of earlier results, especially on the genetics of incongruity (Hogenboom 1972; 1975; Hermsen et al. 1974; 1978; Hermsen this conference). For the plant breeder, the naturally and frequently occurring incongruity constitutes a problem, namely a barrier, which can appear in different forms and limits exploitation of the rich sources of genetic material in species related to the crop. But incongruity can also be exploited in plant breeding for regulation of fertilization in hybrid seed production. This paper demonstrates how.

The principle is simple: from a related species, an extra barrier gene is introduced into lines of the crop to be used as female parents of hybrid cultivars. The corresponding penetration gene(s) is (are) introduced into the male parent lines.

Models for introgression of barrier and penetration genes

The following models for the transfer from one species to another of barrier genes and corresponding penetration genes and for the use of these genes in hybrid seed production are concerned with diploid plants.

In the letter models for the matching genic systems of pistil and pollen and for incongruity in the relationship (Hogenboom 1973, 1975), we deal with the barrier capacity of the pistil, b, based on barrier genes or gene complexes AA to ZZ and with the penetration capacity of the pollen, p, based on penetration genes or gene complexes *AA* to *ZZ*. The capacities are indicated by their first letters only. Absence of a dominant gene can also be indicated by the corresponding small letter.

In intimate partner relationships, four gene systems of interaction may be found: (1) gene-for-gene; (2) genes-for-gene; (3) gene-for-genes; and (4) genes-for-genes. The models to be treated hereafter will consider only simple situations that are most likely to have practical application.

Model 1

In the simplest case, the barrier capacities of the two related species differ by only one gene, which corresponds to one dominant penetration gene. The course of the program of introgression is then as follows.

The two species, P_1 (the crop species) and P_2 (the wild relative), showing unilateral incongruity, are crossed and the F_1 is backcrossed to P_1:

P_1 b:aa,p: *aa* × P_2 b:AA,p: *AA* → F_1 b:Aa,p: *Aa*
P_1 b:aa,p *aa* × F_1 b: Aa,p: *Aa* → B_1 $\frac{1}{4}$ b: Aa,p: *Aa* + $\frac{1}{4}$ b: Aa,p: *aa* + $\frac{1}{4}$ b:aa,p: *Aa* + $\frac{1}{4}$ b:aa,p: *aa*

For the development of the female parent lines, the second genotype in B_1, the only one showing self-incongruity, is selected. It is backcrossed to P_1:

P_1 b:aa,p: *aa* × B_1-selection b:Aa,p: *aa* → B_2 $\frac{1}{2}$.b:Aa,p: *aa* + $\frac{1}{2}$ b:aa,p: *aa*

The first genotype in B_2, showing self-incongruity, is selected and the series of backcrosses to P_1 is continued. The result is a line isogenic to P_1 with b:Aa,p: *aa*. It shows self-incongruity and can serve as a female parent for hybrid seed production. For its maintenance, one may choose backcrossing to P_1, or self-fertilization after temporary inactivation of the introduced barrier gene A by environmental influence, or vegetative reproduction. In the first case the following genotypes are obtained: $\frac{1}{2}$ b:Aa,p: *aa* + $\frac{1}{2}$ b:aa,p: *aa*, in the second: $\frac{1}{4}$ b:AA,p: *aa* + $\frac{2}{4}$ b:Aa,p: *aa* + $\frac{1}{4}$ b:aa,p: *aa*. Selection of the self-incongruous plants would imply that one has to wait until anthesis. This disadvantage can be overcome by selecting a seedling marker gene linked to the barrier gene A.

For the development of the male parent lines for hybrid seed production in B_1

the first genotype (b:Aa,p: *Aa*) is selected. It is the only one showing the combination of self-congruity and unilateral incongruity with P_1. This type is backcrossed to P_1:

P_1 b:aa,p: *aa* × B_1-selection b:Aa,p: *Aa* → B_2 $\frac{1}{4}$ b:Aa,p: *Aa* + $\frac{1}{4}$ b:Aa,p: *aa* + $\frac{1}{4}$ b:aa,p: *Aa* + $\frac{1}{4}$ b:aa,p: *aa*

Again the first genotype is selected on the basis of the combination of self-congruity and unilateral incongruity with P_1 and the series of backcrosses to P_1 is continued, resulting in a line isogenic to P_1 with b:Aa,p: *Aa*. Self-fertilization then gives the next six genotypes (pollen with p: *a* not taking part in fertilization):

$\frac{1}{8}$ b:AA,p: *AA* + $\frac{1}{8}$ b:AA,p: *Aa* + $\frac{2}{8}$ b:Aa,p: *AA* + $\frac{2}{8}$ b:Aa,p: *Aa* + $\frac{1}{8}$ b:aa,p: *AA* + $\frac{1}{8}$ b:aa,p: *Aa*

The last two genotypes can be selected as only these accept pollen of P_1. One of the two, b:aa,p: *AA* can further be selected by the method of '2-allele congruity' (i.e. under ultraviolet microscope, using plants with b:AA or b:Aa as female tester, one selects those plants of which all pollen grows through).

The result of this procedure is a line isogenic to P_1 with b:aa,p: *AA*. It can be maintained by selfing and be used as a male parent line for hybrid seed production.

In the above procedure for the development of the male parent, in B_1 b:Aa,p: *Aa* is selected and the presence of barrier gene A is used in the further program to test the presence of penetration gene *A*. But this is not the only way. In the backcross progenies, one could also select plants of which pollen is accepted on P_2. These have p: *Aa*. After the series of backcrosses, it is probable that only b:aa,p: *Aa* plants are left. After selfing, the b:aa,p: *AA* plants can be selected as above.

The genetic constitution for barrier and penetration capacities of the hybrid varieties to be produced depends on how the female parent is maintained and will be

b:Aa,p: *aa*× b:aa,p: *AA* → F_1 $\frac{1}{2}$ b:Aa,p: *Aa* + $\frac{1}{2}$ b:aa,p: *Aa* or
($\frac{1}{3}$ b:AA,p: *aa* + $\frac{2}{3}$ b:Aa,p: *aa*) × b:aa,p: *AA* → F_1 $\frac{2}{3}$ b:Aa,p: *Aa* + $\frac{1}{3}$ b:aa,p: *Aa*

For certain crops, in which the pollen is very important for production, we see the disadvantage that part of the pollen does not function in some plants.

Somewhat more complicated but not essentially different is the program of introgression if the barrier capacities of the two species differ by more than one gene.

Model 2

If the barrier capacities of the species P_1 and P_2 differ by two genes, each corresponding to one penetration gene, the program is as follows:

P_1 b:aabb,p: *aabb* × P_2 b:AABB,p: *AABB* → F_1 b:AaBb,p: *AaBb*

In B_1 ($P_1 \times F_1$), we get combinations of 1 gamete type from P_1 with 16 from the F_1, giving the following genotypes:

b:AaBb,p: *AaBb*	self-congruity	not accepting pollen from P_1
b:AaBb,p: *Aabb*	self-incongruity	
b:AaBb,p: *aaBb*	self-incongruity	
b:AaBb,p: *aabb*	self-incongruity	
b:Aabb,p: *AaBb*	self-congruity	
b:Aabb,p: *Aabb*	self-congruity	
b:Aabb,p: *aaBb*	self-incongruity	
b:Aabb,p: *aabb*	self-incongruity	
b:aaBb,p: *AaBb*	self-congruity	
b:aaBb,p: *Aabb*	self-incongruity	
b:aaBb,p: *aaBb*	self-congruity	
b:aaBb,p: *aabb*	self-incongruity	
b:aabb,p: *AaBb*	self-congruity	accepting pollen from P_1
b:aabb,p: *Aabb*	self-congruity	
b:aabb,p: *aaBb*	self-congruity	
b:aabb,p: *aabb*	self-congruity	

From these genotypes we take those with a barrier capacity differing by only one gene from that of P_1. Thus the subsequent program is made as simple as the one in Model 1. Therefore we select the genotypes that combine self-congruity, non-acceptance of pollen from P_1 and incongruity as male on P_2. These are b:Aabb,p: *Aabb* (1) and b:aaBb,p: *aaBb* (2).

The program is continued with genotype 1 and/or 2. The choice depends on the barrier qualities of genes A and B. For the development of the female parent lines the penetration gene *A*, or *B*, must be removed from genotype 1 or 2, respectively. This can be done in the next backcross generation:

P_1 b:aabb,p: *aabb* × genotype (1) from B_1 b:Aabb,p: *Aabb* → B_2 $\frac{1}{4}$ b:Aabb,p: *Aabb* + $\frac{1}{4}$ b:Aabb,p: *aabb* + $\frac{1}{4}$ b:aabb,p: *Aabb* + $\frac{1}{4}$ b:aabb,p: *aabb*

The second genotype in B_2, the only one showing self-incongruity, is selected and further treated as in Model 1.

For the development of the male parent lines we take the same genotype 1 or 2 from B_1 and treat it as in Model 1. Alternatively, from the backcross progenies, select those plants of which pollen is accepted on P_2 and after selfing select plants with p: *AABB*.

In Models 1 and 2, we treated the species P_1 and P_2 as fully homozygous. Especially in cross-fertilizing species a certain heterozygosity for certain barrier genes may be expected. This may lead to segregation of different barrier capacities in F_1. Therefore in F_1, the plants should be tested for self-congruity and for non-acceptance of pollen from P_1. Plants showing this combination will then be treated as in Model 1.

Model 3

If between the species P_1 and P_2, there is a one-gene difference in barrier capacity and the gene corresponds to two penetration genes, the program of introgression is as follows:

P_1 b:aa,p: $a_1a_1a_2a_2$ × P_2 b:AA,p: $A_1A_1\ A_2A_2 \rightarrow F_1$ b:Aa,p: $A_1a_1A_2a_2$

In B_1 (P_1 × F_1), we obtain the next 8 genotypes:

$$\left.\begin{array}{lll}\text{b:Aa,p:} & A_1a_1A_2a_2 & \text{self-congruity}\\ \text{b:Aa,p:} & A_1a_1a_2a_2 & \text{self-incongruity}\\ \text{b:Aa,p:} & a_1a_1A_2a_2 & \text{self-incongruity}\\ \text{b:Aa,p:} & a_1a_1a_2a_2 & \text{self-incongruity}\end{array}\right\}\ \text{not accepting pollen from } P_1$$

$$\left.\begin{array}{lll}\text{b:aa,p:} & A_1a_1A_2a_2 & \text{self-congruity}\\ \text{b:aa,p:} & A_1a_1a_2a_2 & \text{self-congruity}\\ \text{b:aa,p:} & a_1a_1A_2a_2 & \text{self-congruity}\\ \text{b:aa,p:} & a_1a_1a_2a_2 & \text{self-congruity}\end{array}\right\}\ \text{accepting pollen from } P_1$$

For the development of the female parent lines, one takes one of the genotypes showing self-incongruity and continues the backcross program to P_1. In B_x the most probable combination of genotypes (because of a high probability of loss of penetration gene A_1 or A_2) is: $\frac{1}{2}$ b:aa,p: $a_1a_1a_2a_2$ + $\frac{1}{2}$ b:Aa,p: $a_1a_1a_2a_2$; the second genotype, showing self-incongruity, is used as female parent line for hybrid seed production.

To develop the male parent lines, in B_1 one selects the genotype with the combination of self-congruity and non-acceptance of pollen from P_1. This is b:Aa,p: $A_1a_1A_2a_2$. The backcross program is continued as in Model 1 and then b:Aa,p: $A_1a_1A_2a_2$ is selfed, giving rise to 12 genotypes, only pollen with p: A_1A_2 taking part in fertilization:

$\frac{1}{16}$ b:AA,p: $A_1A_1A_2A_2$ + $\frac{1}{16}$ b:AA,p: $A_1a_1A_2A_2$ + $\frac{1}{16}$ b:AA,p: $A_1A_1A_2a_2$ + $\frac{1}{16}$ b:AA,p: $A_1a_1A_2a_2$ + $\frac{2}{16}$ b:Aa,p: $A_1A_1A_2A_2$ + $\frac{2}{16}$ b:Aa,p: $A_1a_1A_2A_2$ + $\frac{2}{16}$ b:Aa,p: $A_1A_1A_2a_2$ + $\frac{2}{16}$ b:Aa,p: $A_1a_1A_2a_2$ + $\frac{1}{16}$ b:aa,p: $A_1A_1A_2A_2$ + $\frac{1}{16}$ b:aa, p: $A_1a_1A_2A_2$ + $\frac{1}{16}$ b:aa,p: $A_1A_1A_2a_2$ + $\frac{1}{16}$ b:aa,p: $A_1a_1A_2A_2$

A quarter of this material accepts pollen from P_1. From these plants, one selects those of which on P_2 all pollen tubes take part in fertilization. These plants have the genotype b:aa,p: $A_1A_1A_2A_2$. Again the alternative is to select from the backcross progenies the p: $A_1a_1A_2a_2$ plants by testing acceptance of pollen on P_2. The b:aa,p: $A_1A_1A_2A_2$ genotype can then be selected as above. If several penetration genes correspond to one barrier gene, the possible disadvantage of non-functioning of pollen in the hybrid is more serious. For instance, in the example, in half of the hybrid plants only 25% of the pollen functions.

Accordingly as the desired extra barrier gene in P_2 corresponds to a higher number of penetration genes, in the F_1 the frequency of pollen with complete penetration capacity will be lower. This means that the degree of self-congruity in F_1 may be lower and that in B_1 the probability of finding the combination of self-congruity and non-acceptance of pollen from P_1 is smaller.

Model 4

If the barrier capacities of the species P_1 and P_2 differ by 2 genes, A_1 and A_2, which correspond to one penetration gene A (implying complementary action of barrier genes A_1 and A_2), the program of introgression is as follows:

P_1 b:$a_1a_1a_2a_2$,p: aa × P_2 b:$A_1A_1A_2A_2$,p: AA → F_1 b:$A_1a_1A_2a_2$,p: Aa

In B_1 (P_1 × F_1), we obtain 8 genotypes:

$$\left.\begin{array}{l} \mathrm{b{:}}A_1a_1A_2a_2,\mathrm{p{:}}\ Aa \text{ self-congruity}\\ \mathrm{b{:}}A_1a_1A_2a_2,\mathrm{p{:}}\ aa \text{ self-incongruity}\end{array}\right\}\text{ not accepting pollen from } P_1$$

$$\left.\begin{array}{l} \mathrm{b{:}}A_1a_1a_2a_2,\mathrm{p{:}}\ Aa \text{ self-congruity}\\ \mathrm{b{:}}A_1a_1a_2a_2,\mathrm{p{:}}\ aa \text{ self-congruity}\\ \mathrm{b{:}}a_1a_1A_2a_2,\mathrm{p{:}}\ Aa \text{ self-congruity}\\ \mathrm{b{:}}a_1a_1A_2a_2,\mathrm{p{:}}\ aa \text{ self-congruity}\\ \mathrm{b{:}}a_1a_1a_2a_2,\mathrm{p{:}}\ Aa \text{ self-congruity}\\ \mathrm{b{:}}a_1a_1a_2a_2,\mathrm{p{:}}\ aa \text{ self-congruity}\end{array}\right\}\text{ accepting pollen from } P_1$$

For the development of the female parent lines, the program is continued with the genotype showing self-incongruity as in Model 1.

To develop the male parent lines, the program is continued with the combination of self-congruity and non-acceptance of pollen from P_1, as in Model 1. At the end of the program, after selfing the last backcross progeny, one selects the plants with p: AA by the method of two-allele congruity. Again, the p: AA plants can also be developed by testing acceptance of pollen on P_2, as in the other models.

For each genetic situation, a model can thus be given. They all result in one or more axtra barrier genes in the female parent line and the corresponding penetration gene(s) in the male parent lines. Table 1 lists frequencies of the wanted genotypes in the first backcrosses for some simple genetic constitutions of parents, differing by 1, 2 and 3 genes in barrier capacities which genes correspond to 1, 2 and 3 penetration genes, respectively. More complicated situations were not treated as many suppositions are then necessary.

In the models, we dealt only with dominant penetration genes, although in the model for incongruity (Hogenboom 1973; 1975) it was supposed that penetration genes could be recessive too. The models could all be rewritten for recessive penetration genes. Model 1, for instance, will then, if one barrier gene A corresponds to one penetration gene a, give the following program:

P_1 b:aa,p: AA × P_2 b:AA,p: aa → F_1 b:Aa,p: Aa

P_1 b:aa,p: AA × F_1 b:Aa,p: Aa → B_1 $\frac{1}{4}$ b:Aa,p: AA + $\frac{1}{4}$ b:aa,p: AA + $\frac{1}{4}$ b:Aa,p: Aa + $\frac{1}{4}$ b:aa,p: Aa

Table 1. Barrier and penetration capacities of parent species P_1 and P_2 and some frequencies of genotypes in the first back-crosses to P_1. Barrier and penetration genes taken as dominant.

				Development of ♀ parent lines					Development of ♂ parent lines				
P_1		P_2		genotype		expected frequency in			genotype		expected frequency in		
b:	p:	b:	p:	b:	p:	B_1	B_2	B_3	b:	p:	B_1	B_2	B_3
aa	*aa*	AA	*AA*	Aa	*aa*	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	Aa	*Aa*	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
aa	$a_1a_1a_2a_2$	AA	$A_1A_1A_2A_2$	Aa	$a_1a_1a_2a_2$	$\frac{1}{8}$	$\frac{1}{3}$	$\frac{5}{12}$	Aa	$A_1a_1A_2a_2$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$
					$a_1a_1A_2a_2$	$\frac{1}{8}$	$\frac{1}{12}$	$\frac{1}{24}$					
					$A_1a_1a_2a_2$	$\frac{1}{8}$	$\frac{1}{12}$	$\frac{1}{24}$					
aa	$a_1a_1a_2a_2a_3a_3$	AA	$A_1A_1A_2A_2A_3A_3$	Aa	$a_1a_1a_2a_2a_3a_3$ $A_1a_1a_2a_2a_3a_3$ $A_1a_1A_2a_2a_3a_3$ $A_1a_1a_2a_2A_3a_3$ $a_1a_1A_2a_2a_3a_3$ $a_1a_1A_2a_2A_3a_3$ $a_1a_1a_2a_2A_3a_3$	7x $\frac{1}{16}$	$\frac{1}{2}$	$\frac{1}{2}$	Aa	$A_1a_1A_2a_2A_3a_3$	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
$\mathrm{a_1a_1a_2a_2}$	*aa*	$\mathrm{A_1A_1A_2A_2}$	*AA*	$\mathrm{A_1a_1A_2a_2}$	*aa*	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\mathrm{A_1a_1A_2a_2}$	*Aa*	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$
aabb	*aabb*	AABB	*AABB*	Aabb	*Aabb*	$\frac{1}{16}$	$\frac{1}{4}$[1]	$\frac{1}{2}$[1]	Aabb	*Aabb*	$\frac{1}{16}$	$\frac{1}{4}$	$\frac{1}{4}$
				aaBb	*aaBb*	$\frac{1}{16}$	$\frac{1}{4}$[1]	$\frac{1}{2}$[1]	aaBb	*aaBb*	$\frac{1}{16}$	$\frac{1}{4}$	$\frac{1}{4}$
$\mathrm{a_1a_1a_2a_2a_3a_3}$	*aa*	$\mathrm{A_1A_1A_2A_2A_3A_3}$	*AA*	$\mathrm{A_1a_1A_2a_2A_3a_3}$	*aa*	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{8}$	$\mathrm{A_1a_1A_2a_2A_3a_3}$	*Aa*	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$

[1] In B_1 p: *aabb* is selected, see Model 2.

Table 2. Results of tests with F_1 (*L. esculentum* cv. Stupicke polni × *L. parviflorum*) and backcrosses to *L. esculentum* for development of ♀ parent lines, after pollination with *L. esculentum* pollen (x P_1) and after self-pollination (⊗). SI = self-incongruity; SC = self-congruity; (n.)a. = (non-) acceptance of pollen from P_1; ? = doubtful result.

Material		Treatment	Distribution of plants over incongruity classes in mean number of pollen tubes per stylar base										Number of plants showing			
			0	5	10	15	20	25	30	40	50		SI + n.a.	SI + a.	SC + n.a.	SC + a.
F_1	731090	x P_1	3	14	1	2							–	–	18	–
		⊗						3	4	8	3					
B_1	731107	x P_1		9	7	6	9	2	3	6	9	96	7	5?	23	112
		⊗		5	4	3	2	4	2	1	4	144				
B_2	742400	x P_1	1	6	8	2			1	3	9	131	5	–	12	146
		⊗		6								176				
B_3	762150	x P_1										53	–	–	–	53
		⊗										57				
B_3	762151	x P_1			1		1			3		129	1	–	1	132
		⊗		1								141				
B_4	78148	x P_1		1	1	4		2		3	3	68	4	2?	5	69
		⊗		7								77				

Table 3. Results of tests with backcrosses of F_1 (*L. esculentum*, mutant bs bs × *L. parviflorum*) to *L. esculentum* for development of ♂ parent lines, after pollination with *L. esculentum* pollen (x P_1) and after self-pollination (⊗). Abbreviations as in Table 2.

Material	Treatment	Distribution of plants over incongruity classes in mean number of pollen tubes per stylar base										Number of plants showing			
		0		5	10	15	20	25	30	40	50	SI + n.a.	SI + a.	SC + n.a.	SC + a.
B_1 731116	x P_1		4		2			1	3	2	3				
	⊗	1	1		2	5	2	3	5	4	4				
B_2 742401	x P_1	5	13	2	2		1	2	2	7	26	2	1?	20	38
	⊗		3								90				
B_3 761017	x P_1				1				8	3	39	–	–	1	50
	⊗										57				
B_3 761018	x P_1				1	1		1	4	8	35	–	–	2	48
	⊗										56				

To develop the female parent lines, the genotype b:Aa,p: *AA* (self-incongruity + non-acceptance of pollen from P_1) is selected and further backcrossed and treated as in Model 1. For the development of the male parent lines, b:aa,p: *Aa* is selected and further treated. In the same way the other models can be rewritten for recessive penetration genes. Also the possibility of certain penetration genes being dominant and others recessive can be taken into account.

Results with accessions of Lycopersicon Mill

In the genus *Lycopersicon*, we are trying to exploit the unilateral incongruity found between the self-compatible and self-fertilizing cultivated tomato, *L. esculentum* Mill. and accession LA 1045 66L827 of the self-compatible relative *L. parviflorum* Rick sp. nov. The unilateral incongruity in this accession is expressed during pollen tube growth, resulting in a gradually thinning bundle of pollen tubes. Because of a sometimes incomplete stop of pollen tube growth, the material may not be ideal for our purpose. Tables 2 and 3 give the results of the tests for self-(in)congruity and for (non-)acceptance of pollen of *L. esculentum*. Methods used were described earlier (Hogenboom 1972). To develop female parent lines, we selected in each backcross the combination of self-incongruity and non-acceptance of pollen from P_1 (Table 2), and for development of the male parent lines the combination of self-congruity and non-acceptance of pollen from P_1 (Table 3).

In the F_1, all plants showed seld-congruity and non-acceptance of pollen from P_1. In B_1, clear-cut segregations were not always found, although results of different seasons differed. All degrees of self-congruity and of acceptance of pollen from P_1 were present. In B_2, the segregation for both characters seemed clearer. In B_3, the extra barrier genes were lost or scarce. In B_4, we found clear segregation of a higher frequency of self-incongruity and non-acceptance of pollen from P_1. In the backcross program, we could maintain the combination of self-incongruity and non-acceptance, for development of female parent lines, as well as that of self-congruity and non-acceptance, for development of male parent lines.

Discussion and conclusions

Until now, breeders have used male sterility, self-incompatibility and femaleness as tools in hybrid seed production. These principles could generally only be applied after much research effort, looking for suitable material, analysing the genetics of the systems and selecting those genes with the maximum expression. Each system has advantages and disadvantages, in the origin of the character (intraspecific or interspecific), the genetics of the character, the detectability of the wanted genotypes and the expression of the desired genes. It is difficult to weigh these advantages and disadvantages as they depend largely on specific crop characters.

An advantage of incongruity may be its general applicability. Many crops have related species or populations, with which they show some form of incongruity. Many of these situations are already known. Another advantage of this principle may be that only one gene is needed per crop. Also when incongruity is based on more genes, one of these can be selected during the backcross program on the basis of its barrier quality and ultimately used. Probably a one barrier gene-for-one penetration gene situation can often be found or created.

A disadvantage of incongruity may be its interspecific origin. Interspecific crosses often result in complex genetical situations and abnormal interactions and disturbing principles may well cause serious deviations from expected results. For instance, the loci for extra barrier genes in P_2 may not always be available in P_1, giving rise to structural hybridity. Segregations of genes, also in relation to incongruity, depend on degree of recombination and disturbance by modifying factors. The applicability of incongruity will mainly depend on the expressivity of the introduced barrier gene. As this gene meets a different genetic background, its behavior may be strongly influenced. The results so far with *Lycopersicon* accessions cannot be fully explained by the given models. The results in F_1 agree with all models. That no clear segregations were obtained in B_1 may not be surprising. Yet, for B_1 731107, in an experiment in 1978 a clear segregation was obtained of 4 plants with combination SI + n.a., O SI + a., 17 SC + n.a. and 53 SC + a. These data suggest a possibility not considered in any of the models: two complementary barrier genes corresponding to one or two penetration genes. If so, $\frac{1}{4}$ of the plants in B_1 would be n.a. Of these, a quarter would then show self-incongruity. Three quarters of all plants would be SC + a.

That the segregations became clearer in further backcrosses may be expected, but the low frequency of self-incongruity and non-acceptance in these generations is still difficult to explain. Significantly the wanted combinations of characters (SI + n.a. and SC + n.a.) could be maintained. Further research should reveal whether the obtained results depend largely on the interspecific situation or whether other models apply. Although much remains unknown, the scope for application of incongruity and the results obtained sofar justify further research.

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Factors controlling interspecific crossability and their bearing on the strategy for breaking barriers to intercrossing of tuber-bearing Solanum species

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Index words

Interspecific crossability, reciprocal differences, 2*n* gametes, bridging species, potato, *Solanum*.

Summary

New evidence is presented that interspecific crossability is controlled by the genotypes of both parental species. Some results from intercrossing *Solanum verrucosum* and diploid *S. tuberosum* suggest a gene-for-gene relationship between barrier genes in the pistil parent and penetration genes in the pollen parent, only matching genotypes allowing normal growth of pollen tubes in the style.

Numerically unreduced (or 2*n*) gametes occur in many plant species. Such gametes may be a disadvantage in those interspecific hybrids, the chromosome number of which has to be doubled before further crosses are successful.

The use of bridging species, though complicating the breeding programme, is sometimes unavoidable. The procedure can be made more efficient by accumulating valuable genes in the wild species ('pre-breeding') before starting interspecific crosses. Some examples are presented of limitations and difficulties that the breeder may face in remote hybridization.

Wide crosses may require specific sets of environmental conditions to succeed. So it is recommended to continue to attempt difficult crosses for a long time.

The main conclusions from the foregoing considerations are presented as a series of propositions on interspecific hybridization.

Introduction

Many crops need related species to improve them. If species cannot be hybridized with a crop, such species are worthless for improvement. Crossability between species is primarily under genetic control, but environmental conditions also affect it. In addition to genotype and environment, the following factors are relevant to crossability: fertility and vigor of parent species and the nature and level of their ploidy, age and morphology of flowers and gametes, and last but not least the closeness of genic and genomic relationships between the parents.

Crossability may be defined as the probability of fertilization after pollination. However, such definition does not take into account barriers after fertilization. The definition 'probability of obtaining viable hybrids after pollination' is to be preferred. When the probability is zero, there is non-crossability or incongruity. In practice, it is difficult to prove unequivocally that two related species are uncrossable. The statement 'these related species cannot be intercrossed' has to be qualified by adding: with the available genotypes, under the prevailing environmental conditions, with or without special devices in vivo or in vitro. For the same reasons, one has to be careful in drawing conclusions from a study of inheritance of crossability between species. Genetic and cytological research on interspecific crossability may shorten the way to success, because it may reveal more efficient crossing procedures, create genotypes bridging gaps between species and also deepen the insight into general principles underlying interspecific barriers. These considerations constitute the background of the present paper.

Material and methods

The crop to be improved was the cultivated autotetraploid potato, *Solanum tuberosum* L. ($2n = 4x = 48$) and its dihaploids ($2n = 2x = 24$). Dihaploids can be produced from potato cultivars through induction of parthenogenesis and are designated 2x S. *tuberosum*. The species used in the tests belong to the tuber-bearing relatives of the potato. These species produce tubers, except *S. brevidens* and *S. etuberosum*, which only on the basis of morphological similarities are included in the section of tuber-bearing *Solanum* species.

Solanum species are usually indicated by three-letter abbreviations as proposed by Simmonds (1963). The following species (abbreviations in brackets) are mentioned in this article:

Polyploids ($2n = 4x$ or $6x$)

S. acaule (*acl*, allotetraploid)
S. demissum (*dms*, allohexaploid)
S. stoloniferum (*sto*, allotetraploid)
S. tuberosum (*tbr*, autotetraploid)

Diploids ($2n = 2x = 24$)

S. brevidens (*brd*)
S. bulbocastanum (*blb*)
S. chacoense (*chc*)
S. etuberosum (*etb*)
S. gourlayi (*grl*)
S. phureja (*phu*)
S. pinnatisectum (*pnt*)
S. vernei (*vrn*)
S. verrucosum (*ver*)

Crosses were attempted over a period of 3–4 months in a greenhouse in which too high a temperature and too low humidity was avoided by passing air through a film of cooled water by means of big fans. Flowering time of potato species is normally several weeks but may be extended to several months by grafting them onto tomato rootstock and regularly removing axillary shoots.

Genetic control of reciprocal differences in interspecific crossability

Reciprocal differences in crossability are rather common in interspecific hybridization. Different factors may cause reciprocal differences. When intercrossing short-styled *acl* with long-styled *blb*, the cross was successful only with *acl* as a female.

Table 1. Three types of reciprocal crosses involving *S. verrucosum (ver.)*. + = successful cross; – = cross failed; +, – = success with some; failure with other species.

	Crossability		
	Type 1	Type 2	Type 3
ver ♀ x species	+	+	+
species x *ver* ♂	+	–	+, –

Presumably pollen from the short-styled *acl* cannot form long pollen tubes, which are needed to grow through the long styles of *blb*. However, in addition to this morphological factor Hermsen (1966) found that both *acl* and *blb* fell into 'crossability groups': the groups of *acl* differed significantly in their crossability as females with *blb*, whereas the *blb* groups differed clearly in crossability as males with *acl*. Although no detailed genetic analysis was made, the group differences are presumably genetically controlled.

The genetics of interspecific crosses involving *ver* was examined in detail. Three types of crossability were detected (Table 1), of which Type 2 was most common. Reciprocal crossability with *ver* (Type 1) occurred with spp. *grl* and *vrn*. Type 3 was found with spp. *chc* and 2*x tbr*. The characteristic of Type 3 is that within *chc*, and within 2*x tbr* some genotypes, called acceptors, accept *ver* pollen, whereas other genotypes (non-acceptors) reject it. It is questionable whether the species showing Types 1, 2 and 3, respectively, with *ver*, are basically different. The occurrence of species with Type 3 suggests, that the differences between Types 1 and 2 are only quantitative in that among the species with Type 1 the genotypes accepting *ver* pollen are predominant, whereas among species with Type 2 such acceptor genotypes have not yet been discovered.

Type 3 species, in which both acceptors and non-acceptors of *ver* pollen are available, are suitable for genetic analysis of one-way crossability. Such an analysis was made in *chc* by Grun & Aubertin (1966) and by Hermsen et al. (1974) in 2*x tbr*. In both, several dominant genes control inhibition of *ver* pollen, acceptors of *ver* pollen being homozygously recessive at these loci. Corresponding results were obtained by Hogenboom (1972) in crosses between *Lycopersicon peruvianum* and *L. esculentum*, but also by Lein (1943a, b) in crosses between wheat and rye.

In nearly all such tests only the effect of the genotype of the female parent was considered. However, if crossability be genetically determined, it is illogical to assume that only the genotype of the pistil parent is decisive. Therefore the author studied systematically the effect on crossability of different genotypes of the species *ver* as male parents with different genotypes of 2*x tbr* as pistil parents. Whereas the application of pollen mixtures of *ver* to 2*x tbr* pistils allowed the detection of only acceptors and non-acceptors, the separate use of pollen from different *ver* genotypes gave surprising results. The acceptor genotypes of 2*x tbr* fell apart into different new types, some genotypes accepted pollen from all *ver* clones used, but other genotypes of 2*x tbr* accepted pollen of some, but rejected that of other *ver* clones. So both female and male genotypes determine crossability between species.

Table 2. Reaction pattern with tentative genotypes for barriers (sporophytic and dominant) and for ability to penetrate (gametophytic and recessive). A, acceptors of all *ver* pollen; NA, non-acceptors of all *ver* pollen; DP, differentials for pollen types; a point in the genotypic formula, either dominant or recessive (From Hermsen et al., 1977). Parental species 2*x* *tbr* female; *ver* male.

Pistil		Pollen genotypes and plant numbers			
phenotypes	genotypes	$P_1P_2P_3$	$p_1P_2P_3$	$P_1p_2P_3$	$p_1p_2P_3$
		43	43 x 45	49	45
A	$b_1b_1b_2b_2b_3b_3$	+	+	+	+
DP Type 1	$b_1b_1B_2.\ b_3b_3$	–	–	+	+
DP Type 2	$B_1.\ b_2b_2b_3b_3$	–	+	–	+
NA	$.\ \ .\ .\ .\ B_3.$	–	–	–	–

Table 3. Complete model of a gene-for-gene relationship based on three dominant barrier genes and three corresponding recessive penetration genes. The non-functional alleles (the recessive barrier alleles and the dominant penetration alleles) are indicated by zero.

♀			♂							
			000	p_100	$0p_20$	$00p_3$	p_1p_20	p_10p_3	$0p_2p_3$	$p_1p_2p_3$
00	*00*	*00*	+	+	+	+	+	+	+	+
$B_1.$	*00*	*00*	–	+	–	–	+	+	–	+
00	$B_2.$	*00*	–	–	+	–	+	–	+	+
00	*00*	$B_3.$	–	–	–	+	–	+	+	+
$B_1.$	$B_2.$	*00*	–	–	–	–	+	–	–	+
$B_1.$	*00*	$B_3.$	–	–	–	–	–	+	–	+
00	$B_2.$	$B_3.$	–	–	–	–	–	–	+	+
$B_1.$	$B_2.$	$B_3.$	–	–	–	–	–	–	–	+

Table 2 summarizes preliminary results and a model, which is based on the following assumptions:

– each barrier in the pistil is controlled by one dominant gene and all the 'barrier genes' have a sporophytic action

– overcoming a barrier requires one monogenic recessive gene in the pollen and all the 'penetration genes' have a gametophytic action.

The code DP in Table 2 indicates those 2*x* *tbr* genotypes that react differently to pollen with different penetration genotypes. In other words, DP plants can distinguish different penetration genotypes of the male parent. The different DP data in Table 2 were found experimentally. A complete model of gene-for-gene relationship based on three barrier and corresponding penetration loci is presented in Table 3. The reaction pattern is analogous to host–parasite relations. General acceptance corresponds with susceptibility, all other pistil genotypes functioning as differentials to the pollen types ('races').

Not all new data fit this simple gene-for-gene model, although the basic state-

Table 4. Different ploidy in interspecific F_1 due to functional *n* gametes and 2*n* gametes. The higher ploidy in each F_1 is due to 2*n* gametes either from the male or the female parent.

Crosses	Ploidies F_1	2*n* gametes from
4*x* – *acl* x 2*x* – *blb*	3*x*, 4*x*	male
2*x* – *ver* x 2*x* – *blb*	2*x*, 3*x*	male
2*x* – *ver* x 6*x* – *dms*	4*x*, 5*x*	female
2*x* – *tbr* x 6*x* – *dms*	4*x*, 5*x*	female
4*x* – *tbr* x 2*x* – *phu*	3*x*, 4*x*	male
8*x* – *acl* x 2*x* – *phu*	5*x*, 6*x*	male
4*x* – {(*acl* x *blb*)^2 x *phu*} x 4*x* – *tbr*	4*x*, 6*x*	female

ment that the genotypes of both parental species determine interspecific crossability holds true. Further research is being concentrated on F_1 from crosses between characteristic pistil parents and between characteristic pollen parents.

Implications of 2n gametes in interspecific hybridization

Numerically unreduced or 2*n* gametes have the somatic number of chromosomes and may arise either from incomplete first meiotic division (= first division restitution, FDR) or from incomplete second meiotic division (= second division restitution, SDR). 2*n* gametes are known to occur in many plant species. Harlan & De Wet (1975) mention 60 species and Den Nijs (1972) demonstrated their frequent occurrence in tuber-bearing *Solanum* species. The production of 2*n* gametes has been found to be genetically controlled (Mok & Peloquin, 1975, which gives further references). Genetic determination is confirmed by the observation that in interspecific hybridization certain introductions or rather genotypes of a species produce functional 2*n* gametes, whereas others do not. A few examples from our work are listed in Table 4.

In ploid crosses within a species, e.g. tetraploid × diploid, 2*n* gametes have a great potential value to plant breeding, particularly so, when they originate from FDR (Mok & Peloquin 1975). However in certain interspecific crosses, 2*n* gametes may create a barrier to further crossing. So 4*x* *acl* × 2*x* *blb* may give rise either to 3*x* or 4*x* F_1 hybrids, both being sterile. The triploid hybrids after colchicine treatment became fully fertile hexaploids, whereas the tetraploid hybrids that arise from 2*n* gametes of *blb* remained sterile after colchicine-doubling.

Generally 2*n* gametes seem a disadvantage in those interspecific F_1 hybrids that have to be doubled by colchicine before they can be used for further crossing.

Use of bridging species

Third species are commonly used for bridging the gap between a crop and valuable but remote species that cannot be crossed directly with that crop. This circuitous approach may be made more efficient:

- if also the bridging species have desirable characters

– if the number of crossable genotypes within each species is sufficiently large to avoid restriction of genetic variation in progeny and loss of desired genes
– if as many valuable genes as possible have been accumulated by in the starting material by pre-breeding.

Let me stretch some typical limitations and difficulties, which the breeder may come across in remote hybridization, with experimental results in *Solanum*. For abbreviations of species names see the chapter on material and methods on page 312.

Example 1. Making the large number of valuable genes from *blb* and *pnt* accessible to breeding.

Summary of first procedure. $\{(acl \times blb)^2 \times phu\} \times tbr$ cultivars. Step 1. *acl* × *blb*. There is one-way crossability and even in the successful direction seed and berry set poorly. Offspring F_1 are fully sterile. Their sterility can be overcome by colchicine-doubling of the triploid, but not that of the tetraploid hybrids (see above under implications). Step 2. $(acl \times blb)^2 \times phu$. The hexaploid *acl* × *blb* hybrids (colchicine!)can be crossed with *phu*. However this cross was successful with only a few F_1 plants and is also dependent on the *phu* used. Besides parthenogenetic trihaploids occurred among the progeny of the triple cross. Step 3. $\{(acl \times blb)^2 \times phu\} \times tbr$ cultivars. This last step is a serious limitation, because 18 000 pollinations had to be made to obtain only 40 hybrid plants. These hybrid plants were partly tetraploid, partly hexaploid (2*n* gametes) and partly had 65–66 chromosomes (2*n* gametes and chromosome elimination) (Hermsen & Ramanna 1973). The quadruple hybrids obtained in this way could easily be intercrossed and further hybridized with cultivars.

An improvement in the first procedure is briefly as follows: $\{acl \times (pnt \times blb)^2\} \times tbr$ cultivars.

Step 1. *pnt* × *blb*. There is one-way crossability, but seed and berry set of the successful cross are fairly good. Step 2. Colchicine doubling of *pnt* × *blb* leads to allotetraploid $(pnt \times blb)^2$. This artificial allotetraploid hybridizes well with *acl*, which is allotetraploid as well. Step 3. Crossing of the triple hybrids with *tbr* cultivars forms a serious limitation for genes, as in the first procedure. The advantages of the second procedure over the first are fewer limitations for genes, inclusion of both *blb* and *pnt* and a larger contribution of these species to the progeny.

Example 2. Making resistance genes of non-tuber bearing *Solanum* species accessible to potato breeding.

Two non-tuber bearing species, *brd* and *etb*, were crossed extensively with 11 tuber-bearing species by Dr L.M. Taylor during a stay at our Institute. With *brd* and *etb* as a male, no hybrids were obtained. With *brd* as a female, many parthenocarpic berries were produced, but not one hybrid. With *etb* as a female, success was achieved but only with certain genotypes of the species *pnt*. Detailed data will be published in due course.

Also from example 2 it is apparent, that crossability is genetically determined: the incidental use of particular genotypes of one particular species broke the crossability barriers between tuber bearing and non-tuber bearing *Solanum* species.

Environmental conditions and interspecific hybridization

Many tests and many plant species have shown that environmental conditions in the broadest sense play an important part not only in plant growth, but also in interspecific hybridization. Crosses between remote species are especially sensitive to environment. Each difficult cross may have its own specific requirements of the environment, in other words set of environmental conditions, to be successful. Such optimum conditions can hardly be determined experimentally because of the large number of environmental variables and their interaction.

The following practical approach to this problem, which is most pronounced in wide crosses, is being applied at our Institute with *Solanum*: extend flowering time and intensity by grafting potato species onto tomato rootstock and pollinate until success has been achieved. The premise behind this procedure is that some time during a period of 3–4 months the optimum environmental conditions will be achieved by nature.

Statements on interspecific hybridization

Natural crossing by using many genotypes of both parental species of each cross is efficient, even if crosses seem impossible.

Optimum environmental conditions can be achieved by carrying out crosses over a long time interval, i.e. under a large range of different environmental conditions.

Growth chambers are too small and too expensive for large-scale crosses in different environments.

Somatic hybridization is of little use in making genes from remote species or genera accessible to plant breeders. When natural crosses carried out in a responsible way are not successful, somatic hybridization will usually not be any further help to plant breeding. Intra-specific somatic hybridization is useful for adding the chromosomes of highly selected complementary genotypes and for hybridizing sterile, sublethal, non-flowering plants in young stages of growth.

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Further investigations on partial incompatibility in crosses between Hordeum vulgare L. and H. bulbosum L.

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Index words

Hordeum vulgare, Hordeum bulbosum, barley, incompatibility, interspecific hybridization, doubled haploids.

Summary

Evidence of partial incompatibility between *H. vulgare* × *H. bulbosum* has previously been reported and methods employed in attempts to overcome this incompatibility are described. The interspecific hybridization technique is being used to broaden the genetic base of material used in the barley breeding programme and additional cultivars exhibiting incompatibility have been discovered. Variation within stocks of *H. vulgare* for incompatibility and its genetic control are discussed.

Introduction, material and methods

Whilst producing doubled haploid spring barleys by means of interspecific hybridization as described by Kasha & Kao (1970) and Jensen (1975), Pickering & Hayes (1976) observed reduced seed-sets when certain cultivars of *Hordeum vulgare* derived from *H. distichum* var. *laevigatum* (e.g. cv. Vada) were pollinated with *H. bulbosum*. As this restricted the choice of *H. vulgare* parents to those with high seed-sets, the following attempts were made to overcome the incompatibility in crosses between cv. Vada × *H. bulbosum* (stock S1) encountered under glass day temperature 12–35 and night temperature 5–15°C for summer and winter, respectively; 16 h day-length. Except where otherwise stated, the crossing technique and origin of stocks were as previously described (Pickering & Hayes 1976) and post-pollination crossing bags were similar to those referred to by Pickering (1977, 1978). Direct comparison of two male genotypes was possible by brushing pollen from each onto emasculated florets on different sides of the same spike.

Results

Attempts to overcome incompatibility

– Fluctuations in seed set throughout the year have been recorded on Vada (Feb. 1977, 17.7%; Aug. 1977, 42.9%) and, although there were no significant differences in an experiment at controlled temperatures, further investigation is required to establish the optimum temperature for seed set (Pickering 1978).

– Significant improvements in success rate have been achieved after screening several new accessions of *H. bulbosum* genotypes (Pickering 1978).

None of the following techniques were successful in improving seed set.

– Application of exogenous growth substances gibberellic acid (GA_3); GA_3 + indoleacetic acid (IAA) to emasculated florets both before and after pollination, or to growing plants at different growth stages before ear emergence (GA_3 only).

– Pollination on successive days.

– Pollination after excision of the upper branches of the stigmas/styles.

– Pollination of emasculated florets on intact spikes, and on unemasculated male-sterile plants of Vada.

Therefore the best chance of success in overcoming incompatibility would seem to depend on screening for the most suitable pollinators and the establishment of the optimum environmental conditions under which they could be used.

Broadening the genetic base of barley by interspecific hybridization

Interspecific hybrids (genome VB) from crosses between *H. vulgare* and *H. bulbosum* that are sterile as a result of failure of the *bulbosum* chromosomes to be eliminated are produced more commonly during the winter (Pickering 1978). Fertility of these diploids can be restored by colchicine treatment and three stable hybrids with 28 chromosomes (VVBB) are being maintained from crosses carried out between cvs Emir, Universe and Vada × *H. bulbosum* (stock S1).

When Vada has been pollinated by any of these VVBB hybrids, seed sets are similar to those obtained on compatible cultivars (Table 1).

Normally, selfed progeny from VVBB hybrids tend to revert back to their *vulgare* parent (Lange & Jochemsen 1975). This has been confirmed by screening several hundred such progeny from Emir VVBB for brown rust (*Puccinia hordei* Otth.) reaction type at the 3-leaf stage. Very few resistant plants were obtained and none maintained their resistance in subsequent generations. At maturity, these plants looked like their *vulgare* parent.

Nevertheless root-tip counts on selfeld seedlings from Vada VVBB hybrids showed a greater proportion of cells with 28 chromosomes than those from Uni-

Table 1. Seed sets obtained on barley Vada and Emir pollinated with hybrids (genome) VVBB (August-September 1977).

♀ Parent	♂ Parent	Number of florets pollinated	Seed set (%)
Vada	Vada VVBB	316	72.8
Vada	Emir VVBB	32	46.9
Vada	Universe VVBB	107	54.2
Emir	Vada VVBB	158	55.7
Emir	Emir VVBB	790	68.9
Emir	Universe VVBB	192	73.0

verse and Emir. Thus, besides temperature (Humphreys 1978), chromosome elimination in the hybrid material seems to depend to some extent on genotype.

Meiosis in VVBB hybrids was shown to be disturbed (Lange 1971), although a 27-chromosome hybrid was reported as having regular first and second anaphase at meiosis (Kasha & Sadasivaiah 1971). Furthermore, in spite of a degree of homeology and pairing between *vulgare* and *bulbosum* chromosomes (Kasha & Sadasivaiah 1971), the transfer of characters from the wild species has proved difficult, even with irradiation (Report of the Agricultural Research Institute of Ontario, Ottawa).

Should further results confirm that selfed seed from the Vada VVBB hybrids consistently contain 28 chromosomes, it is intended to irradiate these with a ^{60}Co source (Aung et al. 1977) or thermal neutrons (Laster & Elliott 1956) in an attempt to break chromosomes and transfer chromosome segments from *bulbosum* to *vulgare* chromosomes, but this may be difficult to accomplish in a diploid species such as *Hordeum.*

Additional cultivars exhibiting incompatibility with H. bulbosum

Since the first report of partial incompatibility was published (Pickering & Hayes 1976), several other cultivars of barley have given low seed-sets in crosses with *H. bulbosum* (stock S1) (Table 2).

The strong incompatibilities of CIV 298 and CB 7432 (Cebeco 7432) contrast with that of Tyra, which may not prove difficult to overcome with a suitable pollinator (Table 2). An F_1 hybrid involving Tyra also exhibits a similar percentage seed-set to Tyra itself, thus indicating dominant genetic control, the nature of which will be determined by progeny tests on crosses of F_2 plants with *H. bulbosum.*

Amongst all the alleged parents of Tyra, only one out of six plants of Monte Cristo has shown any incompatibility (Table 3).

Since these crosses were made, the pedigree of Tyra has been questioned and as it is now known that Algerian (CI 1179) rather than Monte Cristo has donated its

Table 2. *Seed sets on various cultivars of H. vulgare* pollinated with *H. bulbosum* (stock S1) (February-April 1978). Origin of stock Cb 2920/4 – selection from bulk population provided by prof. C.J. Jensen, Agric. Res. Dept. Danish Atomic Energy Commission, Research Establishment Risø, 4 000 Roskilde, Denmark.

H. vulgare parent	Number of florets pollinated	Seed set (%)
CIV 298	323	15.2
CB 7432	334	13.2
Tyra	576	42.5
(Tyra x Goldmarker) F_1	623	53.8
(Tyra x Goldmarker) F_1 x *H. Bulbosum* (stock Cb 2920/4)	551	94.0
Vada (control)	447	17.0
Rika + Herta + Drost (controls) (total and mean respectively)	999	87.7

Table 3. Seed set on Monte Cristo after pollination by *H. bulbosum* (stock S1) (February-March 1978).
Origin of stocks:
EBDN-European barley disease nursery 1977.
Cb 826-dr E.A. Favret, Centro de Investigacions en Ciencias Agrónomices, Castelar, Argentine 1959.

Stock No	Plant No	Number of florets pollinated	Seed set (%)
EBDN 77-31	1, 2 and 3	183	80.3
Cb 826	1 and 3	103	86.4
Cb 826	2	50	12.0
Cb 826 x EBDN 77-31	2 x 2	8	100.0

mildew resistance to this cultivar (Wolfe & Wright 1978), test-crosses between Algerian and *H. bulbosum* are being carried out.

A full diallele involving CIV 298, CB 7432, Tyra and Vada, has been completed (normal seed sets being obtained) and F_2 progenies crossed with *H. bulbosum* will determine whether the same gene is responsible for incompatibility. It is also hoped to include Monte Cristo (Cb 826/2) at a later date after screening F_1 and F_2 plants from Cb 826/2 × EBDN 77–31/2.

Because of the variation in seed set which exists in certain stocks such as Monte Cristo, Universe (see below, Variation in *H. vulgare*), it could be useful to screen a range of potential parents that may form part of the doubled haploid program and select for high seed-set where possible.

Stebbins (1957) pointed out that self-fertilizing species are probably derived from cross-fertilized ancestors and Lewis & Crowe (1958) suggested a stepwise progression from self-incompatibility to self-compatibility. Therefore further crosses involving a worldwide range of *H. vulgare* genotypes (such as those comprising 'Composite Cross No. V') might clarify these evolutionary aspects and allow an assessment of the distribution of incompatibility genes.

Variation in H. vulgare in seed set and isoenzymes

In addition to the results achieved with Monte Cristo, variation in seed-setting has also been observed between five different stocks of cv. Universe (Abed 3371 × Vada) when pollinated with *H. bulbosum* (stock S1) (Pickering 1978). During March 1977, two stocks gave mean values of 87.9% and 92.7% and a third 3.0%. The two remaining stocks, which ranged from 4.0–42.9% and 7.5–44.0%, were rechecked during March and April 1978 when more consistent respective seed-sets were obtained of 3.9–17.0% and 10.4–13.3%.

When all stocks were examined for isoenzyme banding patterns, no qualitative differences were observed between

– differently aged leaves from adult plants (acid phosphatase, phosphoglucoisomerase (PGI), esterase)

– 4th leaves from plants ranging from Feekes' growth-stages 4–5 to 10.3 (same enzymes)

– seedling leaves and roots (glutamate oxaloacetate transaminase, phosphoglucomutase, acid phosphatase).
However, such differences were recorded in seedling-leaf esterase and seedling-root PGI both between and within certain stocks.

Polymorphism has also been reported for esterase loci in seedling leaves of a range of barley cultivars (Allard et al. 1969; Kahler & Allard 1970).

Pandey (1967) proposed that each *S* allele controlling self-incompatibility in *Nicotiana alata* had specific peroxidase isoenzymes. Also by means of immunochemical techniques (Nasrallah & Wallace 1967) and isoelectric focusing of stigmatic extracts of *Brassica oleracea* (Nishio & Hinata 1977), the nature of the *S*-specific proteins involved in self-incompatibility have been partly characterized.

In order to investigate the molecules involved in the incompatibility reported here, it is hoped to test the total protein spectrum of stigmas (both before and after pollination) of two isogenic stocks of cv. Universe, which differ only in seed set achieved in crosses with *H. bulbosum*.

Genetics of the H. vulgare × H. bulbosum incompatibility

Unlike the commonly occurring unilateral incompatibility described by Lewis & Crowe (1958) between self-incompatible and self-compatible species (SI × SC), the system described here and that found in certain wheat × rye crosses (Lange & Wojciechowska 1976) takes place between SC × SI species. However, whereas a single dominant *H. vulgare* gene controls crossability with *H. bulbosum* (Pickering & Hayes 1976), two dominant wheat genes with additive effects give three levels of seed setting in wheat × rye crosses (Lange & Wojciechowska 1976).

In contrast to the gametophytic self-incompatibility in graminaceous species where pollen tubes fail to penetrate the stigma surface to any extent (de Nettancourt 1977; Hayman 1956), in both these interspecific crosses pollen-tube growth is arrested mainly within stylar tissues. Although high seed-setting between barley (including cv. Vada) ♀ × rye ♂ (Thomas & Pickering in press) is common, no seed from the reciprocal has been obtained.

De Nettancourt (1977) reviewed the genetic models proposed for interspecific incompatibility, which are basically those related to (Lewis & Crowe 1958) or unrelated to (Hogenboom 1973) the *S* self-incompatible allele. Hermsen (1977) pointed out that if the latter model (incongruity) is correct, one might expect reciprocal interspecific crosses to exhibit contrasting seed-sets. Although little information concerning rye ♀ × wheat ♂ crosses is available, two reports (Meister & Tjumjakoff 1928; Boyes & Thompson 1937) indicated differences in seed set in reciprocal crosses between these two species.

However, this was not shown to be true with *H. vulgare* × *H. bulbosum* crosses where cv. Vada × *H. bulbosum* and the reciprocal cross gave low seed sets (Pickering & Hayes 1976). Nevertheless, having recently repeated hybridizations between *H. bulbosum* (stock S1) ♀ × Vada ♂, similar percentage seed sets to *H. bulbosum* × non-Vada derivatives were obtained.

According to the pattern of seed sets on individual spikes and the great range in percentage seed-set between heads, stigma age at pollination would seem the critical factor in these reciprocal crosses and could explain the discrepancies.

Further investigation will involve a large number of crosses between *H. bulbosum* (stock S1) and *H. vulgare* cv. Vada and will be undertaken in a controlled environment.

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In vitro induction of gynogenesis in higher plants

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Index words

Hordeum vulgare, barley, in vitro induction, gynogenesis, cytoplasm.

Summary

Haploid barley plants were produced by in vitro induction of gynogenesis. Each plant produced was a chlorophyllous haploid.

Introduction

In vitro androgenesis has been developed as a technique to produce haploid plants of several species among higher plants. However in vitro induction of haploid tissues from female gametophyte has rarely been reported (Tulecke 1964; Uchimiya et al. 1971). This paper shows a way to obtain haploid plants by in vitro gynogenesis for *Hordeum vulgare* L.

Materials and methods

We use a spring barley (cv. Berenice) an alternative barley (cv. Mammie), a winter barley (cv. Sympa) and a gynogenetic doubled haploid from 'Sympa' (obtained before). Plants were cultivated in greenhouses with long-day conditions (photoperiod 16h; temperature 27 ± 3 °C).

Spikes were collected when they were still in the sheath and when the tips of the awn were just coming out of the sheath. They were sterilized with calcium hypochlorite solution (50 g/l) for 5 min, then rinsed twice with sterile demineralized water. In a sterile environment, the whole ovaries were excised and cultured on a nutrient medium solidified with 1% agar. This nutrient medium contains Miller's major nutrients and trace nutrients, Fujii's vitamins, EDTA-Fe (II) at substance concentration 10 $mol.m^{-3}$, 2, 4-D at mass concentration 2 $g.m^{-3}$, sucrose 100 g/l, pH adjusted to 6 before autoclaving. The cultures were kept in growth chambers at 27 ± 1 °C with a 16-h photoperiod (3 klx).

Some of the cultured ovaries were dissected after 4 weeks to free the proem-

Table 1. Number and proportion of ovaries giving haploid chlorophyllous plants within 8 – 10 weeks of culture: A,'Berenice'; B, 'Sympa'; C, 'Mammie'; D, gynogenetic doubled haploid from 'Sympa'.

Cultivars	Number of ovaries cultured	Number of ovaries producing					Total	Proportion %
		1 plant	2 plants	4 plants	6 plants	8 plants		
A	1 103	3	3	–	–	–	6	0.5
B	1 996	6	4	1	–	1	12	0.6
C	997	1	1	–	–	–	2	0.2
D	785	3	3	1	1	–	8	1.1

bryos; these were then subcultured on the previous nutrient medium but without auxins and with a lowering of sucrose from mass concentration 100 to 20 g/l. The rest were maintained under the first culture conditions till plantlets appeared; as soon as they emerged, plantlets were transferred to the medium for proembryos.

Chromosomes counts were made on root tips of plantlets before potting.

Results

The best ovary stage for culture was the immature one. We obtained best results with ovaries from florets where pollen grains were either binucleate or young trinucleate (younger ovaries give no results) (San Noeum 1976). At that stage, the embryo sac contains the egg, the two polar nuclei and ten or so antipodial cells, some of them in mitosis.

In culture, these ovaries distinctly increased in size, became green as early as the third day and then ovary walls darkened during the fourth week. Within 8 to 10 weeks, 2 to 6 cultivar ovaries per thousand and 11 doubled haploid ovaries per thousand produced plantlets emerging from a split in the ovary wall (Table 1). Each embryogenic ovary frequently gave one or two plantlets, more rarely up to eight.

After 4 weeks of culture, 'Berenice' and 'Sympa' ovaries were dissected under a binocular microscope; about 19% of them were embryogenic; they contained one or several proembryos (Table 2). Transferred to the 2nd medium, 5.3% of the

Table 2. Number and proportion of ovaries giving proembryos after 4 weeks of culture and number of regenerated plants. A, 'Berenice'; B, 'Sympa'. Proembryos coming from: a, 1 – egg; b, 2 – egg and antipodial cells; c, 1 – antipodial cells; d, 2 – egg and 1 synergid; e, 3 – egg and 2 synegids; f, 1 or 2 – 1 or 2 synergids; g, 2 or 3 – 1 or 2 synergids and antipodial cells; h, 3 or 4 – 1 or 2 synergids, egg and antipodial cells.

Cultivars	Number of dissected ovaries	Number of ovaries producing proembryos								Total number of embryogenic ovaries	Proportion	Number of regenerated plants
		a	b	c	d	e	f	g	h			
A	300	35	8	6	2	1	1	2	1	56	18.6	3
B	580	68	16	10	7	5	2	1	3	112	19.5	6

proembryos regenerated green plantlets; the others died or gave only roots. Proembryos from various tissues regenerated plantlets; but we obtained best result with proembryos from egg or antipodial cells; synergids gave only proliferation of callus type.

In our experiments, we obtained neither albino plants nor calluses or embryos from somatic cells of ovary and ovule walls. All plants obtained by this technique were chlorophyllous and haploid.

Conclusions

Every plant obtained was a chlorophyllous and haploid one. However the proportion of embryogenic ovaries regenerating plants remained low. The proportion varied with genotype (per thousand, 2 for Mammie, 5 for Berenice, 6 for Sympa); the gynogenetic doubled haploid shows a really more superior gynogenetic ability than did the original cultivar (6 for Sympa against 11 for doubled haploid). Otherwise, these plants are produced by proliferation of one (more often from egg) or several haploid cells of the embryo sac.

By dissection of ovaries after 4 weeks of culture, we never observed more than 4 proembryos per ovary (Table 2); however since an ovary can produce 8 plantlets (Table 1), we suppose that some proembryos can give rise to several plantlets.

All cells of the same embryo sac, egg, synergids, polar nuclei and antipodial cells contain the same genome. Since they have a different issue, can we assume they have a different cytoplasm? If so, a plantlet coming for example from egg and another from antipodial cells or synergid would be not identical.

Miller's major nutrients and trace nutrients mass concentrations in g. m^{-3}: KH_2PO_4 300; KNO_3 1 000; NH_4NO_3 1 000; $Ca(NO_3)_2$ 347; $MgSO_4$35; KCL 65; KI 0,8; $ZnSO_4$ 1,5; H_3BO_3 1,6; $MnSO_4$ 4,4.

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Future: advanced and new breeding methods

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Advanced and new breeding methods

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Index words

Advanced breeding methods, new breeding methods, protoplast fusion, somatic hybridization, hybrid cell, organelle transplantation, cell culture, segregation.

Summary

Until recently the only means of hybridization in plants was by sexual crossing. The use of plant protoplasts has, however, in recent years enabled fusion to take place between the somatic cells of plants, and the resultant somatic hybridization has enabled genetic recombination to take place by a process other than the standard sexual cycle. Somatic hybridization has been described by Haldane as an 'alternative to sex'. Such 'alternatives to sex' whether they be in fungi, animals or plants have almost invariably been revealed by a genetic approach using large numbers of cells, coupled with powerful selection methods.

The consequences of protoplast fusion, and the manipulation and selection of cultured hybrid cells, are basically specific aspects of the in vitro culture of plant somatic cells. The general objective is the application of the methodology, and general rationale, of microbial genetics to specific breeding problems in crop plants. Such an approach will be dependent on a basic foundation for the crop plants of callus and suspension culture, haploid plant culture from pollen, protoplast isolation culture and fusion and totipotency in the plant cell cultures. The genetical approach will also involve transfer of genetic information, through uptake of bacterial plasmids, such as those of *Agrobacterium*. Interspecific hybridization will also be involved as well as organelle transplantation by fusion.

The realities of such genetic manipulation of plant cell cultures for plant breeding are still somewhat distant. Many problems still exist, the basic one of which is the adequate growth in culture of cells and tissues of most of the crop plants, and their regeneration into whole plants.

Introduction

It is always difficult to introduce a general topic, which is concerned with future developments because as we all know predictions about the future often prove wrong. The introduction of new breeding methods will depend on two major factors: first the development of the new technology and, secondly, the decision by plant breeders to utilize such new methods. Advanced and new breeding methods may be available but for various sound reasons, economic and otherwise, they may not be implemented.

New methods of genetic manipulation fall into two major categories. The first utilizes plant protoplasts, and in recent years the use of protoplasts has allowed fusion to take place between the somatic cells of plants and the resultant somatic hybridization has allowed genetic recombination to take place by a process other than the standard sexual cycle. The other, which is at a much earlier stage of development, will involve transfer of genes for specific functions by use of bacterial plasmids together with plant protoplasts – in particular the use of *Agrobacterium* plasmids as vectors (Davey et al. 1978).

It must be stressed, however, that the realities of such genetic manipulation of plant cell cultures for plant breeding are still remote. Many problems still exist, the basic one of which is the adequate growth in culture of cells and tissues of most of the crop plants, and their regeneration into whole plants.

General culture problems

While there is no problem isolating large numbers of healthy protoplasts in crop plants, it is their response in culture which has been the barrier in subjecting them to somatic hybridization, or improving their types through the introduction of desirable genetic materials. Leguminous protoplasts have shown some encouraging responses in culture. They often divide and subtain divisions, yielding a callus tissue. However the callus fails to regenerate whole plants. The response of cereal protoplasts in culture is more discouraging. The mesophyll protoplasts of cereals may regenerate a wall and remain viable for weeks, but they rarely divide regularly. The protoplasts isolated from actively growing callus or suspension cultures do exhibit some division.

The tissue cultures of cereals and other graminaceous members exhibit tremendous potentiality for rooting. On all media that support its growth, the callus develops roots. In the presence of high concentrations of 2, 4-D, (1 mg/lt and over) the tissue may remain unorganized to begin with, but, in the long term, the callus starts forming roots. On a medium lacking 2,4-D, or with it at a lower concentration, rooting occurs in young subcultures. Replacement of 2,4-D by indoleaceticacid, naphthylaceticacid or a cytokinin promotes rooting. Often the rooting is so profuse that the entire callus is transformed into a nest of roots.

The differentiation of shoots in tissue cultures of cereals is less frequent, and often sporadic and short-lived. The major cereals have been reported to differentiate shoots. However, the frequency of this phenomenon is extremely poor, and mostly the differentiation of shoots cannot be ascribed to any known growth substance. Like roots, shoots also differentiate when the callus is transferred from a medium containing 2,4-D to a medium having the auxin at a very low concentration, or lacking it.

Much work is needed on tissue culture of cereals, and other crop plants, in order to be able to employ profitably the technique of crop improvement through the protoplast system. With legumes, the problem is of regenerating whole plants from callus. The problem is much more serious with cereals.

Fusion and selection of somatic hybrids

Protoplast fusion involves the establishment of close contact (adhesion) between the plasma-membranes of adjacent protoplasts. Simultaneously the nature of the membrane is altered in such a way so as to initiate limited fusion of the membrane. The mechanism of membrane fusion in plant cells is not clear, but all fusion-inducing agents alter the charge on the membrane surface, thus facilitating close contact. An expansion of local cytoplasmic bridges (similar to plasmodesmata) results in coalescence of the respective cytoplasms to form, for protoplasts of different species, a heterokaryon. Several factors combine to prevent the production of hybrid cells proper. These can include:

- instability of one or both components after fusion
- inherently low plating efficiency of one or both parental protoplast systems
- asynchronous nuclear division
- incomplete mixing of the cytoplasms, through the presence of vacuoles
- chromosome loss during successive mitoses
- the inability of hybrid cells to survive at low concentration.

Little can be done to alter the first four factors but the design and operation of selection methods can be such as to minimise the problem of low density survival. It seems that the minimum plating density for leaf protoplasts of *Petunia* and *Nicotiana*, in the absence of nurse tissue, is 6×10^3 per ml. At that density, the plating efficiency is less than 0.5%. It seems therefore that the recovery of somatic hybrids is often going to be a rare event, and that the extent of protoplast fusion achieved by any method may bear little relationship to the number of somatic hybrids recovered. If selection is rigorous, then the extent of fusion may be of secondary importance when considering the many factors, operating at the culture level, which will reduce the numbers of surviving hybrid cells.

A complementation selection procedure has been developed at Nottingham which involves fusing albino protoplasts from suspension culture with leaf mesophyll protoplasts of the other species, and selecting green colonies formed as a result of complementation and selective growth. This selection procedure should be readily applicable to a wide range of interspecific fusions, including those of sexually incompatible species (Cocking et al. 1977).

Consequences of protoplast fusion – relevance to plant breeding

As we have seen it is now becoming increasingly clear that the actual fusion of isolated protoplasts is not a major obstacle to the implementation of somatic hybridization. However, protoplasts from different species differ in the readiness with which they will fuse, and they also differ greatly in their sensitivity to the various fusion-inducing agents currently employed. Extensive and rapid fusion often results in loss of viability. Unfortunately, all proven selection procedures, developed so far in other laboratories, have been based on some knowledge of an existing sexual hybrid counterpart, or have necessitated the use of special mutant plants, and for this and other reasons, are limited in their general applicability. However, the recent development of procedures for the selection of somatic hybrids by the Agricultural Research Council Group at Nottingham (Cocking et al. 1977), which is not dependent on a knowledge of any sexual hybrid, or the use of special mutant plants other than albino mutants (many of which are naturally occurring or can be readily induced), now means that it is possible to utilize somatic hybridization, which should be of more general applicability.

It would be naïve to suggest, even with somatic hybridization and the technique developed at Nottingham, that the consequences of fusion between protoplasts of different species would necessarily be fertile somatic amphidiploid plants. Many factors could operate to preclude this possibility. Throughout their evolution, plants have evolved a variety of protective mechanisms to maintain the integrity of species – morphological, physiological and biochemical. The plant breeder may circumvent some of these difficulties by embryo culture. Sexual incompatibility in plants may be analogous to immunochemical systems in animals, and the use of immunosuppressant drugs has been proposed. Whatever the limitations of somatic hybridization eventually prove to be, it is clear that pre-zygotic and endosperm-embryo relationships will not be amongst the incompatibility factors operating in this new method of hybridization.

Two considerations will be uppermost in the mind of the plant breeder, first the extent to which any selected somatic hybrid plants will be sterile, and second the extent to which chromosomes will be directionally eliminated. After somatic hybridization between cells of different mammalian species, there is often an undirectional loss of chromosomes, probably as a result of genome incompatibility. To what extent genome incompatibility will be a problem in somatic hybridization of plant cells is not yet clear. Some recent work, on sexual crosses in barley and wheat has indicated that directional chromosome elimination in interspecific hybrids in angiosperms may be more frequent than has been generally realized. Examination of the consequence of fusion of petunia and boston ivy protoplasts suggested that such complete elimination of chromosomes, from one of the parents, may occur in somatic hybrid cells on prolonged culture (Power et al. 1975). Numerous examples of interspecific hybrids in plant evolution suggest that genome incompatibility is not always a serious problem but, of course, present investigations are restricted to sexually derived hybrids. Experimentally, the extent of chromosome elimination or repression of gene function, in interspecific somatic plant hybrids, particularly any obtained from sexually incompatible species, can only be determined by the use of genetic markers, or specific chromosome identification. Though the interactions of nuclear genes in interspecific somatic hybrids is largely unknown, it is even less clear what the extent of cytoplasmic interaction might be after fusion. Cytoplasmically inherited traits are of widespread use in plant breeding, particularly as a source of male sterility; and it is likely that somatic hybridization may be used to transfer cytoplasmic features, such as male sterility from one species to another. Indeed interspecies transfer of male sterility within the tobaccos has recently been reported (Belliard et al. 1977).

Normally, the plant breeder produces amphidiploids by doubling the chromosome number of F_1 interspecific hybrids; and when the two genomes are highly divergent such amphidiploids are often fertile, and reasonably stable, both cytologically and genetically. There may be considerable advantage in using haploids in plant-cell genetics, and for the production of homozygous lines. It could also be argued that the fusion of haploids would be analogous to sexual fertilization. Nevertheless, there appear to be no real advantages in the use of haploids in somatic hybridization, particularly since, to achieve fertility after somatic hybridization between haploids of diverse species, the chromosome complement of the fusion product would have to be doubled.

The extent to which the plant breeder will utilize somatic hybridization will depend on a number of factors. One of the main considerations will be the extent to which the breeder cannot achieve hybridization sexually (including use of embryo culture), where the breeder has a requirement to produce a hybrid between two species. It would therefore be unrealistic to consider somatic hybridization as a panacea for plant breeding, but rather as an additional method of hybridization to be attempted when the sexual method is impossible or undesirable. Some crops such as banana, potato and sugar-cane are of course propagated vegetatively, and a sexual cycle is used merely to increase genetic diversity in a program of crop improvement. Here somatic hybridization may be particularly useful, thereby advantageously bypassing the need for such a sexual cycle.

Many plants of horticultural interest are also propagated vegetatively. Somatic intrageneric hybrids produced by protoplast fusion may prove of particular importance there.

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Expression of cryptic genetic factors in vivo and in vitro

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Index words

Lettuce, *Lactuca sativa*, tomato, *Lycopersicon esculentum*, in vitro tissue culture, variation, vigour.

Summary

In vitro cultures with tissues of homozygous lettuce and tomato were carried out to produce calluses and, after a while without buds, regenerated plants.

After analysing these plants and their successive descendants from selfing, and comparing them with a control, we concluded that a given genome can express itself in different ways leading to new phenotypes; these are heritable and do no segregate.

For lettuce, the reciprocal crosses between descendants of regenerated plants and with the control, expressed an effect which looked like hybrid vigour.

Extra-nuclear changes are supposed to be induced or revealed in the different cells during tissue culture; these changes could involve gene-demasking, explaining the observed phenomenon.

In vitro tissue culture of plants like lettuce (*Lactuca sativa*) can lead to callus formation and to bud regeneration.

Cotyledons of a homozygous plant were cultured with this technique so as to produce calluses without any regeneration. After a few cycles, buds were induced on these cultures.

The regenerated plants P_0 presented phenotypic variations and abnormalities in shape (development of axillary buds) and type or structure of their leaves, though their chromosome numbers were unchanged.

When compared with a control, the selfed progenies P_1 showed:

– on the one hand classical nuclear gene mutations, expressed by segregating P_1 families

– on the other hand a few families that we named 'phenovariants or variants', in which new characters were fixed: flatness of the leaves was observed for one family, changes in coloration (yellowish or darker) for others.

Biometric analysis showed a decrease in weight, length and width of the widest leaf, development of axillary buds; these characters as well as 'narrow leaf' are similar to those found in wild types; there was no segregation in their behaviour and they remained for, at least, six generations with selfing.

This led us to conclude that no mutation did affect the genome of the P_0 plants relative to each line.

Diallel crosses including the control showed mostly maternal inheritance for 'flat leaf', but surprising was the expression of an effect looking like hybrid vigour for the progenies of crosses, though the genome of the progenitors were said to be alike. These progenies were named 'cytohets' or 'cytoheteroplasmics' (Sager 1972).

This vigour, measured in term of leaf area, reached about 144% of the selfed progenies. The intensity of its expression depended on the chosen parents and sometimes on the direction of the crosses.

The interpretation of these phenomena, is that in vitro tissue culture may have induced or revealed a new expression of the genes, or set in action usually masked genes.

The changes may have occurred in either cytoplasmic or 'epigenic' factors (Demarly 1974), that is to say in those that differ from nuclear DNA.

In vitro tissue culture was made from tomato cotyledons (*Lycopersicon esculentum*) and phenovariants seemed to be confirmed in P_1 plants: the intensity of anthocyanin coloration and jagged leaves has already been observed for a few fixed lines.

Further studies on the stability of these phenomena may offer the prospect of fixing variability in homozygous plants, perhaps an improvement in their vigour after crosses to the same genome, the ability to get better nucleoplasmic relations in interspecific crosses that show incompatibility, and may be the releasing of 'linkat' tensions (Demarly 1972), permitting more or new types of crossing-over in heterozygous plants.

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Transfer of genes into plants via the Ti-plasmid of Agrobacterium tumefaciens[1]

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Index words

Ti-plasmid, gene transfer, transformation, *Agrobacterium tumefaciens*, genetic colonization.

Summary

Agrobacterium tumefaciens is a gram-negative bacterium with the unique capacity to induce neoplasmic transformations in dicotyledonous plants.

Recently, both the mechanism and the biological significance of this transformation have been elucidated. *Agrobacterium tumefaciens* strains contain a large extrachromosomal DNA plasmid (the Ti-plasmid). This Ti-plasmid is responsible for the oncogenic properties of *Agrobacterium* strains. A particular segment of the Ti-plasmid which contains information determining the tumorous growth pattern and the synthesis of so-called 'opines', e.g. 'octopine' (N^2-(D-1-carboxyethyl)-L-arginine) and 'nopaline' (N^2-(1,3-dicarboxypropyl)-L-arginine), is transferred to and stably maintained and expressed in the transformed plant cells. This phenomenon can be understood as a 'genetic colonization' of the plant cells by bacterial plasmid DNA so that the transformed plant cell will produce, and secrete into the medium, amino acid derivatives (the opines) that Ti-plasmid carrying agrobacteria can selectively use as carbon and nitrogen sources.

By in vivo genetic manipulations, we have recently been able to introduce a bacterial antibiotic resistance transposon, Tn7, in the Ti-DNA segment that is transferrable to plant cells. In this way we hope to demonstrate that the Ti-plasmid can be used as a general vector for introducing 'foreign' DNA into plants.

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[1] Full text will be published in the Proceedings of the 12th FEBS, Dresden, 1978.

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Concluding remarks

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Plant breeding has produced genetic structures that are able to give high yields to crop plants. Disturbance of those structures (gene blocks, linkats) can give new useful recombinations, but will decrease yield.

So plant breeding faces a dilemma: it is afraid of new recombinations, but new genetic structures are wanted because of the genetic poverty of many field crops.

We had two case studies: potato and sugar-beet. In the latter crop cytoplasmic poverty certainly threatens because of the high proportion of hybrid cultivars bred with male-sterile female lines.

That we can enrich the gene pools of our crops with not too distant relatives is illustrated by *Neo-tuberosum*.

The need to collect landraces, primitive cultivated relatives and wild relatives of our crops is clear. Several papers emphasized the necessity of more attention to landraces (some already lost) and primitive cultivated relatives of our highly improved crop plants. This need was stressed by the papers on quantitative characters. For qualitative characters as resistance the wild relatives are more important.

A strategy for collection is urgently wanted. Not only is a thorough knowledge of plant geography and centres of diversity needed, but also species relationships, evolution after domestication, crop ecology and above all good sampling techniques and population genetics.

Collecting is still mainly an empirical part of science, but we have heard reports about a more experimental approach.

The building up and maintenance of a collection is one thing, evaluation another. A big problem is to eliminate duplications and to evaluate all the agricultural traits of accessions.

We can compare a gene bank with a library. With adequate funds it is not so difficult to order books and periodicals or to collect them. But one has to make the right choice. When one has acquired the books, indexes must be made: for instance by subject or by author. And that is laborious, time consuming and demands a skilled staff. A gene bank without evaluation is like a library with a good collection of books without subject or author indexes. It is nice to have them, but hardly possible to extract the information, and material, that we need.

What can we expect from the collected material as parent to our existing cultivars? Biosystematic research of the material is necessary. This is a major problem of wide crosses. First of all we need pre-breeding, i.e. parental line-breeding. Mostly we need a recurrent backcross-procedure to return to the growers standard. Good examples are *Lycopersicon*, *Solanum* and *Triticinae*.

Another crucial point is the overcoming of barriers. We have made good progress in this field. And the better understanding of the barrier-mechanism makes it maybe even possible to use them as a help in the breeding of hybrid cultivars.

This takes us to a major aspect of broadening the genetic base: the improvement of breeding methods such as enhancement of induction of haploids, and changes in mating systems.

So the wild material can be useful on three levels:

1. direct introgression of certain oligogenic characters
2. introgression of polygenic complexes
3. facilitating plant breeding procedures.

In addition to the conventional methods in vivo, we see a quick development of methods in vitro:

- induction of parthenogenesis
- interference of linkat tensions
- in vitro pollination of cultured ovules.
- protoplast fusion, which opens a new range of wide crosses and introduces two types of cytoplasm into one cell
- revolutionary developments of gene transfer by bacterial messengers, which are followed with close interest by plant breeders.

Conclusions

1. Let us collect and maintain what we can catch, primarily the close relatives (including landraces) of our crops. Plant breeders prefer narrow crosses in order to maintain the yield capacity. This is a matter of experience and is now backed up by theoretical approaches.
2. Let us, in close cooperation, evaluate accessions to gene banks starting today.
3. In the meantime, researchers should investigate the more distant crosses, selecting new gene blocks or linkats that can compare with existing yields.

We, plant breeders, benefit greatly from cooperation with the basic research in vitro and at molecular level. The prospects are exciting and we must be ready to jump in when these findings are ready for application.

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