Trans. Nat. Acad. Science & Tech. (Phils.)
1988: 10: 229-259

APICAL DOMINANCE IN *VIGNA UNGUICULATA* (L.) WALP: STUDIES ON THE ROLE OF ABSCISIC ACID

Gilda C. Rivero
Assistant Professor
Institute of Biology, College of Science
University of the Philippines
Diliman, Quezon City
Philippines

ABSTRACT

The effects of exogenously applied abscisic acid (ABA) on apical dominance in intact and decapitated seedlings of *Vigna unguiculata* (L.) Walp. were investigated. ABA was introduced on intact shoot apices or cut surfaces of decapitated seedlings, on the axillary buds of the second trifoliate leaf, or on both sites. Axillary buds of the second trifoliate leaf were released from apical dominance through decapitation or direct application of ABA on the shoot apex. Transmission electron microscope examinations showed that released buds had cells rich in cytoplasmic inclusions and with reduced heterochromation materials. Soluble protein levels in the axillary bud of the second trifoliate lead in intact and decapitated seedlings were markedly reduced by direct ABA treatments. The electrophoretic patterns of the soluble proteins on acrylamide gels reflected qualitative changes as well as reduction in band number of protein extracts from lateral buds directly treated with ABA in decapitated seedlings. The foregoing observations strongly support the concept that the inhibitory effects of ABA rest on its influence on protein synthesis in lateral buds.

Introduction

The hormonal physiology of this phenomenon has been the subject of much work during the past six decades and has been reviewed by Philipps (1969, 1975) and Walton (1980). Although much attention has been directed towards the controlling influence of auxins, there is evidence for the involvement of other hormones like cytokinins, gibberellins and growth inhibitors (Hillman, 1970). Little attention though has been paid on the role of abscisic acid (ABA) on apical dominance and of the few reported studies, conflicting data were presented. In *Pisum* , ABA inhibited the growth of both apical and lateral buds (Arney and Mitchell, 1969; White and Mansfield, 1977), while in *Phaseolus* (Bellandi and Dorffling, 1974; Hartung and

Steigerwald, 1977; and Hillman, 1970) and *Vicia faba* (Everat-Bourbouloux and Charnay, 1982), ABA has been observed to stimulate the growth of the axillary bud. However, available data are not strictly comparable since the condition and age of the plant at the time of the experiment, the site of application of the hormone, the concentration of the hormone and the medium used for exogenous application of the hormone were different in the various experiments.

Review of Literature

Snow (1973) postulated that a growth inhibitory substance plays an important role in apical dominance. After its isolation and identification in the latter part of the 1960's (Addicott and Lyon, 1969), abscisic acid has been suggested (Arney and Mitchell, 1969; Hillman, 1970) as the likely candidate for the inhibitory substance postulated by Snow (1937). This inhibitory substance had to be something other than IAA, for it had to be readily transported basipetally as well as acropetally though the plant. ABA satisfies this requirement and furthermore, ABA is also known to influence lateral and growth (Arney and Mitchell, 1969).

Abscisic acid has been reported to exhibit little effects on buds of *Phaseolus vulgaris* L. seedlings when applied singly; while, greatest inhibition was obtained when applied in combination of indoleacetic acid and kinetin (Hillman, 1970). A comparative study of the effect of ABA on the growth of lateral and apical buds in seedlings of *Pisum sativum* L. and *Lens culminaris* L. showed that ABA directly applied to the lateral buds of decapitated seedlings had strong inhibitory effects on the bud (Bellandi and Dorffling, 1974). In addition, when ABA was applied to the apical bud of intact seedlings, its growth was inhibited while the lateral buds started to develop (Bellandi and Dorffling, 1974).

In a similar effort to establish that ABA might act as a correlative inhibitor of lateral bud growth, its effects on *Pisum sativum* and *Phaseolus vulgaris* seedlings were compared (White and Mansfield, 1977). Axillary bud growth in *Pisum sativum* was inhibited by two μg of ABA, while *Phaseolus vulgaris,* 10 μg of ABA was found to be still ineffective for axillary bud growth inhibition, suggesting that ABA might involved in the mechanism of correlative inhibition in *Pisum sativum* but not in *Phaseolus vulgaris.*

Several studies as reviewed by Jacobsen (1977) have attempted to explain the mode of action of ABA as it affects a number of physiological processes, such as dormancy, leaf senescence, growth, flowering, and seed germination. Sparse information exists on the possible mode of action of ABA in contrast to other phytohormones (Leshem, 1973). Of the different studies reported, the proposed mode of action of ABA basically involved the inhibition of nucleic acid synthesis (Chrispiels and Varner, 1967; Van Overbeek *et al.*, 1967; Madison and Rappaport, 1968; Villiers, 1968; Chen and Osborne, 1970; Shih and Rappaport, 1970; Evins and Varner, 1972).

Researches on the possible role of ABA in apical dominance have thus releaved a number of contradictory results. Studies since Snow's (1973) remarkable findings have been mainly done on legumes and the present work intends to contribute to the further understanding of the nature of apical dominance in legumes and its control on lateral bud growth as influence by ABA.

The present study aims to investigate the role of abscisic acid in the correlative process of apical dominance of *Vigna unguiculata* (L.) Walp. (stringbeans or 'sitaw') seedlings. The objectives of this study are: 1) to determine the effects of different concentrations of abscisic acid exogenously applied on intact and decapitated seedlings 24, 48, and 72 hours after ABA treatment on the length of axillary buds; 2) to compare the general morphological features and ultrastructure of released and inhibited buds; and 3) to determine the effects of exogenously applied ABA on the quantity and quality of soluble proteins in the axillary buds.

Materials and Methods

Axillary bud release

The seeds of *Vigna unguiculata* (L.) Walp. cv. Pole Sitaw used in this study were obtained from the Bureau of Plant Industry. They were surface-sterilized with 0.05% sodium hypochlorite solution, rinsed thrice with distilled water, and soaked in distilled water for 17-20 hours. They were then germinated in plastic trays (22 x 28 x 4 cm) half-filled with vermiculite and moistened with half-strength complete Hoagland solution (Hoagland and Arnon, 1938). After 1-2 days, seedlings of approximately similar size and vigor with roots measuring 1-3 cm in length were transplanted into plastic cups (7-cm diam) filled with vermiculite and irrigated up to saturation point on alternate days with half-strength complete Hoagland solution and with distilled water. Seedlings were grown in a greenhouse for 25 days before treatment with ABA. At this developmental stage, the second trifoliate had fully expanded and its lateral bud was on the average 2.7-5.9 mm long.

Three concentrations of ABA were used in the experiments, namely, 25, 50, and 100 ppm in 0.05% Tween 80 aqueous solution. Distilled water containing 0.05% Tween 80 served as the control. There were two sets of plant materials used, the intact and decapitated seedlings. In the latter group, seedlings were decapitated with a sharp razor blade one cm. above the base of the axillary bud of the second trifoliate. ABA was applied separately on intact seedlings at three different sites: the decapitated surface, the axillary bud, and both the decapitated surface and axillary bud. In both cases, 0.25 ml of ABA or control solution was introduced for three consecutive days using a 2.5 ml-disposable syringe. There were 10 replicates for each treatment. The length of the bud on the axil of the second trifoliate was measured daily from its point of attachment to the main stem to its apex with a Vernier caliper for seven days (Hartung and Steigerwald, 1977; White and Mansfield, 1977). Elongation was reported as the mean of the sample data for each treat-

ment. A multiple comparison of the Least-Squares Means of the axillary bud length for selected days and a simple ANOVA were undertaken to determine the statistical significance of difference among samples (Steel and Torrie, 1960; Walpole, 1974).

Preparation of buds for observation under the transmission electron microscope (TEM)

The apical region of lateral buds were trimmed off 24 hours after decapitation: one, untreated (control) and the other treated with 100 ppm ABA. The buds were prepared for electron microscopy following the technique of Hayat (1981). Stained copper grids containing the ultrasections were examined under the transmission electron microscope and electron micrographs were taken at 5000X.

Soluble Protein Determination

Lateral buds subtended by the second fully expanded trifoliate from 25-day old *Vigna unguiculata* seedlings were excised and collected 24, 48, and 72 hours after exogenous treatment with varying concentrations of ABA as described above and immediately stored in the freezer for one week. Soluble proteins were extracted using the methods of Steward *et al.* (1985) with some modifications. Soluble protein content was determined by Lowry's Folin test (Lowry *et al.*, 1951) as modified by Garney *et al.* (1977) and Rodriguez *et al.* (1983) (Appendix 1). The protein content of a sample was estimated from a standard curve (Appendix 2) of crystalline bovine serum albumin (Sigma). A simple ANOVA was undertaken to determine the statistical significance of differences among samples (Steel and Torrie, 1960; Walpole, 1974).

Electrophoresis of Soluble Protein Extracts

The extracts prepared earlier for protein content analysis were also used for the electrophoretic runs in polyacylamide gels. The procedures followed were adapted from those of Davis (1964), Nerenberg (1966), and Steward *et al.* (1965) as modified by Tabbada and Flores (1981). The stock solutions (Appendix 3) for the preparation of the gels were stored in brown bottles and refrigerated. Each gel was transferred to a 10-ml test tube and stored in 7% acetic acid. The number and intensity of bands in each gel were then recorded and diagrammed using an adjusted mobility scale (Rf) with a range of 100. Band intensity was described as dark, light, and faint, depending on their staining intensities.

Results and Observations

Axillary Bud Release

The pattern of the growth of the lateral buds at the axil of the second trifoliate of *Vigna unguiculata* seedlings in response to 25-100 ppm ABA applied on the

intact apical bud or decapitated surface is illustrated in Figure 1. The application of ABA on decapitated surfaces significantly reduced bud elongation. Such an inhibition was most evident in seedlings treated with 100 ppm ABA. There was, however, no significant difference between those treated with 25 and 50 ppm (Table 1). It may also be noted that the axillary buds of intact seedlings whose apical buds were treated with 100 ppm ABA elicited some significant growth response after seven days (Table 1a).

The growth of axillary buds treated directly with 25-100 ppm ABA in intact and decapitated seedlings is shown in Figure 2. Only decapitated seedlings not treated with ABA exhibited significant elongation of the axillary bud on day seven (Table 2). The buds of intact seedlings treated with ABA as well as those of the control group failed to grow, i.e., they remained inhibited and showed no significant changes during the experiment.

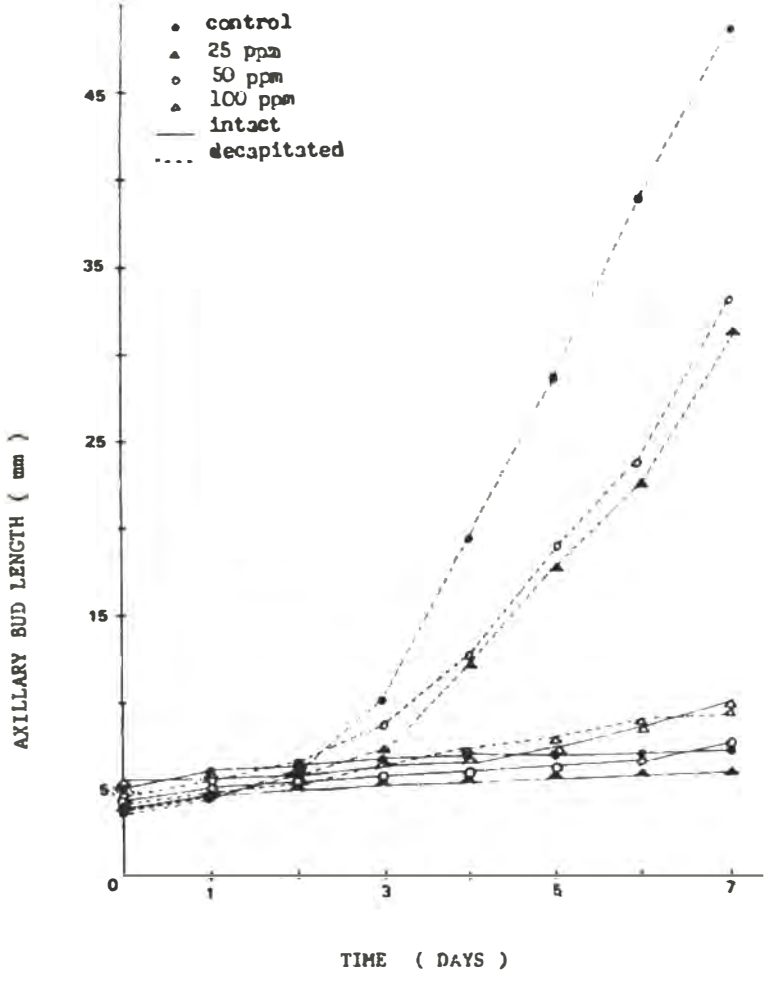

Figure 1. The effect of ABA on the growth of the axillary bud when applied on the stem apex or the decapitated surface.

Table 1. Multiple comparison of Least-Squares Means of second axillary bud length on day 7 in decapitated seedlings treated with ABA at the decapitated surface.

ppm ABA (mean bud length, mm)	*0 (48.613)*	*25 (31.228)*	*50 (32.858)*	*100 (9.468)*
0 (48.613)	–	–	–	
25 (31.228)	***	–	–	–
50 (32.858)	**	ns	–	–
100 (9.468)	***	***	***	–

Table 1a. Multiple comparison of Least-Squares Means of second axillary bud length on day 7 in intact seedlings treated with ABA at the apical bud.

ppm ABA (means bud length, mm)	*0 (7.028)*	*25 (5.818)*	*25 (7.190)*	*100 (9.728)*
0 (7.028)	–	–	–	–
25 (5.818)	ns	–	–	–
50 (7.190)	ns	ns	–	–
100 (9.728)	*	**	ns	–

ns = not significant $p > .05$
* $.05 \geq p > .01$
** $.01 \geq p > .001$
*** $.001 \geq p > .0001$

When ABA was applied simultaneously on the lateral and the apical buds of intact seedlings, there was no axillary bud release or growth even after seven days (Figure 3). The simultaneous treatment of the decapitated seedlings with 25 and 50 ppm ABA delayed bud release which was evident only on day five. There was however no significant differences between these and the control seedlings on days

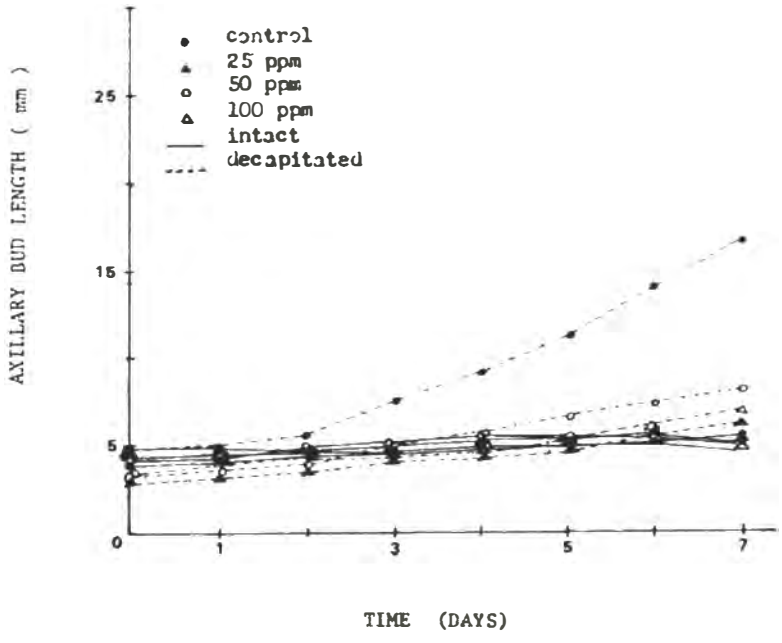

Figure 2. Multiple comparison of Least-Squares Means of length of ABA-treated second axillary bud on day 7 in decapitated seedlings.

Table 2. Multiple comparison of Least-Squares Means of length of ABA-lenth second axillary bud on day 7 in decapitated seedlings.

ppm ABA (mean bud -length, Mm)	*0 (16.530)*	*25 (6.385)*	*50 (8.325)*	*100 (6.960)*
0 (16.530)	–	–	–	–
25 (6.385)	**	–	–	–
50 (8.325)	*	ns	–	–
100 (6.960) –	**	ns	ns	–

ns = not significant $p > .05$ $p > .05$
* $.05 \geq p > .01$
** $.01 \geq p > .001$

Table 3. Multiple comparison of Least-squares Means of second axillary bud length on day 5 in decapitated seedlings simultaneously treated with ABA at the decapitated surface and the lateral buds.

ppm ABA (mean bud length, mm)	*0 (13.423)*	*25 (8.525)*	*50 (7.710)*	*100 (3.808)*
0 (13.423)	—	—	—	—
25 (8.525)	ns	—	—	—
50 (7.710)	ns	ns	—	—
100 (3.808)	ns	ns	ns	—

ns = not significant $p > .05$

Table 3a. Multiple comparison of Least-Squares Means of second axillary bud length on day 5 in intact seedlings simultaeously treated with ABA at both the apical and lateral buds.

ppm (mean bud length, mm)	*0 (6.093)*	*25 (6.068)*	*50 (4.505)*	*100 (5.578)*
0 (6.093)	—	—	—	—
25 (6.068)	ns	—	—	—
50 (4.505)	ns	ns	—	—
100 (5.578)	ns	ns	ns	—

Table 4. Multiple comparison of Least-Squares Means of second axillary bud length on day 7 in decapitated seedlings simultaneously treated with ABA at the decapitated surface and the lateral buds.

ppm ABA (mean bud length, mm)	*0 (20.530)*	*25 (13.538)*	*50 (12.138)*	*100 (4.238)*
0 (2.530)	—	—	—	—
25 (13.538)	ns	—	—	—
50 (12.138)	ns	ns	—	—
100 (4.238)	*	ns	ns	—

ns = not significant $p > .05$
* $.05 \geq p > .01$

Table 4a. Multiple comparison of Least-Squares Means second axillary bud length on day 7 in intact seedlings simultaneously treated with ABA at both the apical and lateral buds.

ppm ABA (mean bud length, mm)	*0 (6.270)*	*25 (6.260)*	*50 (5.123)*	*100 (5.643)*
0 (6.270)	—	—	—	—
25 (6.260)	ns	—	—	—
50 (5.123)	ns	ns	—	—
100 (5.643)	ns	ns	ns	—

Table 5. ANOVA Table for length of second axillary buds on day 7 in intact (IN) and decapitated (DE) seedlings treated with ABA at the apical bud or decapitated surface.

SV	SS		df		MS		f	
	IN	DE	IN	E	IN	DE	IN	DE
C	2.145	1434.050	3	3	9.382	478.017	5.93**	23.31***
D	38.373	5293.778	7	7	5.482	756.254	3.47**	36.88***
C X D	10.086	1614.888	21	21	0.480	76.899	0.30n2	3.75***
ERROR	50.620	656.158	32	32	1.582	20.505	–	–
TOTAL	127.225	8998.874	63	63	–	–	–	–

ns = not significant $p > .05$
** $.01 \geq p > .001$
*** $.001 \geq p > .0001$
C = ABA concentration (ppm)
D = day

Table 6. ANOVA Table for length of second axillary buds on day 7 in intact (IN) and decapitated (DE) seedlings treated with ABA at the lateral bud.

SV	SS		df		MS		f	
	IN	DE	IN	DE	IN	DE	IN	DE
C	0.743	213.085	3	3	0.248	71.028	0.17ns	5.99**
D	5.238	250.946	7	7	0.748	35.849	0.51ns	3.02*
C X C	1.460	119.396	21	21	0.070	5.686	0.05ns	0.48ns
ERROR	46.830	379.613	32	32	1.643	11.863	–	–
TOTAL	54.272	963.040	63	63	–	–	–	–

ns = not significant $p > 0.5$
* $.05 \geq p > .01$
II $.01 \geq p > .001$
C = ABA concentration (ppm)
D = day

Ultrastructure of an Axillary Bud 24 hr. after Decapitation

Cells from the apical meristem of the axillary buds were found to be rich in cytoplasmic inclusions (Figure 4). Food reserves were abundant and conspicuously located within vacuoles of varying sizes (Figure 4 and 5). The nucleus possessed small dense areas (heterochromatin) mostly distributed near the nuclear membrane

Table 7. ANOVA Table for length of second axillary buds on day 7 in intact (IN) and decapitated (DE) seedlings simultaneously treated with ABA at the apical bud or decapitated surface and the lateral buds.

SV	*SS*		*df*		*MS*		*f*	
	IN	*DE*	*IN*	*DE*	*IN*	*DE*	*IN*	*DE*
C	25.716	290.560	3	3	8.572	96.853	20.87***	2.45^{ns}
D	11.640	667.411	7	7	1.663	95.344	4.05**	24*
C X D	1.677	277.349	21	21	0.080	13.207	0.19ns	0.33^{ns}
ERROR	13.144	1264.245	32	32	0.411	39.508	–	–
TOTAL	52.177	2499.565	63	63	–	–	–	–

ns = not significant $p > .05$
* $.05 \geq p > .01$
** $.01 \geq p > .001$
*** $.001 \geq p > .0001$
C = ABA concentration (ppm)
D = day

(Figure 5). The cells of released buds showed increasing vacuolation with a lot of small vacuoles tending to coalesce (Figure 6 and 7) and finally forming a big central vacuole (Figure 8). The vacuoles contain both osmiophilic food reserves (Figures 4, 5 and 8) and amorphous material (Figures 6 and 7) and in some cases, vesiculate materials (Figure 7).

Ultrastructure of ABA-treated Buds in Decapitated Seedlings

Conspicuous vacuoles were noted among cells in the apical mieristem of ABA-treated axillary buds (Figures 9-12). Food reserves were also abundant, but these remained mainly outside the vacuoles (Figures 9-12). The vacuoles were found to be commonly filled only with amorphous materials (Figures 10-12). In a few sections, osmiophilic bodies which were noted to be enclosed within a vacuole were smller than those found within the vacuole of released, untreated buds. The nuclei possessed large dense areas (heterochromatin) which were dispersed throughout the nuclear region (Figures 9, 10 and 12).

Soluble protein levels in axillary buds

The analysis of variance for the mean soluble protein content in the second axillary buds of all treated seedlings showed that the site of ABA application significantly affected soluble protein levels (Table 8). The analysis of variance for the mean soluble protein content in the second lateral bud of all treated decapitated seedlings revealed that prolonged application of higher levels of ABA significantly influenced the soluble protein content (Table 8).

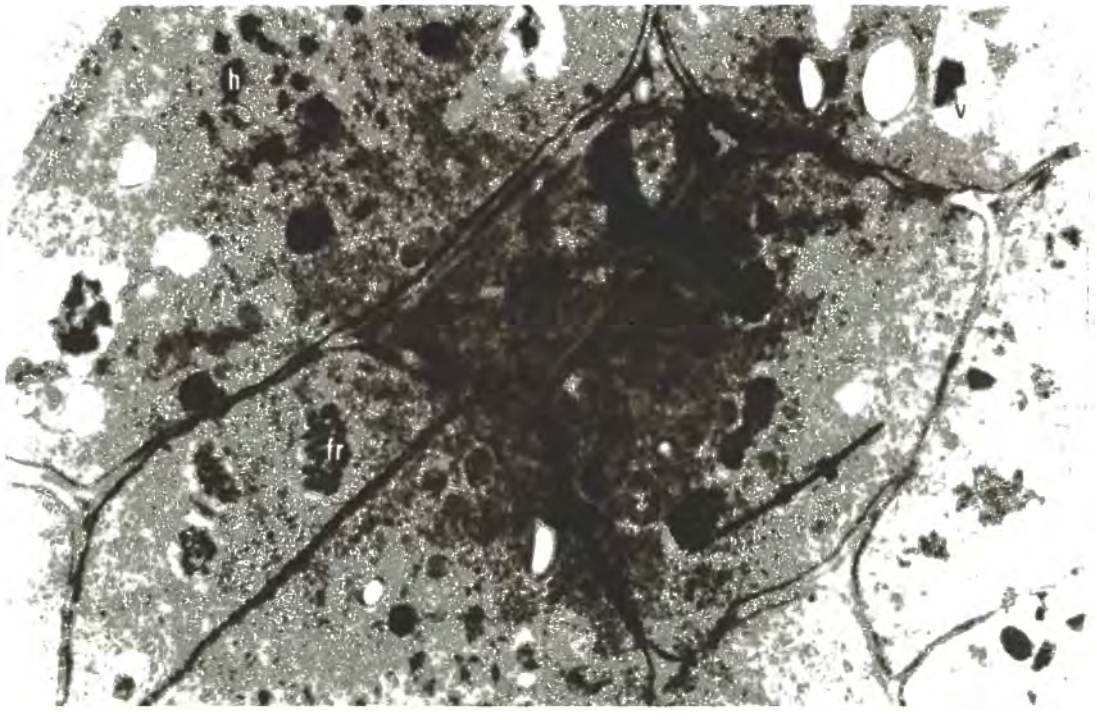

Figure 4. Cells of released, untreated axillary bud showing numerous small vacuoles (v) with lots of food reserves (fr); heterochromatin material (h) minimal (10,000X).

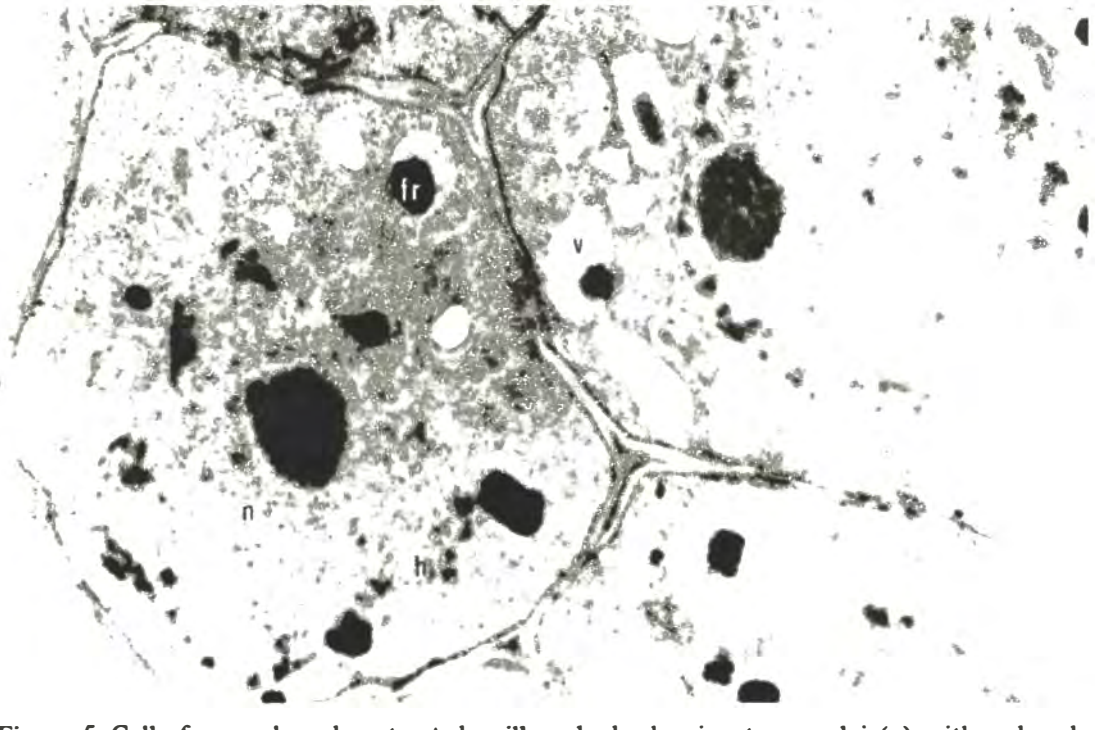

Figure 5. Cells from released, untreated axillary buds showing two nuclei (n) with reduced amounts of heterochromatin material (h) confined to the peripheral area of the nuclei. Vacuoles (v) present with food reserves (fr) (10,000X).

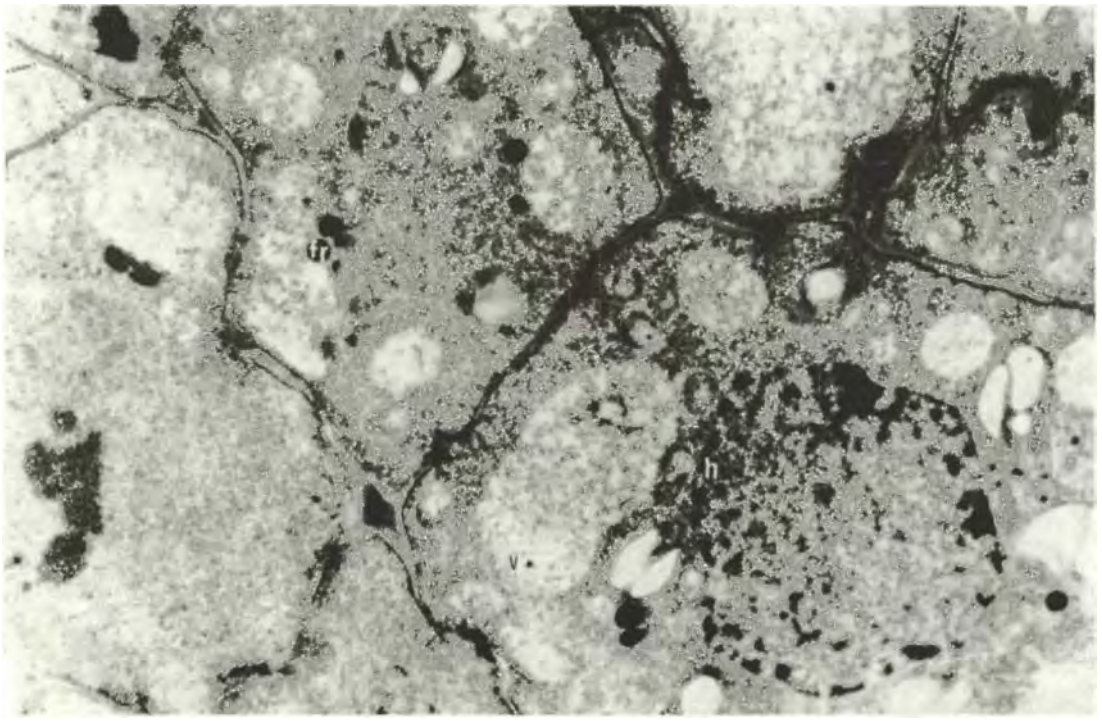

Figure 6. Vacuoles (v) are about to coalesce; food reserves (fr) mobilized to the vacuoles; nucleus with small heterochromatin bodies (h) (10,000X).

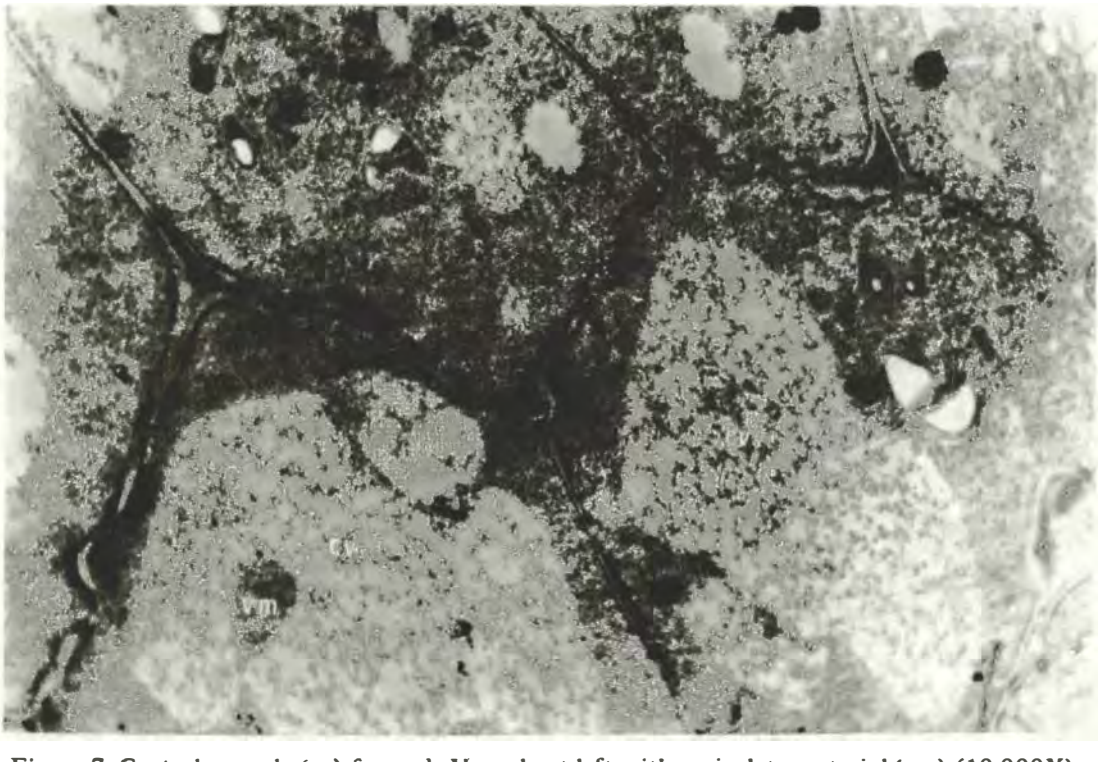

Figure 7. Central vacuole (cv) formed. Vacuole at left with vesiculate material (vm) (10,000X).

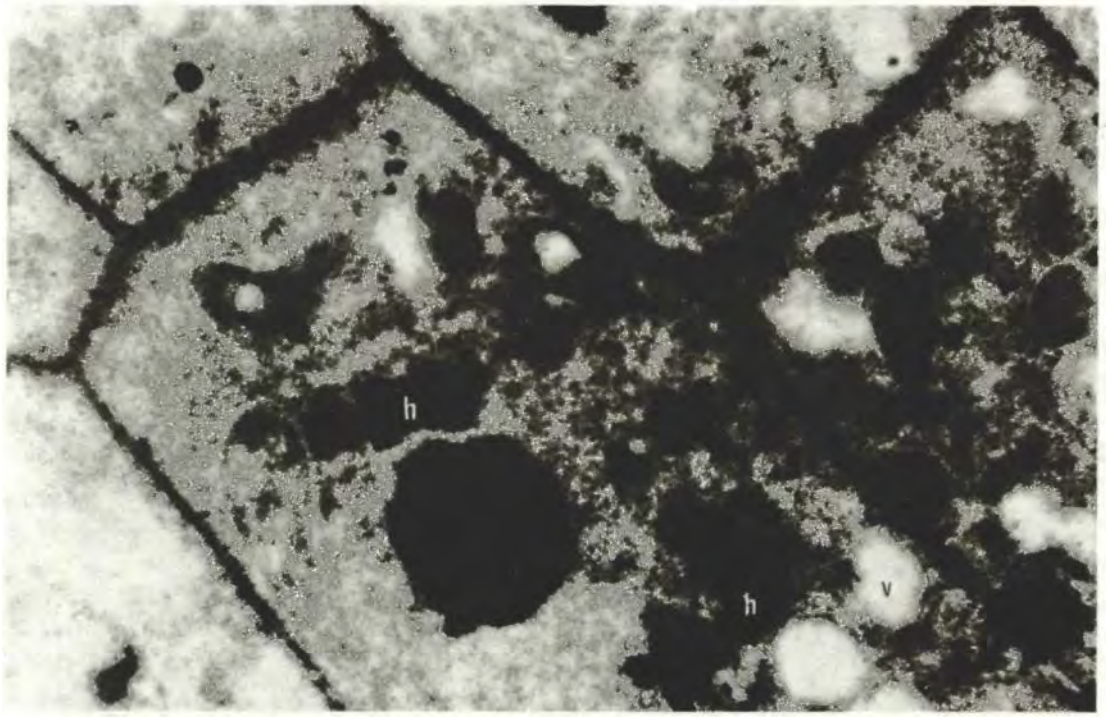

Figure 8. Central vacuoles (cv) with incorporated food reserves (fr) found in cells from released untreated axillary bud (10,000X).

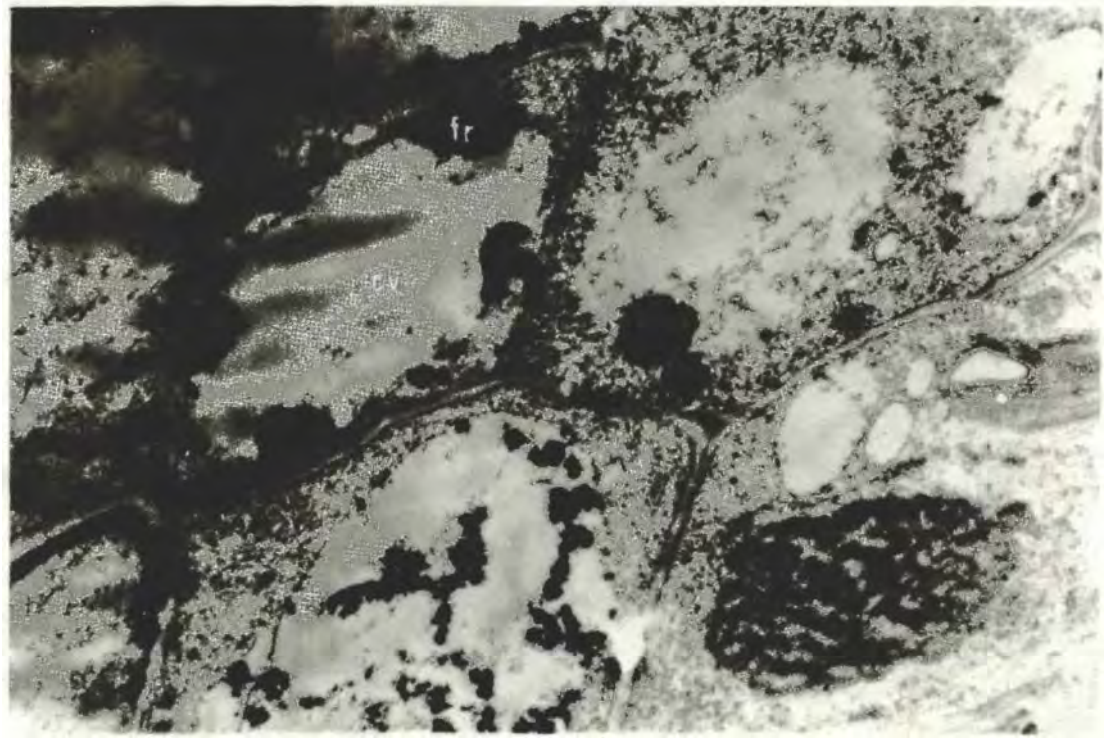

Figure 9. Cells of inhibited axillary bud, treated with 100 ppm ABA. Note vacuolation (v) and large heterochromatin bodies (h) (10,000X).

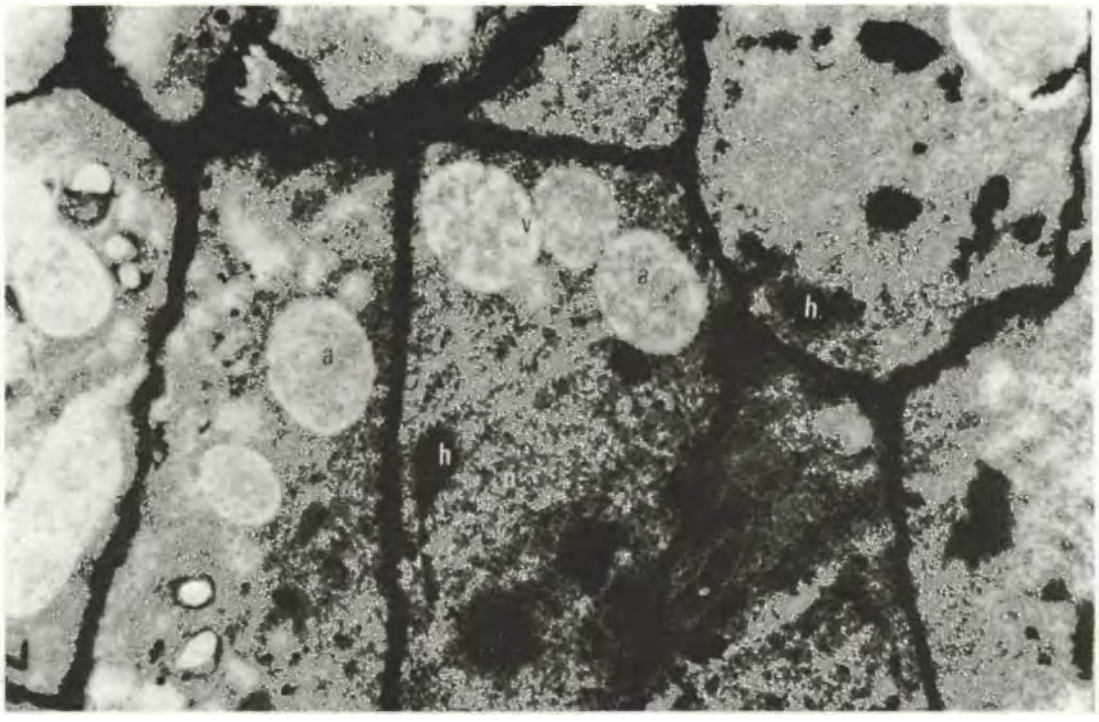

Figure 10. Increase in vacuolation (v). Vacuoles contain amorphous materials (a). Note large heterochromatin areas (h) in nucleic (n) (10,000X).

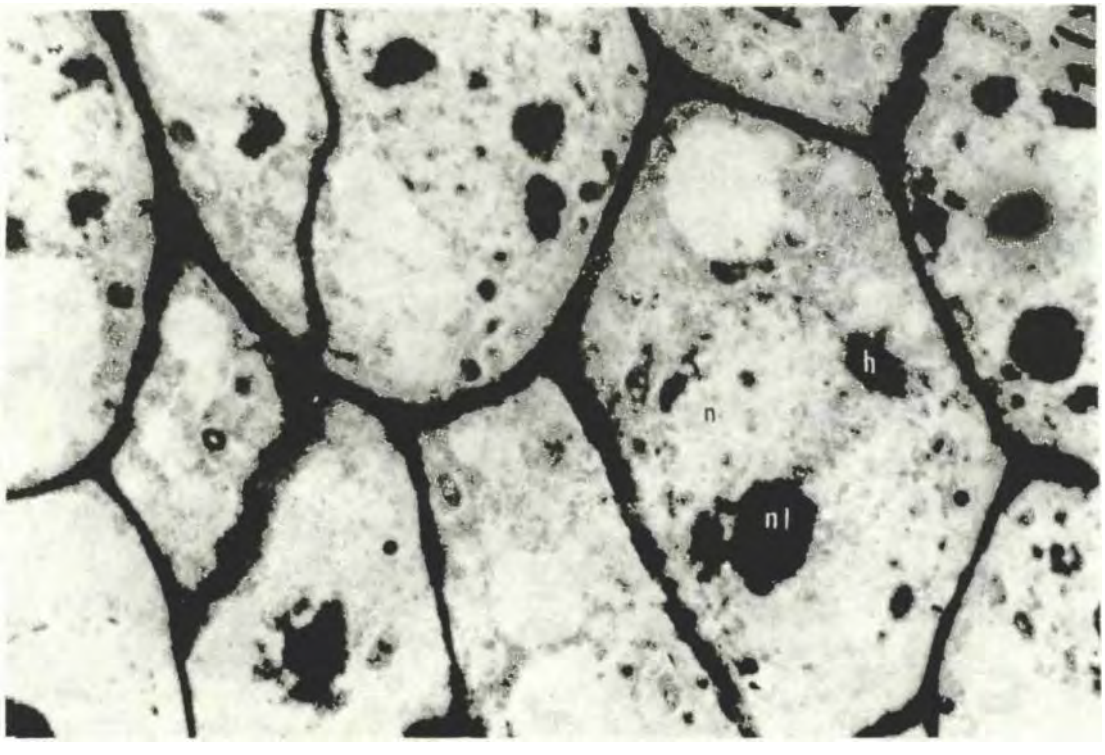

Figure 11. Nuclei (n) showing large heterochromatin bodies (h); nucleous (nl) (10,000X).

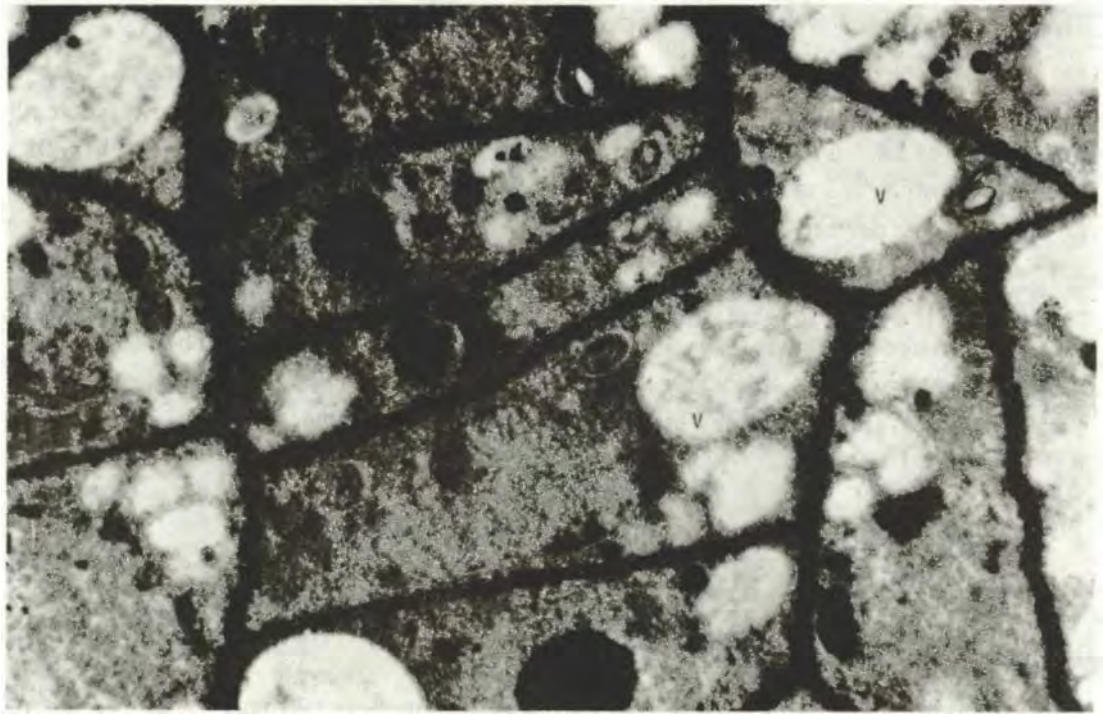

Figure 12. Highly vacuolated cells to ABA treatment (vacuoles, v) (10,000X).

Table 8. ANOVA Table for soluble protein content of the second axillary bud in intact (IN) and decapitated (DE) seedlings.

SV	*SS*		*df*		*MS*		*f*	
	IN	*DE*	*IN*	*DE*	*IN*	*DE*	*IN*	*DE*
C	78.124	147.952	3	3	26,041	49.317	0.209^{ns}	$1,189^{ns}$
S	12,710.658	196.737	2	2	6,355.329	98.363	51.054**	2.365^{ns}
T	529.258	129.963	2	2	264,529	64.991	2.126^{ns}	1.563^{ns}
C X S	2,096,655	519.314	6	6	349.442	86.552	2.807^{ns}	2.082^{ns}
C X T	585.970	910.092	6	6	97.661	151.682	0.785^{ns}	3.648*
S X T	551.928	198.299	4	4	137.982	49.574	1.108^{ns}	1.192^{ns}
ERROR	1,493.797	498.919	12	12	124.483	41.576	–	–
TOTAL	18,046.393	2,601.278	25	25	–	–	–	–

ns = not significant $p > .05$
* $.05 > p > .01$
** $p < .01$

C = ABA concentration (ppm)
S = site of ABA application
T = time (hr)

Electrophoretic patterns of soluble proteins

The electrophoretic patterns of soluble protein extracts from lateral buds on the axil of the second trifoliate of intact (Figures 13, 15, 17) and decapitated seedlings (Figures 14, 16, 18) variously treated with 25-100 ppm ABA are diagrammatically represented in Figures 13-18. Bands varied in staining intensity depending both on whether the seedlings were intact or decapitated and on the concentration of the applied ABA. Bands were darkest from control intact seedlings after 48 and 72 hours (Figure 13) and were faintest from decapitated seedlings where their axillary buds were directly treated with higher ABA concentrations (Figure 16). Bnads were noted to decrease in number in extracts from decapitated seedlings (Figures 14, 16, 18) and from intact seedlings in which the axillary buds were directly treated with ABA (Figure 15).

Discussion

Axillary Bud Release

The presence of an intact apex inhibits the development of lower lateral buds and, hence, decapitation leads to their growth or release from apical dominance. A decrease in the endogenous levels of ABA in the different parts of the stem following decapitation has been noted in broad bean (*Vicia faba*) plants (Eve-

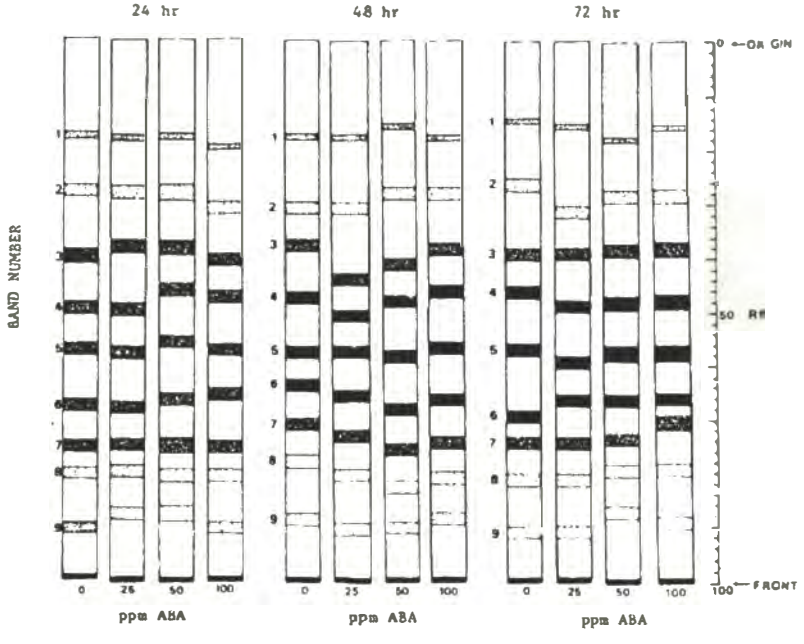

Figure 13. Diagrammatic patterns of the electrophoretic separation of soluble proteins of intact seedlings treated at the apical bud; control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100); after 24 hr; 48 hr; and 72 hr. Band intensities are described as: very dark; light; and faint.

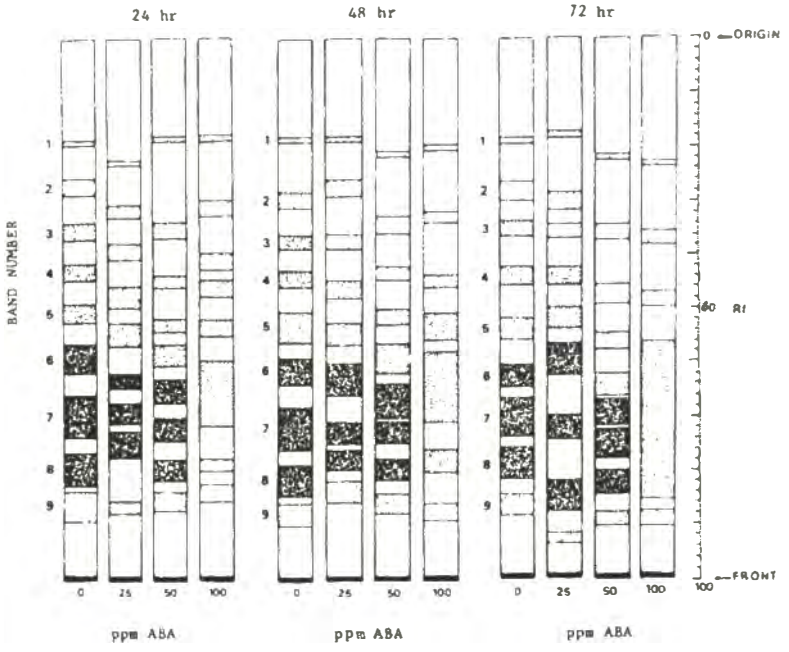

Figure 14. Diagrammatic patterns of the electrophoretic separation of solube proteins of decapitated seedlings treated at the decapitated surface; control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100); after 24 hr; 48 hr; and 72 hr. Band intensities are described as dark; light; and faint.

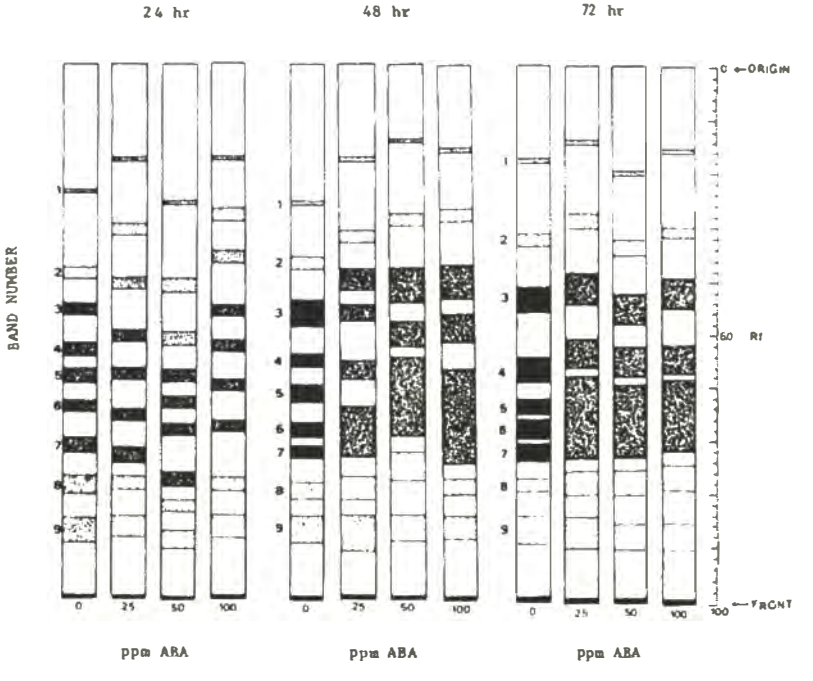

Figure 15. Diagrammatic patterns of the electrophoretic separation of soluble proteins of intact seedlings treated at the lateral bud: control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100); after 24 hr; 48 hr; and 72 hr. Band intensities are described as: very dark; dark; light; and faint.

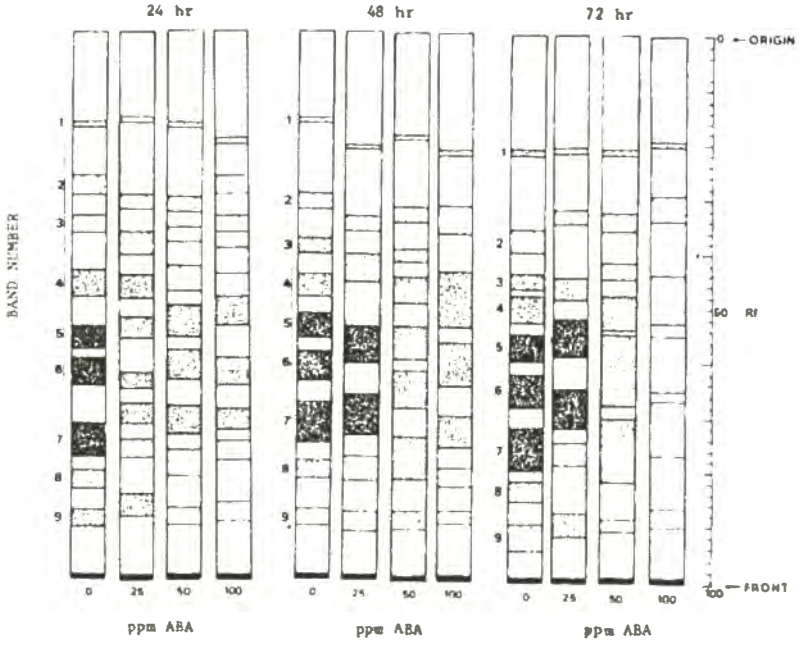

Figure 16. Diagrammatic patterns of the electrophoretic separation of soluble proteins of decapitated seedlings treated at the lateral bud: control control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100); after 24 hr; 48 hr; and 72 hr. Band intensities are described as: dark; light; and faint.

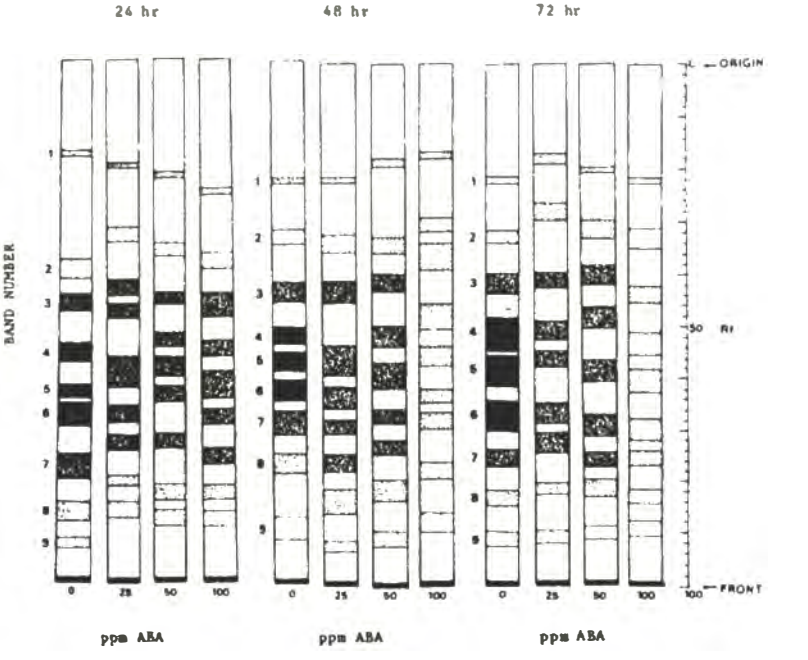

Figure 17. Diagrammatic patterns of the electrophoretic separation of soluble proteins of intact seedlings treated simultaneously at the apical and lateral buds: control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100); after 24 hr; 48 hr; and 72 hr. Band intensities are described as: very dark; dark; light; and faint.

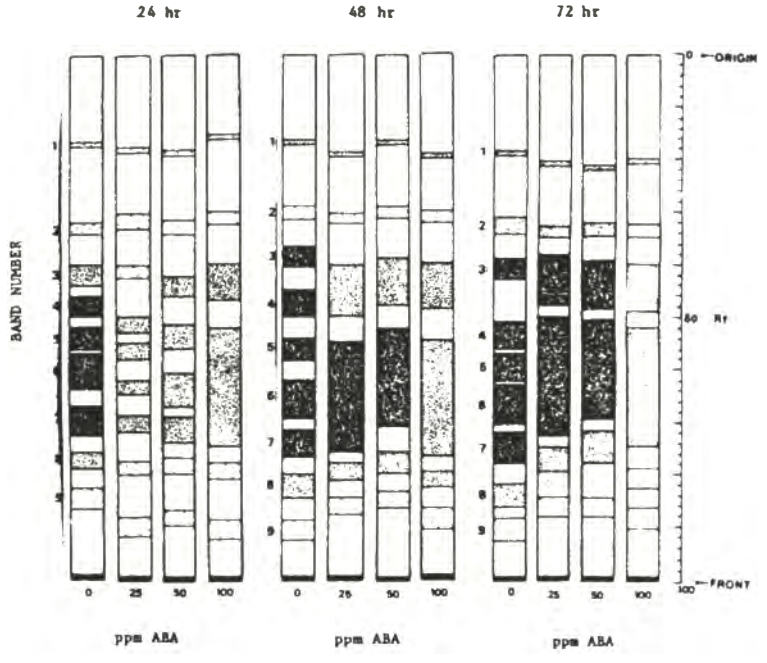

Figure 18. Diagrammatic patterns of the electrophoretic separation of soluble proteins of decapitated seedlings treated simultaneously at the decapitated surface and the lateral bud: control (0); 25 ppm ABA (25); 50 ppm ABA (50); 100 ppm ABA (100; after 24 hr; 48 hr; and 72 hr. Band intensities are described as: dark; light; and faint.

rat-Bourbouloux and Charnay, 1982). This may account for the release of axillary bud in decapitated *Vigna* seedlings. Knox and Wareing (1984) reported a 60-80% and 40-60% decrease in the amount of ABA in the lateral buds and stem tissues, respectively, of *Phaseolus vulgaris* seedlings 24 hours after decapitation.

The exogenous application of adequate amounts of ABA on an intact apex apparently inhibited its growth and subsequently weakened its capacity to inhibit the growth of lower axillary buds. This was quite evident in *Vigna* seedlings treated for three days with 50 and 100 ppm ABA. The observation that those treated with 25 ppm ABA elicited responses similar to those of the control group would indicate that the level of applied ABA was largely inadequate in inhibiting apical development so as to effect lateral bud release. These observations are consistent with the reported dependence of the degree of apical and lateral bud inhibition on the amount of applied ABA (Bellandi and Dorffling, 1974).

Bellandi and Dorffling (1974) and Hartung and Steigerwald (1977) also reported that ABA applied on lateral buds *in situ* is hardly transported to other tissues. Hence, the growth of treated tissues in both intact and decapitated seedlings was *per se* inhibited by the application of ABA. This was quite evident whenever the axillary buds were treated with varying levels of ABA. Decapitation likewise failed to release the axillary buds of the second trifoliate when these were treated with ABA.

Ultrastructural changes in released buds

During the early stages of bud release (24 hours) the food reserves were apparently mobilized within cells and were incorporated into the vacuole to provide substrates for the production of energy needed by the growing cell (Lott, 1980). The food reserves could have been degraded within the vacuoles where a variety of hydrolytic enzymes are located (Matile, 1978; Boller and Kende, 1979; Nishimura and Beevers, 1979). Some degradation products were utilized for structural purposes of the metabolic cell (Lott, 1980). The reduced amounts and sizes of heterochromatin material within the nucleus further indicate that transcription was occurring. It has been demonstrated that transcriptionally active chromatin materials tend to be more diffused (Jordan *et al.*, 1980). Hence, the condition of the nucleus, the increase in vacuolation, and the change in vacuolar contents suggest that the cells of the buds undergoing release were actively growing (Booker and Dwivedi, 1972).

Ultrastructural changes in ABA-treated buds

The electron micrographs of cells from the apical meristem of axillary buds from decapitated seedlings of *Vigna unguiculata* showed an apparent increase in vacuolation after 24 hours of ABA treatment. A similar observation had been described into potato tuber buds wherein vacuolation was evident an hour after treatment with ABA (Shih and Rappaport, 1970). That the vacuoles contained mainly amorphous materials is typical of inhibited cells (Booker and Dwivide, 1972). Since the food was largely confined within the cytoplasm, no mobilization of food reserves for the growth or release of the axillary bud could have occurred as earlier proposed by Lott (1980). Furthermore, the presence of relatively large dense areas (heterochromatin) in the nuclei of inhibited cells is suggestive of reduced or no transcriptional activity which according to Lott (1980) is characteristic of cells that are not actively growing.

Soluble protein levels

The marked reductions in the soluble protein content of the axillary buds of the second trifoliate in both ABA-treated as well as untreated *Vigna* seedlings when their apices were decapitated might be attributed to the elimination or removal of the source of substances, e.g., endogenous growth regulators, which are transported down to influence protein synthesis in lower tissues, including the axillary buds. It had been reported that the removal of the stem apex of *Vicia faba* L. seedlings affected the distribution of auxin in the lower tissues of the stem (Everat-Bourbouloux and Bonnemain, 1980).

Moreover, exogenously applied auxin has been reported to prevent the decrease of ABA content of the lateral bud and stem tissues during bud release after decapitation of *Lycopersicum esculentum* (Tucker, 1978) and *Phaseolus vulgaris* (Knox and Wareing, 1984) and of isolated *Pisum* stem tissues when incubated with

IAA (Eliasson, 1975). Auxin thus acts to maintain high levels of ABA which is responsible for the inhibition of bud growth. The removal of the stem apex accompanied with exogenous application of ABA only served to reduce soluble protein levels in decapitated *Vigna* seedlings. This suggests that inhibitory effect of ABA is on protein synthesis. Some investigators have suggested that ABA may affect protein synthesis at the transcriptional level (Villiers, 1968; Pearson and Wareing, 1969) or at the translational level or in post-transcription processes (Chen and Osborne, 1970; Jacobsen, 1977). Since the ultrastructural analyses have revealed reduced heterochromatin materials in nuclei of cells from released buds, it is possible that ABA affected soluble protein synthesis at the transcriptional level.

The application of ABA on the decapitated apex leads to a reduction in soluble protein content of the axillary bud of the second trifoliate. Since decapitation eliminated the shoot apex, no inhibitory effects were exerted on the lateral buds resulting in their elongation. The levels of soluble proteins were reduced because these proteins were utilized by the lateral buds for elongation (Robinson and Brown, 1952). Directly applied ABA to the apical bud generally resulted into an increase in soluble protein in the lateral bud of 25 ppm- and 50 ppm-treated seedlings. These concentrations were inadequate to inhibit the apical bud and bring about the release of lateral buds. Since the lateral buds were inhibited, soluble protein levels were high because they were not being utilized. However, 100 ppm ABA significantly inhibited the apical bud, so that the lateral buds were released (Figure 1 and Table 2a). This would explain the reduced levels of soluble protein which were in effect being utilized for lateral bud growth. ABA was observed to likewise inhibit the growth of the apical bud of intact *Psium sativum* and *Lens culinaris* seedlings (Bellandi and Dorffling, 1974).

Direct ABA treatment of the lateral buds in intact seedlings decreased soluble protein content. This suggests that ABA can directly inhibit soluble protein synthesis in intact seedlings. The general increase in soluble protein content of the lateral buds in decapitated seedlings may be attributed to their release from the untreated decapitated seedling (Table 2), the treated seedlings nevertheless elicited some degree of elongation over the intact set of seedlings. This would suggest that somehow, certain soluble proteins were synthesized. But since exogenous application of ABA inhibited their further growth, these soluble proteins accumulated and eventually lead to an increase in their levels. After 72 hours, the greatest percentage increase was with 25 ppm. Relatively low levels of ABA, between one and 25 ppm, directly applied to the axillary buds of decapitated *Phaseolus vulgaris* (Hillman, 1970), *Pisum sativum* (Bellandi and Dorffling, 1974), and *Phaseolus coccineus* (Hartung and Steigerwald, 1977) seedlings were reported to be ineffective in causing complete inhibition of lateral bud growth. The degree of inhibition depended on the amount of ABA applied, e.g., 0.1 μg had a significant effect and 10 μg completely prevented growth of lateral buds in *Pisum* (Bellandi and Dorffling, 1974). Hence the levels of soluble proteins extracted from the lateral buds

of decapitated seedlings treated simultaneously at the cut of surfaces and the lateral bud were likewise reduced.

Electrophoretic patterns of Soluble proteins

That exogenously applied ABA possibly influenced protein synthesis in the lateral buds of Vigna seedlings is reflected in the variations in the acrylamide gel electrophoretic patterns of extracted soluble proteins.

In untreated seedlings, the change in the staining intensity of three to five bans (#3-7) might indicate an accumulation of specific protein fractions (Steward *et al.*, 1965; Edelman and Singh, 1967). Although seedling decapitation induced the lateral buds not treated with ABA to elongate (Rivero and Tabbada, 1984), it is highly possible that either no new soluble proteins were formed since there was no change in the number of bands or any newly synthesized proteins could have been utilized during bud growth (Madison and Rappaport, 1986). That the levels of newly synthesized proteins associated with bud release after decapitation were not detectable in the gels cannot likewise be discounted (Steward *et al.*, 1965). A comparison of the nine electrophoretic bands of soluble proteins in axillary buds from intact and decapitated but untreated seedlings also reflect distinct differences in their staining intensity, i.e, more light and wider bands were evident in the gel patterns of bud soluble proteins from decapitated seedlings. This suggests that the soluble proteins were being utilized consistent with the earlier observations on the soluble protein levels and on the ultrastructural examinations.

Different protein components or the changes in concentration of the various protein fractions distinguished an inhibited lateral bud in an untreated intact seedling from a released one in an untreated decapitated seedling. The wider bands reflected in electrophoregrams from decapitated seedlings suggest not only possible changes in the levels of the soluble protein but also changes in the quality of soluble proteins as a consequence of cell elongation (Street and Cockburn, 1972). The differences in electrophoretic patterns between intact and decapitated control seedlings may in part reflect the difference in the growth activities of their lateral buds.

In decapitated and untreated seedlings, there were no significant variations in the electrophoretic patterns of soluble proteins from axillary buds after 24, 48, and 72 hours, during which period the buds were released from apical dominance. When the decapitated surfaces were treated with 25 and 50 ppm ABA, not only were the buds also released but the gel patterns were also similar during the 72-hour growth period. When decapitated surfaces were treated with 100 ppm ABA, the axillary buds were not released and the corresponding electrophoretic pattern of the soluble proteins was light in contrast with those of the control and 25 and 50 ppm ABA-treated seedlings. ABA has been reported to be transported basipetally in cotton seedlings (Shindy *et al.*, 1973) and at high concentrations may inhibit protein synthesis (Jacobsen, 1977). Hence, it seems likely that when decapitated stem apices of *Vigna* seedlings were treated with 100 ppm ABA for

three consecutive days, adequate levels of the growth regulator reached the lateral buds through basipetal transport so as to inhibit or reduce soluble protein synthesis and cause inhibition in the release of the bud. The levels of ABA reaching the lateral bud when seedlings were given only 25 and 50 ppm, however, have been insufficient to reduce protein levels and inhibit the growth of the axillary buds.

When ABA was applied on the axillary buds of intact seedlings, there were also changes in the electrophoretic patterns of bud soluble proteins although no bud release was noted during the 72-hour observation period. The changes in the gel patterns of untreated or control seedlings after 48 and 72 hours also were not associated with bud release. Hence, the observed differences in the electrophoregrams would merely indicate possible changes in the soluble protein synthesis of the buds exposed to varying levels of ABA which are all characteristics of inhibited lateral buds of *Vigna unguiculata*. As mentioned earlier, ABA is known to inhibit protein synthesis (e.g., Chen and Osborn, 1970; Jacobsen, 1977). Hence, the reduction in the number of soluble protein bands from nine to seven after 48 and 72 hours in ABA-treated buds might be a reflection of the inhibitory effects of ABA on protein synthesis.

The electrophoregrams of soluble protein extracts from lateral buds directly treated with ABA in decapitated seedlings were supportive of the strong inhibitory effect of ABA on bud soluble protein synthesis. The profiles for each of the treated group were reflective of the varying degrees of the inhibitory effect of different ABA levels on soluble protein synthesis. The effects were mostly evident after 72 hours, after which time, the number of bands decreased from nine to six for 100 ppm ABA-treated buds. The staining patterns likewise reflect the relative effectiveness of varying ABA levels on bud soluble protein synthesis. The synthesis of particular soluble proteins could have likewise been inhibited by ABA at this level since afer 72 hours, all bands turned faint.

The simultaneous treatment of the apical and lateral buds in intact seedlings produced electrophoregrams with profiles similar to buds from intact seedlings treated at the apical bud: nine distinct bands, although none from the treated groups intensified in staining 48 and 72 hours after ABA application. Hence, even if the axillary buds were likewise not released, differences in their electrophoretic profiles could still be noted. At 100 ppm, ABA was found to reduce soluble protein levels after 48 hours and the synthesis of certain soluble protein fractions could also have been inhibited since the bands turned light. Bud soluble protein levels and the synthesis of certain fractions were apparently affected by the increasing levels of ABA through 100 ppm direct applications concommitant with increase due to basipetal transport of ABA.

The electrophoretic patterns depicted by extracts from lateral buds of decapitated seedlings simultaneously treated at the cut surfaces and lateral buds appeared similar to those derived from extracts of directly treated buds in intact seedlings. That the axillary buds remained inhibited even after 72 hours might be indicative of the inhibitory effects of ABA on soluble protein levels and on the synthesis

of certain soluble protein fractions since a decrease in band number in all ABA-treated groups was noted. Moreover, after 48 hours, all treated groups yielded electrophoregrams in which the band numbers 3 to 7 were fused indicating further, the decrease or absence of certain protein meoities possibly due to their utilization. In addition, the electrophoregrams of extracts from seedlings treated with 100 ppm ABA yielded only six bands which stained lightly, as early as 24 hours after treatment. Band numbers 3 to 7 were likewise fused as early as 24 hours after ABA application and hence, might be a reflection of the inhibitory effect of ABA on soluble protein synthesis.

Summary and Conclusions

The primary effect of ABA applied to the apical bud of intact "sitaw" seedlings is growth inhibition of the apical bud itself which may lead to diminished apical dominance and these subsequently promoted the elongation of lateral buds. ABA inhibited the elongation of the lateral bud when applied at concentrations of 25 to 100 ppm to the lateral bud alone or when applied simultaneously to both the stem apex or decapitated surface and the lateral bud. The application of ABA to the apical bud simulated the effect of decapitation on lateral bud elongation.

Ultrastructural analysis of the released lateral buds showed apparent mobilization of food reserves into the vacuoles. The nuclei of cells from released buds contained less distinct heterochromatin materials.

Ultrastructural analysis of the axillary buds inhibited by exogenously applied ABA revealed vacuolation in their cells. These vacuoles, however, contained only amorphous materials. The nuclei were noted to possess more prominent heterochromatin materials distributed throughout the nuclear area in contrast with those observed in cells from released buds.

The quality of soluble proteins and their levels were observed to be different for inhibited and released lateral buds. Soluble protein levels generally were reduced when buds were released. The absence of certain bands and reduction in intensity of staining in the electrophoregrams of the soluble proteins suggest that inhibitory effect of ABA affected the synthesis of certain protein fractions. The electrophoregrams also served to confirm reduction in soluble protein levels. Reduced number of bands were most evident in electrophoregrams derived from directly treated buds from decapitated seedlings. These bands were often indistinct and lightly stained. The inhibitory effect of ABA therefore rests on its influences on the soluble protein synthesis in the lateral buds.

Acknowledgment

Acknowledgment is due the University of the Philippines for the Fellowship. The support of the UP – Natural Science Research Institute and the Charley Barretto Dissertation Grant is also acknowledged.

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Appendix 1. Lowry Reagents

The reagent formulas given here are based on the method of Lowry *et al.* (1951) as modified by Garney *et al.* (1977) and Rodriguez *et al.* (1980).

Reagent A:

Reagent A is a mixture of three stock solutions (A-1, A-2, A-3) which are combined shortly before use. This combined reagent must be discarded after one day.

Reagent A-1

$Na_2C_4H_4O_6 \cdot 2H_2O$		2.0 g
distilled water	to	100.00 mL

Dissolve the sodium tartrate in a 100-mL volumetric flash containing about 40 mL of distilled water. Make up to 100 mL.

Reagent A-2

$CuSO_4 \cdot 5H_2O$		1.0 g
distilled water	to	100.0 mL

Reagent 3

Na_2CO_3		20.0 g
NaOH, 0.1 N,	to	1000.0 mL (1 liter)

Dissolve the Na_2CO_3 in a 1-liter volumetric flash containing about 400 mL of 0.1N NaOH. Make up to 1 liter with the same solvent.

To prepare a volume of Reagent A sufficient for 25 determinations, deliver the stock solutions into a 125-mL Erlenmeyer flask in the following amounts and sequence:

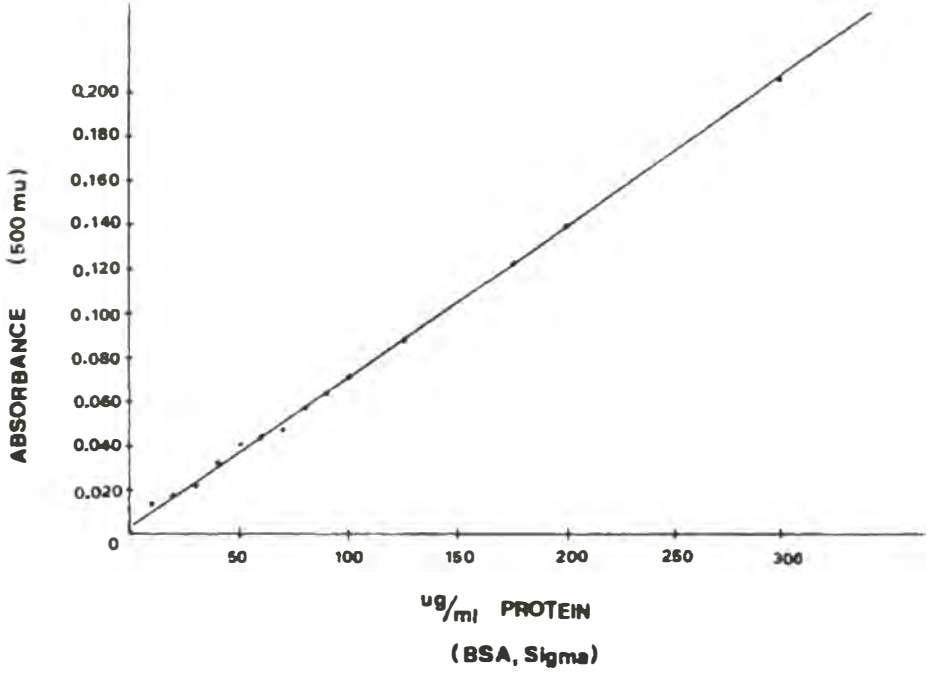
Reagent A-1	0.5 mL
Reagent A-2	0.5 mL (mix)
Reagent A-3	50.0 mL (mix)

Reagent B:

Phenol reagent (Folin-Ciocalteu), 1.0 N
(purchased as a 2.0 N reagent, Merck)

1 part Folin Reagent: 1 part distilled water.

All the above stock solutions were kept in brown bottles and stored in the refrigerator.



Appendix 2. Standard absorbance curve of different concentrations of bovine serum albumin solution.

Appendix 3. Stock Solutions for Electrophoresis

Solution A	1 N CHl	–	48.0 ml
	TRIS	–	36.6 g
	TEMED	–	0.23 ml
	Distilled Water to		100.0 ml
Solution B	1 N HCl	–	48.0 ml
	TRIS	–	5.98 g
	TEMED	–	0.46 ml
	Distilled Water to	–	100.0 ml
Solution C	Acrylamide	–	28.0 g
(Monomer)	BIS	–	0.735 g
	Distilled Water to	–	100.0 ml
Solution D	Acrylamide	–	10.0 g
(Monomer)	BIS	–	2.5 g
	Distilled Water to	–	100.0 ml
Solution E	Riboflavin	–	0.004 g
(Catalyst)	Distilled Water to	–	100.0 ml
Solution F	Ammonium persulphate	–	0.14 g
(Catalyst)	Distilled Water to	–	100.0 ml
Solution G	TRIS	–	6.0 g
(Buffer)	Glycine	–	28.8 g
	Distilled Water to	–	1000.0 ml (1 liter)
Solution H	Amido Black	–	0.5 g
(Stain)	7% Glacial acetic acid	–	500.0 ml
Solution I	Bromophenol blue	–	0.005 g
(Tracking dye)	Distilled Water to	–	100.0 ml
Solution J	Glacial acetic acid	–	70.0 ml
(Destaining/ Storing Solution)	Distilled Water to	–	1000.0 ml (1 liter)

Stock solutions were stored in brown bottles in the cold for several months without appreciable deterioration. Prior to use, stock solutions must be warned to room temperature.

TRIS – Tris (hydroxymethyl) amino-methane
TEMED – N, N, N′, N′ – Tetramethylethylenediamine
BIS – N, N′, – Methylene-bisacrylamide