CHAPTER - 6: RESPONSES OF SHOOT CUTTINGS

6.1 General introduction to chapter 6.

Whenever GA_3 was applied it promoted growth of the main shoot and a few of the laterals (Section 4.7.5). The GA_3 promoted laterals correlatively inhibited the growth of the other laterals (Section 5.9). Therefore it was hypothesised that the lack of reversal of PBZ inhibition of lateral shoot growth when GA_3 was applied simultaneously was due to the confounding effect of increased apical dominance or correlative inhibition (Section 5.9). A further complication is the reduction in lateral shoot number with simultaneously applied PGRs (Section 4.7.4 and 5.8.4). This can also lead to correlative inhibition and/or compensatory growth between shoots.

One way of avoiding apical dominance by the main shoot or correlative effects between lateral shoots, would be to use separate shoots i.e. shoot cuttings. It was hypothesised that in separate shoot cuttings GA_3 should reverse the effects of PBZ (Section 5.9).

Three experiments are reported in this chapter, where the growth pattern of main and lateral shoot cuttings were observed more closely with or without PGRs (Section 6.2 and 6.3) and in the last experiment an effort was also made to understand the process of PBZ induced growth inhibition and the reversal of the same effect in cuttings (Section 6.4).

6.2 Study on the growth pattern and GA_3 application.

6.2.1 Introduction:

Devlin (1969) proposed that the highest remaining lateral bud after decapitation normally replaces the position and function of the main shoot and imposes dominance over the rest of the laterals. However, none of the laterals from decapitated SDP plants showed dominance in earlier experiments unless they were treated with PGRs (Table 5.3c and 5.4c). Moreover, the main and lateral shoots responded differently to applied PGRs (Sections 3.5; 4.2; & 7.2). Therefore there was a need to clearly understand the growth pattern and the hormonal balance required for main and lateral shoot growth.

Accordingly, two experiments were initiated with main and lateral shoot cuttings. The objectives of these experiments were to determine:

- the growth pattern of these two types of shoots,
- are there any inherent differences in growth pattern between these shoots ?
- does GA_3 promote or inhibit the growth of the cuttings ?

6.2.2 Materials and Methods:

Experiment #1: Four (4) treatments including cuttings from main and lateral shoots treated with or without GA_3 (500ppm) were applied in this experiment. The experiment was laid out in a randomised complete block design with 5 replications.

The seeds were sown on 11.04.94 and seedlings were allowed to grow under glasshouse conditions for about 2 months. Cuttings were made on 07.06.94 from main and lateral shoots of these plants. Cuttings were placed under mist with bottom heating (25°C constant) for about 3 weeks. When the cuttings had produced enough roots (on 01.07.94), they were again transferred to the same glasshouse conditions. These plants were allowed to grow another 2 weeks to adjust to the new environment then PGRs were applied on 13.07.94.

For quick and easy rooting, a mixture of equal parts of (2000ppm) NAA + IBA prepared in 50% ethanol solution was used as a quick dip treatment (Williams and Taji, 1987).

A large population of the cuttings were produced from the homogeneous stock plants and the required numbers were selected from that population again on the basis of homogeneity. Because of these rigorous selection procedures less variability was observed among these cuttings, therefore only 5 replications were used for these experiments. Main and lateral shoot cuttings were selected with 3 leaves per cuttings. Main shoot cuttings were 4 cm long and lateral shoot cuttings were 6 cm.

Vegetative data were recorded 4 weeks after GA_3 application. The angle data were collected from the deflected angle produced by the primary cuttings from 90° axis not the angle which was formed by the subsequent laterals. This was the variation from the normal system used in other experiments of this current project. The rest of the procedures were followed as per section 3.2.

The terms "primary" and "secondary" growth used in this current chapter (Chapter 6) specifically refer to the growth of the original tip cuttings (of main or lateral shoots) from a stock plant and the subsequent lateral growth from this cuttings respectively.

Experiment #2: Two treatments with 5 replications were used in this experiment. The treatments were with or without decapitation of lateral shoot cuttings.

Decapitation of the cuttings was done on 15.08.94. The rest of the procedures were followed as per experiment #1 of section 6.2.2.

6.2.3 Results:

Experiment #1: Primary growth: GA_3 increased the height of main shoot cuttings compared to untreated main shoot cuttings. On the other hand, GA_3 was unable to increase the length of lateral shoot cuttings (Figure 6.1; Plate 6.3). Although the length of the lateral shoot cuttings ($\pm$ GA_3) was statistically similar with similar internodes (number and length), the added GA_3 tended to have an inhibitory effect on these parameters (Figure 6.1 and 6.2).

The main shoot cuttings grew upright like normal main shoots of intact plants but the lateral shoot cuttings grew almost horizontal just like laterals of intact plants (Plates 6.1 and 6.2).

The survival rate was also different for these two sets of cuttings, 50% of the main shoot cuttings survived compared to 80% of the lateral shoot cuttings. The lateral shoot cuttings produced earlier roots than the main shoot cuttings.

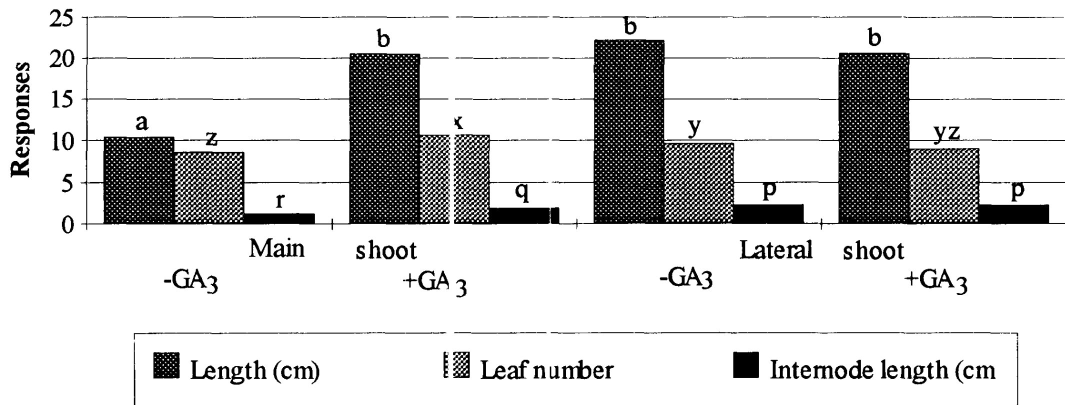


Figure 6. 1 Effect of GA_3 application on the growth pattern and elongation of shoot cuttings

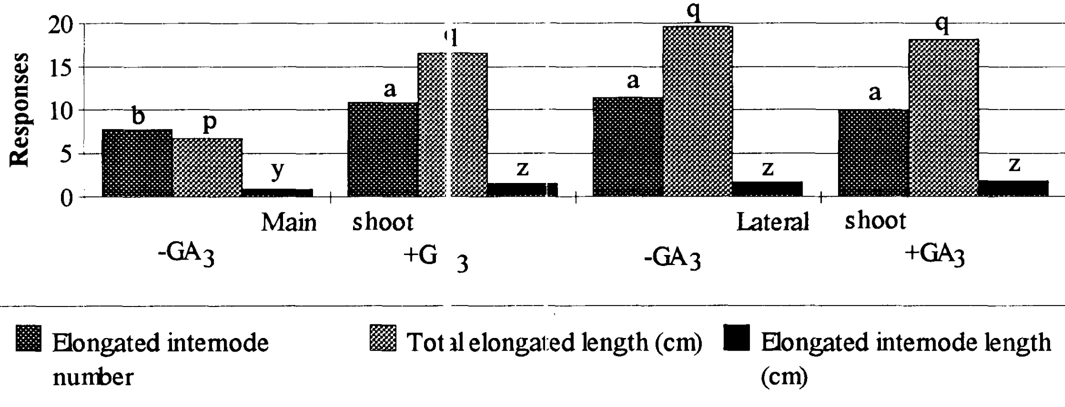


Figure 6. 2 Effect of GA_3 application on the growth pattern and elongated internode of shoot cuttings

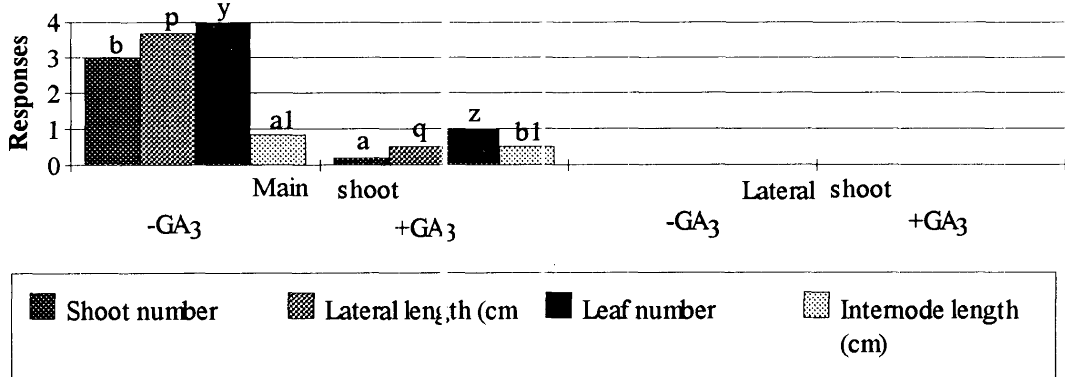
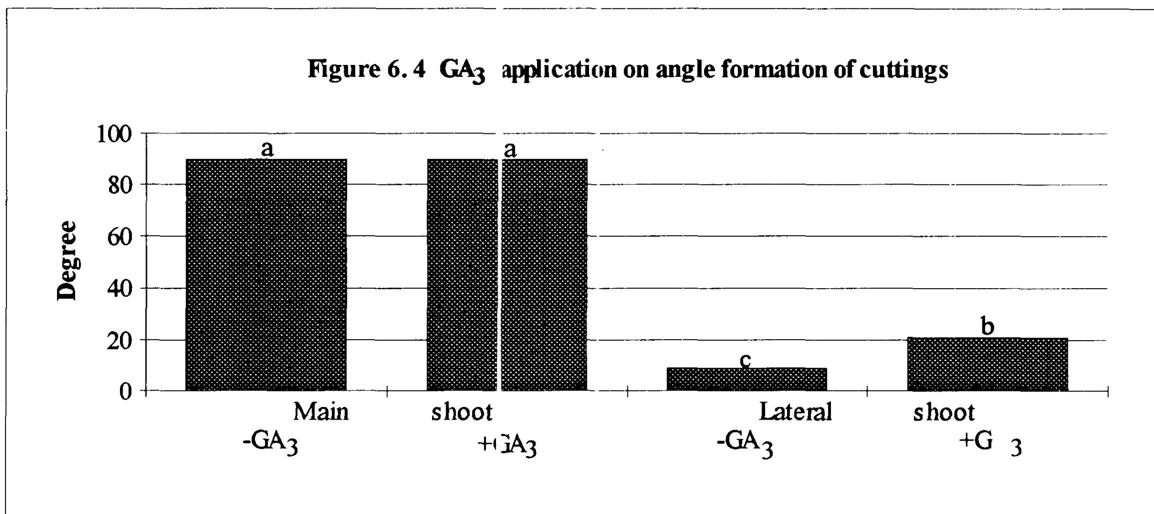
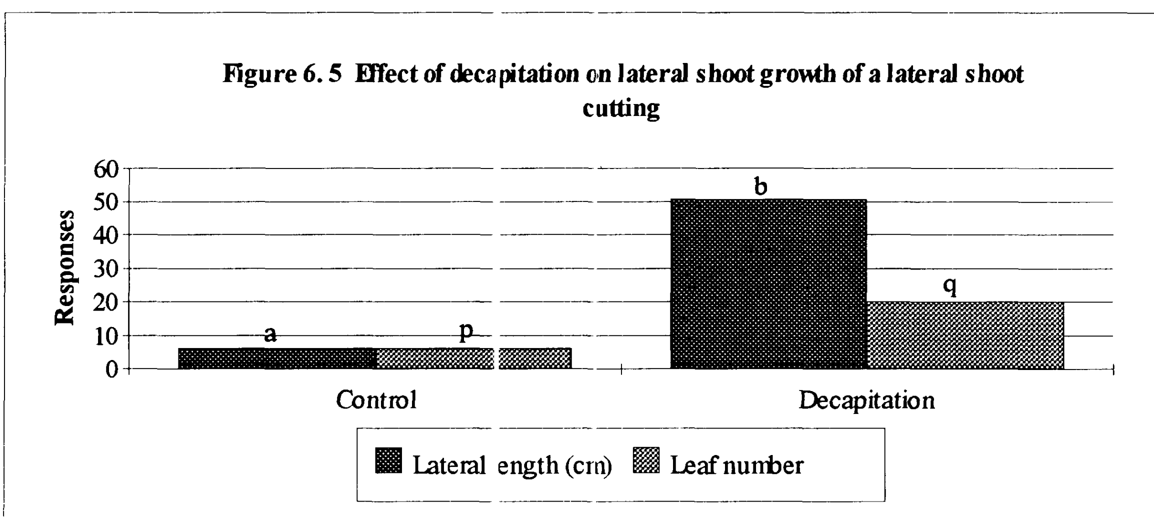
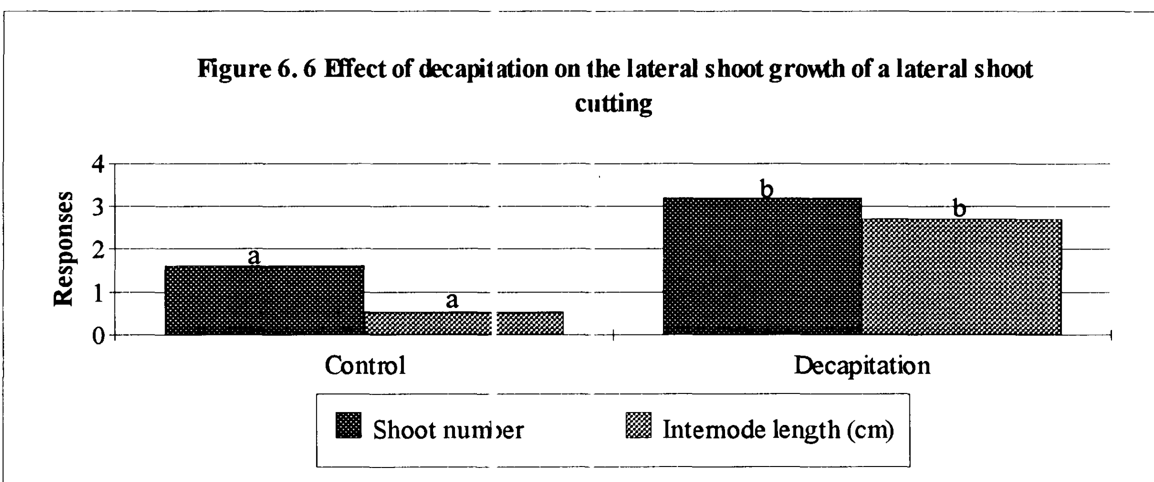


Figure 6. 3 Effect of GA_3 application on the growth pattern and secondary growth of the cuttings

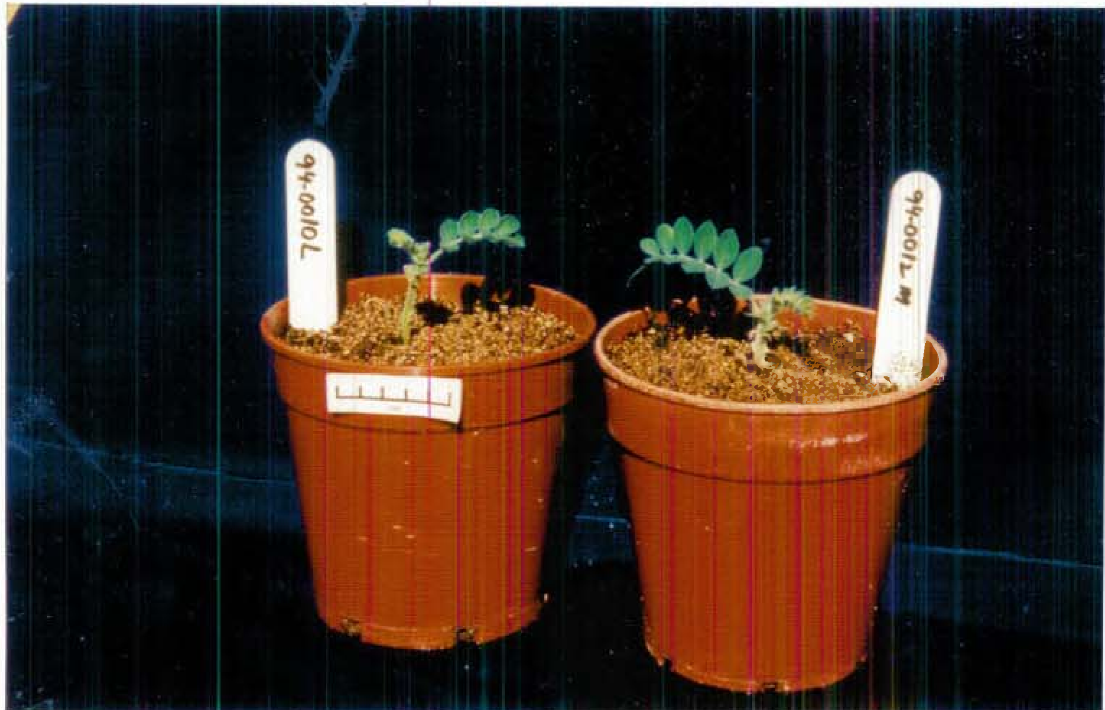






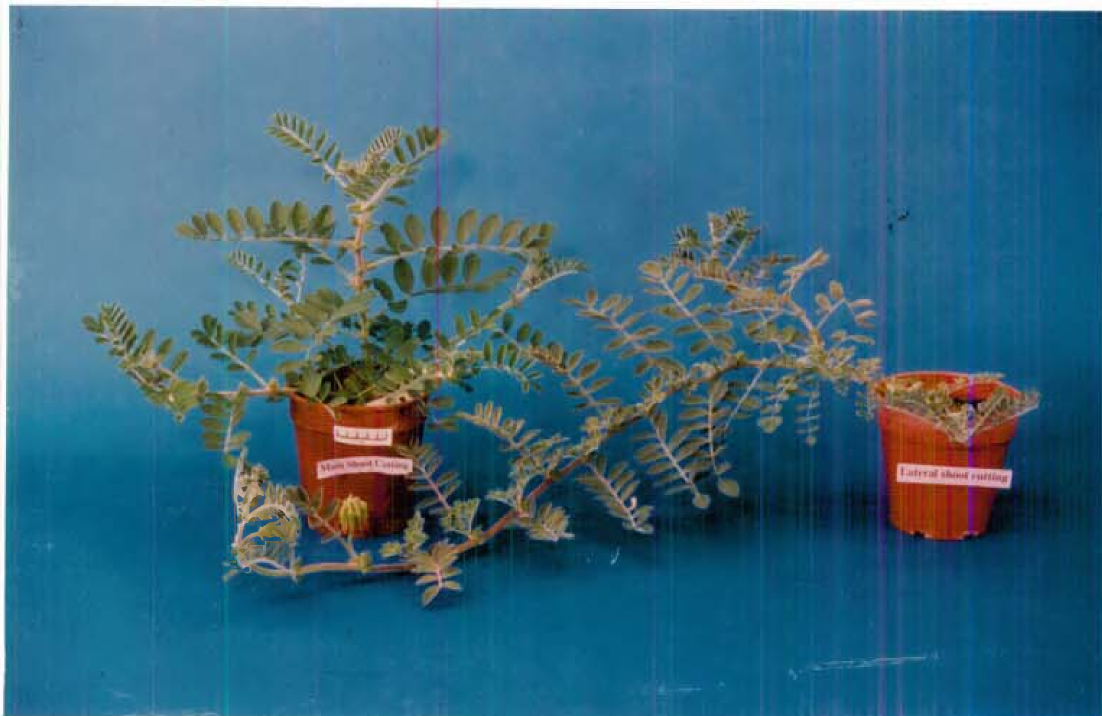
Secondary growth: There were no lateral shoots on GA_3 treated or untreated lateral shoot cuttings and the GA_3 treated main shoot cuttings almost had no secondary growth (Figure 6.3).

Cutting angle: The main shoot cuttings grew vertically producing a 90° angle with ground level with or without GA_3 but lateral shoot cuttings had a tendency to grow vertically only with the GA_3 application (Figure 6.4).



(a) (b)

Plate 6.1 Rooted lateral (a) and main (b) shoot cuttings.



(a) (b)

Plate 6.2 Mature plants from main (a) and lateral (b) shoot cuttings.

(a) (b) (c) (d)

Plate 6.3 Effect of GA_3 on shoot cuttings: (a) Lateral shoot cutting + GA_3; (b) Main shoot cutting + GA_3; (c) Main shoot cutting; (d) Lateral shoot cutting.

Experiment #2: Secondary growth: Decapitation of lateral shoot cuttings increased lateral shoot number, lateral length and lateral leaf number along with their internode length (Figure 6.5 and 6.6).

Cutting angle: The decapitated lateral shoot cuttings produced secondary lateral shoots which again grew like a laterals of an intact plant (data not presented).

6.2.4 Discussion:

Experiment #1: Primary growth: The lateral shoot cuttings of SDP plants had a plagiotropic pattern of growth. This difference in pattern of growth could explain the different responses of main and lateral shoots to the applied PGRs (Section 6.2.1). Walter and Cocking (1969) reported that plagiotropism is not inherent in lateral branches and that plagiotropic organs could become orthogeotropic. The orientation of the lateral shoots was changed from plagiotropic to orthogeotropic when they were treated with GA_3 in earlier experiments in both intact (Table 3.2c) and decapitated plants (Table 5.3c). In this present study, GA_3 was unable to change the orientation of the lateral shoot cuttings completely but had a slight tendency to change. It suggests that something required by the lateral shoots to respond to added GA_3 was not optimum or missing in the lateral shoot cuttings. This missing factor may be auxin because in other experiments GA_3 and auxin acted together to promote elongation when there

was a suitable balance (Section 2.2 5.1). Probably *in situ* laterals in earlier experiments (Sections 3.4 and 5.3) had auxin supplied from other parts of the plant to interact with exogenous GA_3.

The present results further indicate that the main shoot cuttings were dependent on exogenous GA_3 for their extra elongation but the lateral shoot cuttings did not respond to added GA_3. The lateral shoot cuttings might lack the required amount of auxin to interact with added GA_3 and cause extra elongation. However, in a preliminary experiment IAA (20 mg a.i./plant) along with GA_3 (500ppm) had no effect on the elongation of lateral shoot cuttings (data not presented) probably due to inappropriate amount of auxin supplied by IAA or its basipetal mode of transport. Application of the proper type and concentration of auxin precursors (e.g. tryptophan) to the lateral shoot cuttings might cause elongation with exogenous GA_3 by supplying proper auxin at a proper place.

The inhibitory effect of GA_3 on elongation of lateral shoot cuttings might also mean that laterals contain more endogenous GA so that added GA_3 increased the total GA to supra-optimal level. Higher endogenous GA in laterals was also proposed earlier (Section 3.8 and 4.8). It was also reported earlier (Section 3.5.5) that shoot elongation depends on the balance between GA and auxin; a lack of auxin (preceding para) and excess GA probably confounded their inhibitory effect.

Secondary growth: The inhibited secondary growth in both GA_3 treated or untreated lateral shoot cuttings further suggested that lateral shoot cuttings already had enough endogenous GA (Section 3.8 and 4.8) to inhibit the growth of the secondary laterals.

Cutting angle: While it was proposed that laterals have a high level of endogenous GA, compared to the main shoot, this level was still inadequate for the formation of a vertical shoot angle in lateral shoot cuttings. But GA_3 application produced vertical angle in laterals of the intact (Table 3.2c) or decapitated plants (Table 5.3c). However, for lateral shoot cuttings, GA application only increased shoot angle slightly (Figure 6.4). The lack of response may be due to a lack of the auxin in the laterals required for the GA effect on branch angle. As in intact or decapitated plants auxin could be supplied from the other parts of the plants (Section 6.2.4).

Experiment #2: Secondary growth: Even though the lateral shoot cuttings showed distinctly different growth patterns compared to the main shoot cuttings (Section 6.2.3; Experiment #1), the lateral shoot cuttings expressed the characteristics of the main shoots when decapitated, because they produced more lateral shoot growth. The mechanism of this change is not known.

Cutting angle: The plagiotropic growth of secondary laterals of a decapitated lateral shoot cutting suggests an inherent mechanism which controls the plagiotropic growth in lateral shoots of SDP.

6.2.5 Conclusions:

* Lateral shoots had a plagiotropic growth pattern.

* Lateral shoot cuttings might have enough endogenous GA but lack auxin or its precursor, and therefore are unable to respond to added GA_3.
* Application of auxin or its precursor along with GA_3 might cause further elongation of the lateral shoot cuttings.
* An adequate supply of auxin is required for vertical angle formation with the exogenously supplied GA_3 in lateral shoot cuttings.

6.3 Interaction of tryptophan and GA_3.

6.3.1 Introduction:

It was hypothesised (Section 6.2.4) that the applied GA_3 was unable to promote elongation of the lateral shoot cuttings possibly due to the lack of auxin precursors. It was proposed that application of the right type and concentration of auxin precursors would promote elongation of lateral shoot cuttings by producing auxins and thereby interacting with exogenous GA_3. Accordingly, the effects on shoot elongation of different auxin precursors at different concentrations, alone or with GA_3, were studied on the main and lateral shoots in the following 2 experiments.

6.3.2 Materials and Methods:

Experiment #1: Fourteen (14) treatments were arranged in a randomised complete block design with 5 replications. The treatments consisted of 3 different concentrations (0.3, 3.0 and 6.0 mg/kg of soil) of each of L and D-tryptophan, alone or in combination with GA_3 (500ppm). Main shoot cuttings were used in this experiment. The weight of the potting mixture was 1500 gm (air dried weight). The required amount of tryptophan was calculated on the basis of this weight.

The seeds were sown on 15.12.94 and vegetative data were recorded 4 weeks after PGR application. The cuttings were made, selected and PGRs were applied as per section 6.2.2. The rest of the procedures were followed as per general methodology described in section 3.2.

Experiment #2: Lateral shoot cuttings were used in this experiment. The rest of the experimental procedures were followed as per experiment #1 (Section 6.3.2).

6.3.3 Results:

Experiment #1: Primary growth: L-tryptophan 0.3 mg /kg of soil (alone) had no effect but together with GA_3 gave the greatest elongation of the main shoot cuttings. D-tryptophan (all concentrations) alone or with GA_3 had an inhibitory effect on main shoot elongation compared to the untreated and GA_3 treated control plants respectively (Table 6.1).

Secondary growth: L and D-tryptophan (alone) had no effect on lateral shoot number or their elongation, but when GA_3 was added both reduced shoot number and their length (Table 6.1).

Table 6.1 Effect of L-tryptophan (L-T, mg/kg of soil), D-tryptophan (D-T, mg/kg of soil) and GA_3 (500ppm) on main shoot cuttings.

Treatments	Primary growth			Secondary growth			
	Plant height (cm)	Leaf number	Internode length (cm)	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)
Control	12.80f*	13.20cdef	0.97e	**3.20a**	**13.60a**	**9.60c**	0.96b-e
L-T (0.3)	13.20f	13.80bcd	0.96e	**2.80ab**	**11.80a**	**9.20c**	1.25de
L-T (3.0)	12.40f	13.60bcde	0.92e	**2.80ab**	**12.80a**	**9.20c**	1.27e
L-T (6.0)	13.00f	13.40b-f	0.97e	**3.00a**	**13.00a**	**11.20c**	1.31e
L-T (0.3) + GA_3	**25.80a**	**15.40a**	**1.68a**	1.00f	0.50b	1.00a	**0.50a**
L-T (3.0) + GA_3	16.72de	**14.20abc**	1.18d	1.40ef	0.90b	1.80ab	**0.50a**
L-T (6.0) + GA_3	18.00cd	12.40def	1.45b	1.00f	0.50b	1.00a	**0.50a**
D-T (0.3)	11.00g	12.40def	0.89e	**3.00a**	**5.90ab**	**7.40c**	**0.76a-d**
D-T (3.0)	10.40g	12.20ef	0.85e	**3.00a**	**7.90ab**	**9.20c**	0.88b-e
D-T (6.0)	10.40g	12.00f	0.87e	**3.00a**	**7.50ab**	**8.00c**	0.94cde
D-T (0.3) + GA_3	16.20e	13.00c-f	1.25cd	2.00cd	1.30b	2.40b	**0.53ab**
D-T (3.0) + GA_3	19.00bc	13.20c-f	1.44b	1.00f	0.50b	1.00a	**0.50a**
D-T (6.0) + GA_3	18.00cd	13.80bcd	1.33bc	2.00cd	1.60b	2.80b	**0.54ab**
GA_3	20.20b	**14.80ab**	1.37bc	1.80de	1.70b	2.60b	**0.60abc**
					t	t	t

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Table 6.2 Effect of L-tryptophan (L-T, mg/kg of soil), D-tryptophan (D-T, mg/kg of soil) and GA_3 (500ppm) on lateral shoot cuttings.

Treatments	Primary growth			Secondary growth			
	Plant height (cm)	Leaf number	Internode length (cm)	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)
Control	34.00b*	**14.00b**	**2.44b**	3.00c	2.20cd	4.00b	0.55abc
L-T (0.3)	33.80b	15.00c	**2.26b**	4.00d	10.40g	15.00d	0.73b-f
L-T (3.0)	31.80d	**14.00b**	**2.28b**	4.20d	10.60g	14.40d	0.74b-f
L-T (6.0)	31.40de	14.20c	2.21c	5.40e	11.20gh	15.20d	0.75b-f
L-T (0.3) + GA_3	32.20cd	15.00c	2.15c	4.00d	4.50e	8.40c	0.54ab
L-T (3.0) + GA_3	**35.40a**	15.00c	**2.36b**	4.40de	6.70f	10.00cd	0.67abcd
L-T (6.0) + GA_3	30.00f	15.60c	1.92f	4.20d	5.30ef	8.20c	0.64abcd
D-T (0.3)	30.60ef	**14.00b**	2.20c	4.40de	12.00gh	14.40d	1.01efg
D-T (3.0)	31.60de	15.40c	2.06d	5.00de	12.30gh	12.80cd	1.02fg
D-T (6.0)	26.00g	14.20c	1.84g	4.60de	14.50h	14.20d	1.11g
D-T (0.3) + GA_3	30.60ef	11.40a	2.69a	**2.00b**	**1.40b**	**2.80b**	0.50a
D-T (3.0) + GA_3	27.20g	15.60c	1.75h	**2.00b**	**2.70d**	**3.60b**	0.82b-g
D-T (6.0) + GA_3	32.00cd	15.80c	2.03e	**1.20a**	**0.60a**	**1.20a**	0.50a
GA_3	33.00bc	14.80c	**2.24b**	**2.20b**	**1.70bc**	**3.00b**	0.55ab
		t		t	t	t	t

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Experiment #2: Primary growth: L-tryptophan 3.0 mg/ kg of soil plus GA_3 caused a slight but significant increase in elongation of the lateral shoot cuttings. However, the rest of the treatments were either ineffective or inhibitory to the elongation of the primary lateral shoot cuttings (Table 6.2).

Secondary growth: GA_3 alone or with D-tryptophan reduced the secondary shoot growth, other treatments increased the growth (Table 6.2).

6.3.4 Discussion:

Experiment #1: Primary growth: All concentrations of L-tryptophan alone were ineffective. This might be due to inadequate endogenous GA in main shoot, as a role of GA_3 has been proposed for the conversion of L-tryptophan to auxin (Section 2.2.2.3). However, L-tryptophan at 0.3 mg/kg of soil in presence of GA_3 (500ppm) promoted elongation growth of main shoot cuttings (Table 6.1). Applied GA_3 (500ppm) may have promoted the conversion of L-tryptophan to auxin and in turn provided a suitable balance of GA to auxin for elongation in this present experiment. As it is known that both GA and auxin are important for elongation growth (Section 2.2.5.1). The inability of applied GA_3 to induce elongation with higher L-tryptophan (3.0 or 6.0 mg/kg of soil) compared to GA_3 500ppm alone might mean that more GA_3 is needed or these higher concentrations might be supra-optimal for elongation.

D-tryptophan is also known to produce auxin but without any involvement of GA_3 (Section 2.2.2.3). D-tryptophan (all concentrations) alone or with GA_3, reduced plant height compared to the respective controls in this present experiment. The inhibition of growth could be due to supra-optimal auxin availability compounded by a lack of endogenous GA_3 (for D-tryptophan only) or lack of exogenous GA_3 supply (for D-tryptophan + GA_3). These results might suggest that different quantities and types of auxins are synthesised by L and D-tryptophan. They might also suggest a different GA_3 requirement for L and D-tryptophan for an appropriate balance for elongation growth.

Secondary growth: The ineffectiveness of all L-tryptophan concentrations (alone) on the growth of the subsequent lateral shoots was again probably due to inadequate endogenous GA. On the other hand, all D-tryptophan concentrations proved inhibitory to main shoot growth but were unable to cause inhibition in secondary laterals. This result suggests a higher requirement or tolerance of auxin by the laterals. Irrespective of type and concentration of precursor used added GA_3 reduced lateral growth probably because of GAs tendency to reinforce apical dominance (Section 2 1.2.1). Very little free auxin was often found in dormant meristematic tissues (Leopold, 1960). The free auxin increment with tryptophan in the presence of GA_3 application in this present experiment might mainly occur at the apex and in turn cause elongation of the main shoot cuttings.

The data for the lateral branch angle were not recorded as some of the laterals were not long enough to measure the angle. This inhibition of growth could be due to the reinforcement of apical dominance by auxin and GA_3. This is consistent with the involvement of auxin and GA_3 in the reinforcement of apical dominance.

Experiment #2: Primary growth: Although the increased elongation in lateral shoot cuttings with L-tryptophan 3.0 mg/kg soil plus GA_3 500ppm was significant, the promotion was very small. The concentrations 0.3 and 6.0 mg/kg of soil proved sub or supra-optimal. Therefore further research is needed to test the validity of these results. However, this present experiment at least identified that for the elongation of a lateral shoot with exogenous GA_3, presence of appropriate auxin is required. It was also revealed from this present experiment that the requirement for auxin was higher for elongation of the primary lateral shoot cuttings (3.0 mg/kg of soil) compared to main shoot cuttings (0.3 mg/kg of soil) or to the main shoot of intact plants (0.3 mg a.i./kg of soil; Section 3.5). In *Pinus radiata* seedlings, when the auxin level was suboptimal for the lateral shoots, the same level was inhibitory for the terminal shoot (Little and Pharis, 1995).

Secondary growth: The secondary laterals of the lateral shoot cuttings had increased growth with all concentration of L-tryptophan with or without GA_3 but added GA_3 had an inhibitory effect. Perhaps because of the active apices on the laterals the hormonal balance was more favourable for growth in general in the secondary growth region.

6.3.5 Conclusions:

* Conversion of L-tryptophan to auxin is probably dependent on GA_3 but D-tryptophan did not require GA_3 for auxin production.
* Different types of auxins are probably formed by L and D-tryptophan.
* For elongation growth the balanced presence of both auxin and gibberellins is necessary.
* The increment of auxin from tryptophan in the presence of GA_3 probably occurred at the main shoot apex thereby promoted main shoot growth.
* Lateral shoot cuttings require higher quantities of auxin for elongation compared to main shoots.

6.4 <u>Interaction of auxin, GA_3, tryptophan and PBZ on lateral shoot cuttings.</u>

6.4.1 Introduction:

The reversal of PBZ induced inhibition of lateral shoot growth by GA_3 did not occur in the presence of an active apex (Section 4.4.4) or correlative inhibition from other laterals (Section 5.7.5). That was why the application of PGR to cuttings was proposed to reverse PBZ induced inhibition (Section 5.9). PBZ is mainly a GA-biosynthesis inhibitor (Section 2.2.6.4). At least a part of the reversal should be accomplished with exogenous GA_3. But the lack of auxin in lateral shoot cuttings was proposed earlier as a reason for the inability to respond to applied GA_3 (Section 6.2.4). The application of IAA plus GA_3 did not promote the elongation of lateral shoots (Section 6.2.4). Auxin precursor L-tryptophan (3.0 mg/kg of soil) plus GA_3 (500ppm) caused a significant but slight increment in elongation of the lateral shoot cuttings in an earlier experiment (Section 6.3.4).

Finally, to accommodate these ideas and to test a new hypothesis an experiment was formulated to test or verify:

- that simultaneous application of GA_3 with auxin precursor, L-tryptophan would reverse PBZ induced growth inhibition in lateral shoot cuttings;
- the effect of IAA on the reversal of PBZ induced inhibition of lateral shoot cuttings;
- that L-tryptophan (3.0 mg/kg of soil) + GA_3 (500ppm) treatment causes elongation of the lateral shoot cuttings.

6.4.2 Materials and Methods:

Twelve(12) treatments were applied to lateral shoot cuttings (Table 6.3). The treatments consisted of PBZ (10 mg a.i./plant); GA_3 (500ppm); IAA (20 mg a.i./plant); L-tryptophan (3.0 mg/kg of soil) and their different combinations. The treatment PBZ + GA_3 + L-tryptophan + GA_3 had double the quantity of GA_3 500ppm. These treatments were arranged in a randomised complete block design with 5 replications. The weight of the potting mixture was 1500 gm (air dried weight).

The seeds were sown on 05.06.95. The cuttings were made, selected and PGRs were applied as per section 6.2.2. Vegetative data were recorded 4 weeks after PGR application. The rest of the procedures were followed as per section 3.2.

6.4.3 Results:

Primary growth: GA_3 alone did not affect elongation growth but with L-tryptophan (3.0 mg/kg of soil) it produced significant elongation of the lateral shoot cuttings compared to the rest of the treatment combinations (Table 6.3). PBZ alone, with auxin (IAA) or with auxin precursor, inhibited elongation of the lateral shoot cuttings.

Table 6.3 Effect of PBZ (10 mg a.i./plant) in combination with GA_3 (500ppm), L-tryptophan (3.0 mg/kg soil) and IAA (20 mg a.i./plant) on lateral shoot cuttings.

Treatments	Primary growth		
	Elongation (cm)	Leaf number	Internode length (cm)
Control	22.60ef*	14.60b	1.55bc
PBZ	12.4b	11.40d	1.09a
GA_3	21.00de	13.60bc	1.55bc
IAA	16.80c	12.60c	1.33b
L-tryptophan	19.20d	14.00b	1.38b
L-tryptophan + GA_3	34.00h	17.00a	2.00de
PBZ + GA_3	24.00f	13.80bc	1.74cd
PBZ + IAA	12.00b	10.40d	1.16a
PBZ + L-tryptophan	10.40a	10.40d	1.00a
PBZ + GA_3 + IAA	10.80a	10.40d	1.05a
PBZ + GA_3 + L-tryptophan + GA_3	28.60g	13.40bc	2.14e
PBZ + GA_3 + L-tryptophan	22.00ef	13.60bc	1.62c
	t		

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Plate 6.4 Effect of L-tryptophan and GA_3 on lateral shoot cuttings.

Plate 6.5 Effect of IAA alone or with GA_3 on PBZ treated lateral shoot cuttings.

Plate 6.6 Reversal of PBZ induced growth inhibition by GA_3 (alone) on lateral shoot cuttings.

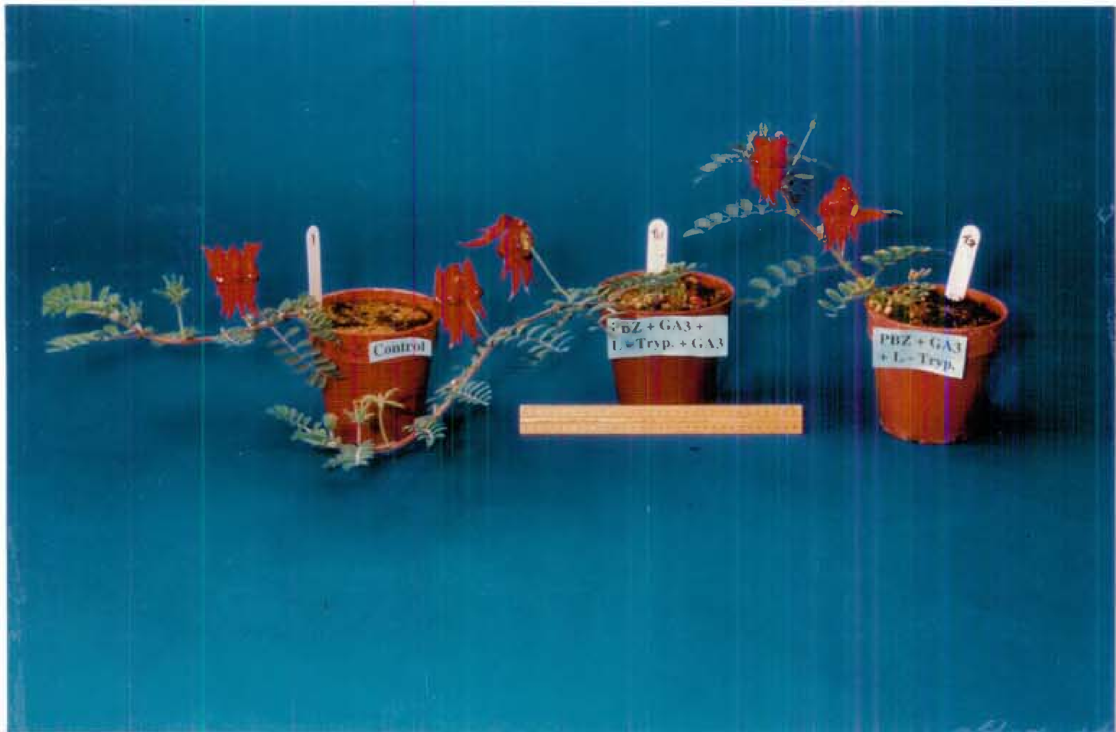


Plate 6.7 Reversal of PBZ induced growth inhibition on lateral shoot cuttings with L-tryptophan + GA_3 or with L-tryptophan + GA_3 + GA_3.

The reversal of the PBZ inhibited lateral shoot growth elongation was achieved by the application of GA_3 alone or its combination with auxin precursor (L-tryptophan) but not with auxin (IAA). The reversed plants had statistically similar numbers of leaves but similar or longer internodes compared to untreated controls (Table 6.3).

6.4.4 Discussion:

Primary growth: The present results support the earlier findings (Section 6.3.4), where L-tryptophan (3.0 mg/kg of soil) + GA_3 (500ppm) increased elongation of the lateral shoot cuttings. However, in this present experiment the growth increment was much greater. This increased growth promotion in lateral shoot cuttings could be due a difference in overall plant vigour in response to seed source and growing season. The seasonal dependence of SDP plant responses to applied PGRs has also been observed earlier (Section 4.5.4 and 8.7).

The inhibited growth following application of PBZ alone or its combinations with IAA or L-tryptophan could be due to inadequate endogenous GA level because of PBZ inhibition of GA biosynthesis. On the other hand, the inability of GA_3 alone to cause elongation of the lateral shoot cuttings could be due to the higher endogenous level of GA and lack of auxin or its precursor (Section 6.2.4).

The reversal of PBZ induced inhibition of lateral shoot growth with L-tryptophan + GA_3 is consistent with the formulated hypothesis (Section 6.4.1) and could be explained on the basis of a balanced supply of auxin (supplied from L-tryptophan and induced from PBZ) and GA_3 for the elongation of the lateral shoot cuttings.

The reversal of PBZ inhibition by GA_3 alone was probably due to the increased level of available auxin induced by PBZ which in turn interacted with supplied GA_3 to cause elongation in the absence of an intact apex or other laterals. The presence of an intact apex or other laterals might direct auxin to the apex. Increased auxin availability due to PBZ was also proposed earlier (Section 4.5.5).

The inability of GA_3 along with IAA to reverse PBZ inhibition could be due to an inappropriate amount of auxin or its basipetal mode of transport (?). This result also suggests that auxin synthesised by L-tryptophan could have a different mode of action to IAA. L-tryptophan might have been transported to the apex of the lateral shoots, where it became auxin in the presence of GA_3 and also interacted with GA_3 to elongate the lateral shoots but IAA was probably transported basipetally (immediately after application) and was unable to promote elongation.

The present results also suggest that GA_3 (500ppm) alone was enough to produce auxin from L-tryptophan and to interact with this auxin and also with the auxin induced by PBZ.

6.4.5 Conclusions:

* GA_3 alone or in combination with L-tryptophan reversed PBZ induced growth inhibition in lateral shoot cuttings probably because of the balanced supply of auxin and GA_3 to the apex.

* Applied IAA and auxin synthesised from L-tryptophan might have different modes of action.
* GA_3 500ppm might be enough to produce auxin from L-tryptophan and also to interact with this auxin and auxin induced by PBZ.

6.5 General conclusions from chapter 6.

* Conversion of L-tryptophan to auxin probably is dependent on GA_3 but D-tryptophan did not require GA_3 for auxin production.
* GA_3 alone or in combination with L-tryptophan reversed PBZ induced growth inhibition in lateral shoot cuttings probably because of the balanced supply of auxin and GA_3 to the apex.
* GA_3 500ppm might be enough to produce auxin from L-tryptophan and also to interact with this auxin and auxin induced by PBZ.
* Applied IAA and auxin synthesised from L-tryptophan probably produce different concentrations in the tissues and might have different modes of action.
* An adequate supply of auxin is required for the vertical angle formation with the exogenously applied GA_3 in lateral shoot cuttings.

It is apparent from this chapter that PBZ probably works through inhibition of GA biosynthesis and through increased auxin availability (Section 6.4.4). It also became apparent that SDP lateral shoots respond differently to added PGRs probably because of their:
- plagiotropic pattern of growth (Section 6.2.5),
- higher content of endogenous GA (Section 6.2.5) and
- higher requirement for auxin (Section 6.3.5).

CHAPTER - 7: ANATOMICAL RESPONSES

7.1. General introduction to chapter 7.

The objective of this present chapter was to determine the anatomical changes in the main and lateral shoots (if any) in relation to the different growth responses of these two types of shoots clearly observed in earlier experiments (Section 3.8; 4.8; 5.9 and 6.5). This information may help to increase our understanding of the mechanism of PBZ control of SDP growth. No previous work has been done to determine the changes in SDP stem anatomy following PGR application.

The 1st section (Section 7.2) reports the anatomical responses recorded at 2 different growth stages of the plant following PGR application. The other 2 sections (Section 7.3 and 7.4) present anatomical results from treatments involving the reversal of PBZ inhibition of main and lateral shoot growth in intact plants, decapitated plants or plants obtained from cuttings.

7.2 Interaction of PBZ and GA_3 on stem anatomy at different growth stages.

7.2.1 Introduction:

The experiments reported in this section (Section 7.2) is supplementary to the experiments conducted earlier (Section 4.3 and 4.4) in intact plants. Apart from presented results of the effects of different PGRs on the reversal of PBZ inhibition both on the main and lateral shoots, the effects of time for recording anatomical data at different growth stages of the plants (vegetative and flowering) will also be presented in this section.

7.2.2 Materials and Methods:

Experiment #1: This was a supplementary experiment to that of section 4.3. The same plants and experimental methodology were used but only 2 replications were selected for anatomical observations. The treatments were: i) Control, ii) GA_3 500ppm and iii) GA_3 (1000ppm) + PBZ (10 mg a.i./plant).

Both main and lateral shoots were used for anatomical work. The samples were taken from the 4th internode of mature plants at the flowering stage (i.e. approximately 12 weeks after sowing). From each sample, longitudinal sections (LS) and transverse sections (TS) were made. The initial length of the samples was about 1 cm, taken from the middle of the selected internodes. After that, smaller hand sections were cut. These smaller sections (both LS and TS) were rapidly frozen in excess water in a freezer for about 6 hours and then freeze-dried overnight (in a Lab Vac freeze drier). Freeze dried sections were then mounted on brass stubs using Supa-glue (Sellys, Australia), coated with gold/palladium in a sputter-coater. The mounted sections were then viewed on a scanning electron microscope (JEOL, JSM-35; Japan).

Micrographs were taken of the ultrastructure of the stem sections. Measurements of the cell lengths and diameters were made from these contact micrographs using a metric

measuring scale. The length was measured for LS and the diameter [(Length + Breadth)/2] for TS. Ten (10) cells were measured from each photograph by choosing 2 cells from those nearest the four corners and 2 cells from near the middle of each micrograph. The average of these 10 cells were considered as a replication.

The cortical (parenchyma) cells were measured and the average vertical cell number per internode was calculated by dividing mean internode length with the mean parenchyma cell length. The % of increase or decrease in cell length or number was calculated from those recorded means compared to a control.

Experiment #2: This experiment was supplementary to the experimental section 4.4. The same plants and experimental methodology were used but with only 10 selected treatments and 3 replications. The treatments were: Control, PBZ (1 and 10 mg a.i./plant), GA_3 (100, 500 and 1000ppm), GA_3 (100ppm) + PBZ (1 mg a.i./plant), GA_3 (1000ppm) + PBZ (1 mg a.i./plant), GA_3 (100ppm) + PBZ (10 mg a.i./plant), and GA_3 (1000ppm) + PBZ (10 mg a.i./plant).

The samples were collected 4 weeks after PGR application (i.e. 8 weeks after sowing). The rest of the procedures were followed as per section 7.2.2 (Experiment #1).

7.2.3. Results:

Main and lateral shoot transverse section: There were no consistent differences between any of the treatments in cell diameter in either of the experiments. Therefore, TS data from main and lateral shoots are not presented.

Main shoot longitudinal section: At the vegetative stage (Table 7.3; Plate 7.1), PBZ (alone) reduced both cell length and cell number but at the flowering stage (Table 7.1; Plate 7.3), cell number was reduced up to 51% while cell length was slightly increased for PBZ treated internodes compared to untreated (control) plants.

Higher concentrations of GA_3 (500 and 1000ppm) alone increased both cell length and cell number, at the vegetative stage (Table 7.3). However, at the flowering stage GA_3 (500ppm) caused more elongation of the cells than a PBZ alone (81%) and untreated control plants (87%) and reduced cell number by 12% of control plants (Table 7.1).

All GA_3 concentrations when applied together with PBZ negated the inhibitory effect of PBZ and increased cell length and cell number compared to control plants at the vegetative stage (Table 7.3). Similarly GA_3 also negated PBZ's effect at the flowering stage and gave about 26% more elongation of the cells along with a 25% increase in cell number compared to the untreated control plants (Table 7.1).

Lateral shoot longitudinal section: At the vegetative growth stage (Table 7.4; Plate 7.2) all concentrations of GA_3 alone or GA_3 1000ppm in combinations with PBZ or PBZ - 1 mg a.i./plant (alone), increased cell number. The rest of the treatments at the vegetative stage reduced cell number while all treatments reduced cell length. At flowering stage (Table 7.2; Plate 7.4) all treatments (GA_3, or PBZ $\pm$ GA_3) increased cell number but reduced cell length.

Table 7.1 Effect of PGRs on main shoot anatomy (LS) at flowering stage: GA_3 (500ppm); PBZ (10 mg a. i./plant) and GA_3 (1000ppm) + PBZ (10 mg a. i./plant).

Treatments	Internode length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Control	1.07b*	108.00a		99.00b	
PBZ	0.55c	114.00b	6.00	48.00d	-51.00
GA_3	1.77a	202.00d	87.00	88.00c	-12.00
PBZ + GA_3	**1.68a**	**136.00c**	**26.00**	**124.00a**	**25.00**
		t			

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Table 7.2 Effect of PGRs on lateral shoot anatomy (LS) at flowering stage: GA_3 (500ppm); PBZ (10 mg a. i./plant) and GA_3 (1000ppm) + PBZ (10 mg a. i./plant).

Treatments	Internode length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Control	1.46bc*	179.00d		81.00a	
PBZ	0.60a	56.00a	-69.00	107.00c	32.00
GA_3	1.77c	170.00c	-5.00	104.00c	29.00
PBZ + GA_3	**1.07ab**	**121.00b**	**-33.00**	**89.00b**	**10.00**
		t		t	

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
ns = Not significant.
t = Analysis was done on the transformed data.

Table 7.3 Effect of PGRs on main shoot anatomy (LS) at vegetative stage: GA_3 (ppm); PBZ (mg a. i./plant) and their combinations.

Treatments	Internode length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell numbers (%) deviated from control
Control	0.79c*	163.00e		49.00e	
GA_3 (100ppm)	1.19e	164.00e	1.00	73.00bc	49
GA_3 (500ppm)	1.39f	172.00c	6.00	81.00a	66
GA_3 (1000ppm)	1.55g	188.00b	15.00	82.00a	70
PBZ (1 mg)	0.46b	118.00f	-28.00	39.00f	-20
PBZ (10 mg)	0.39a	112.00g	-31.00	35.00g	-28
PBZ (1 mg) + GA_3 (100ppm)	**1.18e**	**169.00d**	**4.00**	**70.00c**	**44**
PBZ (1 mg) + GA_3 (1000ppm)	**1.51g**	**198.00a**	**22.00**	**76.00b**	**57**
PBZ (10 mg) + GA_3 (100ppm)	**0.92d**	**172.00c**	**6.00**	**54.00d**	**10**
PBZ (10 mg) + GA_3 (1000ppm)	**1.17e**	**168.00d**	**3.00**	**70.00c**	**44**

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).

Table 7.4 Effect of PGRs on lateral shoot anatomy (LS) at vegetative stage: GA_3 (ppm); PBZ (mg a. i./plant) and their combinations.

Treatments	Internode length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Control	1.09d*	219.00a		50.00c	
GA_3 (100ppm)	1.09d	170.00c	-22	64.00g	29
GA_3 (500ppm)	0.99c	155.00d	-29	64.00g	29
GA_3 (1000ppm)	1.03d	161.00cd	-26	64.00g	29
PBZ (1 mg)	0.72a	136.00e	-38	53.00d	7
PBZ (10 mg)	0.51a	116.00f	-47	44.00b	-12
PBZ (1 mg) + GA_3 (100ppm)	**0.75b**	**167.00c**	**-24**	**45.00b**	**-10**
PBZ (1 mg) + GA_3 (1000ppm)	**1.02b**	**182.00b**	**-17**	**56.00e**	**12**
PBZ (10 mg) + GA_3 (100ppm)	**0.54a**	**150.00d**	**-32**	**36.00a**	**-28**
PBZ (10 mg) + GA_3 (1000ppm)	**0.47a**	**78.00g**	**-64**	**60.00f**	**21**
				t	

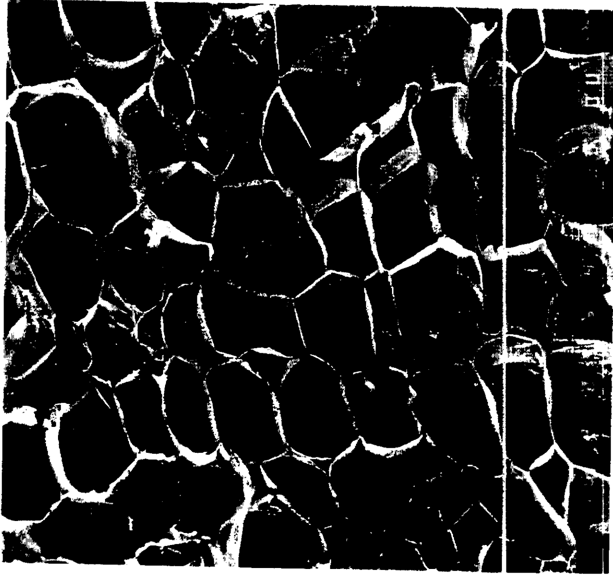
* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

General observations: In most of the GA_3 treated stems central pith cells have degenerated causing a hollow cavity in the center of the stem. This effect was partly reduced by PBZ application.

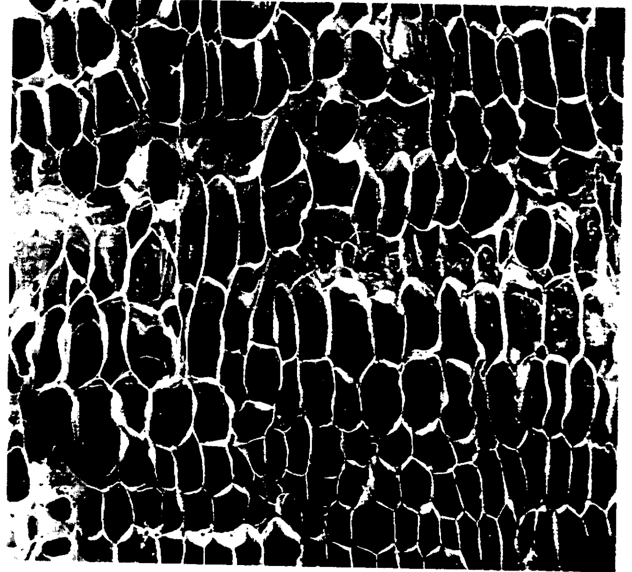
7.2.4 Discussion:

Main shoot longitudinal section: The present results revealed that PBZ at vegetative stage reduced internode length by reducing both cell length and cell number (Table 7.3). This is consistent with PBZ inhibition of GA biosynthesis (Section 2.2.6.1) because GA_3 promoted both of these processes (Table 7.3). In this present experiment (Table 7.3) PBZ showed a slight trend towards reduction of both cell length and cell division with the increased PBZ concentration. Davis *et al.,* (1988) reported that, at low dosages, GA biosynthesis inhibitors may restrict cell elongation but at high dosages may inhibit cell division by inhibiting sterol biosynthesis. Sterols have specific influences on cell membranes (Cooke *et al.,* 1994; Taton *et al.,* 1994). In this case (Table 7.3) the concentration dependent effect of PBZ on cell elongation and cell division in main shoot could be due to PBZ's inhibition of GA-biosynthesis as well as sterol biosynthesis. Irrespective of the concentrations (1 or 10 mg a.i./plant) PBZ reduced both cell length and cell number (Table 7.3), which suggests that both the doses are probably in the higher range and have ability to inhibit both GA and sterol biosynthesis. However, with time (i.e. at the flowering stage) the inhibitory effect of PBZ on cell length was reduced (Table 7.1). Therefore, reduced cell number was the only cause of retardation of the plant at the flowering stage.

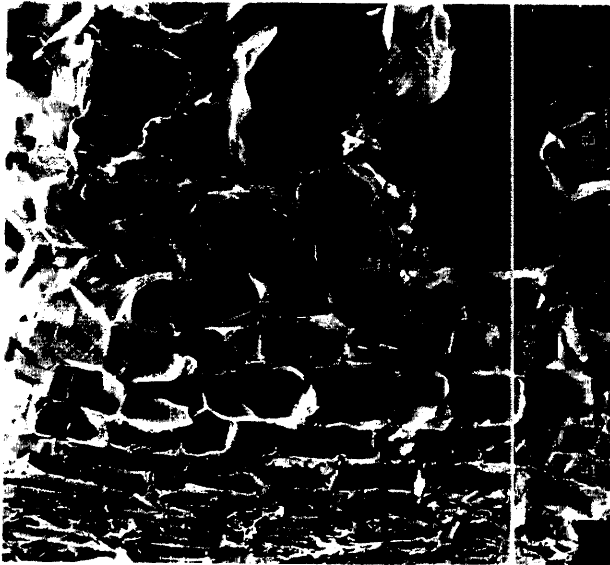
The slight increase in cell length in PBZ treated internodes compared to untreated plants at the flowering stage might be related to PBZ induced probable auxin availability and



(a) Control



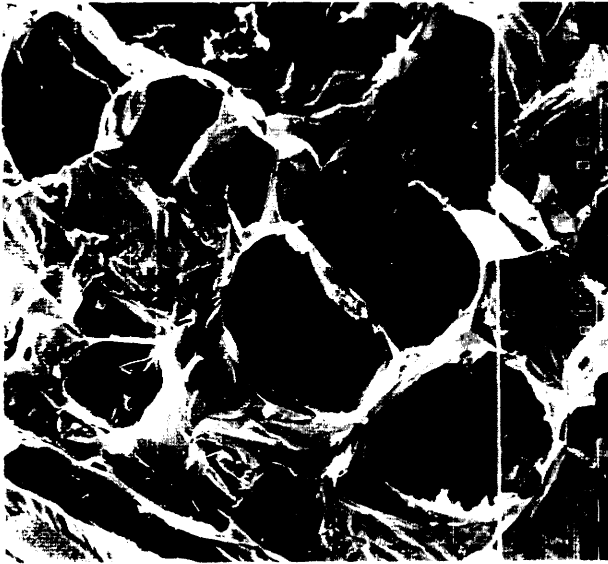
(b) PBZ



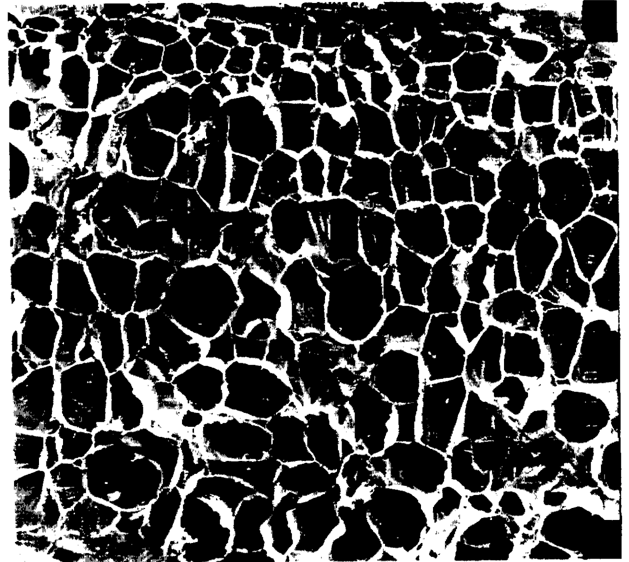
(c) GA_3

(d) PBZ + GA_3

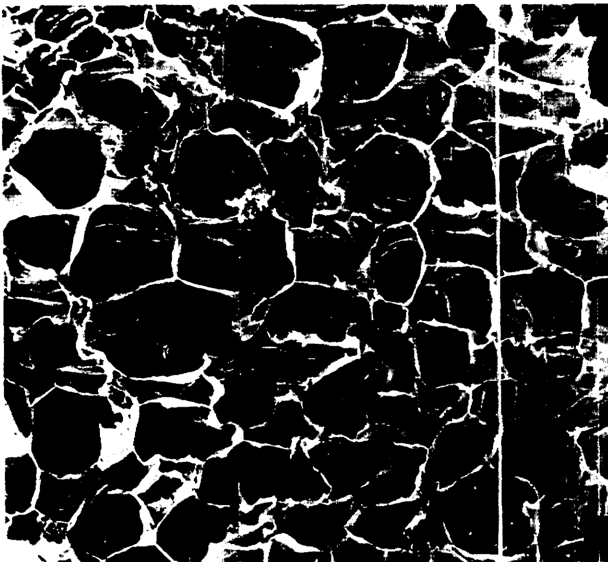
Plate 7.1 Longitudinal sections of main shoot internode of an intact plant following PGRs application, recorded at 8 weeks after sowing: PBZ = 10 mg a.i./plant; GA_3 = 500ppm; PBZ (10 mg a.i./plant) + GA_3 (1000ppm). Scale 7 mm = 100 μm



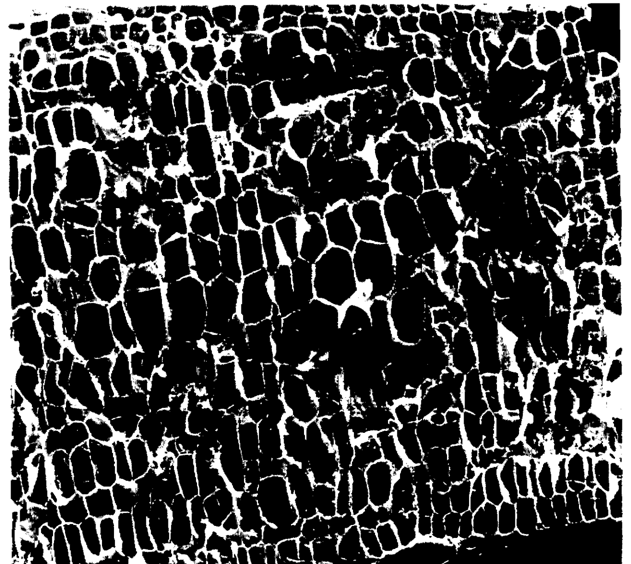
(a) Control



(b) PBZ

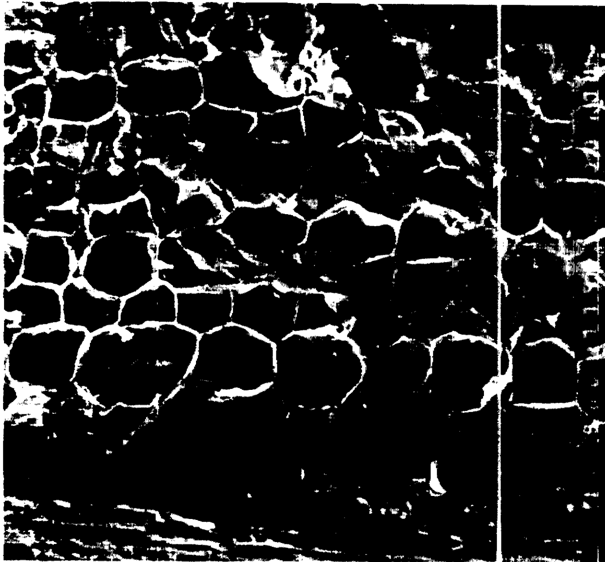


(c) GA_3



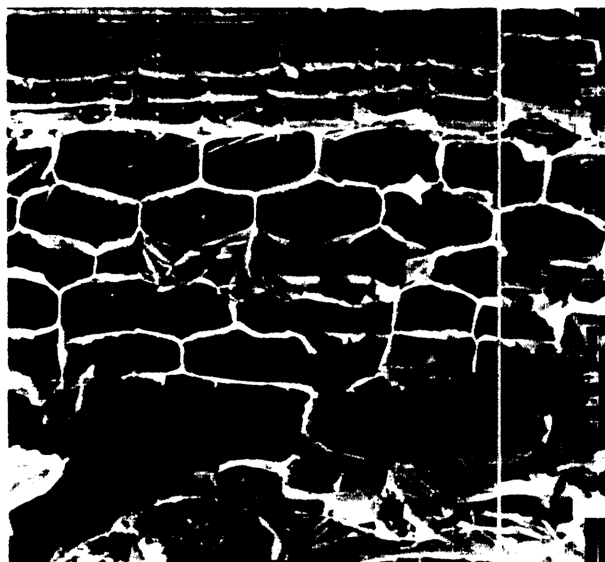
(d) PBZ + GA_3

Plate 7.2 Longitudinal sections of lateral shoot internode of an intact plant following PGRs application, recorded at 8 weeks after sowing: PBZ = 10 mg a.i./plant; GA_3 = 500ppm; PBZ (10 mg a.i./plant) + GA_3 (1000ppm). Scale 7 mm = 100 μm.



(a) Control

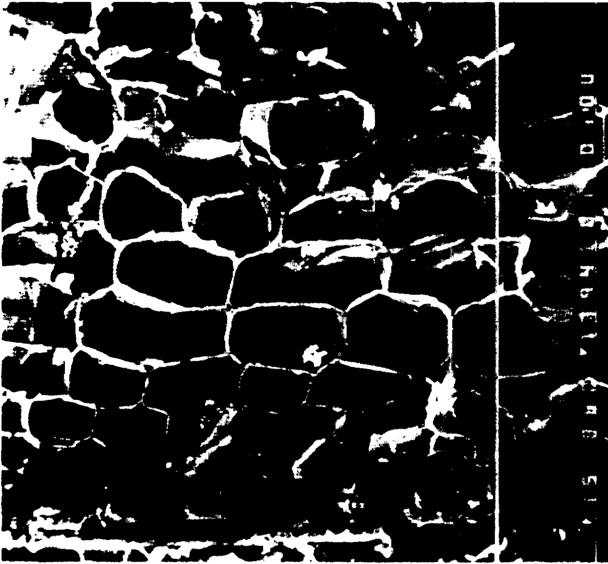
(b) PBZ



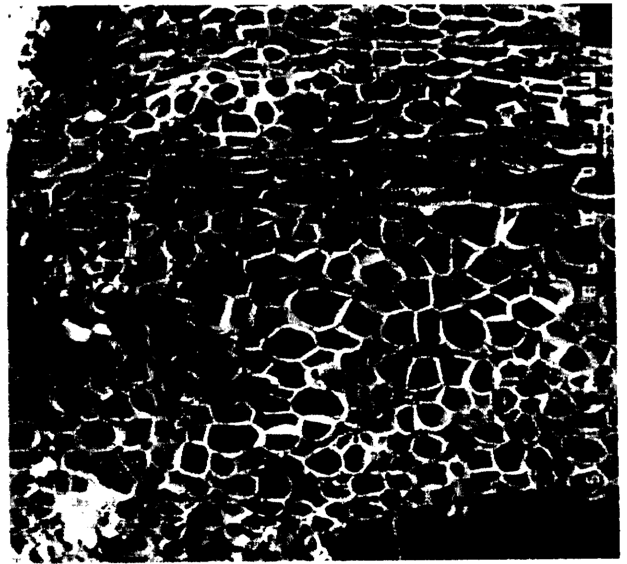
(c) GA_3

(d) PBZ + GA_3

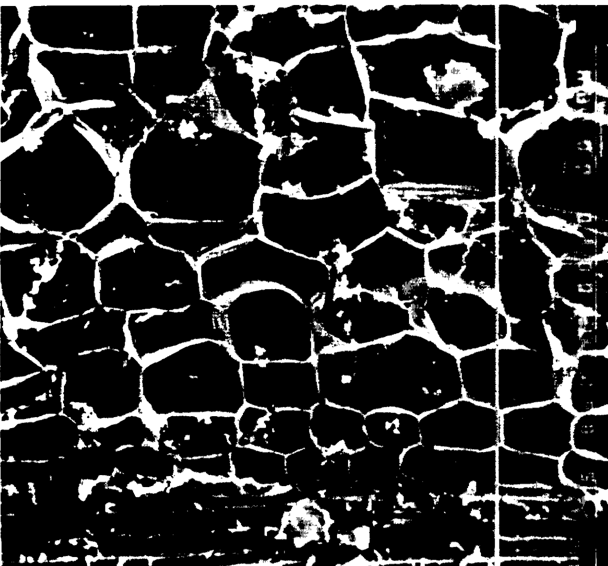
Plate 7.3 Longitudinal sections of main shoot internode of an intact plant following PGRs application, recorded at 12 weeks after sowing. PBZ = 10 mg a.i./plant; GA_3 = 500ppm; PBZ (10 mg a.i./plant) + GA_3 (1000ppm). Scale 7 mm = 100 µm.



(a) Control



(b) PBZ



(c) GA_3

(d) PBZ + GA_3

Plate 7.4 Longitudinal sections of lateral shoot internode of an intact plant following PGRs application, recorded at 12 weeks after sowing: PBZ = 10 mg a.i./plant; GA_3 = 500ppm; PBZ (10 mg a.i./plant) + GA_3 (1000ppm). Scale 7 mm = 100 μm.

thereby cell elongation. It was proposed earlier that PBZ might have an effect on increased auxin availability in SDP plants (Section 4.5.5) and auxin in general has an effect on cell elongation in many plants (Section 2.2.2.2). However, this elongation was not found at the vegetative stage. One explanation for this may be that the induced auxin might require more time to restore cell elongation from PBZ inhibition. However, the increased auxin at the flowering stage was unable to cause greater cell elongation in PBZ only treated plants compared to control plants probably due to the lack of available GA_3.

The present results at the vegetative stage are similar to that of Potter *et al.*, (1993), where in safflower, PBZ reduced both cell number and size. But the results at flowering stage are similar to those of Dalziel and Lawrence (1984) where, in suspension cultures of Paul's scarlet rose, PBZ prevented increment of cell number but increased cell size. The variable anatomical responses were also reported with PBZ in *Chrysanthemum* (Burrows *et al.*, 1992) and they added that those variations might be related to the method of application, concentrations, age of the plant materials used etc.

GA_3's mode of action seems dependent on the stage of plant growth as well as the concentration. Cell division was observed at the early vegetative stage irrespective of the concentration used (Table 7.3). Cell elongation was also promoted by GA_3 at this stage at high concentrations (500 and 1000ppm) but not at 100ppm. This may be explained on the basis that the level of GA required for cell division is higher than for cell elongation. At the early stage, cell division predominates so that at the low concentration of 100ppm it occurs at the expense of cell elongation. As the GA is metabolised, the concentration may fall below the threshold for cell division as well as for cell elongation. Cell elongation continues longer at the high rates of application since the level in the tissues remains high enough for a longer period. This idea is consistent with that of Goodwin (1978). He reported that GA growth response is saturated at lower doses which stimulated growth for a short duration in a number of species (0.01µg/plant in dwarf peas) but higher concentrations increased the duration of response and hence the final height.

The flowering data (Table 7.1) also supported the idea of GA_3's involvement in cell elongation at a later stage of plant growth. The cell elongation may ultimately cease because of the total metabolism of GA_3 at the senescence stage. However, the reason for reduced cell division in response to GA_3 alone at flowering stage is not understood. The unexpected results at the flowering stage might be explained mainly on the basis of two different growing seasons and the variation between the time of assessment for experiment #1 and experiment #2.

Stem elongation by GA due to cell elongation was reported in lettuce (North, 1990) but in *Samolus parviflorus*, GA induced stem elongation was mainly due to increased cell number (Sachs and Lang, 1961). In other cases GA increased both cell length and cell number (Cleland, 1969; Taiz and Zeiger, 1991). This variability in response to GA was noted by Nickell (1982) who stated that GA elongated the primary stalk by increasing cell length, an

increase in the rate of cell division, or a combination of these two, depending on the age and types of tissue treated.

GA_3 prevented PBZ inhibition of cell number immediately (Table 7.3) and eventually increased both cell length and cell number (Table 7.1) thereby stimulated main shoot growth. However, cell division by GA_3 alone in the main shoot was not found at the later stages of plant growth (Table 7.1). The additional supply of auxin induced by PBZ may have interacted with supplied GA_3 to stimulate cell division and cell elongation and thereby increased plant height over the control plants (Figure 4.7). The increased plant height with the agency of auxin and GA was reported earlier in many plants (Section 2.2.5.1).

Lateral shoot longitudinal section: Similarly like main shoot (at the vegetative stage), PBZ (10 mg a.i./plant) reduced both cell length and cell number at the early stage of a lateral shoot growth (Table 7.4) supporting PBZ's role in both GA and sterol biosynthesis inhibition (Davis *et al.,* 1988). But PBZ 1 mg a.i./plant only reduced cell elongation and was unable to inhibit cell division, which might mean that PBZ 1 mg a.i./plant was enough to inhibit GA-biosynthesis inhibition but not the sterol biosynthesis in lateral shoots. In main shoot at the vegetative stage both 1 and 10 mg a.i./plant PBZ reduced cell division as well as cell elongation. This present data further suggests that lateral shoots might have higher endogenous GA as compared to their main shoot.

Unlike in the main shoot the reduction in cell length with PBZ was not restored with time (at the flowering stage) (Tables 7.1 and 7.2). The proposed auxin induced by PBZ was unable to cause cell elongation in lateral shoots. It could be that supra-optimal concentration of auxin (endogenous from apex + PBZ induced) might have inhibitory effect on cell elongation in laterals. The main shoot probably maintains an optimum auxin level through basipetal transport thus enabling cell elongation in main shoot. Increased cell division in the laterals with PBZ at flowering stage could also be accounted for by supra optimal auxin concentration. Alternatively a higher auxin level may be required for cell division in lateral shoots but not for the cell elongation.

GA_3's mode of action in lateral shoots (Table 7.2 and 7.4) was different compared to the main shoot (Tables 7.1 and 7.3). In lateral shoots, at the flowering stage, GA_3 increased cell division and reduced cell elongation but GA_3 in the main shoot increased cell elongation and reduced cell division. Again at the vegetative stage GA_3 still increased cell division and reduced elongation in a lateral shoots but increased both cell division and cell elongation in main shoot. These results highlighted the difference in responsiveness of the main and lateral shoots to applied plant hormones and PGRs.

PBZ and GA_3 both reduced lateral shoot cell length when applied alone and when applied together their effect on cell length was still inhibitory (Table 7.2 and Table 7.4). This negative effect on cell length may explain why GA_3 was unable to reverse PBZ inhibition of lateral shoot growth in intact plants (Figure 4.11 and Table 4.2.) even though the number of cells per internode was increased (Table 7.2 and 7.4). It should be mentioned here that growth is always accompanied by cell enlargement (Section 2.1.1). This inhibitory effect of GA_3 +

PBZ on cell length in lateral shoots of an intact plant could be due to GA_3's translocation to the main shoot and interaction with auxin (from endogenous or PBZ induced) in favour of the elongation at that place. It was also revealed that, higher concentration of GA_3 was required to cause cell division when applied along with PBZ (Table 7.4).

General observation: Similar hollowing of the pith was also reported in bean plants with GA_3 (Takano *et al.,* 1995). They attributed this to excess elongation of the central pith cells by GA_3 and further added that the hollowing effect was independent of other factors like ethylene.

7.2.5 Conclusions:

* Anatomical responses depend on different variables including shoot type (main or lateral), growth stage, PGRs type, their concentrations and also on the growing season.
* In the main shoot at the vegetative stage, PBZ (1 and 10 mg a.i./plant) probably inhibited both GA and sterol biosynthesis and gave reduced cell elongation and cell division. But in laterals, at the vegetative stage, the lower PBZ concentration (1 mg a.i./plant) only inhibited cell elongation (i.e. GA biosynthesis) probably due to higher endogenous GA levels.
* In main shoots, PBZ reduction of cell length decreased with time possibly due to PBZ induced increased auxin supply.
* In lateral shoots at the flowering stage, increased (supraoptimal) level of auxin (endogenous + probable auxin induced) probably reduced cell length but increased cell division.
* Higher GA_3 concentration (1000ppm) along with PBZ (10 mg a.i./plant) can cause cell division at all stages, probably through an interaction with auxin (induced by PBZ).
* In main shoots, added GA_3 reversed PBZ inhibition by increased cell elongation and cell division.
* In lateral shoots of intact plants, PBZ and GA_3 alone or in combination reduced cell length thereby prevented reversal of PBZ inhibition.

7.3 <u>Anatomical responses to delayed GA_3 application to PBZ treated plants.</u>

7.3.1 Introduction:

Reversal of the main shoot growth inhibition caused by PBZ was possible by applying GA_3 at the same time as PBZ application or by delaying GA_3 application after PBZ application (Section 4.7.5). On the other hand, the reversal of the lateral shoot growth inhibition caused by PBZ was only possible with delayed GA_3 application (Section 4.7.5 and Section 5.8.5).

The objective of this present experiment was to investigate the anatomical basis of the reversal of PBZ induced inhibition of main and lateral shoots of intact plants and laterals of decapitated plants by delayed GA_3 application. Do main and lateral shoots still differ in their anatomical responses to PBZ inhibition and its reversal by GA_3? What hormonal balance was involved in reversing PBZ's anatomical effect?

7.3.2 Materials and Methods:

The same plants and experimental procedures were used as in section 5.8 but 9 selected treatments from that experiment, with 3 replications, were used in this present experiment. The selected treatments were: a) Control, b) PBZ (10 mg a.i./plant) and c) PBZ (10 mg a.i./plant) + GA_3 (500ppm; applied 8 weeks after sowing i.e. 4 weeks after PBZ application). These same treatments were applied to 3 different sets of plants. These sets were: i) Main shoot of intact plants; ii) Lateral shoots of intact plants and iii) Lateral shoots of decapitated plants. The samples were collected 8 weeks after PBZ application i.e. 4 weeks after GA_3 application. The 9th internode was used from the main and lateral shoot for anatomical examination because of the delayed GA_3 application. The rest of the procedures were followed as per section 7.2.2 (Experiment #1).

7.3.3 Results:

Main shoot longitudinal section: PBZ (alone) applied to the main shoot had no effect on the length of parenchyma cells but reduced cell number per internode by 57% (Table 7.5). However, addition of GA_3 along with PBZ reduced the inhibitory effect of PBZ by increasing both cell length (49%) and cell division (from -57% to -5% only) (Table 7.5).

Lateral shoot longitudinal section: PBZ alone produced shorter cells and fewer cells per internode, both in lateral shoots of intact plants and in the lateral shoots of decapitated plants (Table 7.6). In plants treated with PBZ and GA_3, the cell length was increased in the lateral shoots of intact or decapitated plants compared to PBZ only treated plants but cell number was increased compared to the respective control and PBZ only treated plants (Table 7.6).

Main and lateral shoot transverse section: PBZ reduced cell diameter in the main (Table 7.7) and lateral shoots (Table 7.8) of intact plants. GA_3 added to these PBZ treated intact plants increased their diameter compared to the control plants. But in decapitated PBZ treated lateral shoots, PBZ had no effect on cell diameter while added GA_3 reduced the diameter.

General observation: GA_3 again produced hollowing of the central pith cells.

Table 7.5 Anatomical response (LS) of delayed GA_3 application (500ppm) on PBZ (10 mg a.i./plant) treated main shoots.

Treatments	Internode length (cm)	Cell length (µm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Main shoots	1.02b*	94.00a		109.00b	
Main shoots + PBZ	0.44a	94.00a	0.00	47.00a	-57.00
Main shoots + PBZ + GA_3	**1.45b**	**140.00b**	**49.00**	**104.00b**	**-5.00**
		t		t	

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Table 7.6 Anatomical response (LS) of delayed GA_3 application (500ppm) on PBZ (10 mg a.i./plant) treated lateral shoots.

Treatments	Internode length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Laterals of intact plants	2.22b*	170.00bc		131.00c	
Laterals of intact plants + PBZ	0.80a	110.00a	-35	73.00f	-45
Laterals of intact plants + PBZ + GA_3	**2.59b**	**151.00b**	**-11**	**173.00a**	**32**
Laterals of decapitated plants	2.32b	193.00c		122.00d	
Laterals of decapitated plants + PBZ	1.19a	140.00b	-27	86.00e	-30
Laterals of decapitated plants + PBZ + GA_3	**2.66c**	**190.00c**	**2**	**140.00b**	**15**
		t			

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

Table 7.7 Anatomical response (TS) of delayed GA_3 application (500ppm) on PBZ (10 mg a.i./plant) treated main shoots.

Treatments	Cell diameter (μm)	Cell diameter (%) deviated from control
Main shoots	79.00b	
Main shoots + PBZ	72.00c	-9.00
Main shoots + PBZ + GA_3	**88.00a**	**11.00**

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).

Table 7.8 Anatomical response (TS) of delayed GA_3 application (500ppm) on PBZ (10 mg a.i./plant) treated lateral shoots.

Treatments	Cell diameter (μm)	Cell diameter (%) deviated from control
Laterals of intact plants	113.00d	
Laterals of intact plants + PBZ	94.00b	-17.00
Laterals of intact plants + PBZ + GA_3	**120.00e**	**7.00**
Laterals of decapitated plants	102.00bc	-
Laterals of decapitated plants + PBZ	102.00bc	0.00
Laterals of decapitated plants + PBZ + GA_3	**80.00a**	**-22.00**
	t	

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

7.3.4 Discussion:

Main shoot longitudinal section: The dwarfing of PBZ treated main shoots was only due to fewer cells per internode but their reversal was mainly due to increased elongation of the cells. Although, cell division was increased in PBZ + GA_3 treated plants compared to PBZ alone treated plants, cell number did not exceed the control (Table 7.5). In earlier experiments (Table 7.1 and Table 7.3) cell division did exceed the control plants when PBZ + GA_3 treatments were applied simultaneously in intact main shoots. One explanations for this may be that the plants lost their ability to cause excessive cell division with added GA_3 at that stage of growth (8 weeks after sowing). In the earlier experiment GA_3 was applied at the appropriate growth stage of the plants to cause cell division (4 weeks after sowing).

Lateral shoot longitudinal section: The reversal of the PBZ inhibition of lateral shoot growth by late GA_3 application to intact plants (Table 7.6) was mainly due to an increase in cell number and partly due to cell elongation (compared to PBZ only treated plants). But in lateral shoots of decapitated plants, it was the increment of both cell length (compared to PBZ only treated plants) and cell number. These results suggest that in intact plants cell division could increase (at a higher rate) in the laterals following PGR application but the main shoot did not respond at the same rate (Section 7.3.4; Main shoot longitudinal section). That is, cell division in the main shoot ceases earlier than in the laterals. This is consistent with the normal growth pattern of a plant.

In decapitated PBZ treated plants, inhibition of cell length in lateral shoots was less than in intact plants. This reduced inhibition of cell length in decapitated plants was probably due to the absence of an intact apex. Decapitation possibly changed the hormonal balance favourable for the growth of the lateral shoots (Section 2.1.2.3) and therefore addition of PBZ was less effective than on laterals of the intact plants.

Although PBZ + GA_3 when applied simultaneously to intact plants in an earlier experiment (Table 7.2) had a similar effect to that found in this experiment, there was no overall morphological reversal of lateral shoot growth (Table 7.2), probably because of the presence of an apically dominant intact apex. In this present experiment delayed GA_3 application to PBZ pre-treated plants probably elongated more buds because there were more buds released due to PBZ induced auxin (Section 5.8.4). However, the mechanism of bud release does not appear to be related to the PBZ induced increased cell division in this present experiment (Table 7.6). The proposed induced auxin only increased cell elongation in the main shoot (Section 7.2.4). Elongation was not found in lateral shoots but cell division increased at flowering stage (Section 7.2.4).

Main and lateral shoot transverse section: The reduced diameter of PBZ treated cells both in the main and lateral shoots of intact plants might be related to the reduced cell number/internode (Table 7.5 and 7.6). GA_3 added to an intact plant increased cell diameter but reduced it in a decapitated plant (Table 7.7 and 7.8). GA_3, PBZ alone or in combination altered the diameter of both main and lateral shoot parenchyma cells in this present experiment but the effects of these treatments were insignificant in the earlier experiment (Section 7.2).

These differences might be related to the difference in the node studied and time of plant hormones and PBZ application or even seasonal variations between the experiments.

7.3.5 Conclusions:

* The reversal of PBZ inhibition of main shoot growth by delayed GA_3 application was mainly due to cell elongation but in lateral shoots of an intact plant it was due to cell division. However, in lateral shoots of decapitated plants it was due to both cell elongation and cell division.

* Cell division in the main shoot ceases earlier than in the laterals.

* Stem anatomical response following PGRs application varies with the time of application, and the position of the node.

7.4 Anatomical responses of lateral shoot cuttings to PBZ, GA_3 and L-tryptophan.

7.4.1 Introduction:

In an earlier experiment with intact or decapitated plants delayed GA_3 application reversed PBZ inhibition of lateral shoot growth (Section 5.8). Anatomically, delayed GA_3 application also counteracted PBZ inhibition of growth in intact or decapitated plants (Section 7.3.5).

Simultaneous application of GA_3 or GA_3 plus the auxin precursor L-tryptophan also reversed PBZ inhibition of lateral shoot cuttings (Section 6.4.5). The objective of this present experiment was to study the anatomical responses of these reversed lateral shoot cuttings following simultaneously applied GA_3 or GA_3 plus auxin precursor.

7.4.2 Materials and Methods:

Anatomical observations were made on 3 replications of the following seven treatments selected from the section 6.4. The treatments were: i) Control, ii) PBZ, iii) GA_3, iv) L-tryptophan, v) PBZ + GA_3, vi) PBZ + GA_3 + L-tryptophan and vii) PBZ + GA_3 + L-tryptophan + GA_3. The treatment # vii had double the amount of GA_3 500ppm. The rates of application were: PBZ = 10 mg a.i./plant; GA_3 = 500ppm and L-tryptophan = 3.0 mg/kg of soil.

The samples were taken from the 5th internode of the lateral shoot cuttings, 4 weeks after PGRs application. The scanning electron microscope used for this experiment was a Jeol, JSM- 580LV (Japan).

Other procedures were followed as per section 7.2.2 (Experiment #1).

7.4.3 Results:

Cell length and number: PBZ + GA_3 + L-tryptophan or PBZ + GA_3 + L-tryptophan + GA_3 increased cell length but had no effects on the number of cells per internode. L-tryptophan or PBZ alone, or PBZ in combination with GA_3, reduced cell length but increased the number of cells per internode (Table 7.9). GA_3 alone increased cell length but reduced cell

number per internode. The plate (7.5) shows the effects of different PGRs on the individual cell length (LS).

Cell diameter: PBZ + GA_3 + L-tryptophan + GA_3 slightly increased cell diameter but the rest of the treatments reduced cell diameter (Table 7.10).

General observation: GA_3 again caused hollowing of the central pith and PBZ partly reduced that effect.

Table 7.9 Anatomical response (LS) of lateral shoot cuttings to PGRs: PBZ (10 mg a.i./plant), GA_3 (500ppm), L-tryptophan (3.0 mg/kg of soil) and their combinations.

Treatments	Internode Length (cm)	Cell length (μm)	Cell length (%) deviated from control	Calculated cell number	Cell number (%) deviated from control
Control	1.55bc*	170.00d		90.00d	
PBZ	1.09a	95.00f	-44	116.00c	29
GA_3	1.55bc	215.00b	26	72.00e	-20
L-tryptophan	1.38b	114.00e	-33	121.00b	35
PBZ + GA_3	**1.74cd**	**90.00g**	**-47**	**193.00a**	**114**
PBZ + GA_3 +L-tryptophan	**1.52c**	**179.00c**	**5**	**91.00d**	**1**
PBZ + GA_3 +L-tryptophan + GA_3	**2.14e**	**232.00a**	**36**	**92.00d**	**3**

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).

Table 7.10 Anatomical response (TS) of lateral shoot cuttings to PGRs: PBZ (10 mg a.i./plant), GA_3 (500ppm), L-tryptophan (3.0 mg/kg of soil) and their combinations.

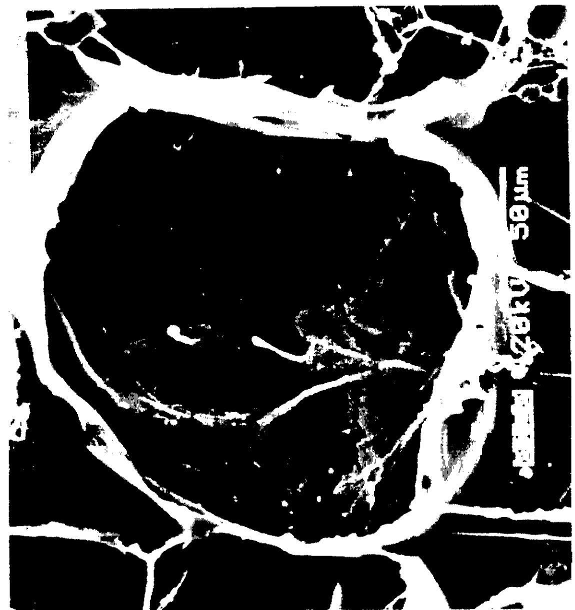
Treatments	Cell diameter (μm)	Cell diameter (%) deviated from control
Control	124.00e	
PBZ	102.00d	-18
GA_3	82.00a	-34
L-tryptophan	98.00c	-21
PBZ + GA_3	90.00b	-27
PBZ + GA_3 +L-tryptophan	98.00c	-21
PBZ + GA_3 +L-tryptophan + GA_3	**134.00f**	**8**
	t	

* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
t = Analysis was done on the transformed data.

7.4.4 Discussion:

Cell length and number: The anatomical results from this present experiment revealed that the reversal of PBZ inhibition of lateral shoot growth by GA_3 alone (Section 6.4.4) was accompanied by increased cell number per internode (Table 7.9). The same reversal of PBZ inhibition with GA_3 + L-tryptophan or GA_3 + L-tryptophan + GA_3 was mainly due to increased cell length. It is clear from these results that the reversal of PBZ inhibition by GA_3 requires a supply of auxin (either from PBZ induced or from auxin precursor).

(a) Control



(b) PBZ

(c) GA_3

(d) PBZ + GA_3

Plate 7.5 Anatomy of the single cells (LS) of lateral shoot cuttings following PGRs application: PBZ = 10 mg a.i./plant; GA_3 500ppm; PBZ (10 mg a.i./plant) + GA_3 (1000ppm).

PBZ alone increased cell division in this present experiment (Table 7.9). Similar increase in cell number by PBZ in the lateral shoots was observed earlier (Table 7.2). However, it was reduced in other experiments (Table 7.4 and Table 7.6) with intact or decapitated plants respectively. These differences could be mainly explained in terms of the differences in the position of the nodes studied and growth pattern of the intact or decapitated plants vs lateral shoot cuttings.

L-tryptophan alone was unable to cause elongation in lateral shoot cuttings (Table 6.2). But anatomically L-tryptophan alone increased cell division in this present experiment. L-tryptophan might have some interaction with endogenous GA and form auxin and probably had effect on increased cell division, but which was not enough to cause significant elongation of the lateral shoot cuttings. GA_3 alone, on the other hand, caused cell elongation and therefore elongated some of the internodes but was not enough to increase the mean internode length (Table 7.9). However, GA_3 alone reduced cell elongation in the lateral shoots of intact plants in earlier experiments (Table 7.2 and 7.4). GA_3 induced reduction of cell division in this present experiment was unexpected because cell division was increased in earlier experiments in the laterals of intact plants (Section 7.2 and 7.4). These unexpected results highlight the differences in hormonal balance of the lateral shoots of intact plants compared to lateral shoot cuttings. As for example lateral shoot cuttings possibly devoid of enough auxin (Section 6.2.4).

Cell diameter: As in decapitated plants (Table 7.8), PBZ + GA_3 reduced cell diameter in this present experiment (Table 7.10) but the cell diameter of lateral shoots was increased by PBZ + GA_3 application in intact plants (Table 7.8). The different responses of intact plants and lateral shoot cuttings could be again interpreted on the basis of differences in hormonal balance of the laterals in intact plants and lateral shoot cuttings.

7.4.5 Conclusions:

* Supply of auxin along with GA_3 is important to reverse PBZ inhibition of lateral shoot growth.

* The reversal of PBZ inhibition of lateral shoot cuttings, was only due to increased cell division with GA_3 alone, but due to increased cell elongation in GA_3 + L-tryptophan or GA_3 + L-tryptophan + GA_3.

7.5 General conclusions from chapter - 7.

* Shoot anatomical responses depend on different variables including shoot type (main or lateral) PGR type, time of application, concentration, age of the plant and also the growing season.

* Cell division in the main shoot ceases earlier than in the laterals.

* In main shoots, PBZ induced reduction of cell length was reduced with time possibly due to PBZ induced increased auxin supply.

* In main shoot at the vegetative stage, PBZ (1 and 10 mg a.i./plant) probably inhibited both GA and sterol biosynthesis and gave reduced cell elongation and cell division. But in laterals, at the vegetative stage, the lower PBZ concentration (1 mg a.i./plant) only inhibited cell elongation (i.e. GA biosynthesis) but unable to reduce cell division (i.e. sterol biosynthesis) probably due to higher endogenous GA levels.
* The reversal of PBZ inhibition of main shoot growth by delayed GA_3 application was mainly due to cell elongation but in lateral shoots of an intact plant it was due to cell division. However, in lateral shoots of decapitated plants it was due to both cell elongation and cell division.
* A balanced supply of auxin (either PBZ induced or from auxin precursors) along with GA_3 is essential to have complete reversal of PBZ induced growth inhibition.
* The reversal of PBZ inhibition of lateral shoot cuttings, was only due to increased cell division with GA_3 alone, but due to increased cell elongation in GA_3 + L-tryptophan or GA_3 + L-tryptophan + GA_3.

CHAPTER - 8: INTEGRATION

To understand the mechanism of PBZ action on SDP shoot growth, 4 key issues were identified. These are:

- Does PBZ have a similar inhibitory effect on both main and lateral shoot ? (Section 8.1)
- Is there any involvement of apical dominance in the response to PBZ ? (Section 8.2)
- What is the relationship between PBZ and other hormones in SDP shoot growth ? (Section 8.3)
- How does PBZ affect stem anatomy? What is the effect of PBZ on cell elongation and cell division ? (Section 8.4)

The knowledge gained from these studies is integrated in sections 8.1, 8.2, 8.3 and 8.4. The conclusions drawn from the whole study are presented in section 8.5 and the directions for the future research are presented in the section 8.6.

8.1 Effect of PBZ on main and lateral shoots:

PBZ induced growth retardation of main and lateral shoot is probably mediated through inhibition of GA-biosynthesis and also perhaps through increased auxin availability in plants (Section 4.8). A similar decrease in GA and increase in auxin was also proposed for *Ligustrum vulgare* L. (Rauscherova and Tesfa, 1993). However, different growth control mechanisms of SDP main and lateral shoots were suggested since higher retardant concentrations were required to reduce lateral shoot growth. Lateral shoots may have a higher GA synthesis or supply (Section 3.8).

The simultaneous application of GA_3 alone or in combinations with other PGRs in a PBZ treated plant reversed PBZ inhibition of main shoot growth but was unable to reverse the inhibition of lateral shoot growth (Section 4.2.5) probably due to the re-establishment of the apical dominance in intact plants and correlative inhibition in decapitated plants (Section 8.2).

8.2 PBZ and apical dominance:

PBZ reduced main shoot growth but increased lateral shoot number by reducing apical dominance (i.e by inhibiting GA biosynthesis). Gibberellin enhanced apical dominance and reduced lateral growth (Martin, 1987) in several plant species. PBZ and GA had similar modes of actions but exerts different effects (Section 4.8).

PBZ inhibition of main shoot was released by simultaneous or delayed GA_3 application but in laterals only delayed GA_3 application was successful. The inability of GA_3 to reverse PBZ inhibition of lateral shoot growth was interpreted on the basis of re-establishment of apical dominance when GA_3 was applied to intact plants (Section 4.5.4). Again, addition of any PGRs (Table 5.5) to PBZ reduced lateral length (without GA_3) or reduced total lateral growth (shoot number and lateral length compared to untreated controls) due to induced correlative inhibition (with GA_3) in decapitated plants. It was postulated that after releasing

apical dominance, application of GA_3 alone or in combination with other PGRs would reverse PBZ inhibition of lateral shoot growth (Section 4.5.4).

Decapitation normally eliminates apical dominance (Cline, 1991). But decapitation and addition of GA_3 was unable to reverse PBZ inhibition in lateral shoots because of the induction of correlative inhibition by other released laterals (Section 5.7).

Delayed GA_3 application after the release of apical dominance or correlative inhibition (i.e 4 weeks after PBZ application) reversed PBZ inhibition of lateral shoot growth in intact plants (Figure 4.31) and in decapitated plants (Section 5.8). Perhaps at that stage buds were released due to a balance between PBZ induced auxin and applied GA (Section 5.8.4). However, the problem of reversing PBZ inhibition of lateral shoot growth by simultaneously applied GA_3 and other PGRs remained unresolved.

8.3 Relationship between PBZ and other hormones:

Section 8.1 proposed a difference in the growth control mechanism of main and lateral shoots. Experiments with cuttings from main and lateral shoots supported this view. GA_3 was unable to elongate lateral shoot cuttings (Section 6.2). It was hypothesised that the lack of lateral shoot elongation in response to exogenous GA_3 might be due to inadequate auxin levels in the laterals or higher endogenous GA resulting in supra-optimal levels after GA_3 was applied (Section 6.2.4).

This hypothesis was based on the idea that lateral shoots of intact plants, or in decapitated plants (compared to cuttings), are still supplied with auxin from the other terminals of the plant. Moreover they have an established root system which could also supply their auxin requirements (Taiz and Zeiger, 1991). These auxins, along with added GA_3, may cause elongation of the main shoot in intact plants or in 1 or 2 laterals of decapitated plants thereby enhancing apical dominance or correlative inhibition. On the other hand, cuttings lack these other sources of endogenous auxins and therefore are unable to elongate in response to exogenous GA_3. The requirement for a suitable ratio of auxin : GA for elongation was also reported earlier (Hasan *et al.,* 1994; Okamoto *et al.,* 1995). Changing that hormonal balance had inhibitory effect on lateral shoot elongation in SDP (Section 3.5.5).

If this rationale is correct supplying exogenous auxin to lateral shoot cuttings, along with the GA_3 should stimulate elongation. In fact, it did not promote elongation in lateral shoot cuttings in a preliminary experiment (data not presented). The balance between GA_3 and auxin levels appears to be critical. The supply of IAA (20 mg a.i./plant) may have been too high causing an oversupply of auxin. Alternatively, basipetal transport of IAA (Section 2.2.2.4) may mean that the applied auxin accumulated at the base of the cuttings and was not available to the subapical region of growth. Endogenous auxin may be in a different form or distributed differently.

An alternative approach was to supply auxin precursor (L-tryptophan) rather than IAA. Since GA_3 is thought to be involved in the regulation of auxin synthesis, supplying L-tryptophan may result in formation of a "natural" form of auxin, at the appropriate site (i.e.

where GA_3 is located). Another possibility is that basipetal transport is avoided (i.e. the precursor or "natural" form of auxin is not subject to basipetal transport as is applied IAA). If any of these alternatives are true, supplying L-tryptophan + GA_3 should reverse the PBZ inhibition of lateral shoot cutting growth even though IAA was unsuccessful (Section 6.4.1).

Accordingly, different combinations were applied to lateral shoot cuttings and, at last, simultaneously applied GA_3 + L-tryptophan or GA_3 + L-tryptophan + GA_3 successfully reversed PBZ induced inhibition of lateral shoot cuttings (Section 6.4.5). This was interpreted on the basis of a balanced supply of auxin and GA_3, where there was no effect of one shoot on another. However, addition of GA_3 along with PBZ in lateral shoot cuttings also successfully reversed PBZ inhibition. PBZ might have increased the level of auxin (Section 4.5.5) and in the absence of the intact main shoot or other laterals, this auxin might have interacted with the added GA_3 to reverse PBZ inhibition of lateral shoot cuttings (Section 6.4.4).

8.4 PBZ and stem anatomy:

The anatomical responses (i.e the effect of PBZ on the cell elongation and cell division) were found to depend on a number of factors including shoot type (main or lateral), plant age, growing season, PGRs type and concentrations.

In the main shoot at the vegetative stage, PBZ probably reduced cell elongation and cell division by inhibition of both GA and sterol biosynthesis. But in laterals, at the vegetative stage, the lower PBZ concentration (1 mg a.i./plant) only inhibited cell elongation (i.e. GA biosynthesis) probably due to higher endogenous GA levels in lateral shoots. The slight increase in cell elongation in PBZ treated internodes compared to untreated plants at the flowering stage might be due to PBZ induced auxin availability (Section 7.2.4).

The reversal of PBZ inhibition of the main shoot by simultaneously applied GA_3 involved both increased cell division and cell elongation due to a balance supply of auxin : GA (Section 7.2.4 and 7.3.4).

The reversal of PBZ inhibition of lateral shoot cuttings, was due to increased cell division with GA_3 alone, but due to increased cell elongation both in GA_3 + L-tryptophan or GA_3 + L-tryptophan + GA_3 (Section 7.4.5) treated plants. A balanced supply of auxin (either PBZ induced or from auxin precursors) along with GA_3 is also essential to have reversal of PBZ induced growth inhibition in lateral shoots (Section 7.4.4). Similarly delayed GA_3 application reversed PBZ induced growth inhibition of lateral shoots probably by an interaction with PBZ induced auxin (Section 7.3.4).

8.5 Conclusions:

PBZ inhibition of shoot growth in SDP is probably mediated through reduced GA and sterol biosynthesis and increased auxin level. The response differences between the SDP main and lateral shoots to applied PGRs could be due to:

- the plagiotropic pattern of lateral shoot growth (Section 6.2.5),

- higher content of endogenous GA in lateral shoots (Section 6.2.5) and
- higher requirement for auxin in lateral shoots (Section 6.3.5).

Apart from improving our understanding of the mechanism of PBZ induced growth control, the knowledge gained through this research could be used to improve the marketability of SDP plants, at least until better varieties are bred. Growers can use PBZ 10 mg a.i./plant applied 30 days after sowing to produce a compact SDP pot plant with more lateral shoots. On the other hand, they can use GA_3 500ppm (applied at the same time or 60 days after sowing) to produce taller plants with less lateral growth suitable for producing cut flowers. However, GA_3 500ppm applied 4 weeks after PBZ can also be used as an antidote (if required) to reverse the effect of PBZ 10 mg a.i./plant.

8.6 Directions for future research:

While in this present study, it was not proved that PBZ increased auxin levels in shoots, the evidence from other experiments is consistent with this idea. If it was increased, then through what mechanism(s) ? Was that by enhanced synthesis through enhanced enzymatic action or by enhanced transport. Similarly reduced sterol levels by PBZ in plants was not proved but circumstantial evidence supports this idea.

Measuring hormonal balance as well as sterols and their relationship with PBZ could give a clear understanding of the PBZ control of growth in future. The increased auxin (if any) and reduced GA and sterol could be determined by measuring the levels using different techniques. These include: gas chromatography (GC), mass spectrometry (MS), selected ion monitoring (SIM), gas chromatography-mass spectrometry (GC-MS), gas chromatography-selected ion monitoring (GC-SIM), high performance liquid chromatography (HPLC) or high performance liquid chromatography-mass spectrometry (LC-MS) etc.

The proposed differences in the transport modes for auxin and its precursor could also be tested through the use of radio labelled materials.

The presence of the type and availability of the soil microbe populations were not recorded but microbe populations in the soil can alter the amount of auxin produced from its precursor (Normanly *et al.,* 1995). In future experiments, the measurements of the microbe populations in the glasshouse pots and in the field could provide some fruitful information.

The preliminary results from this study at least indicate that there are some differences at the anatomical level following PGRs application. A thorough investigation and understanding of these differences at anatomical level should help to understand the total mode of PBZ's action.

Apart from GA_3 and IAA, the interaction(s) of other hormones and sterols were mostly overlooked. But GA-biosynthesis inhibitors can affect the levels of other hormones (Davis and Curry, 1991) and sterols (Lenton *et al.,* 1994). To ascertain a total picture of the hormonal balance and PBZ induced growth control in SDP, in the future, interaction of other hormones (e.g. Cytokinins, ethylene, ABA etc.) and sterols with PBZ could also be thoroughly investigated.

Molecular biotechnology techniques to understand the hormone physiology and biochemistry are being successfully used in different plants. Use of such techniques could provide a clear insight into the PBZ induced growth control mechanism in SDP.