CHAPTER - 4 : INTERACTIONS BETWEEN PGRs

4.1 General introduction to chapter 4.

PBZ was selected as the best growth retardant because of its consistent inhibitory effect on total plant growth and also because of its desirable side effects (Section 3.6.5). In many plants PBZ inhibition of growth was reversed by the application of gibberellic acid (Section 2.2.6.4) and hence GA biosynthesis inhibition is regarded as the primary biochemical mechanism of PBZ action. Other than GA biosynthesis inhibition, reports are also available where PBZ interfered with the level of sterols and other plant hormones (Section 2.2.6.5).

The main objective of the experiments in this chapter was to study the interaction between PBZ and the plant hormones in intact plants, particularly the reversal of PBZ induced inhibition by the application of GA_3. Out of 6 reported experiments, 2 experiments (Section 4.2 and 4.4) were aimed at determining the appropriate concentrations of PBZ and GA_3 for reversing PBZ induced growth inhibition. In section 4.3 attempts were made to study the effects of PBZ and GA_3 on the additional growth parameters of SDP. The probable involvement of IAA and BAP for reversing PBZ's effect was also investigated in the 4th experiment (Section 4.5). The 5th experiment investigated the interaction of PGR using a clinostat (Section 4.6) for releasing apical dominance. The effect of time of PGR application relative to plant age was investigated in the last experiment (Section 4.7).

4.2 Interaction of PBZ and different GA_3 concentrations.

4.2.1 Introduction:

PBZ is mainly a GA biosynthesis inhibitor (Steffens *et al.*, 1985). It does not block the action of either endogenous or exogenous gibberellins (Steffens and Wang, 1986), therefore applied GA should overcome the growth inhibitory effect of PBZ. However, controversy exists about the GA_3 reversal of PBZ inhibition of shoot growth. Tromp (1987) stated that the growth reduction caused by PBZ in apple was hardly counteracted or was unaffected by GAs (GA_3 or GA_{4+7}). But the dwarfing effect of PBZ was completely reversed by applying GA_3 in poinsettia (Cox, 1993).

The interaction between PBZ and GA_3 in the reversal of shoot growth inhibition in SDP, was investigated in the following 2 experiments.

4.2.2 Materials and Methods:

Experiment #1: The 8 treatments were: water (control), PBZ 10 mg a.i./plant alone and its combination with 6 low concentrations of GA_3 (10, 50, 100, 250, 500 & 750ppm).

Experiment #2: Since 750ppm of GA_3 did not reverse the inhibitory effects of PBZ (10 mg a.i./plant) on lateral shoots, higher GA_3 concentrations were used in this second experiment. The 6 treatments were: water (control), PBZ 10 mg a.i./plant alone and its combination with high GA_3 concentrations (1000, 1500, 2000 & 2500ppm).

The seeds were sown on 2?.09.93 (for experiment #1) and on 16.02.94 (for experiment #2). Both experiments were laid out in a randomised complete block design with 10 replications. Vegetative data were recorded 4 weeks after PGRs application. The rest of the procedures were followed as per section 3.2.

4.2.3 Results:

Toxicity: The highest concentration of GA_3 (2500ppm) along with PBZ (10 mg a.i./plant) caused toxicity (slight leaf burn and deformed leaf) the day after application but the plants had partly recovered by about 2 weeks after application. Other treatments had no sign of phytotoxicity.

Figure 4.1 PBZ and low GA_3 concentrations on main shoot growth

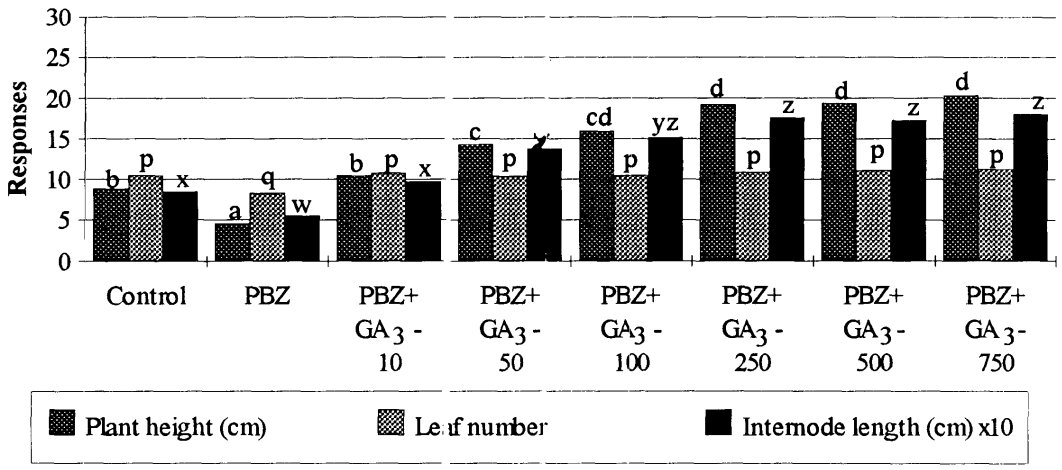


Figure 4.2 PBZ and high GA_3 concentration on main shoot growth

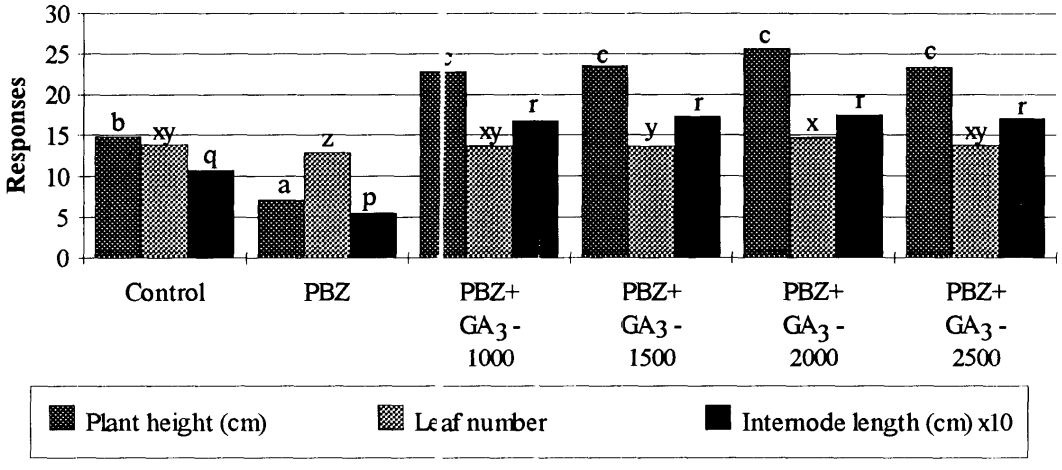


Figure 4.3 PBZ and low GA_3 concentrations on lateral shoot growth

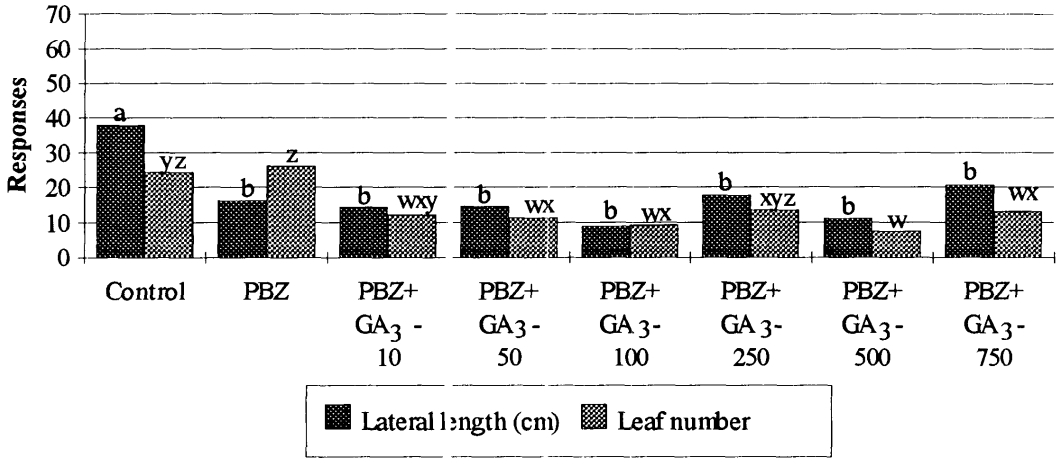


Figure 4.4 PBZ and low GA_3 concentrations on lateral shoot growth

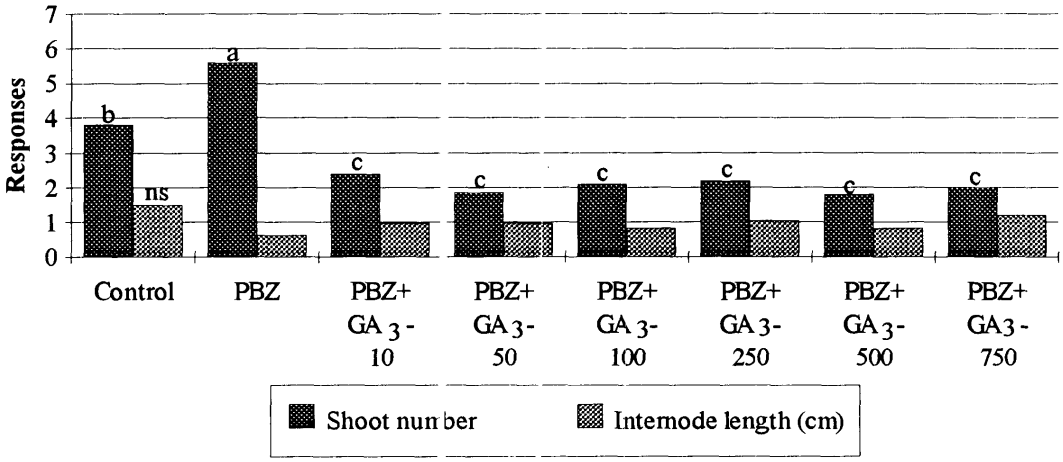


Figure 4.5 PBZ and high GA_3 concentrations on lateral shoot growth

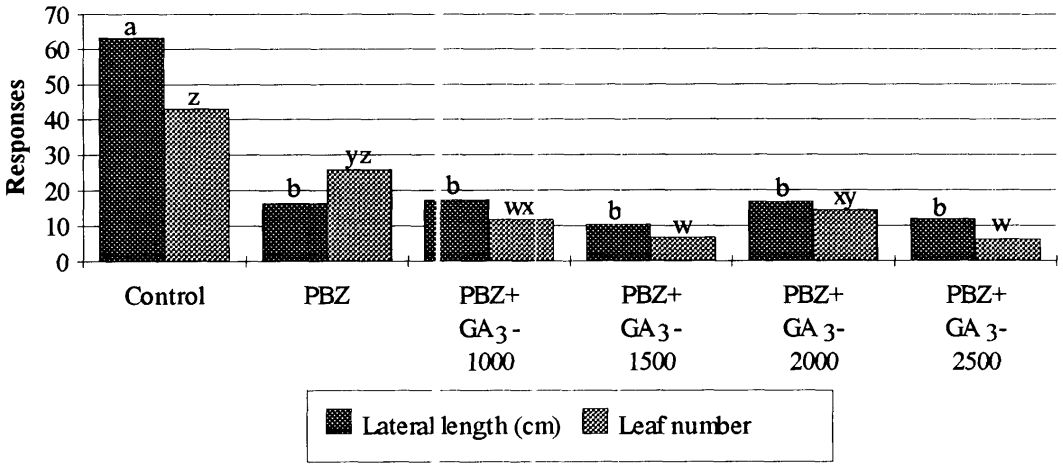
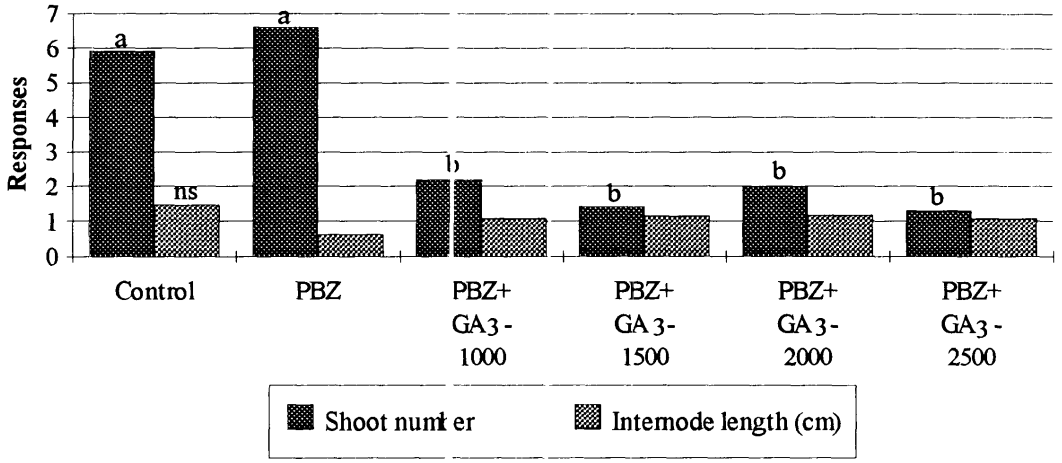


Figure 4. 6 PBZ and high GA_3 concentrations on lateral shoot growth



Main shoot growth: In both experiments, PBZ alone reduced plant height, internode length and leaf number. When PBZ was applied together with GA_3 it reversed the effect of PBZ and stimulated main shoot height and internode length (except with GA_3 10ppm) over the control (Figure 4.1 and 4.2).

Lateral shoot growth: PBZ alone increased shoot number in both the experiments. All PBZ + GA_3 treated plants produced significantly lower numbers of lateral shoots compared to PBZ alone or untreated control plants (Figure 4.4 and 4.6). The lateral length was significantly less than the control for PBZ alone or for all of the GA_3 + PBZ treated plants (Figure 4.3 and 4.5). The total lateral leaf number was similar for PBZ only treated plants with the control plants but addition of GA_3 reduced that number (Figure 4.3 and 4.5).

General observation: About 8 weeks after PGRs application, all GA_3 + PBZ treated plants in experiment #1 had an umbrella like appearance at the top of the main shoot and at the terminals of the laterals. A similar appearance occurred but was delayed in experiment #2.

4.2.4 Discussion:

Toxicity: GA_3 2500ppm was too high and toxic for SDP shoot growth. Toxic plants were also reported earlier (Barth, 1989) but with lower concentrations (100-250ppm) of GA_3 or GA_{4+7}.

Main shoot growth: The simultaneous application of GA_3 (10ppm) was enough to overcome the inhibitory effect of 10 mg a.i./plant of PBZ on the main shoot but was not enough to stimulate main shoot growth relative to the control (Figure 4.1). GA_3 concentrations more than 10ppm stimulated main shoot growth when applied simultaneously with PBZ (Figure 4.1 and 4.2). Horrell *et al.*, (1990) also stimulated growth of *Pennantia corymbosa* by applying 0.1 mg/plant GA_3 on a weekly basis for 4 weeks through a wick along with 1.25 mg/plant PBZ applied daily as a drench for 20 days. This result suggests that the effect of PBZ could be reversed and growth stimulated in 2 different ways. It could be by applying higher GA_3 concentration once at the same time of the PBZ application (in this

experiment) or by providing small quantities of GA_3 on a regular basis (Horrell *et al.*, 1990). Higher concentration of GA can stimulate growth by prolonging its effect (Goodwin, 1978) and lower concentrations with a regular supply, on the other hand, can stimulate growth by being available regularly.

It is clear from the whole discussion that the exact amount of GA_3 required for the reversal of PBZ in main shoot is very small and the excess GA_3 applied might stimulate growth.

In this experiment PBZ reduced leaf number in main shoot and addition of GA_3 with PBZ reduced that inhibitory effect on leaf number and produced statistically similar number of leaves to that of control plants. It is revealed from this experiment that reversal of PBZ inhibition in main shoot was achieved by GA_3's effect on increased leaf number (i.e. internode number) compared to PBZ alone treated plants and increased elongation of the internodes compared to the control plants (Figure 4.1 and 4.2). In an earlier experiment GA_3 (alone) also increased both internode number and length (Table 3.1a). Similar increment of internode length and number was found in hybrids of *Leucospermum concarpodendron* X *L. cordifolium* (Napier *et al.*, 1986) and in *Clerondendrum thomsonae* (Hildrum, 1973).

Lateral shoot growth: Shoot numbers were increased by PBZ application in both the experiments. Increased number of runners in SDP were also found by Jusaitis and Schmerl (1993) following PBZ drench. However, bud development in 'Tifblue' rabbiteye blueberry was reduced with increased levels of soil applied PBZ (Spiers, 1988). In strawberry both the formation of runners and development of daughter plants were significantly reduced by PBZ (Nishizawa, 1993). In this present experiment, PBZ alone or in combination with GA_3 reduced lateral length significantly compared to the control plants (Figures 4.3, and 4.5). In PBZ treated plants this was due to less elongation of the released shoots but in PBZ + GA_3 treated plants, this was achieved by less elongation and fewer shoots. The lateral internode length for PBZ alone or with GA_3 although insignificant, had a trend towards reduction compared to the controls in both experiments (Figure 4.4 and 4.6). Stewart (1991) also found a reduction in lateral shoot growth with PBZ application to different Australian native plants.

Higher GA_3 concentrations (experiment #2) were also unable to reverse PBZ inhibition of lateral shoot growth. The reason for this inability of GA_3 concentrations to reverse PBZ induced inhibition could be due to any of the following factors: a) insufficient GA relative to the level of PBZ, b) hormonal imbalance imposed by PBZ (by affecting the level of auxin, cytokinin or their combinations with GA_3), and c) improper timing of GA_3 application.

GA_3 concentrations can not be increased any more because GA_3 2500ppm caused toxicity on plants but the effect of different ratios of PBZ and GA_3 could be tested for reversing PBZ induced lateral shoot growth inhibition (Section 4.4). Factors other than decreased GA biosynthesis can cause dwarfism in response to growth retardants and there are species in which GA does not completely relieve the inhibition (Graebe and Roppers, 1978; Davis and Curry, 1991). So the addition of plant hormones (auxins, cytokinins alone or in combinations with GA_3) might reverse this inhibitory effect (Section 4.5). Again there are

certain reports where complete utilizat on of GA_3 was observed on plants already inhibited by PBZ by delaying GA_3 application bu: not if applied at the same time of PBZ application. Probably a part of the GA_3 might have been metabolised before the establishment of inhibition (Steffens, *et. el.* 1985). Delaying GA_3 application could also allow the plants to be released from apical dominance through ageing otherwise simultaneous application could reinforce that process (Section 2.1.2.1). This hypothesis is tested in section 4.7.

General observation: After 8 weeks, GA_3 was probably metabolized by microbial degradation in potting medium or conjugated to an inactive form in the plants (Section 2.2.3.3) and this may be why the effec: of PBZ was evident again. The delayed appearance of umbrella like growth in experiment #2 might mean that the effect of higher concentrations of GA_3 lasted longer. The umbrella like appearance was also found in *Vicia faba* when Huang *et al.*, (1989) applied daminozide along with GA. The effect of PBZ on apple seedlings was reversed by GA_3 for 20 days before he growth was inhibited again (Steffens *et al.*, 1985). The long term effect of PBZ was mos: probably due to its retention in the shoot apex and its spreading in the vascular system below the growing points (Lever, 1986).

4.2.5 Conclusions:

* GA_3 reversed the effect of PBZ on the main shoot but not on the laterals.
* The reversal of PBZ inhibition of la:eral shoot growth probably depends on the PBZ : GA ratio, the inclusion of other PGRs or th e time of GAs application.

4.3 Interaction of PBZ and GA_3 on different growth parameters.

4.3.1 Introduction:

This experiment repeats sorne of the important treatments from the previous experiment (Section 4.2) but with the objective of determining the effects on additional growth parameters.

4.3.2 Materials and Methods:

Four (4) treatments (control, GA_3 500ppm, PBZ 10 mg a.i./plant and GA_3 1000ppm + PBZ 10 mg a.i./plant) along with 10 replications were laid out in a randomised complete block design.

The seeds were sown on 16.02.94. Vegetative data were recorded at 4 weeks after PGR application and the flowering was also recorded whenever visible. Five replications were used for leaf data recorded at the flowering stage of the plant (leaf area, leaf length and leaf-let numbers) and each of the 5 replications were the average of the 5 largest expanded leaves per plant sampled from the main shoot only. Leaf area was recorded using a leaf area meter (Paton electronic planimeter). Five replications were also used for measuring stem diameter (mature internodes) both from main and lateral shoots, with a slide calliper at the flowering stage of the plant. The shoot fresh weight was measured immediately after harvest. Roots were washed in water and air dried for 48 hr to ascertain root fresh weight. To ascertain dry weight, both

roots and shoots were oven dried (48 hr at 80 C). Shoot to root ratio was calculated by dividing the dry weight of the shoot by the dry weight of the root. Anatomy of the treated plants was also studied and is presented in section 7.2. The rest of the procedures were followed as per section 3.2.

4.3.3 Results:

Main shoot growth: PBZ (alone) reduced plant height, leaf number and internode length while GA_3 (alone) had the opposite effect and increased these parameters. When GA_3 was added together with PBZ, plant height was stimulated as compared to PBZ and control plants but reduced as compared to GA_3 treatment alone (Figure 4.7 and Plate 4.1).

Leaf growth (main shoot): The leaf number was reduced slightly by PBZ (alone) but increased slightly by GA_3 (alone) or in combination with PBZ as compared to control plants (Figure 4.7). PBZ alone significantly reduced the leaf area and leaf length but had no effect on leaf-let number (Figure 4.8; 4.9 and Plate 4.2a). Except a significant reduction in leaf length compared to GA_3 alone, the PBZ + GA_3 combinations increased leaf area and leaf-let number compared to PBZ or control plants but were similar to that of GA_3 alone treated plants.

Figure 4.7 PBZ and GA_3 on main shoot growth

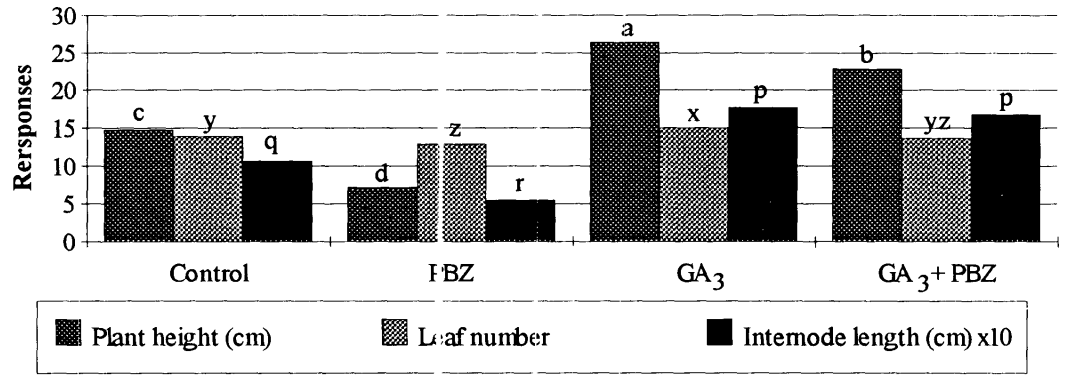


Figure 4.8 PBZ and GA_3 on main shoot leaf area

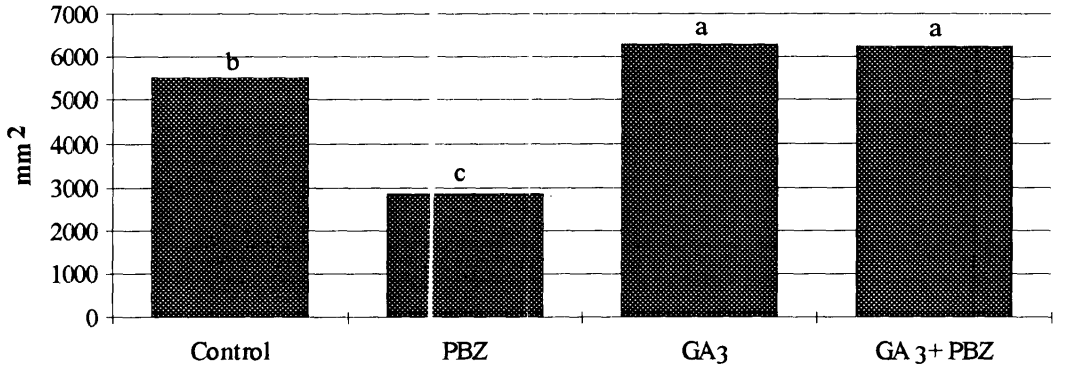


Figure 4. 9 PBZ and GA 3 on main shoot leaf length and leaf-let number

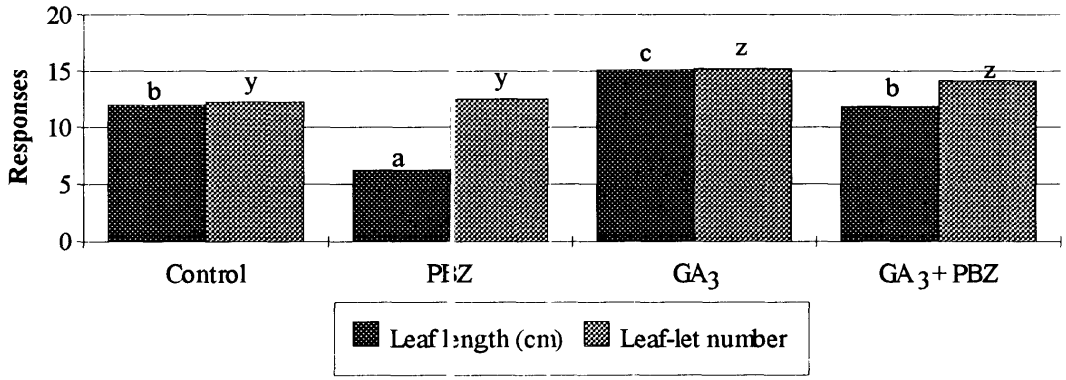


Figure 4. 10 PBZ and GA3 on stem diameter

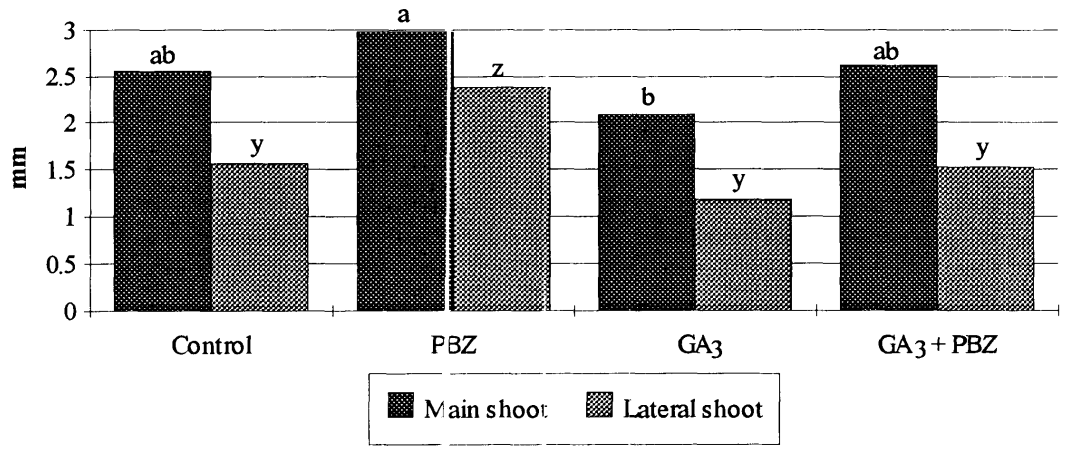


Figure 4. 11 PBZ and GA3 on lateral shoot growth

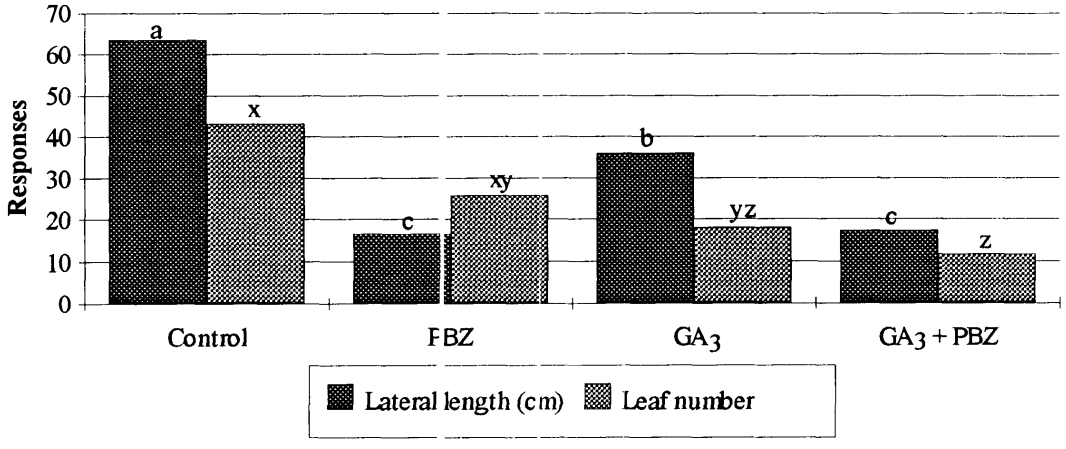


Figure 4. 12 PBZ and GA_3 on lateral shoot growth

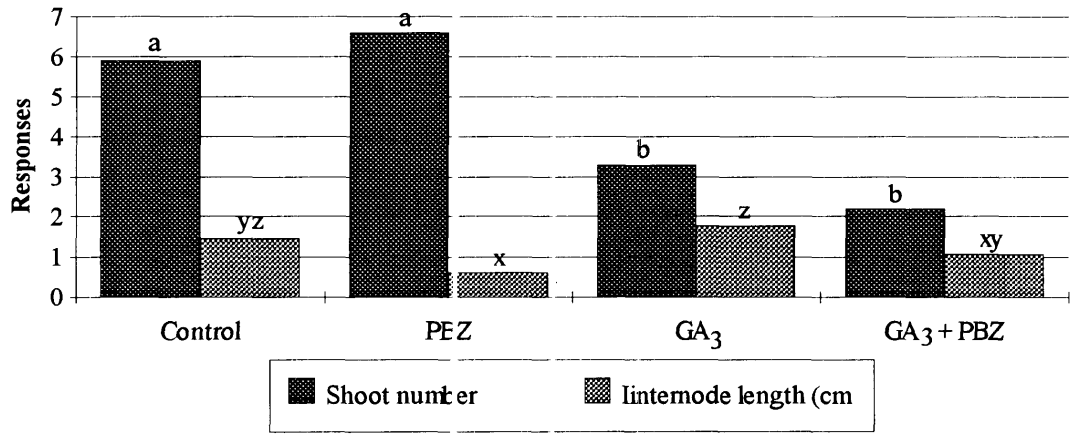


Figure 4. 13 PBZ and GA_3 on total shoot growth

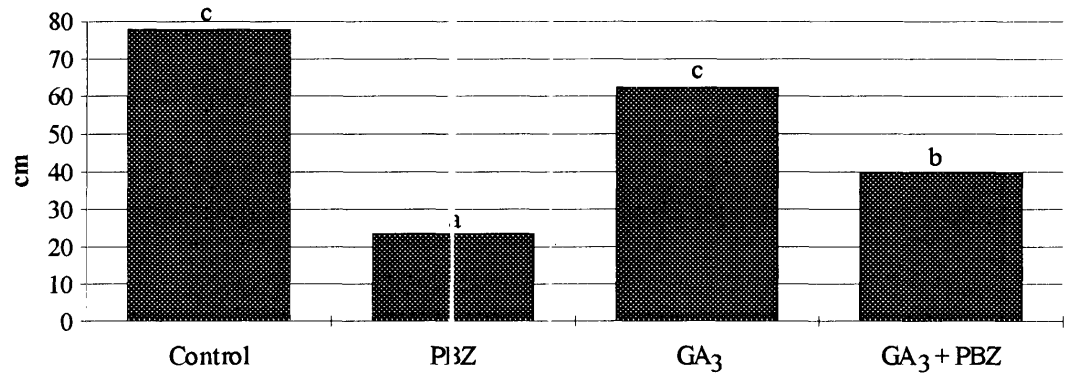
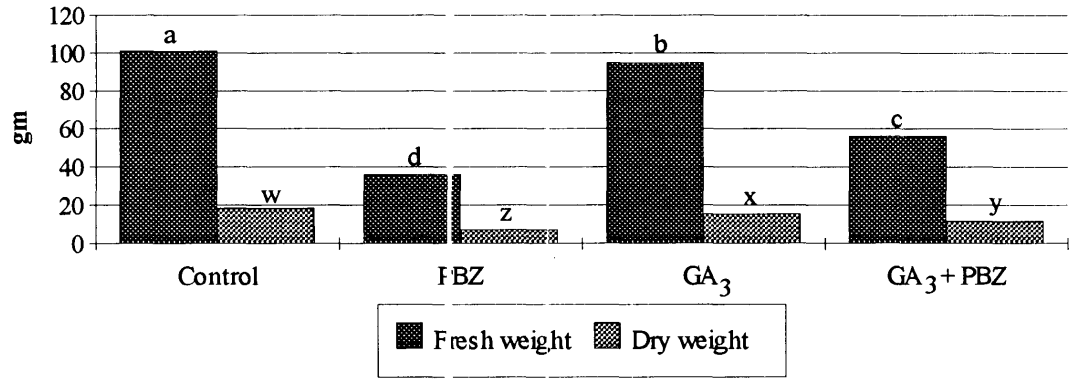


Figure 4. 14 PBZ and GA_3 on shoot weight



Stem diameter: PBZ alone slightly increased the diameter of the main shoot compared to control and PBZ + GA_3 treated plants but significantly increased the diameter of the lateral shoots compared to the rest of the treatments (Figure 4.10). Added GA_3 along with PBZ had no effect on stem diameter.

Figure 4.15 PBZ and GA_3 on root weight

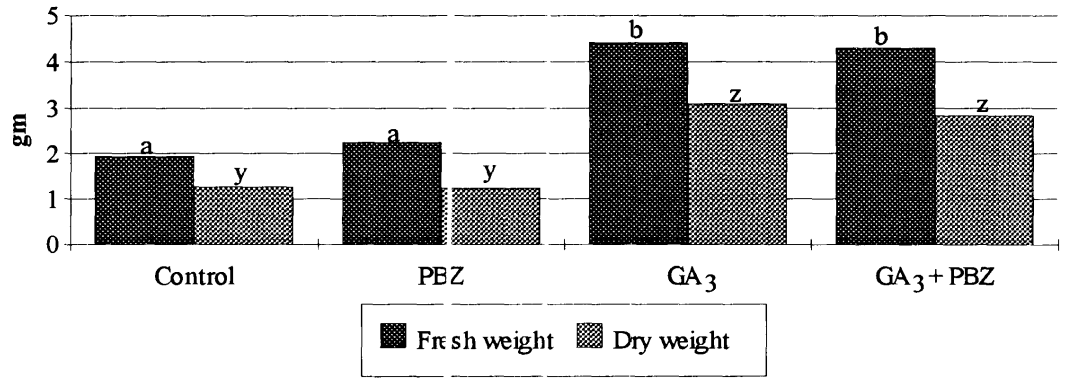


Figure 4.16 PBZ and GA_3 on total dry weight and shoot : root ratio

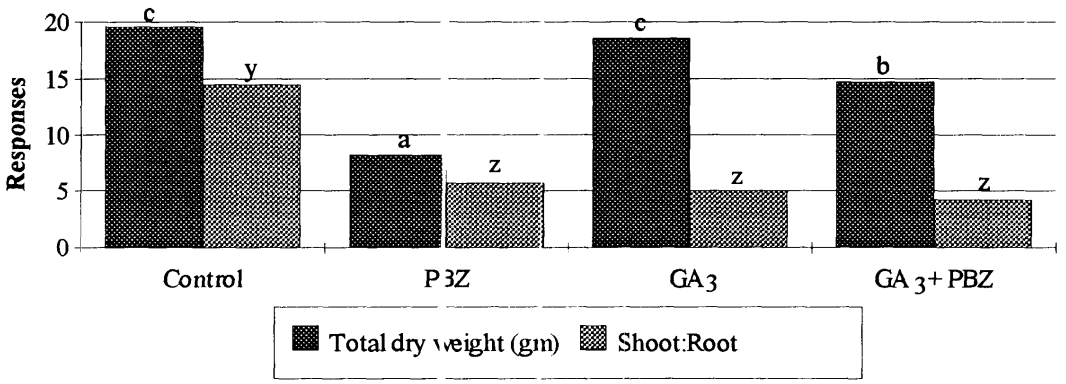
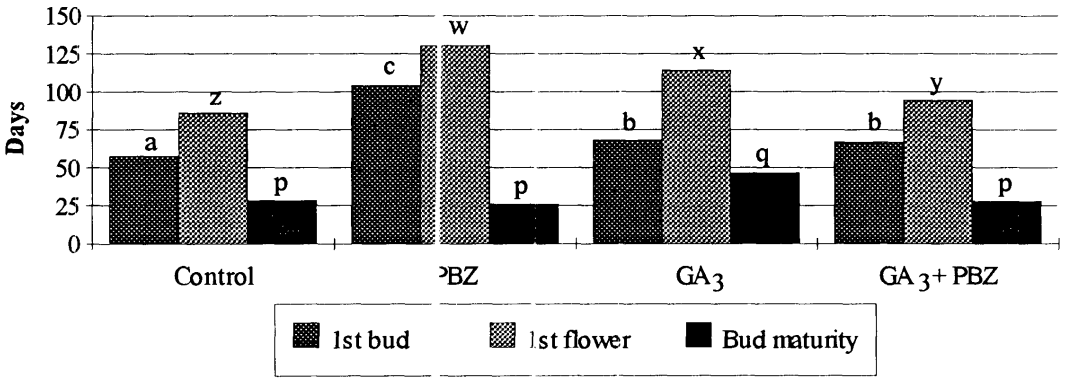


Figure 4.17 PBZ and GA_3 on flowering



Lateral shoot growth: Shoot number was increased but lateral length and internode length were reduced on plants treated with PBZ alone. Shoot number and the lateral length were reduced following GA_3 and (±) PBZ application (Figure 4.11 and 4.12).

Total shoot growth: PBZ reduced total shoot growth, added GA_3 increased that growth but was still less than GA_3 alone or control plants (Figure 4.13).

Fresh & dry weight: Untreated control plants had the highest shoot fresh and dry weight. PBZ (alone) had the lowest shoot fresh and dry weight (Figure 4.14). GA_3 reversed a part of PBZ's effect on shoot weight. PBZ had no effect on root weight but GA_3 alone or with PBZ increased root weight (Figure 4.15).

Shoot to root ratio: In control plants, the shoot : root ratio was about 14 but for the rest of the treatments, the ratio was about 5 (Figure 4.16). The root characteristics following PGRs application are illustrated in plate 4.2b.

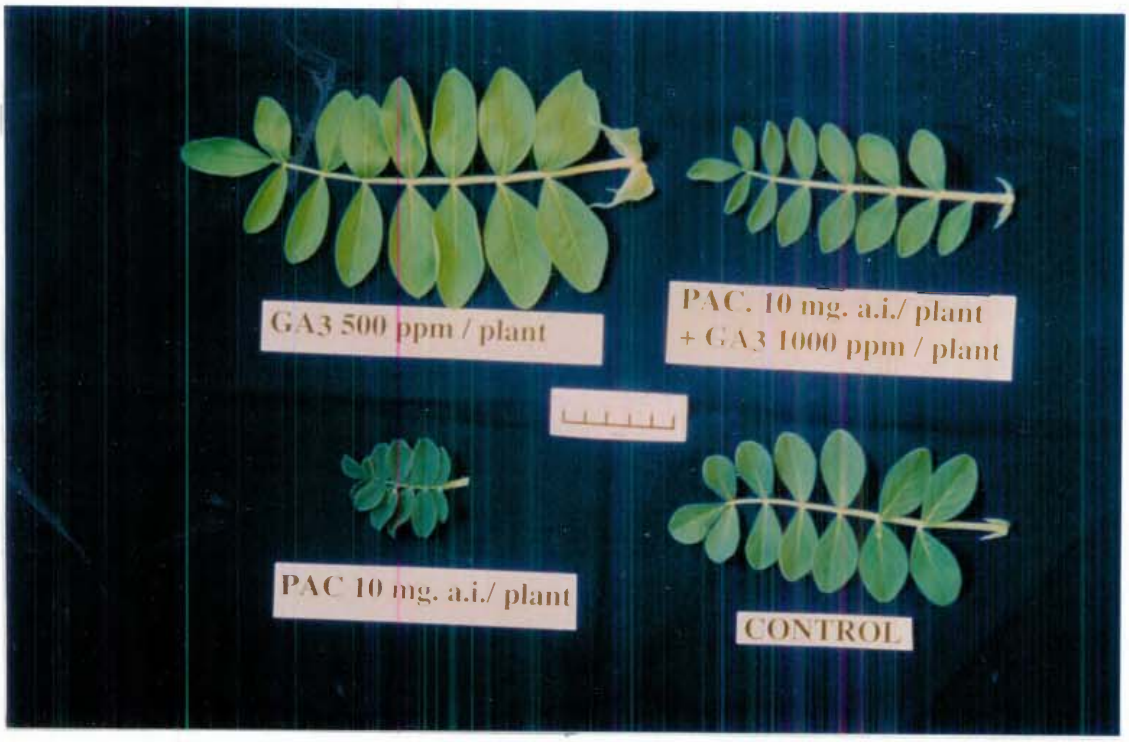
Flowering: Any PGR application (including PBZ) delayed the appearance of the 1st flowering bud and the 1st flower (Figure 4.17); PBZ or GA_3 applied alone took 130 and 104 days respectively compared to 86 days for the control to produce the first flower. However GA_3 reduced the delaying effect of PBZ on the appearance of the 1st flower to about 95 days.

4.3.4 Discussion:

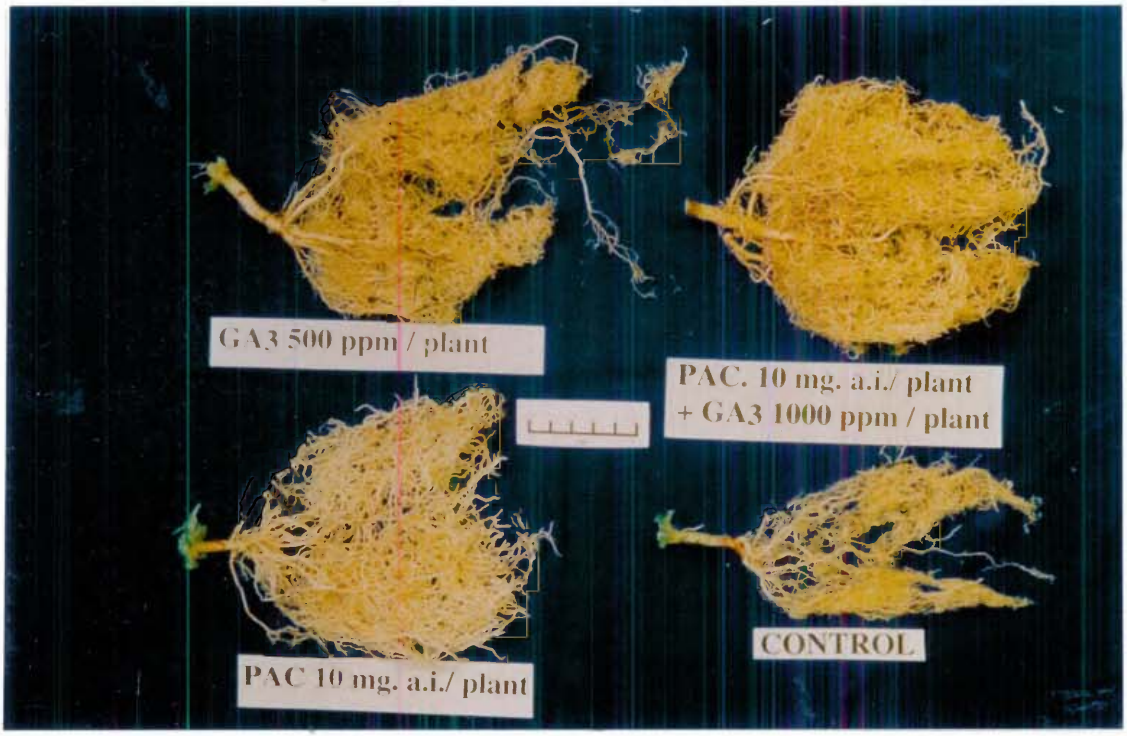
Main shoot growth: GA_3 reversed PBZ inhibition of main shoot growth in this experiment. The dwarfing effect of PBZ was also completely reversed in poinsettia by GA_3 application (Cox, 1993). However, PBZ reduced a part of the GA_3's promotory growth of main shoot when both applied together suggesting that GA_3 was used up to neutralise PBZ's inhibition.

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

Plate 4.1 Effect of different PGRs on SDP shoot growth: (a) GA_3 500ppm; (b) Control; (c) PBZ (PAC) 10 mg a.i./plant; and (d) PBZ (PAC) 10 mg a.i./plant + GA_3 500ppm.



(a)



(b)

Plate 4.2 Effect of PGRs on leaf (a) and root (b) characteristics: GA_3 500ppm; PBZ (PAC) 10 mg a.i./plant; and PBZ (PAC) 10 mg a.i./plant + GA_3 500ppm.

Leaf growth: PBZ alone reduced leaf area and leaf length but had no effect on leaf let numbers. This inhibition was reversed by adding GA_3. The leaf data for the lateral shoots were not recorded but from visual observation it was also found that the PBZ treated lateral shoots had shorter leaves along with reduced leaf area. The reason for reduced growth of PBZ treated plants might be reduced photosynthetic area (shorter leaves). Steffens and Wang (1986) reported similar results, where PBZ was more effective for inhibiting leaf area expansion than for reducing leaf number in apple seedlings. The present result was also similar to the results of Numbere *et al.*, (199?) where PBZ reduced or inhibited leaf expansion. They also proposed that reduced leaf area might reduce photosynthetic capacity of the plant. On the contrary, there are numerous reports of GA (alone) stimulated leaf enlargement and elongation (Goodwin, 1978) but GA_3 (alone) had no effect on 6 cyclamen cultivars for leaf size, leaf count, and for plant size (Widmer *et al.*, 1974).

Stem diameter: The increased stem diameter of the main shoot and lateral shoots following PBZ treatment could also be due to PBZ's inhibition of GA biosynthesis, because GA normally increases longitudinal expansion of the stem cells with a corresponding reduction in cell diameter thereby reducing the diameter of the stem (Shibaoka, 1993). However added GA_3 had no effect on the stem diameter in this experiment and in an earlier experiment (Section 3.7.4) but it was reduced in other experiment (Section 4.5.4). This could be explained on the basis of the difference in the growing environment. Shoot diameter was unaffected by GA_3 applications in hybrids of *Leucospermum concarpodendron* X *L. cordifolium* (Napier *et al.*, 1986). But Taiz and Zeiger (1991) reported decreased stem thickness with GA_3 for many plants.

Lateral shoot growth: Reduced elongation in PBZ treated lateral shoots was because of the reduced total elongation of the released shoots only as PBZ increased shoot numbers. But the reduced lateral length in a GA_3 + PBZ treated plant was due to fewer shoots along with reduced elongation. GA_3 (250ppm) has also previously been found to reduce the number of lateral shoots in SDP (Barth, 1990) but GA_3 (25, 50 or 100ppm) did not alter shoot number in *Zantedeschia* (Funnell *et al*, 1992).

Total shoot growth: Control plants had the greatest total shoot growth because of greater lateral growth. PBZ had the lowest shoot growth because of the low main and lateral shoot growth. GA_3 gave less (but insignificant) total shoot growth than control plants (Figure 4.13). The large increase in main shoot growth by GA_3 was accompanied by an even greater reduction in lateral shoot growth giving a net decrease in total shoot growth.

Fresh & dry weight: PBZ reduced shoot weight but had no effect on root weight (Figure 4.14 and 4.15). Rietveld (1989) reported that foliar sprays might increase root growth, and exposure of the roots to high concentration of PBZ might reduce both root and shoot growth. In *Bouvardia humboldtii* PBZ sprays were more inhibitory for total shoot growth compared to drench (Wilkinson and Richards, 1987). In this present experiment both spraying and drenching were used and GA_3 (±) PBZ enhanced root weight but reduced the shoot weight. Reduced shoot weight could be attributed to the reduced lateral shoot growth

following GA_3 (± PBZ) application (Figure 4.11 and 4.12). In *Chrysanthemum* the fresh and dry weights of foliage and roots were increased by GA_3 (Dahab *et al.*, 1987) but in hybrids of *Leucospermum concarpodendron* X *L. cordifolium,* GA_3 application increased shoot dry weight only (Napier, *et al.* 1986). These differences could be due to differences in the species and/or method and time of PGR application.

Shoot to root ratio: The results suggest that PGR applications either reduced shoot weight (e.g. PBZ + GA_3) or increased root weight (e.g. GA_3) and thereby reduced the shoot to root ratio (Figure 4.14 and 4.15). Reduced shoot to root ratio was also reported earlier with PBZ application in peach and nectarine plants grown in hydroponic system (Avidan and Erez, 1995).

Flowering: In this present experiment, either PBZ or GA_3 delayed the time to produce the 1st flower. But GA_3 + PBZ had a synergistic effect and advanced flowering compared to either PBZ or GA_3. One explanation for this could be that, when PBZ + GA_3 were applied together a part of the GA_3 is neutralized to reverse PBZ's effect and the rest of the GA_3 might offer a suitable hormonal balance with the endogenous hormones or even supplied from PBZ (e.g. auxin, as proposed by Sebanek *et al.*, 1991) in favour of flowering.

Jusaitis and Schmerl (1993) found no significant response of days to flowering of SDP with PBZ. Stewart (1991) also found no response to PBZ in flowering of some Australian native plants. But in geranium, PBZ caused earlier flowering (Cox, 1991). The effects of GA_3 on flowering are also variable. Wilfert and Raulston (1975) found early flowering of statice with GA_3 but Griffin *et al.*, (1993) reported that GAs generally inhibit flowering in woody angiosperms. Jusaitis and Schmerl (1993) found no response to GA in flowering of SDP. Cox (1991) delayed flowering in geraniums by applying PBZ and GA on the same day but no delay was reported when GA_3 was applied 14 or 21 days after PBZ application.

4.3.5 Conclusions:

* PBZ and GA_3 had opposite modes of action on SDP growth.
* Reduction in growth by PBZ could at least partly be due to reduced photosynthetic area.
* Reduced lateral growth due to PBZ is due to reduced elongation of the released shoots only but for GA_3 + PBZ it was due to the reduction of both shoot numbers and their elongation.
* PBZ + GA_3 had a synergistic promoting effect on flowering.

4.4 Interactions of different ratios of PBZ and GA_3.

4.4.1 Introduction:

It was hypothesised (Section 4.2.4) that changing concentration ratios of PBZ : GA_3 might reverse PBZ inhibition of a lateral shoot growth. To test this hypothesis an experiment was initiated with different concentrations of PBZ and GA_3 to study their interactions on main and lateral shoots and also to reverse PBZ induced lateral shoot growth inhibition.

4.4.2 Materials and Methods

Sixteen (16) treatments were used in this present experiment. The treatments were control, PBZ (1, 5 and 10 mg a../plant), GA_3 (100, 500 and 1000ppm) and their combinations. The treatments were arranged in a randomised complete block design with 10 replications.

The seeds were sown on 22.08.94 and the data were recorded 4 weeks after PGR application. The rest of the procedures were followed as per section 3.2.

4.4.3 Results:

Main shoot growth: All concentrations of PBZ alone reduced plant height and with the highest concentration (10 mg a.i./plant) producing the smallest plants with shortest internodes compared to the rest of the treatments (Table 4.1). PBZ had no effect on leaf number.

GA_3 (alone) increased plant height, leaf number and internode length with increasing concentration.

GA_3 reversed the effect of PBZ and produced a net increase in growth but still had lesser growth compared to GA_3 alone (except with PBZ 1 mg a.i./plant).

Lateral shoot growth: All concentrations of PBZ reduced the lateral shoot length and internode length but the reduction was only statistically significant at 10 mg a.i./plant. Lateral shoot number and leaf number were unaffected following PBZ application. All concentrations of GA_3 alone or in combination with PBZ produced fewer lateral shoots. With the exception of 100ppm GA_3 (alone), all GA_3 concentrations alone or in combination with PBZ also produced lesser lateral length than the control and PBZ (alone) treated plants (Table 4.2).

Lateral shoot angle: This data was not analysed because there were insufficient elongated lateral shoots in some of the treatments. Presented data are the means of all available data. PBZ (alone) had no effect on branch angles but GA_3 alone and in combination with PBZ reduced them (Table 4.2).

4.4.4 Discussion:

Main shoot growth: The inhibition of SDP shoot growth by PBZ was concentration dependent. Similar concentration dependent responses for different crops with PBZ were also reported elsewhere (Davis and Curry 1991). For example in different bedding plants (*Salvia splendens, Impatiens wallerana, Tagetes erecta, Petunia hybrida*) PBZ spray @ 10 to 160 mg/L reduced the plant size for all species compared with untreated control plants and the reduction was more with higher concentrations (Barrett and Nell, 1992). Similarly 0, 0.5, 1.0, 2.0 and 4.0 mg a.i./pot PBZ were applied as a drench or spray for the height control of marigold and the height retardation increased linearly with the increasing PBZ concentrations (Keever and Cox, 1989).

GA_3 also caused a concentration dependent effect on main shoot and increased plant height. In geranium, stem elongation was correlated with the GA_3 concentrations (Larson, 1985).

Table 4.1 Effect of PBZ (a.i./plant), GA_3 (ppm) and their combinations on main shoot growth.

Treatments	Plant height (cm)	Leaf number	Internode length (cm)
Control	7.30c*	9.30a	0.79c
PBZ (1mg)	4.30b	9.30a	0.46b
PBZ (5mg)	4.50b	9.50a	0.47b
PBZ (10mg)	3.60a	9.20a	0.39a
GA_3 (100ppm)	14.45g	12.20fgh	1.19e
GA_3 (500ppm)	16.90h	12.20fgh	1.39g
GA_3 1000ppm	19.20i	12.40gh	1.55h
GA_3 (100ppm) + PBZ (1mg)	3.75fg	11.70def	1.18e
GA_3 (500ppm) + PBZ (1mg)	15.60h	11.80efg	1.33fg
GA_3 (1000ppm) + PBZ (1mg)	19.00i	12.60h	1.51h
GA_3 (100ppm) + PBZ (5mg)	9.55d	10.30b	0.93d
GA_3 (500ppm) + PBZ (5mg)	13.90g	11.30cde	1.23ef
GA_3 (1000ppm) + PBZ (5mg)	16.00h	11.80efg	1.36g
GA_3 (100ppm) + PBZ (10mg)	0.25de	11.20cde	0.92d
GA_3 (500ppm) + PBZ (10mg)	10.45e	11.10cd	0.94d
GA_3 (1000ppm) + PBZ (10mg)	12.80f	11.00c	1.17e
	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 4.2 Effect of PBZ (a.i./plant), GA_3 (ppm) and their combinations on lateral shoot growth.

Treatments	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle (°)
Control	4.80i *	19.25i	17.70de	1.09e	90.00
PBZ (1mg)	5.40e	13.90hi	19.40e	0.72abcde	90.00
PBZ (5mg)	5.70e	9.95fghi	16.70de	0.60abcd	90.00
PBZ (10mg)	5.20e	8.20efgh	16.30de	0.51abc	90.00
GA_3 (100ppm)	2.40b	10.55ghi	9.60cd	1.09e	64.37
GA_3 (500ppm)	1.60ab	7.40def	6.20ab	0.99cde	44.37
GA_3 1000ppm	1.80ab	7.25defg	6.70bc	1.03de	40.00
GA_3 (100ppm) + PBZ (1mg)	2.50b	4.10cde	5.50bc	0.75abcde	63.33
GA_3 (500ppm) + PBZ (1mg)	1.80ab	4.00bcd	3.60ab	1.01de	51.00
GA_3 (1000ppm) + PBZ (1mg)	1.40a	7.00bcd	4.80ab	1.02bcde	42.50
GA_3 (100ppm) + PBZ (5mg)	1.80ab	2.30abc	4.20ab	0.49ab	61.79
GA_3 (500ppm) + PBZ (5mg)	1.90ab	2.00ab	3.50ab	0.51abc	49.00
GA_3 (1000ppm) + PBZ (5mg)	1.80ab	2.15abc	3.60ab	0.54abc	50.00
GA_3 (100ppm) + PBZ (10mg)	2.00ab	1.90ab	3.20ab	0.54abc	57.50
GA_3 (500ppm) + PBZ (10mg)	1.80ab	1.00a	2.00a	0.45a	50.00
GA_3 (1000ppm) + PBZ (10mg)	2.00b	1.30a	2.80ab	0.47abc	52.00
	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.

The present results indicate that the main shoot growth inhibition caused by PBZ - 1 mg a.i./plant could be completely overcome by any of the GA_3 concentrations used. Although the promotory effect of all GA_3 concentrations was reduced (Table 4.1) when they were combined mainly with higher PBZ concentrations (5 and 10 mg a.i./plant), all PBZ + GA_3 combinations showed a stimulated growth compared to control plants. The reduction of this promotory effect of GA_3 when applied together with PBZ could be due to PBZ's inhibitory effect on endogenous GA synthesis (Davis and Curry, 1991). Horrel *et al.*, (1990) reported that PBZ caused almost complete stem inhibition in five native New Zealand species but joint application of PBZ and GA_3, depending on the species completely overrode that inhibition or caused stimulation compared to the controls.

Lateral shoot growth: All PBZ concentrations (alone) were inhibitory for the main shoot growth (Table 4.1) but not for the lateral shoot growth. A significant reduction in lateral length and internode length was only noticed by the highest PBZ concentration (10 mg a.i./plant) (Table 4.2). This result suggests that lateral shoots might have a higher supply or synthesis of GA. However, all GA_3 ± PBZ combinations again had an inhibitory effect (except GA_3 100ppm alone on lateral elongation) on lateral shoot growth (shoot number and lateral length) rather than increasing it. The inability of GA_3 to reverse lateral growth inhibition of PBZ was probably because of GA_3 reinforced apical dominance. Application of other PGRs (IAA, BAP etc.) or PGR application to a plant released from apical dominance might reverse PBZ inhibition of lateral shoot growth. The effect of different PGRs in reversing PBZ inhibited shoot growth is investigated in section 4.5.

Lateral shoot angle: The wider angles produced by control or PBZ treated plants could be due to the presence of auxin (endogenous or PBZ induced), because auxin favours wider angle formation (Janick, 1986). Addition of GA_3 to these plants reduces these angles probably because of the interaction of GA_3 and auxin (endogenous and PBZ induced) in favour of an apically dominant plant and also in favour of fewer dominant laterals which probably reduced branch angle. The role of auxin + GA_3 in apical dominance is reported in many plants (Section 2.1.2.1). Horrell *et al.*, (1990) also reported similar branch angle in juvenile *Carpodetus serratus* plants with GA_3 and its combination with PBZ.

4.4.5 Conclusions:

* Different ratios of PBZ : GA reversed the effect of PBZ on the main shoot but not on the lateral shoot.
* Higher PBZ concentrations (5 or 10 mg a.i./plant) prevented a part of GA_3's promotion of main shoot growth.
* Higher PBZ concentrations were required for lateral shoot growth inhibition probably because of higher GA synthesis or supply in the laterals.
* PBZ and GA caused a concentration dependent response in SDP.

4.5 Interaction of PBZ with GA_3, IAA and BAP.

4.5.1 Introduction:

GA_3 was unable to reverse PBZ inhibition of lateral shoot growth (Section 4.2; 4.3 and 4.4) probably because of GA_3's supportive role in apical dominance (Section 4.4.4). It was proposed earlier that apical dominance sometimes could be released by supplying different PGRs (e.g. auxins, cytokinins etc.) even if the apex is present (Phillips, 1971; Prasad and Cline, 1985). On the other hand, PBZ has been found to reduce the amount of endogenous hormones other than GA_3 alone (Graebe and Ropers, 1978; Santakumari and Fletcher, 1987; Davis and Curry, 1991). So, the supply of other PGRs (IAA, BAP etc.) might reinstate the hormonal balance which has been altered by PBZ and also could release apical dominance. GA_3 added with these treatments could reverse PBZ induced lateral shoot growth inhibition because of GA_3's action on the post re ease phase of bud growth (Khan, 1975).

Accordingly 2 different experiments were initiated with PBZ, GA_3, IAA and BAP alone or in combinations.

4.5.2 Materials and Methods:

Experiment #1: Including a control, 8 treatments were used in this experiment. The treatments were: PBZ (10 mg a. i./ plant), IAA (20 mg a.i./plant) and GA_3 (500ppm) applied alone or in combinations.

Experiment #2: Including a control, 10 treatments were used in this experiment. The treatments were: PBZ (10 mg a.i./plant), BAP (25ppm), IAA (20 mg a.i./plant) and GA_3 (500ppm) applied in different combinations.

Both the experiments were laid out separately in a randomised complete block design with 10 replications. The seeds were sown on 11.04.94 (experiment #1) and on 23.01.95 (experiment #2). Vegetative data were recorded 4 weeks after PGRs application. The rest of the practices were followed as per general methodology describe in section 3.2.

4.5.3 Results:

Epinasty: All IAA treated plants (alone or in combination) showed epinasty symptoms immediately after application but recovered about 3-5 days after application.

Main shoot growth: Experiment #1: PBZ alone reduced plant height and internode length. IAA alone increased plant height but when combined with PBZ, growth was reduced more than with PBZ alone (Figure 4.18). IAA + GA_3 gave the tallest plants but PBZ again reduced this effect and produced shorter plants compared to IAA + GA_3.

PBZ alone or with IAA did not affect leaf number but all other treatments increased leaf numbers.

All of the GA_3 treated plants produced slender stems.

Main shoot growth: Experiment #2: PBZ alone reduced plant height, internode length and leaf numbers. BAP alone increased these parameters. Addition of BAP alone or with IAA to PBZ produced dwarfed plants statistically similar to PBZ alone (Figure 4.19). BAP along

Figure 4. 18 IAA with other PGRs on main shoot growth

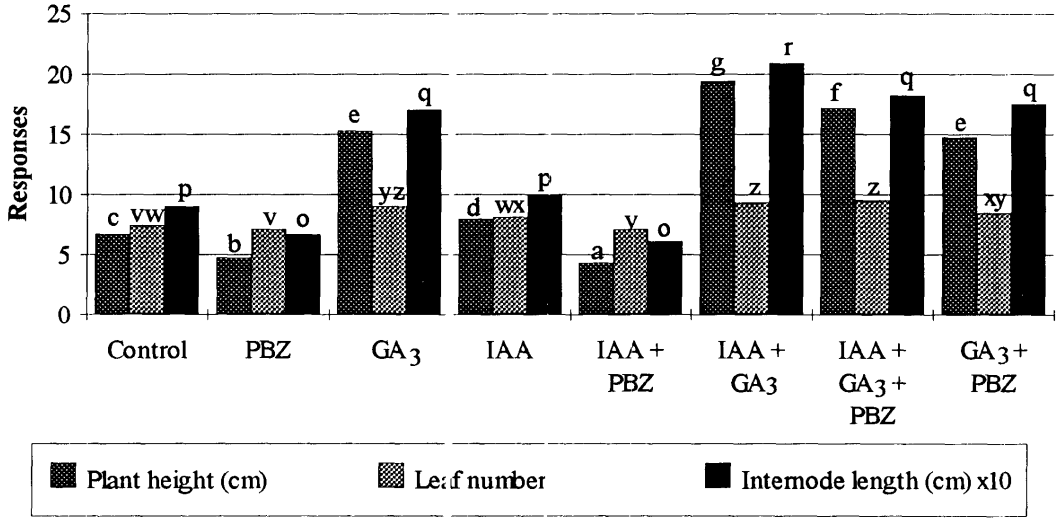
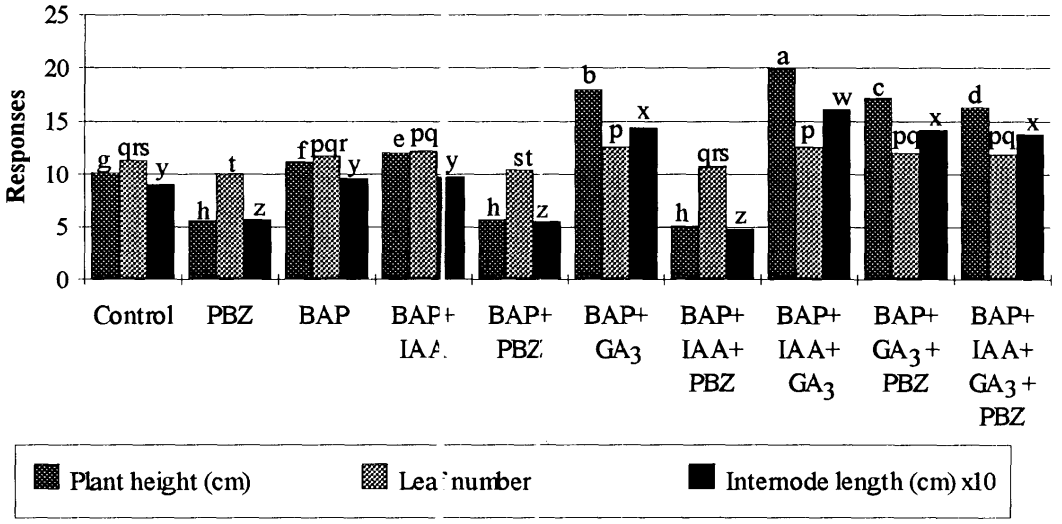


Figure 4. 19 BAP with other PGRs on main shoot growth



with GA_3 or GA_3 + IAA increased plant height, leaf numbers and internode length. Although PBZ reduced these promotory effects, the inhibitory effect of PBZ was completely reversed by these treatments and growth was stimulated as compared to control plants.

Lateral shoot growth: Experiment #1: The highest number of lateral shoots was produced by PBZ alone or its combination with IAA. Addition of GA_3 reduced that number to less than control plant (Figure 4.21). All treatments including PBZ and its combinations with IAA or GA_3 reduced lateral length (Figure 4.20).

Lateral shoot growth: Experiment #2: PBZ alone increased lateral shoot numbers but BAP alone reduced them. Addition of BAP or IAA + BAP to PBZ had no additional effect on shoot number (Figure 4.23). Addition of GA_3 on the other hand, to any of the treatment

Figure 4.20 IAA with other PGRs on lateral shoot growth

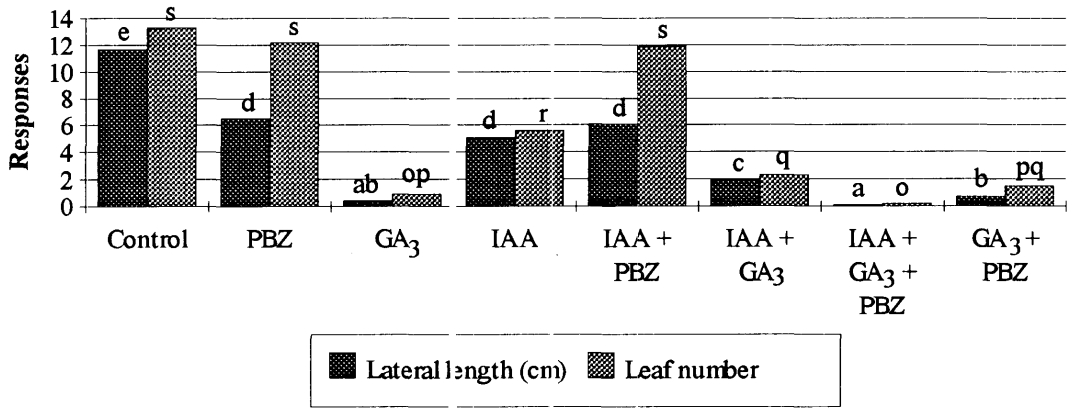


Figure 4.21 IAA with other PGRs on lateral shoot growth

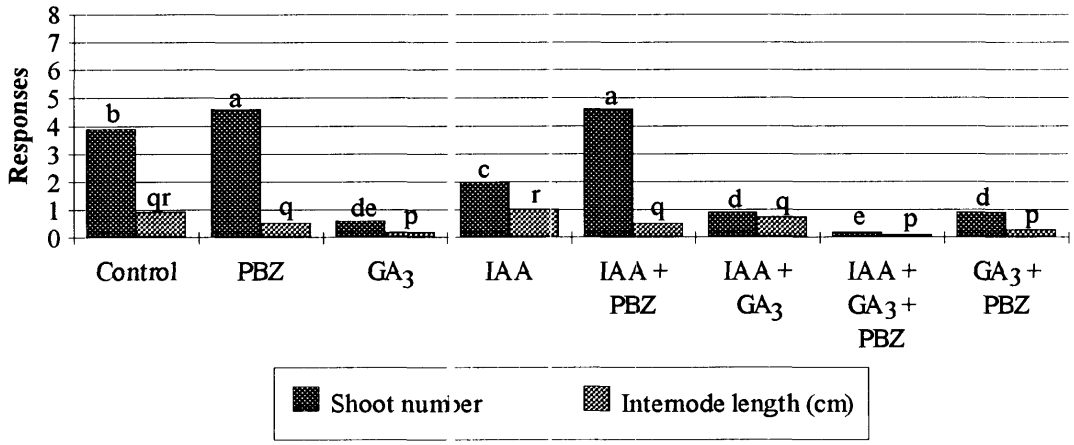


Figure 4.22 BAP with other PGRs on lateral shoot growth

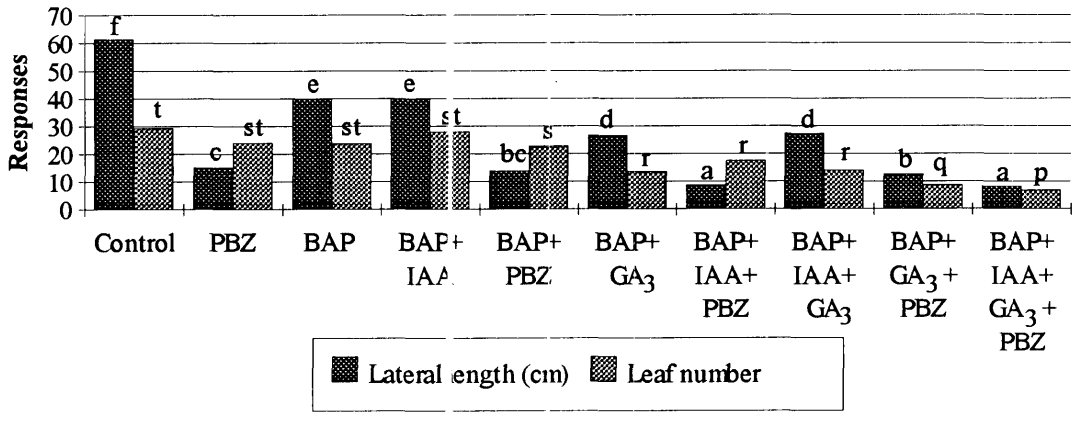


Figure 4. 23 BAP with other PGRs on lateral shoot growth

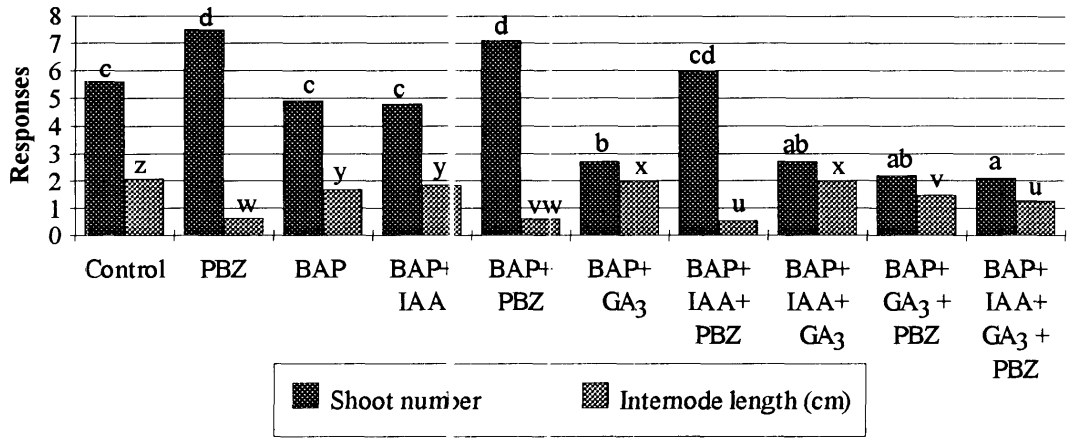
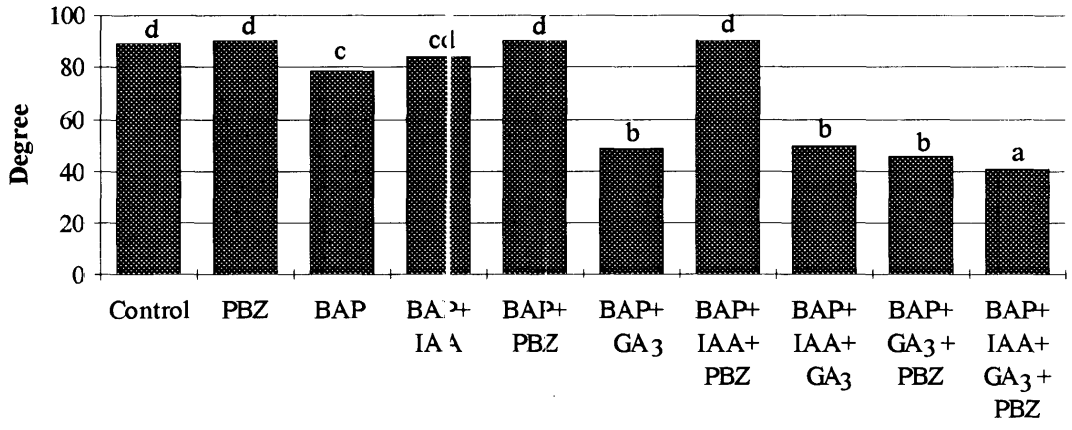


Figure 4. 24 BAP with other PGRs on angle formation



combinations, significantly reduced lateral shoot number. None of the added PGRs were able to reverse PBZ inhibition of lateral shoot elongation (Figure 4.22).

Lateral shoot angle: Experiment #2: PBZ alone or with any other combinations (except with GA_3) resulted in similar wider angles to that of the control. However, BAP alone produced an angle narrower than its combination with PBZ (Figure 4.24). GA_3 added to any of the PBZ combinations produced narrower angles.

4.5.4 Discussion:

Epinasty: Auxin (IAA) produced epinasty in this present experiment. Similar epinasty by auxin (NPA) was also reported by Jusaitis and Schmerl (1993) in SDP. In most plants, epinasty is common immediately after foliar application of auxin (Leopold, 1960).

Main shoot growth: Experiment #1: PBZ reduced main shoot height. The effect increased with the addition of IAA even though IAA alone increased main shoot height. The inhibitory effect of IAA in the presence of PBZ may be due to supra-optimal auxin supply or

availability. Increased auxin levels or activity have been reported following PBZ application to pear (Browning *et al.*, 1992a), *Ligustrum vulgare* (Sebanek *et al.*, 1991) and in melon cotyledons (Leshem *et al.*, 1994). The combination of PBZ induced auxin and endogenous IAA may lead to supra-optimal auxin levels. PBZ treated plants may also be more sensitive to increased auxin levels because of the reduced GA i.e. there may be an imbalance between auxin and GA.

Increased auxin along with reduced GA was also reported earlier in *Ligustrum vulgare* (Rauscherova and Tesfa, 1993). If this is true then added GA_3 should restore the balance and should reverse PBZ inhibition. In fact addition of GA_3 to PBZ + IAA gave taller plants than the control and PBZ + IAA treated plants. For shoot elongation a balance of IAA and GA was found very important (Section 2.2.5 1). In this experiment PBZ probably disrupted that balance by increasing auxin and reducing GA and ultimately caused a reduction in growth. However, it is not clear from this experiment if PBZ increases auxin and if so through what mechanism.

The combination of IAA + GA_3 produced taller plants because of a probable balanced supply of auxin and GA (both endogenous and exogenous) compared to counterparts without PBZ.

All GA_3 treated plants produced slender stems in this present experiment. This effect was not observed earlier (Section 3 7.4). This difference could be due to environmental differences between the two experiments. Weak stems were observed in *Boronia* by Lamont (1985) and by Day (1994).

Main shoot growth: Experiment #2: PBZ alone reduced plant height (Figure 4.19). BAP alone or with IAA was unable to reverse PBZ's inhibitory effect although BAP alone or with IAA increased main shoot height. However, addition of GA_3 to PBZ + BAP or PBZ + BAP + IAA reversed PBZ's effect. These results suggest that presence of GA is essential to the reversal of PBZ induced inhibition. This idea is consistent with the prevailing idea of PBZ's inhibition of GA biosynthesis (Davis and Curry, 1991). Jusaitis and Schmerl (1993) found no interaction of PBZ and BAP on main and lateral shoot growth of SDP plants. But when PBZ was applied together with BAP and NAA the growth of the above ground parts were inhibited and root growth was promoted on regenerated rice plantlets (Zhao *et al.*, 1991).

Addition of GA_3 + BAP or GA_3 + BAP + IAA to PBZ stimulated plant height compared to control or PBZ (alone) treated plants but produced shorter plants compared to their counterparts without PBZ (Figure 4.19). Other than PBZ's effect on increased auxin level this could also be due to the lack of extra GA which would be produced by the plant if PBZ was not there. PBZ does not block the action of either existing GA or of exogenously applied GAs, it only inhibits GA bio-synthesis (Steffens and Wang, 1986).

In this present experiment main shoot leaf number (i.e. internode number) was reduced by PBZ (Figure 4.19). Hagiladi and Watab (1992) also reported a 10% reduction in leaf number in *Cordyline* with higher PBZ concentrations (1000ppm). The results from the leaf data of the preceding experiments (Section 4.2, 4.3, 4.4 and 4.5) revealed that the reduction in

plant height and lateral shoot elongation by PBZ was achieved by 2 different ways. PBZ reduced both internode number (i.e. leaf number) and internode length (Figure 4.2, 4.5 & 4.6, 4.7, 4.11 & 4.12, 4.19, 4.22 & 4.23) or only reduced internode lengths (Table 4.1 & 4.2; Figure 4.18, 4.20 & 4.21). GA_3 by itself, on the other hand always increased internode number (i.e. leaf number) in the main shoot and reduced it in the lateral shoot (Section 4.2, 4.3, 4.4, 4.5).

The reversal of PBZ inhibition of the main shoot by GA_3 in these earlier experiments was accomplished by increased internode number (i.e. leaf number) and internode length (Figure 4.18, Table 4.1) or simply by elongation of the internodes (Figure 4.7, 4.1 and 4.2). As GA_3 was unable to reverse PBZ's effect in lateral shoots, in those experiments, combination of PBZ + GA_3 always reduced leaf number and tended to reduce internode length (Figure 4.3-4.6, 4.11-4.12, 4.20-4.23, Table 4.2). The variable results of PBZ $\pm$ GA_3 could be due to the inherent variability of the SDP plants depending on the seed source (Section 2.3.2) and also due to the variability within the growing environment of these experiments. The dependence of the PGRs on the environment for their effectiveness is well documented (Menhenett, 1979; Tayama *et al.*, 1992).

Lateral shoot growth: Experiment #1: PBZ alone or with IAA increased the number of the shoots and reduced lateral length probably by inhibiting GA synthesis and by increased auxin availability. GA normally has an inhibitory effect on bud growth but promotory effect on the elongation of the released buds (Section 2.1.2.1). On the other hand, increased auxin level increased bud release (Prasad and Cline, 1985). However, the addition of GA_3 to all PBZ treated plants again reduced total lateral growth probably through the reinforcement of apical dominance.

Lateral shoot growth: Experiment #2: BAP or BAP + IAA were unable to reverse PBZ's effect on shoot number and PBZ again increased shoot number but elongation of these shoots was limited due to the absence of GA_3 (Figure 4.22 and 4.23). However, addition of GA_3 to these treatments produced apically dominant plants. It seems that in intact plants, the reversal of PBZ effects on lateral shoots would not be possible with any PGRs combinations because of the active apex. PGRs supply after release of apical dominance (by ageing or by other treatments e.g. gravity, decapitation etc.) might reverse PBZ induced lateral shoot growth inhibition.

Lateral shoot angle: Experiment #2: This result suggests that BAP is unable to reduce the angle formed by PBZ.

4.5.5 Conclusions:

* The growth retardation with PBZ might be due to increased auxin availability or supply and/or reduced GA synthesis in plants.
* IAA or BAP alone or together were unable to reverse PBZ's effect in main or lateral shoots.
* PGR applications increased apical dominance.

4.6 Effect of clinostat and GA_3 application.

4.6.1 Introduction:

It was hypothesised (Section 4.4.4) that application of PGRs after releasing apical dominance would reverse PBZ induced inhibition of lateral shoot growth. Gravity is believed to have a direct role in releasing apical dominance of plants (Section 2.1.3). For example placing *Pharbitis nil* plants horizontally or in an inverted position, increased lateral shoot growth (Prasad and Cline, 1987). Therefore it is probable that the treatments which reduce the effect of gravity would release apical dominance and subsequent application of PGRs would release PBZ induced lateral shoot inhibition.

An attempt was made to release apical dominance of SDP plants, by placing the plants horizontally (Section 5.2). Apical dominance was not released in horizontally grown plants and GA_3 simply reinforced the dominance. The continuous upward gravitropic curvature of the shoot tip due to strong gravimorphic sensitivity of SDP's main shoots might have prevented the release from apical dominance (Section 5.2.4).

In this present experiment a clinostat (Audus, 1967) was used in an attempt to neutralise the force of gravity on the plant. GA_3 was also applied in this present experiment to see if there was any interaction between gravity and GA in their effect on lateral shoot growth and elongation.

4.6.2 Materials and Methods:

Four (4) treatments were used in this experiment: plants grown on the clinostat, plants grown on the clinostat + GA_3 500ppm, plants grown on the glasshouse bench and plants grown on the glasshouse bench + GA_3 500ppm. The clinostat was also placed on the glass house bench. Three replications were used because of the space constrains on the clinostat.

The speed of the vertically rotating clinostat was 4 revolutions per minute. Pots were placed horizontally on the clinostat. The potting mixture used was 2 : 2 : 2 of vermiculite : perlite : peat. This lighter potting mixture (compared to section 3.2) was used to reduce the weight of the individual pots for easy rotation on the clinostat.

The seeds were sown on 25.05.94. Seven days old seedlings were transplanted directly into pots on the clinostat or on the glasshouse bench depending on the treatments. GA_3 was applied 4 weeks after sowing and the vegetative data were recorded 4 weeks after PGR application. The rest of the procedures were followed as per section 3.2.

4.6.3 Results:

Main shoot growth: Irrespective of the gravity treatment, the main shoots of GA_3 treated plants were longer, with longer internodes (Figure 4.25).

Lateral shoot growth: Lateral shoot numbers were not significantly different between the treatments but plants grown without GA_3 had higher means (Figure 4.27). The lateral length and leaf numbers were significantly higher for the untreated control plants compared to GA_3 treated plants (Figure 4.26).

Figure 4.25 GA_3 on clinostated main shoot

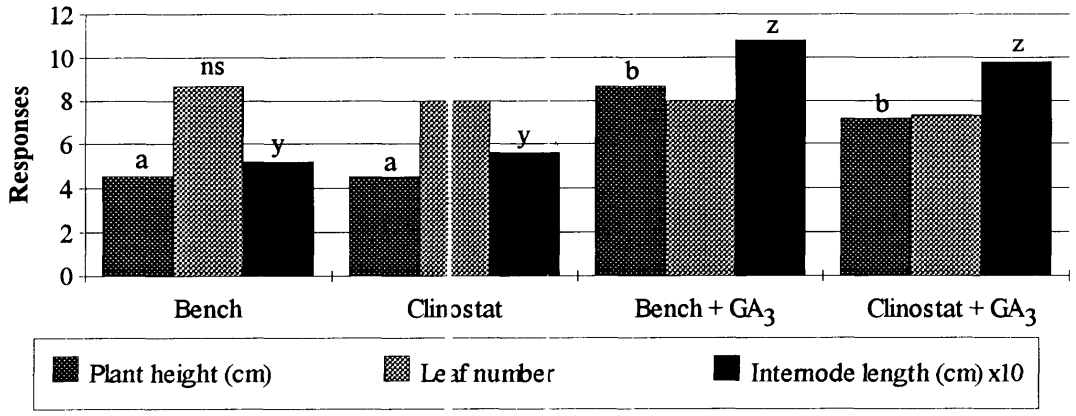


Figure 4.26 GA_3 on clinostated lateral shoot

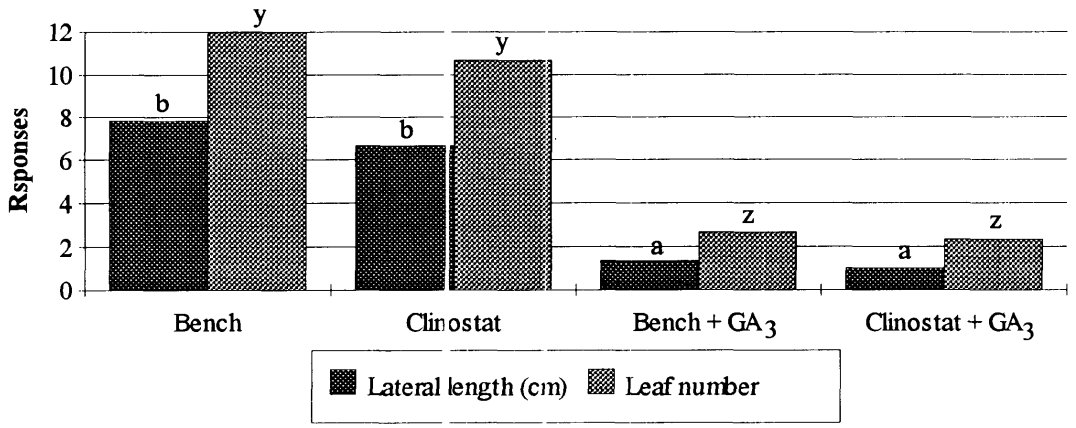
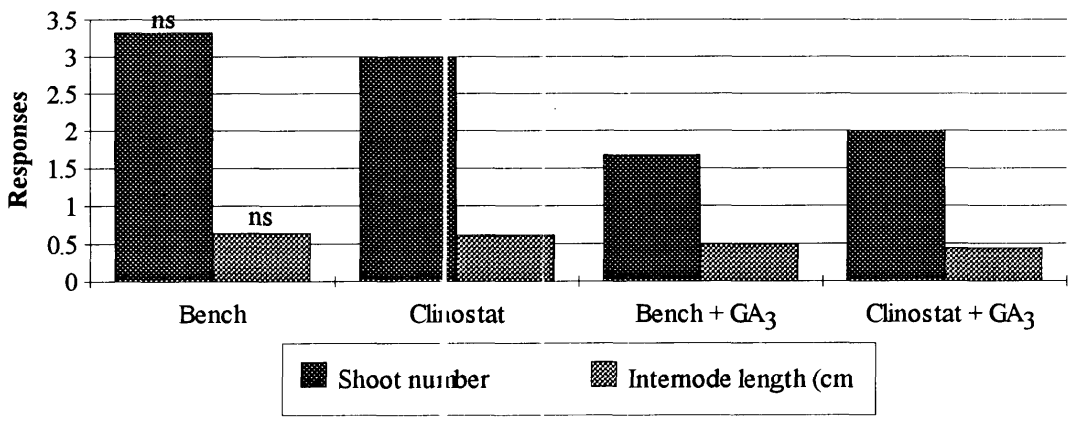


Figure 4.27 GA_3 on clinostated lateral shoot



4.6.4 Discussion:

Main shoot growth: This result suggests that the clinostat had no effect on SDP height and was unable to negate the gravity force and thereby unable to suppress apical dominance.

Irrespective of treatment combinations. GA_3 reinforced the process of apical dominance in this present experiment. However, seedlings of *Torenia* and tomatoes had reduced internodes and weight of the shoots when grown on clinostats for 6 weeks (Lyon, 1965). Again, in horizontally growing fruit trees (e.g. apples, cherries, plums and blackcurrants) the total growth was markedly reduced compared to their vertical controls, and the effect of the horizontal positioning was more pronounced when plants were rotated (Wareing and Nasr, 1958). These differences might be related to the differences in plant species, growing conditions including potting mixture or speed of the rotation.

Lateral shoot growth: It was expected that the clinostat would release the apical dominance enhancing lateral shoot growth and that GA_3 applied to those released laterals, would have an additive effect on production of lateral shoot elongation. The present data did not support that idea. This present data further demonstrated the positive role of GA_3 in the apical dominance.

4.6.5 Conclusions:

* Horizontal rotation on a clinostat was unable to neutralise the effect of gravity in SDP plants.
* GA_3 reinforced the process of apical dominance even when plants were rotated horizontally on a clinostat.

4.7 Effect of different time of GA_3 application on a PBZ treated plant.

4.7.1 Introduction:

In all of the earlier experiments in this chapter (Chapter 4), PBZ alone increased lateral shoot numbers but resulted in less elongation of these shoots presumably due to the lack of available GA, as it is known that PBZ inhibits GA biosynthesis (Steffens and Wang, 1986). Adding GA_3 simultaneously with PBZ always failed to reverse that effect because GA_3 reinforced the process of apical dominance (Section 4.4.4). Ageing normally releases apical dominance (Section 2.1.2.2). On the other hand, PBZ when applied alone releases more buds (Section 4.2 and 4.4) i.e. releases apical dominance of the plant. It has also been reported that GA_3 normally works efficiently at the post release phase of the bud for their elongation but has an inhibitory effect on initial growth of the buds (Section 2.1.2).

It was presumed that delayed GA_3 application would reverse PBZ induced growth inhibition by elongating already released buds (released through ageing or by PBZ's action). An experiment was accordingly initiated with different times of GA_3 application to PBZ treated plants to study the effects of time of GA_3 application on reversal of PBZ induced lateral shoot growth inhibition.

4.7.2 Materials and Methods:

Four (4) treatments with 10 replications were arranged in a randomised complete block design. The treatments were: untreated control, PBZ (10 mg a.i./plant), PBZ (10 mg a.i./plant)

+ GA_3 (500ppm); and PBZ (10 mg a. ./plant; applied at the 3rd leaf stage) + GA_3 (500ppm; applied 4 weeks after PBZ application)

The seeds were sown on 11.08 94 and the PGRs were applied on 16.09.94 for 3rd leaf stage or on 14.10.94 only for the delayed GA_3 application. Vegetative data were recorded 4 weeks after 1st PGRs application and again at 8 weeks after the 1st application (i.e. 4 weeks after delayed GA_3 application). The rest of the procedures were followed as per section 3.2.

4.7.3 Results:

Main shoot growth: PBZ (alone) reduced plant height and internode length but had no effect on leaf number all through the experiment. Irrespective of the time of GA_3 application PBZ + GA_3 gave taller plants with longer internodes and more leaves compared to control and PBZ (only) treated plants (Figure 4.28 and 4.29). GA_3 treated plants had approximately 8-10 elongated internodes compared to 6 in control plants.

Lateral shoot growth: GA_3 applied simultaneously with PBZ gave fewer lateral shoots with the least lateral length compared to the rest of the treatments in both sets of data (Figure 4.30 and 4.31). On the other hand, delayed GA_3 application (i.e. 4 weeks after PBZ application) gave statistically similar lateral shoot number compared to PBZ treated or control plants. In PBZ + GA_3 (delayed) treatment the lateral length and internode length were less at 4 weeks (before GA_3 application; Figure 4.30) but were higher at 8 weeks (after GA_3 application; (Figure 4.31).

Lateral angle: Irrespective of the time of GA_3 application, the branch angle was narrower compared to the rest of the treatments (Figure 4. 32).

4.7.4 Discussion:

Main shoot growth: The present results confirm that growth inhibition of main shoots due to PBZ could be completely reversed and growth could be stimulated at least up to 8 weeks after sowing by simultaneous or delayed (4 weeks after PBZ application) GA_3 application. Quinlan and Richardson (1984) completely overrode the effect of PBZ (500ppm) with GA_3 (200ppm, applied on the same day) in apple seedlings and those seedlings showed no difference to the control plants after 3 weeks. Cox (1993) reported that when GA_3 (50 mg/L) was applied to poinsettia 28 or 42 days after PBZ (0.5 mg/pot drench or 125 mg/L spray) application, there was little or no stimulation of plant height however, the dwarfing effect of PBZ was completely reversed by applying GA_3 on the same day or 14 days after PBZ application.

Lateral shoot growth: GA_3 applied simultaneously with PBZ inhibited total lateral growth (lateral shoot numbers and their elongation). But when GA_3 was applied 4 weeks after PBZ application, it increased the elongation of those laterals released by PBZ treatments (applied 4 weeks before GA_3 application). As a result of that, delayed GA_3 application gave statistically similar lateral length to control plants. GA_3 clearly worked in a post release phase of an axillary bud in a similar way as reported for peas by Tamas (1987).

Figure 4. 28 Simultaneously (S) applied GA_3 and PBZ on main shoot growth

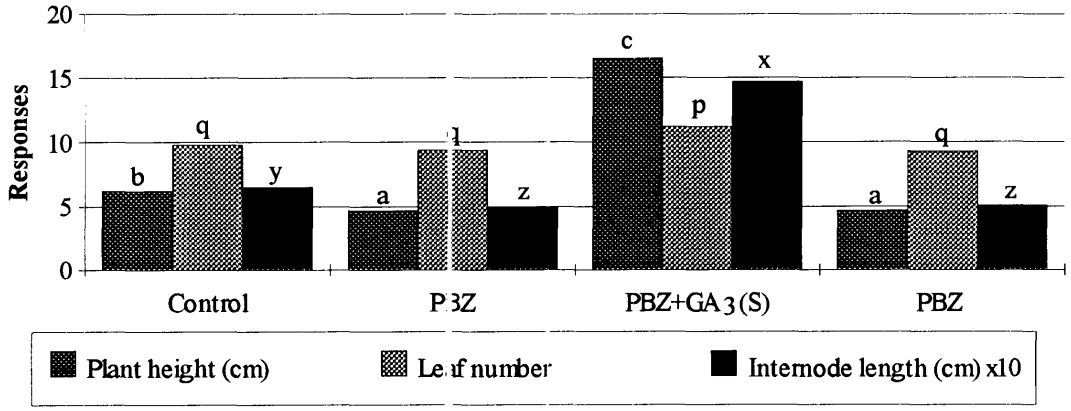


Figure 4. 29 Simultaneous (S) and delayed (D) GA_3 application on a PBZ treated main shoot

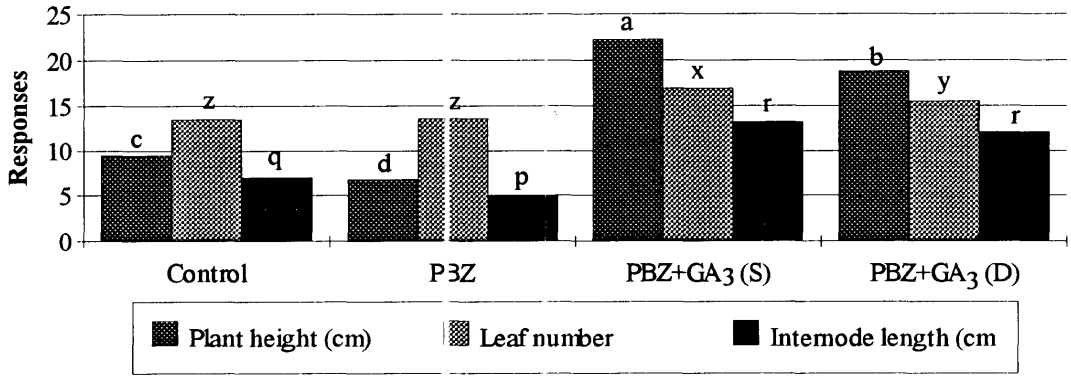


Figure 4. 30 Simultaneously (S) applied GA_3 and PBZ on lateral shoot growth

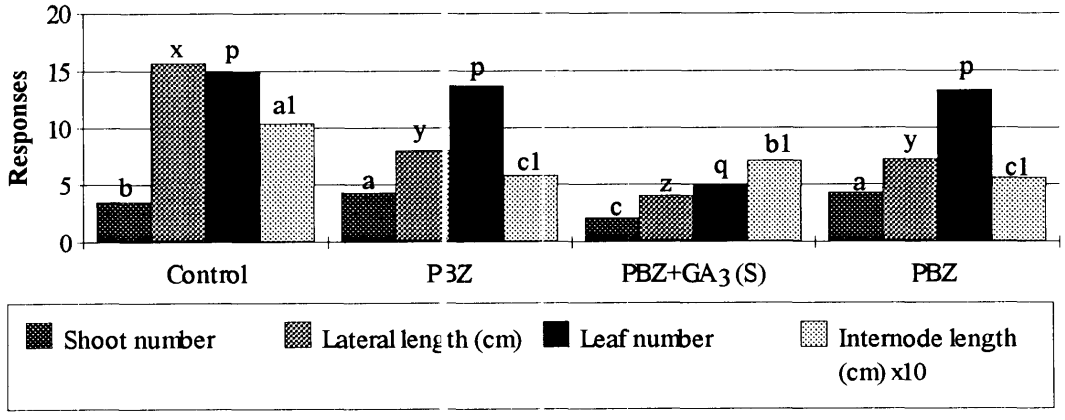


Figure 4.31 Simultaneous (S) and delayed (D) GA_3 application on a PBZ treated lateral shoot

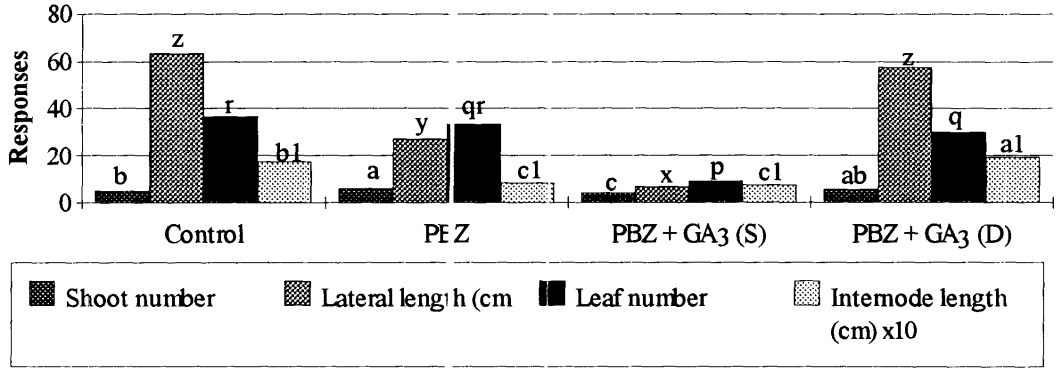
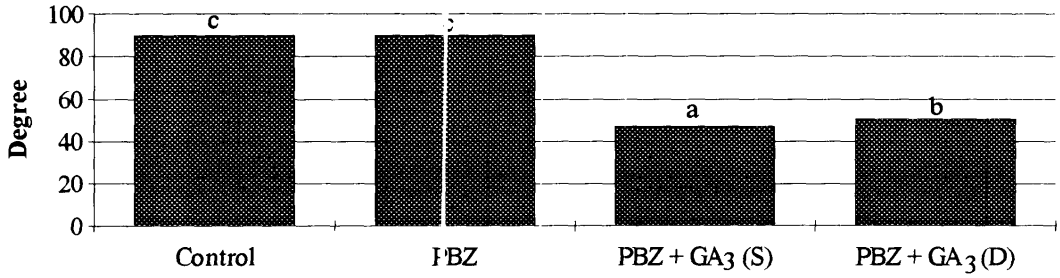


Figure 4.32 Simultaneous (S) and delayed (D) GA_3 application with PBZ on angle formation



Lateral angle: Narrower angle was produced by GA_3 + PBZ irrespective of time of GA_3 application. This narrower angle could be due to GA_3 induced apical dominance in the main shoot along with vigorous and more upright growth for some of the released laterals causing narrower branch angles.

4.7.5 Conclusions:

* PBZ induced inhibition of main shoot growth could be released by simultaneous or delayed GA_3 application but in lateral shoots only delayed GA_3 application was effective.
* Although delayed GA_3 application reversed PBZ induced lateral shoot growth inhibition, GA_3 inhibited further bud growth and elongation at all application times through the expression of apical dominance in the main shoot and correlative inhibition in some of the released laterals.

4.8 General conclusions from chapter 4.

* PBZ induced growth retardation is probably mediated by increased auxin availability or supply and/or reduced GA levels in SDP plants.
* PBZ and GA_3 caused a concentration dependent response in SDP.
* PBZ and GA_3 had opposite modes of actions on SDP growth.

* PBZ + GA_3 had a synergistic and promotory effect on flowering.
* Higher PBZ concentrations were required for lateral shoot growth inhibition probably because of higher GA synthesis or supply in the laterals.
* Reduced lateral growth due to PBZ is due to reduced elongation of the released shoots only but for GA_3 + PBZ it was due to the reduction of both shoot numbers and their elongation.
* PBZ induced inhibition of main shoot growth could be released by simultaneous or delayed GA_3 application but in lateral shoots only delayed GA_3 application was effective.
* Although delayed GA_3 application reversed PBZ induced lateral shoot growth inhibition, GA_3 inhibited further bud growth and elongation at all application times through the expression of apical dominance in the main shoot and correlative inhibition in some of the released laterals.
* The main shoot remains responsive to exogenously applied GA_3 at least up to 8 weeks (after sowing) in regard to the reversal of PBZ induced inhibition.
* GA_3 reinforced the process of apical dominance even when plants were rotated horizontally on a clinostat.

CHAPTER - 5 : RESPONSES OF DECAPITATED PLANTS

5.1 General introduction to chapter 5.

In earlier experiments simultaneous application of GA_3 to intact plants failed to reverse PBZ induced lateral shoot growth inhibition probably because of apical dominance due to the presence of the intact apex (Section 4.2; 4.3; 4.4 and 4.5). Rotation on a clinostat did not even release the plants from apical dominance (Section 4.6).

Since decapitation should release apical dominance (Cline, 1991), PGR application to decapitated plants should release PBZ inhibition of lateral shoot growth (Section 4.5.4). Out of 7 experiments in this chapter, 5 were conducted to study the effects of different PGRs alone or in combinations on the release of apical dominance in decapitated plants (Section 5.2; 5.3; 5.4; 5.5 and 5.6). The last 2 experiments (Sections 5.7 and 5.8) studied the combined effects of PBZ and other PGRs on the reversal of PBZ inhibition of lateral shoot growth in decapitated plants.

5.2 Effect of GA_3 on decapitated, desuckered and horizontally grown plants.

5.2.1 Introduction :

Any treatments (including decapitation or gravimorphic treatments) which restrict terminal growth should release apical dominance (Cline, 1991). On the other hand, desuckering (lateral shoot removal) generally increases main shoot growth and hence apical dominance (Janick, 1986).

In this present experiment GA_3 was applied to decapitated, desuckered and horizontal plants to determine the interactions involved in the hormonal balance associated with apical dominance.

5.2.2 Materials and Methods:

Four (4) treatments were used in this experiment and they were arranged in a factorial RCBD with 10 replications. The treatments were:

i). Control plants, untreated;

ii). Plants grown horizontally;

iii). Desuckered plants (all laterals were removed) and

iv). Plants with the main shoot decapitated.

Each group of treatments were either sprayed with 500ppm GA_3 or with DD water 4 weeks after sowing. The decapitation and desuckering were done on the same day as GA_3 application but desuckering continued all through the experiment.

In the case of horizontal plants, a 4 cm diameter circular hole was made on one side of each pot and plants were transplanted directly into these holes. These pots were kept in their normal vertical position, thus allowing the plants to grow horizontally. Bamboo stakes and ties (15 mm plastic clip) were used to maintain the horizontal growth.

The seeds were sown on 22.02.94. The vegetative data were recorded 4 weeks after GA_3 application. The rest of the procedures were followed as per section 3.2.

5.2.3 Results:

For the main shoot data, the comparison between the control, desuckered and horizontal plants was made because the decapitated plants do not have a main shoot. Conversely, for lateral shoot data desuckering is inappropriate and the comparison was made between the control, decapitated and horizontal plants.

Main shoot growth: Irrespective of any interaction with shoot orientation or desuckering, GA_3 treated plants had taller main shoots with more leaves and longer internodes compared to plants without GA_3 (Table 5.1a). There was no interaction effects on main shoot elongation between the growing systems (main shoot orientated or desuckering) (Table 5.1b) and GA_3 (Table 5.1c).

Table 5.1 Effect of GA_3, growing systems and their interactions on main shoot growth.

(a) GA_3.

Treatments	Plant height (cm)	Leaf number	Internode length (cm)
Control	15.10a*	11.37a	1.33a
GA_3	**25.23b**	**13.00b**	**1.94b**

(b) Growing systems.

Treatments	Plant height (cm)	Leaf number	Internode length (cm)
Control	18.55	12.00	1.54
Desuckered	21.90	12.65	1.70
Horizontal plant	20.05	11.90	1.66
	ns	ns	ns

(c) GA_3 X growing systems.

Treatments	Plant height (cm)		Leaf number		Internode length (cm)	
	00	GA_3	00	GA_3	00	GA_3
Control	13.70	23.40	10.20	12.00	1.18	1.91
Desuckered	16.40	27.40	10.40	13.20	1.40	2.00
Horizontal plant	15.20	24.90	10.90	12.40	1.40	1.92
	ns		ns		ns	

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

Lateral shoot growth: Irrespective of any interaction with shoot orientation or decapitation, application of GA_3 reduced shoot and leaf number and lateral length but had no effect on the lateral internode length (Table 5.2a).

Decapitation increased shoot number, lateral length and the number of leaves compared to the rest of the treatments (Table 5.2b). Although there was no significant

interaction between growing methods and GA_3 on the lateral shoot growth, GA_3 had a tendency to reduce the mean length of lateral shoots in decapitated plants (Table 5.2c). The horizontal plants did not show any sign of decreasing apical dominance and constant upward gravitropic curvature of the shoots was observed in these plants.

Table 5.2 Effect of GA_3, growing systems and their interactions on lateral shoot growth.

(a) GA_3.

Treatments	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)
Control	6.03a*	64.03a	37.87a	1.74
GA_3	**4.17b**	**43.92b**	**24.50b**	1.78
	t		t	ns

(b) Growing systems.

Treatments	Shoot number	Lateral Length (cm)	Leaf number	Internode length (cm)
Control	4.45a*	43.45b	24.50a	1.72
Decapitated	**7.30b**	**81.05a**	**49.70b**	1.70
Horizontal plant	3.55a	37.43b	19.35a	1.86
	t		t	ns

(c) GA_3 X growing systems.

Treatments	Shoot number		Lateral length (cm)		Leaf number		Internode length (cm)	
	00	GA_3	00	GA_3	00	GA_3	00	GA_3
Control	5.30	3.60	53.50	33.40	29.50	19.50	1.78	1.66
Decapitated	**8.40**	**6.20**	**94.10**	**68.00**	60.10	39.30	1.66	1.74
Horizontal plant	4.40	2.70	44.50	30.35	24.00	14.70	1.76	1.95
	ns		ns		ns		ns	

* = 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.

5.2.4 Discussion:

Main shoot growth: Decapitation was the only growing system able to release apical dominance of SDP plants (Table 5. b and 5.2b). Regardless of the treatment, GA_3 again enhanced apical dominance by increasing internode length and number (Table 5.1a). In this present experiment the gravity treatment (horizontal plants) was unable to reduce apical dominance. Moreover, the geotropic force was very strong in SDP because tying down horizontal plants everyday did not keep them growing horizontally. But growing *Pharbitis nil* plants horizontally or inverted reduced apical growth and released apical dominance (Prasad and Cline, 1987).

Lateral shoot growth: In decapitated plants, increased lateral growth was observed presumably due to the release of apical dominance (Table 5.2b). According to King and vanStadan (1990), decapitation brings about changes in the hormonal and nutritional status of plants in favour of the bud release. However, the decapitated GA_3 treated plants (Table 5.2c) also tended to reduce lateral growth (e.g. shoot number, lateral length and leaf number). This

result is different from Phillips (1975), who reported that GA_3 increased the rate of lateral bud growth in many plants.

The inability of GA_3 to increase lateral bud growth in this present experiment might be related to the stage of GA_3 application or stage of decapitation or both. At the time of GA_3 application there might not have been enough shoots released from apical dominance enabling them to elongate or plants might not have reached their optimum stage for lateral growth, when they were decapitated. Therefore in the following two sections (Section 5.3 and 5.4), the effect of delaying decapitation plus simultaneous GA_3 application (Section 5.3) and the effect of delaying decapitation plus delaying GA_3 application (Section 5.4) were tested with a view to achieve more lateral growth.

The horizontally grown plants did not reduce apical dominance in this present experiment which is also different to that of Cline (1991), who reported that several of the inactive lateral buds grew out from horizontal or inverted shoots. However, he also mentioned that horizontal intact pea seedlings did not release bud growth without decapitation. To prevent the constant gravitropic curvature in horizontal plants in this present study, tying down of the shoots every day was necessary. Similar tying down was also required for *Ipomoea* shoots (Cline, 1991). That was why the author was not sure how much of that total stress could be attributed to the gravity force only. A rotating clinostat might negate the gravity force, as suggested by Sachs (Audus, 1967), without introducing the stress of tying down.

5.2.5 Conclusions:

* Horizontal orientation did not have any effect on the release of apical dominance or the subsequent response to GA_3 in SDP plant.
* The strong shoot gravitropism in SDP could not be overcome by the horizontal method of growing.
* Decapitation released apical dominance and increased lateral shoot growth but did not enable GA_3 promotion of lateral growth when simultaneously applied.
* Delayed decapitation and GA_3 application might cause increased lateral growth.

5.3 Effect of stage of decapitation and simultaneous GA_3 application.

5.3.1 Introduction:

GA_3 applied simultaneously with decapitation still reduced the elongation growth of lateral shoots in the preceding experiment (Section 5.2.4). It was hypothesised (Section 5.2.4) that delayed decapitation (after 3rd true leaf stage) and simultaneous GA_3 application might increase total lateral growth. GA normally acts on the post release phase of bud growth (Khan, 1975). Delaying decapitation and GA_3 application until after the plant achieves its optimum physiological stage and releases more buds from apical dominance might increase the promotion of lateral growth by GA_3.

Accordingly, an experiment was set up to test the effect of different times of decapitation and simultaneous GA_3 application on lateral shoot growth of SDP plants.

5.3.2 Materials and Methods:

Six (6) treatments were laid out in a factorial RCBD with 10 replications. The treatments were: 3 different times of decapitation (1st, 3rd or 5th true leaf stage) and 2 GA_3 concentrations (00 and 500ppm). Decapitations were done on 24.06.94 (for 1st true leaf stage); 02.07.94 (for 3rd true leaf stage) or on 13.07.94 (for 5th true leaf stage). GA_3 treatments were applied at the time of decapitation.

The seeds were sown on 25.05.94. Vegetative data were recorded 4 weeks after each GA_3 application. Other procedures were followed as per section 3.2.

5.3.3 Results:

Lateral shoot growth: Irrespective of the stage of decapitation, simultaneously applied GA_3 did not increase lateral length, reduced the number of the lateral shoots but increased lateral internode length (Table 5.3a; P ate 5.1).

As the process of decapitation was delayed, lateral length was increased all through the experiment (Table 5.3b). The interaction of delayed decapitation and simultaneously applied GA_3 significantly reduced shoot and leaf number, and had a reducing trend for lateral length (both at 3 and 5 leaf stage) compared to decapitation without GA_3 application. But lateral internodes were longer than in the untreated controls (Table 5.3c).

Lateral shoot angle: The branching angle of the laterals were narrower in GA_3 treated plants compared to their corresponding untreated control plants (Table 5.3a; 5.3c).

5.3.4 Discussion:

Lateral shoot growth: The amount of lateral growth was dependent on the stage of decapitation (Table 5.3b); late decapitation increased all of the lateral growth parameters.

Simultaneous GA_3 application up to the 5th true leaf stage was generally inhibitory (Table 5.3c) for lateral shoot growth (except for the lateral internode length). GA_3 increased the elongation of the 1 or 2 already released shoots and by inducing correlative inhibition inhibited the growth of the others which actually increased the overall mean lateral internode length (Table 5.3a and 5.3c). This difference in response of released and not released buds (at the time of GA_3 application) suggests that decapitation followed by delayed GA_3 application to the released buds might increase shoot number and lateral length. Cline (1991) reported that for most herbaceous plants, the initiation of bud elongation began within 3 to 10 hours after decapitation. In this present experiment simultaneous GA_3 application with decapitation might have inhibited the growth of the undeveloped buds at that early stage. It is probable that if GA_3 application were made after decapitation it would not inhibit the bud initiation processes. Bud growth stimulation was achieved with GA_3 application to the decapitated shoot stump or

Table 5.3 Effect of simultaneous GA_3 application, stages of decapitation and their interactions on lateral shoot growth.

(a) Simultaneous GA_3 application.

Treatments	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle ( )
Control	5.30a*	33.18	24.23a	1.19a	89.83a
GA_3	**3.63b**	26.97	**15.23b**	**1.59b**	**49.25b**
	t	ns			t

(b) Stages of decapitation.

Leaf stages	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle ( )
1st	2.85a*	9.80a	10.80a	0.91a	68.38
3rd	**4.25b**	**21.45b**	**17.90b**	**1.18b**	71.13
5th	**6.30c**	**58.98c**	**30.50c**	**2.06c**	69.13
	t	t			ns

(c) Simultaneous GA_3 application X stages of decapitation.

Leaf stages	Shoot number		Lateral length (cm)		Leaf number		Internode length (cm)		Mean angle ( )	
	00	GA_3	00	GA_3	00	GA_3	00	GA_3	00	GA_3
1st	3.30b*	**2.40a**	9.00	10.60	11.40c	10.20c	0.77d	**1.05c**	90.00c	**46.75a**
3rd	4.50c	**4.00bc**	24.95	17.95	21.20b	14.60c	1.15c	**1.22c**	90.00c	**52.25b**
5th	8.10d	**4.50c**	65.60	52.35	40.10a	20.90b	1.64b	**2.49a**	89.50c	**48.75ab**
	t		ns						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.

Plate 5.1 Reduced lateral shoot growth following GA_3 application at 1st, 3rd or 5th leaf stage.

to the buds of different plants but this growth response was dependent on plant age and other experimental variables (Tamas, 1987).

Lateral shoot angle: Phillips (1975) reported that decapitation of shoots frequently results in upward, hyponastic movements of leaves, lateral shoots, and even woody branches and application of auxins can counteract these effects. In this present experiment decapitation did not produce narrower branch angle but GA_3 application did.

5.3.5 Conclusions:

* Simultaneous GA_3 application inhibited lateral shoot growth irrespective of the time of decapitation.
* GA_3 probably had an inhibitory effect at the early stage of bud growth.
* A period of time following decapitation may be needed for initiation of bud activity before GA_3 is applied. Simultaneous GA_3 application may inhibit this initiation.

5.4 Effect of stage of decapitation and delayed GA_3 application.

5.4.1 Introduction:

Simultaneously applied GA_3, even on the plants decapitated at different growth stages, was unable to increase lateral growth (Section 5.3.4). It was hypothesised that delayed GA_3 application after decapitation would not inhibit the initial processes of bud release and may therefore increase their growth (Section 5.3.4).

To test this hypothesis GA_3 application to decapitated plants (decapitated at different growth stages) was delayed (not simultaneously).

5.4.2 Materials and Methods:

Six (6) treatments were laid out in a factorial RCBD with 10 replications. The treatments were: 3 stages of decapitation (1st, 3rd and 5th true leaf stage) and 2 levels of GA_3 (00 and 500ppm). The plants were decapitated on 24.06.94 (for 1st true leaf stage), 02.07.94 (for 3rd true leaf stage) or on 13.07.94 (for 5th true leaf stage). After decapitation, there was a waiting period for the first 2 laterals to produce at least 2 leaves each. GA_3 was then applied to the whole plant 18 days after decapitation of the 1st true leaf stage; 8 days after decapitation of the 3rd true leaf stage or 4 days after decapitation of the 5th true leaf stage.

The seeds were sown on 28.06.94. Vegetative data were recorded 4 weeks after each GA_3 application. The rest of the procedures were followed as per section 3.2.

5.4.3 Results:

Lateral shoot growth: Irrespective of other variables, GA_3 application reduced shoot and leaf numbers and increased lateral internode length but had no effect on lateral length (Table 5.4a).

Decapitation at different stages (Table 5.4b) caused a significant increase in lateral growth (shoot number, leaf number, lateral length and internode length). Except for increased

shoot numbers, the rest of the parameters were not affected when decapitation was done at the 3rd or 5th leaf stages of growth (Table 5.4b).

Although there was no significant interaction between different stages of decapitation and delayed GA_3 application (Table 5.4c), Other than increasing growth GA_3 still had a tendency to reduce all parameters (except internode length, which was increased) all through the experiment compared to untreated plants.

Lateral shoot angle: Irrespective of the treatment combinations, GA_3 produced narrower branch angles for the decapitated lateral shoots (Table 5.4a and 5.4c).

Table 5.4 Effect of delayed GA_3 application, stages of decapitation and their interactions on lateral shoot growth.

(a) Delayed GA_3 application.

Treatments	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle ( )
Control	6.63a*	51.02	33.53a	1.44a	89.33a
GA_3	**4.03b**	46.67	**18.90b**	**2.40b**	**47.17b**
		ns		t	t

(b) Stages of decapitation.

Leaf stages	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle ( )
1st Leaf	3.90c*	34.15a	21.80b	1.71a	66.00
3rd Leaf	**5.45b**	**53.35b**	**27.55a**	2.00ab	70.00
5th Leaf	**6.65a**	**59.00b**	**29.30a**	**2.06b**	68.75
		t		t	ns

(c) Delayed GA_3 application X stages of decapitation.

Leaf stages	Shoot number		Lateral length (cm)		Leaf number		Internode length (cm)		Mean angle ( )	
	00	GA_3	00	GA_3	00	GA_3	00	GA_3	00	GA_3
1st	5.30	2.50	34.10	34.20	29.30	14.30	1.14	2.27	89.00b	**43.00a**
3rd	6.30	4.60	54.90	51.85	34.10	21.00	1.58	2.42	90.00b	**50.00a**
5th	8.30	5.00	64.05	53.95	37.20	21.40	1.61	2.52	89.00b	**48.50a**
	ns		ns		ns		ns		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.

5.4.4 Discussion:

Lateral shoot growth: GA_3 treated plants had longer internodes which resulted in statistically similar lateral length to the control even though fewer shoots were released (Table 5.4a), probably because of the increased elongation of those fewer shoots already released. This elongation following GA_3 could be due to an interaction of GA_3 with available auxin

(Section 2.2.5.1) in shoots, leaving not enough auxin for bud growth and elongation. Thereby those vigorously growing released lateral shoots could in turn induce correlative inhibition of undeveloped buds and reduce the number of the shoots. Longer lateral internodes in decapitated plants following GA_3 application were also observed earlier (Table 5.3a).

The trend of lower shoot numbers, lateral length and leaf numbers for GA_3 treated plants in the interaction table (Table 5.4c) suggest that delayed GA_3 application was still inhibitory. To get increased lateral growth (shoot number and lateral length), the waiting period for GA_3 application might need to be more than 2 lateral leaves. Another option would be to have a pre-treatment of those decapitated shoots with auxin because in many systems, gibberellins were found to work in conjunction with auxin and GA alone proved ineffective for elongation growth (Phillips, 1971; Khan, 1975).

Lateral shoot angle: These present results agree with the earlier findings, where GA_3 also induced narrower angle formation (Table 5.3a).

5.4.5 Conclusions:

* GA_3 exerts its effect on elongation only after laterals have been released from apical dominance and hence commenced growth. The increased and upright growth of some of the released lateral shoots by GA_3 may have induced correlative inhibition and reduced total shoot numbers.

* Delaying GA_3 application up to 2 weeks was not sufficient to increase total lateral elongation.

* Interaction of auxin and GA_3 might prove stimulatory for lateral shoot growth.

5.5 Effect of IAA and GA_3 pre-treatments.

5.5.1 Introduction:

Khan (1975) suggested that auxin was the primary stimulator for control of bud growth and growth of tissues in culture whereas gibberellins acted only after auxin had initiated growth in those systems.

It was hypothesised (Section 5.4.4) that, if decapitated plants were pre-treated with auxin prior to GA_3 application, the lateral growth would increase. To test this hypothesis, an experiment was initiated with IAA and GA_3 pre-treatment of decapitated plants to examine their effect on lateral shoot growth.

5.5.2 Materials and Methods:

Five (5) treatments and 10 replications were arranged in a randomised complete block design. The treatments were:

i). Decapitation

ii). Decapitation + simultaneous GA_3 (500ppm) application

iii). Decapitation + simultaneous IAA (20 mg a.i./plant) application

iv). Decapitation + simultaneous GA_3 application followed by IAA one week later and

v). Decapitation + simultaneous IAA application followed by GA_3 one week later.

The seeds were sown on 08. 03. 94. Decapitation was done for all of the treatments at the 3rd true leaf stage of the plants. Vegetative data were recorded 5 weeks after 1st PGR application. The rest of the procedures were followed as per section 3.2.

5.5.3 Results:

Lateral shoot growth: Simultaneous IAA application and decapitation increased the number of lateral shoots compared to the rest of the treatments but with less total elongation of those shoots compared to the control plants (Figure 5.1 and 5.2). IAA pre-treatment followed by GA_3 or any other GA_3 combinations reduced shoot numbers but had no effect on the lateral length (Figure 5.1 and 5.2).

Lateral shoot angle: Simultaneous IAA (only) application did not affect branch angle but GA_3 alone or when added to IAA treated plants (pre or post), reduced it (Figure 5.3).

Figure 5. 1 PGRs pre-treatment on lateral shoot growth of a decapitated plant

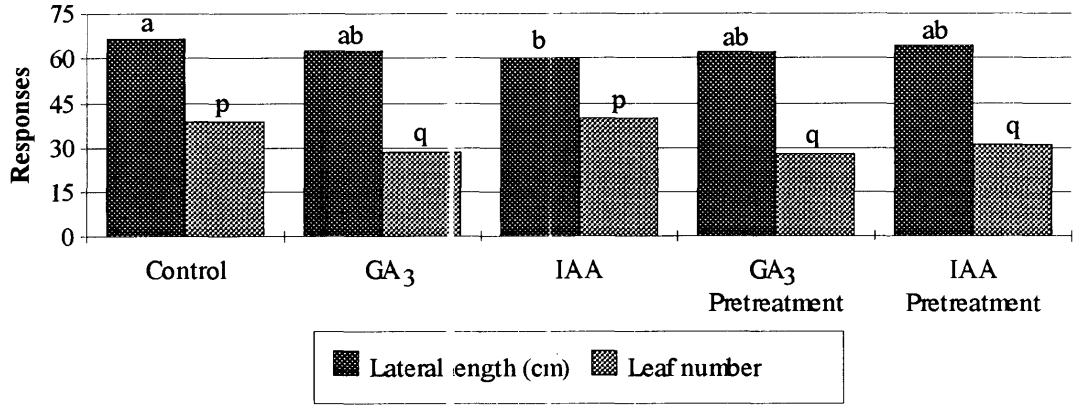


Figure 5. 2 PGRs pre-treatment on lateral shoot growth of a decapitated plant

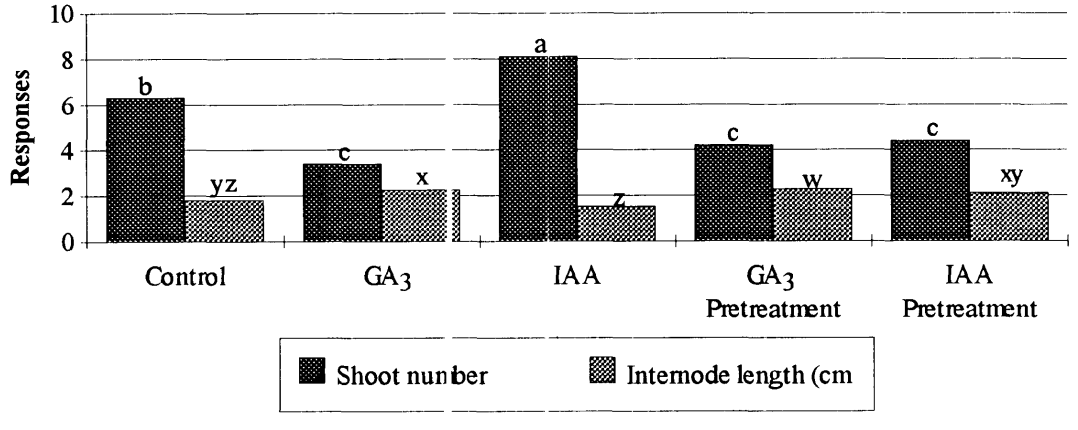
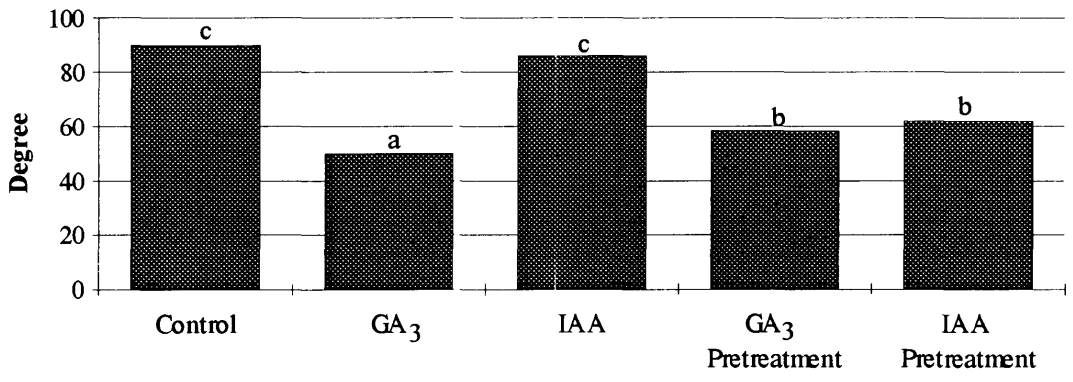


Figure 5. 3 PGRs pre-tre atment on angle formation of a decapitated lateral shoot



5.5.4 Discussion:

Lateral shoot growth: The present results revealed that, irrespective of the time of IAA application (pre or post), GA_3 reduced the total number of lateral shoots and reduced lateral length. A similar trend towards inhibition of shoot number and lateral length by GA_3 (alone) was found earlier with simultaneous (Table 5.3a) or delayed (Table 5.4a) GA_3 application to decapitated plants. This present result did not agree with the earlier findings, where pre-treatment with auxin followed by GA in different systems gave enhanced elongation growth (Galston and Davies, 1970; Phillips, 1971; Khan, 1975). The inability of auxin pre-treatment to enhance lateral growth in this present experiment could be due to in appropriate timing of GA_3 application. As the timing of GA_3 plays an important role for GA induced lateral shoot growth (Tamas, 1987).

Lateral shoot angle: IAA in this present experiment had statistical similar wider angles to that of control plants. Auxin induced wider angles between laterals and the main shoot were reported earlier in different plants (Janick, 1986). GA_3 produced narrower angles irrespective of its time of application (pre or post IAA).

5.5.5 Conclusions:

* GA_3 application resulted in fewer lateral shoots and did not increase lateral length irrespective of (pre or post) IAA combinations.

5.6 Effect of lateral shoot removal and GA_3 application.

5.6.1 Introduction:

In decapitated plants GA_3 generally had no effect or caused a reduction in the lateral length (Sections 5.2; 5.3; 5.4 & 5.5). That insignificant or reduced lateral length following GA_3 application was mainly related to the reduced shoot numbers. To find out the relationship between reduced shoot numbers and the lateral length, 2 different hypotheses were proposed. GA_3 might: (i) directly reduce shoot numbers through an inhibitory effect on bud release

thereby enabling greater elongation of the released shoots or (ii) directly cause elongation of the released shoots which in turn inhibit the release of new buds to form shoots.

To test these hypotheses GA_3 was applied simultaneously to decapitated plants, where the lateral shoots had been removed. The removal of the released lateral shoots in decapitated plants would eliminate any elongation of the already released laterals at the time of the treatment. Moreover by removing all released shoots, GA_3's effect on bud release would also clearly be observed.

5.6.2 Materials and Methods:

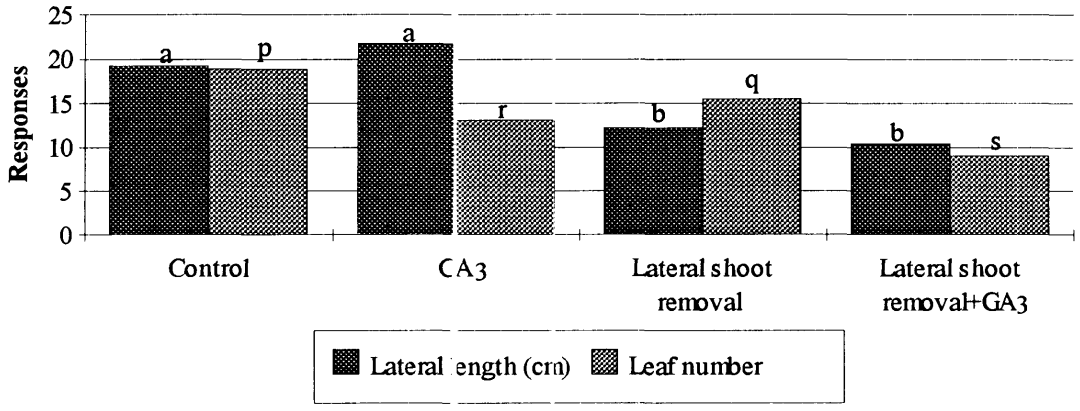
Four (4) treatments were used in this experiment. Plants were decapitated and then divided into 2 groups; released laterals were removed from one group and not from the other. Both groups were then treated with or without GA_3 (500ppm). The treatments were applied on 16.09.94. The treatments were arranged in a RCBD with 10 replications.

The seeds were sown on 11.08.94. Vegetative data were recorded 4 weeks after GA_3 application. The rest of the procedures were followed as per section 3.2.

5.6.3 Results:

Lateral shoot growth: Irrespective of treatment combinations, GA_3 reduced shoot numbers but increased internode length with no net effect on the lateral length compared to their controls (Figure 5.4 and 5.5).

Figure 5.4 Lateral shoot removal and GA_3 application in a decapitated plant

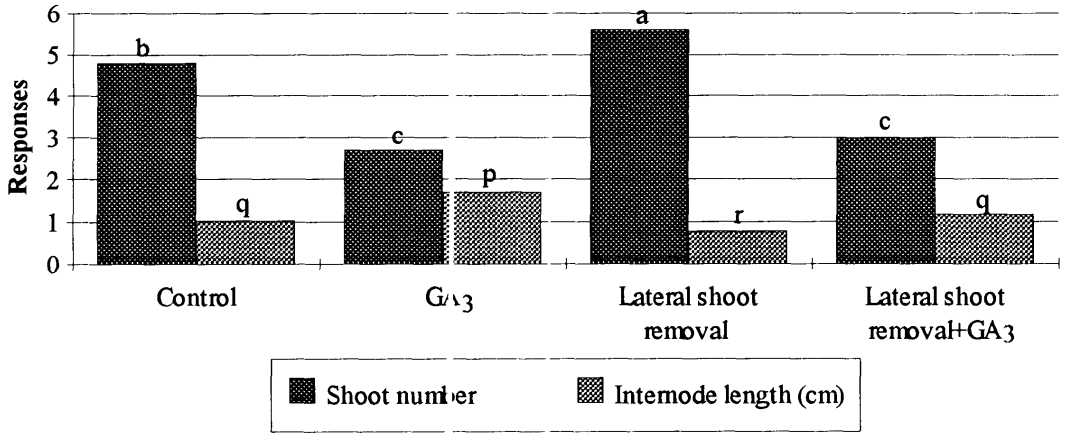


5.6.4 Discussion:

Lateral shoot growth: The present result revealed that GA_3 does not directly inhibit bud growth immediately after application. Few shoots are released and then GA_3 elongates those released shoots and inhibits further bud growth due to correlative inhibition. GA_3 probably initiates bud release of the few advanced buds. Thus the effect of GA_3 depends on the stage or age of the bud growth. GA_3 produced statistically similar lateral length from a

significantly lower number of lateral shoots, which could be due to GA_3 induced vigorous growth of those shoots or in other words GA_3 induced correlative inhibition.

Figure 5. 5 Lateral shoot removal and GA_3 application in a decapitated plant



5.6.5 Conclusions:

* GA_3 allows limited bud release and then increases elongation only in these released shoots and thereby induces correlative inhibition which inhibits further bud growth and elongation.
* The effect of GA_3 depends on the stage of bud growth.

5.7 Interaction of PBZ with GA_3, IAA and BAP.

5.7.1 Introduction:

It was hypothesised earlier (Section 4.5.4) that removal of apical dominance and application of GA_3 or its combination with other PGRs, might reverse PBZ induced lateral growth inhibition in SDP.

PBZ is mainly a GA biosynthesis inhibitor (Steffens and Wang, 1986) but is also found to interfere with the level of other hormones (e.g. auxin, cytokinin) (Davies and Curry, 1991). Therefore reversal of the lateral growth inhibition should be possible with PGR (including GA_3) application. But in earlier experiments decapitation released apical dominance (Section 5.2) but addition of GA_3 alone (Sections 5.2; 5.3; 5.4) or with IAA (Section 5.5) induced correlative inhibition from already released laterals and inhibited the release of new lateral buds. Similarly addition of PGR also induced apical dominance in intact plants (Section 4.5). In some species, gibberellin and/or auxin treatments following initial cytokinin application were required for continued lateral bud outgrowth (Cline, 1991). Again in some plants, the growth of the laterals and apical dominance was controlled by the balance between gibberellins, auxins and cytokinins (Field and Jackson, 1974).

In light of this information, it was hypothesised that if bud release itself was not inhibited, then GA_3 alone or along with any other PGR combinations, might reverse PBZ

inhibition of lateral shoot growth. To test this hypothesis an experiment was formulated with PBZ, GA_3, IAA, BAP and their combinations applied to decapitated plants.

5.7.2 Materials and Methods:

Eighteen (18) treatment combinations were arranged in a randomised complete block design with 10 replications. The PGRs used in this experiment were PBZ (10 mg a.i./plant), GA_3 (500ppm), IAA (20 mg a.i./plant), BAP (25ppm) and their combinations. The plants were decapitated at 4 weeks immediately before PGRs application.

The seeds were sown on 01.12.94. Vegetative data were recorded 4 weeks after PGRs application. The rest of the procedures were followed as per section 3.2.

5.7.3 Results:

Lateral shoot growth: GA_3 alone or in any other combinations reduced lateral shoot numbers. PBZ alone or in any other combinations (except GA_3) increased lateral shoot numbers. All PBZ combinations (including GA_3) reduced lateral length (Table 5.5).

Lateral shoot angle: GA_3 alone or in any combinations reduced the branch angle and reinstated correlative inhibition (Table 5.5).

Table 5.5 Effect of PBZ with GA_3, IAA and BAP on the reversal of PBZ induced lateral shoot growth inhibition.

Treatments	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle ( )
Intact Control	5.80e*	61.65bc	32.50cde	1.90cd	**88.00d**
Intact + PBZ	7.50cd	15.25i	23.95g	0.65f	**90.00d**
Decapitation	7.60cd	**86.45a**	39.70a	2.21b	**87.50d**
Decapitation + PBZ	**10.40a**	29.85fg	35.10bcd	0.84ef	**90.00d**
Decapitation + IAA + PBZ	**10.30 a**	22.20h	32.10cde	0.69ef	**90.00d**
Decapitation + BAP + PBZ	**10.40a**	34.95f	38.60ab	0.91e	**90.00d**
Decapitation + IAA + BAP + PBZ	**10.40 a**	24.50gh	31.40de	0.78ef	**90.00d**
Decapitation + BAP	8.40b	67.00b	39.80a	1.70d	**80.00d**
Decapitation + GA_3	5.50e	**85.00a**	31.10de	2.73a	49.50b
Decapitation + IAA	7.70c	**79.90a**	39.10ab	2.07bc	**85.00d**
Decapitation + IAA + GA_3	4.60f	**82.00a**	30.20ef	2.73a	58.50c
Decapitation + IAA + BAP	7.00d	65.15bc	35.90abc	1.84cd	**81.00d**
Decapitation + GA_3 + BAP	5.70e	**86.00a**	31.70cde	2.73a	50.00b
Decapitation + GA_3 + PBZ	4.50f	59.70c	30.50ef	1.92cd	50.00b
Decapitation + IAA + GA_3 + PBZ	3.10g	51.30d	26.80fg	1.91cd	41.50a
Decapitation + IAA + GA_3 + BAP	5.90e	**86.30a**	32.50cde	2.73a	51.00b
Decapitation + GA_3 + BAP + PBZ	4.40f	48.15de	26.30fg	1.84cd	49.00b
Decapitation + IAA + GA_3 + BAP + PBZ	4.30f	41.95e	23.40g	1.82cd	51.00b
					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.

5.7.4 Discussion:

Lateral shoot growth: All of the PBZ combinations in this present experiment had less lateral elongation probably because of the PBZ induced inhibition of endogenous GA synthesis. GA_3 added to these PBZ combinations gave higher lateral length than without GA_3 but less than control plants. Exogenous GA_3, alone or in any other combinations was not enough to reverse PBZ induced growth inhibition in decapitated plants. Again this would appear to be due to GA_3 induced reinforcement of correlative inhibition through those released laterals.

A suitable ratio of auxin to GA is required for lateral shoot growth and elongation in many plants (Section 2.2.5.1). A balance of auxin and GA was also found important for lateral shoot growth and elongation in SDP (Section 3.5.4). Any exogenous PGR application to SDP would alter this endogenous balance and accordingly the growth would be altered. It is assumed that in a treated plant, PBZ would reduce endogenous GA synthesis and probably increase auxin availability (Section 4.5.5). This increase auxin : GA ratio is probably favourable for bud release but not for elongation (Table 5.5). However, if GA_3 is applied simultaneously thus maintaining the auxin : GA ratio the balance would favour elongation and correlative inhibition will occur (Table 5.5). It was proposed (Section 5.7.1) that if bud release itself was not inhibited then GA_3 alone or in any combinations would reverse PBZ inhibition of lateral shoot growth. Therefore it was again hypothesised that by delaying GA_3 application, PBZ induced increased auxin would promote lateral bud release (Table 5.5) and application of GA_3 at that stage would establish a suitable balance of auxin to GA_3 so that PBZ induced growth inhibition would be reversed through elongation.

Lateral shoot angle: Angle formed by GA_3 alone or its combinations in this present experiment are similar to Phillips (1969) where, in decapitated *Cupressus arizonica* seedlings, GA_3 induced a normal negative geotropic reaction in upper lateral branches. GA_3 applications to growth retardant treated decapitated soybean and redwood seedlings also restored the dominance of upper lateral buds over lower buds. He further suggested that GA_3 possibly restored the vigorous growth to the upper outgrowing lateral shoots resulting in a typical basipetal spread of correlative inhibition from such actively growing shoots.

5.7.5 Conclusions:

* The reversal of the PBZ inhibition of lateral growth in decapitated plants was not possible with simultaneously applied PGRs (IAA, BAP, GA_3 alone or their different combinations) due to the lack of elongation (e.g. without GA_3 treated plants) but mainly due to induction of correlative inhibition by released laterals (in GA_3 treated plants).
* Alteration of auxin to GA balance was proposed for the PBZ induced inhibition of lateral shoot growth.
* In a PBZ pre-treated plant induced auxin level would increase bud growth and GA_3 added at that stage may provide a proper auxin : GA balance for elongation.

5.8 Interaction of delayed GA_3 application to PBZ treated plants.

5.8.1. Introduction:

PBZ alters the level of the hormones other than gibberellin (Section 2.2.6.5). Simultaneous application of PGRs (including GA_3) with PBZ did not reverse the inhibition of lateral shoot growth in decapitated plants (Section 5.7.5). It was proposed (Section 5.7.4) that delayed GA_3 application in a PBZ treated decapitated plant would reverse PBZ inhibition of lateral shoot growth. Reversal of PBZ induced lateral shoot growth inhibition was achieved by delaying GA_3 application until lateral shoots were released by PBZ in intact plants (Section 4.7). Tamas (1987) also reported that GA_3 only works during the post release phase of bud growth.

To test earlier results (Section 4.7) as well as to understand the process of reversal of growth inhibition in decapitated plants more clearly, an experiment was initiated with intact and decapitated SDP plants, where PBZ and different other PGRs were applied earlier than GA_3 application.

5.8.2. Materials and Methods:

Fourteen (14) treatments were arranged in a randomised complete block design with 10 replications. Out of 14 treatments, 4 were applied to intact plants and the rest to the decapitated plants. The PGRs used in this experiment were BAP (25ppm), GA_3 (500ppm), IAA (20 mg a.i./plant), PBZ (10 mg a.i./plant) and their combinations. In the case of decapitation treatments, all plants were decapitated immediately before the 1st PGR application at 4 weeks. GA_3 was only applied at 8 weeks after sowing (4 weeks after 1st PGR application) on specific PBZ treated plants.

The seeds were sown on 23.01.95 and the data were recorded at 4 and 8 weeks after 1st PGR application. At 4 weeks, each of the treatments had 20 plants and 10 randomly selected plants were used for recording data. However, at 8 weeks (i.e. 4 weeks after GA_3 treatment) each treatment had 10 plants again. The rest of the procedures were followed as per section 3.2.

5.8.3. Results :

Main shoot growth: At 8 weeks, irrespective of the PGR combinations, GA_3 produced taller plants with longer internodes compared to intact control or PBZ (alone) treated plants (Table 5.6).

Lateral shoot growth: At 8 weeks, GA_3 alone treated intact plants produced the lowest number of lateral shoots. In decapitated plants, at 8 weeks, PBZ treated and decapitated (control) plants had statistically similar and higher number of shoots compared to GA_3 treated decapitated plants but their elongation growth was variable, depending on the treatment combinations (Table 5.7). GA_3 reversed PBZ induced growth inhibition of the laterals in all of the treatment combinations (except PBZ + IAA) and all GA_3 combinations produced significantly greater lateral length, with longer internodes, compared to their without

GA_3 treatments. Plates 5.2 and 5.3 show reversal of PBZ induced total and lateral shoot growth inhibition in intact and in decapitated plants respectively.

Lateral shoot angle: Irrespective of treatment combination, GA_3 treated laterals produced more upright growth i.e. narrower branch angle than their - GA_3 counterparts (Table 5.8).

Table 5.6 Effect of delayed GA_3 (500ppm) application on the reversal of PBZ (10 mg a.i./plant) induced main shoot growth inhibition.

Treatments	Plant height (cm)		Leaf number		Internode length (cm)	
	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
Intact control	9.80a*	17.80c*	11.10a	17.50a	0.89a	1.02c
Intact + GA_3		**34.60a**		16.90a		**2.05a**
Intact + PBZ	**4.05b**	5.50d	**10.10b**	**11.40b**	**0.40b**	0.48d
Intact + PBZ + GA_3		23.80b		16.60a		**1.45b**

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

Table 5.7 Effect of delayed GA_3 (500ppm) application on the reversal of PBZ (10 mg a.i./plant) induced lateral shoot growth inhibition.

Treatments	Shoot number		Lateral length (cm)		Leaf number		Internode length (cm	
	4 Wks	8 Wks	4 Wks	8 Wks	4 Wks	8 Wks	4 Wks	8 Wks
Intact control	5.80a*	7.90b	61.65b	157.10ef	32.50b	71.80de	1.90d	2.22de
Intact + GA_3		6.00a		**216.15hi**		62.10bc		3.52g
Intact + PBZ	7.50b	8.00b	15.25f	39.85a	23.95c	**49.80a**	**0.65a**	**0.80a**
Intact + PBZ + GA_3		7.50b		147.55e		**57.40ab**		2.59ef
Decap. (only)	7.60b	**10.00c**	**88.05a**	201.80gh	**39.70a**	86.60ef	2.24e	2.32de
Decap. + GA_3		8.00b		**219.00hi**		74.60de		2.95g
Decap. + PBZ	10.40c	**11.00c**	29.85d	89.55c	**35.10ab**	76.00de	0.84bc	1.19b
Decap. + PBZ + GA_3		**10.50c**		**245.25i**		93.50f		2.66f
Decap. + PBZ + IAA	10.30c	**11.00c**	22.20e	76.20b	32.10b	**52.90ab**	**0.69a**	1.44c
Decap. + PBZ + IAA + GA_3		**10.50c**		167.70ef		69.70cd		2.47ef
Decap. + PBZ + BAP	10.40c	**10.60c**	34.95c	109.60d	**38.60a**	63.10bc	0.91c	1.74c
Decap. + PBZ + BAP + GA_3		**10.50c**		190.20fg		90.40f		2.13d
Decap. + PBZ + BAP + IAA	10.40c	**11.30c**	24.50e	95.65c	31.40b	75.80de	**0.78ab**	1.26b
Decap. + PBZ + BAP + IAA + GA_3		**10.70c**		191.10gh		84.20ef		2.29de
	t	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.
Decap. = Decapitation.

5.8.4. Discussion:

Main shoot growth: Application of GA_3 4 weeks after PBZ application completely overcame PBZ induced growth inhibition and stimulated growth of the main shoot. The

reversal of PBZ induced main shoot growth inhibition was also observed when GA_3 was applied simultaneously or 4 weeks after PBZ application in an earlier experiment (Section 4.7).

Table 5.8 Effect of delayed GA_3 (500ppm) application and angle formation in PBZ (10 mg a.i./plant) treated intact and decapitated plants.

Treatments	Mean angle ( )	
	4 Wk's	8 Wk's
Intact control	88.00a	**87.35d**
Intact + GA_3		45.25a
Intact + PBZ	90.00b	**90.00d**
Intact + PBZ + GA_3		48.00b
Decapitation (only)	87.50a	**87.50d**
Decapitation + GA_3		44.25a
Decapitation + PBZ	90.00b	**90.00d**
Decapitation + PBZ + GA_3		47.50b
Decapitation + PBZ + IAA	90.00b	**90.00d**
Decapitation + PBZ + IAA + GA_3		48.75c
Decapitation + PBZ + BAP	90.00b	**87.40d**
Decapitation + PBZ + BAP + GA_3		44.50a
Decapitation + PBZ + BAP + IAA	90.00b	**90.00d**
Decapitation + PBZ + BAP + IAA + GA_3		50.00c
	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.

Lateral shoot growth: Delayed GA_3 application reversed PBZ induced inhibition of lateral shoot growth for all of the PGR combinations (except PBZ + IAA + GA_3). The delay meant that there were more shoots at the time of GA_3 application (Table 5.7). GA_3 did not have any inhibitory effect on bud growth at that stage but it had inhibitory effects in earlier experiments when applied simultaneously with PBZ in decapitated (Table 5.5) or intact plants (Figure 4.12). These results suggest that GA_3 was only responsible for reversal of the lateral shoot growth inhibition after growth had commenced. In PBZ treated plants, once the growth has commenced, the proposed additional auxin induced by PBZ may induce more bud release but those shoots would not elongate because of PBZ's inhibitory effect in GA biosynthesis. However, application of exogenous GA_3 at that stage (i.e. delayed) would interact with endogenous auxins to give a suitable balance for elongation. This idea was also proposed earlier (Section 5.7.4). The non reversal of lateral growth in PBZ + IAA treated plants with delayed GA_3 application might mean that the auxin concentrations in those plants is too high for the exogenous GA_3 to override. In this system, there were probably 3 sources of auxin supply (i.e. initial endogenous supply + exogenous supply + probable PBZ induced supply), which might make the level of auxin supra-optimal for elongation growth. This same treatment combination was also unable to reverse PBZ inhibition in laterals of an intact plants (Figure 4.20 & 4.21) in an earlier experiment.

(a) (b) (c)

Plate 5.2 Reversal of PBZ induced shoot growth inhibition with delayed GA_3 application in an intact plant: (a) Reversed plant; (b) PBZ treated plant; (c) Control plant.

(a) (b) (c)

Plate 5.3 Reversal of PBZ induced lateral shoot growth inhibition with delayed GA_3 application in a decapitated plant: (a) Reversed plant; (b) PBZ treated plant; (c) Control plant.

Lateral shoot angle: The narrower angle by GA_3 similar to this present experiment was also reported earlier in other experiments (Section 5.5.4; 5.7.4). This was because of the more vigorous and upright growth of the released laterals.

5.8.5. Conclusions:

* Application of GA_3 simultaneously with PBZ or at 4 weeks after PBZ can reverse PBZ induced inhibition of main shoot growth.
* Reversal of lateral shoot growth inhibition by GA_3 was possible after the release of apical dominance irrespective of PGR combinations (except PBZ + IAA).
* In PBZ + IAA, treated plants supra-optimal auxin level compared to GA_3 probably prevented reversal of PBZ inhibition.

5.9 General conclusions from chapter 5.

* SDP plants are strongly gravitropic and the effect of gravity could not be overcome by horizontal orientation of the plants.
* GA_3 induced correlative inhibition by enhancing the growth of the fewer released laterals.
* The response to GA_3 depends on the stage or age of bud growth.
* GA_3 application resulted in fewer lateral shoots and did not increase lateral length irrespective of (pre or post) IAA combinations.
* A suitable balance between auxin and GA_3 was vital for the bud growth and elongation.
* Alteration of auxin to GA balance was proposed for the PBZ induced inhibition of shoot growth.
* Application of GA_3 simultaneously with PBZ or at 4 weeks after PBZ can reverse PBZ induced inhibition of main shoot growth.
* The reversal of the PBZ inhibition of lateral growth in decapitated plants was not possible with simultaneously applied PGRs (IAA, BAP, GA_3 alone or their different combinations) due to the lack of elongation (e.g. without GA_3 treated plants) but mainly due to induction of correlative inhibition by released laterals (in GA_3 treated plants).
* Reversal of PBZ inhibited lateral shoot growth by GA_3 was only possible after the release of apical dominance (or correlative inhibition).
* In a PBZ pre-treated plant, induced auxin probably increased bud growth and added GA_3 at that stage gave a proper balance for elongation growth.
* Another approach to investigate the interaction of PGRs and their effects on the reversal of PBZ induced inhibition of lateral shoot growth in the absence of apical dominance or correlative inhibition, could be the use of shoot cuttings. This is the subject of the next chapter (Chapter 6).