CHAPTER - 1: INTRODUCTION

"Men and plants have come a long way together down through the ages. The association has long since changed from the casual contact of a nomad with a quick meal to that of almost complete interdependence. One of the major goals of civilization has always been to improve the usefulness and reliability of plants in promoting human welfare" (McNew, 1961).

Other than food and shelter, human beings have also been trying from the pre-historic ages, to increase the usefulness of plants by manipulating plant growth patterns to satisfy their own aesthetic aspirations. There are several practical methods available for controlling plant growth including plant breeding or biotechnology (Davis and Andersen, 1989), environmental manipulation (Dawson, 1994), reduced water and nutrient supply (White and Holcomb, 1974), brushing (Baden and Latimer, 1992) or even plant shaking (Napoli and Klee, 1993). However these are not always possible or feasible (Davis and Curry, 1991) and chemical control offers an alternative. At least in the short term, plant growth regulators (PGR's) offer a quick, relatively easy and cheap method of achieving a desirable shape, size and form. Luckwill (1981) proposed that there are certain specific benefits of PGRs which could still be used simultaneously with plant breeding methods. Thus PGRs "are complementary, rather than mutually exclusive, methods of crop improvement". Davis and Andersen (1989) also proposed that PGRs cannot be the permanent solution to the problem of growth control.

The use of PGRs is now at stake because of the higher developmental costs and increased anti-chemical attitude of society (Davis and Curry, 1991). They pointed out that chemophobia is "clouded by emotions rather than clarified facts". To redress this serious problem Cutting and Wolstenholm (1993) suggested several strategic steps including more research to understand the hormonal mechanism of action. This may help to optimise or even stop the use of PGR's. For the last few decades, a lot of work has been done to understand the hormonal mechanism of action of growth control of different plants (Cline, 1994). However, because of the variable and unpredictable nature of effects of PGRs, and the many factors involved (Section 2.2.1), scientists are still trying to understand their mode of action for different plants and also for the different environmental conditions.

Australia has a vast and diverse natural gene pool of wild flowers (Watkins and Heggers, 1981) but limited scientific research has been done on them (Fuss and Sedgley, 1991). Scientific data are also lacking in terms of the crop physiology and production of the native plants (Dawson, 1994). Limited work has been done on Sturt's desert pea (SDP), a most spectacular representative of the Australian native flowering plants, to understand its physiology, particularly in relation to hormones and responses to PGRs (Section 2.3.5).

The normal growth habit of this beautiful plant is variable (Section 2.3.2). Moreover, because of the handling difficulties associated with its delicate flower, it was proposed that SDP be marketed as a pot plant rather than a cut flower (Barth 1990a). Paclobutrazol (PBZ),

a broad spectrum growth retardant (Section 2.2.6.3), has been used commercially to produce attractive SDP pot plants (Section 2.3.4).

After an initial screening of the available growth retardants (ancymidol, CCC, daminozide, flurprimidol and PBZ), PBZ was selected for further studies (Section 3.6). PBZ reduces growth mainly through inhibition of GA biosynthesis and it can also affect the level of other plant hormones and sterols in the plants (Section 2.2.6.4).

This thesis seeks to improve our understanding of PBZ induced shoot growth control mechanisms in SDP. In particular:

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

Chapter 2 presents literature review of shoot growth and development, physiology and biochemistry of important plant hormones and their effects on the shoot growth. The general growth pattern along with up to date information on SDP research is also presented in a precise form in this chapter. Chapter 3 presents results from the experiments conducted with plant hormones and plant growth retardants and discuss their mode of action. Chapters 4, 5 and 6 report results and discusses the possible mode of action of different PGRs, applied alone or in combinations to intact or decapitated plants and to plants obtained from shoot cuttings. Chapter 7 reports the anatomical results obtained from different PGR applications. Chapter 8 integrates the whole research work and proposes directions for future work.

CHAPTER - 2: REVIEW OF LITERATURE

2.1 Shoot growth physiology.

2.1.1 Growth and development:

Thimann (1969) defined growth as an irreversible increase in volume; which may or may not be accompanied by cell division but is always accompanied by cell enlargement. The maturing embryo sac increases in total size without any cell division (Salisbury and Ross, 1992). Depending on the situation, growth is assessed as an increase in one or more size parameters (e.g. weight, length, height, width, area or volume) (Taiz and Zeiger, 1991). The unidirectional elongation of stems, coleoptiles and roots, and the multi directional enlargement of fruits and tubers are examples of growth (Thimann, 1969).

Morphogenesis and differentiation are the two important accompanying steps in growth. The development of form or shape of cells and organs is known as morphogenesis and its course mainly depends on the control of the direction of cell expansion and plane of cell division. Differentiation is a process which prepares the cells to undergo biochemical and structural changes to render specialized functions (Taiz and Zeiger, 1991). Development in the broadest sense, is the whole series of changes that an organism passes through its life, which may be equally applied to individual organs or tissues or even to the cells (King, 1991). In the case of shoot development of *in vitro* roses, 3 stages could be identified and these are: bud sprouting, unfolding of the leaves from the bud and formation of new leaves and flower buds (Marcelis-van Acker and Scholten, 1995).

The growth of the plant structures can be determinate, where growth stops after attaining certain size followed by senescence and death (e.g. leaves, flowers and fruits) or indeterminate, where growth continues from meristems and the structures (e.g. vegetative stem and root) remain youthful. Annual plants and the individual parts of both annual and perennial plants, typically display an 'S - shaped growth curve". This curve has 3 phases: (a) logarithmic phase, where size increases exponentially; (b) linear phase, where size increase continues at a maximum rate; c) senescence phase, where growth rate decreases and senescence commences (Salisbury and Ross, 1992).

2.1.1.1 Cellular basis of growth and development:

Most flowering plants have common processes in their cellular method of growth and development, even though they may show distinctly different plant forms. These common processes are: i) cell division - 2 cells are produced from one mature cell; ii) cell enlargement - daughter cells increase in volume and iii) cellular differentiation - cells become specialised in function (Salisbury and Ross, 1992). In stems, roots and in the grass leaf, cell division and elongation occur simultaneously but separated in space. In dicot leaves, flowers and fruits, the cell division and enlargement are probably separated in time but not in space (Cleland, 1969). The elongation growth in most monocots and dicots is due to an uninterrupted meristem extending a few internodes below the apex (Goodwin, 1978).

Plant cells rarely grow in isolation. The nature of growth and development of a cell is clearly and carefully monitored and coordinated by the neighbouring cells throughout its entire life. The cell to cell communication and coordination in a plant normally take place via plasmadesmata (symplastically) or via the cell wall (apoplastically). Hormones are the major carriers of these messages, which allow the plant to grow accordingly (Taiz and Zeiger, 1991). It is probable that different messages are sent from different tissue types depending on their relative requirements for hormones (e.g. GA) and other cell wall materials (e.g. sterols) (Lenton *et al.,* 1994). Okamoto *et al.,* (1995) reported that a linear age gradient exists for the growth of the cells in stem and root of a higher plant. This gradient is considered to be related to the distribution of endogenous plant hormones and/or metabolites. The detailed effects of important hormones on plant growth are discussed later in section 2.2.

Availability of water in addition to absorbable solutes is an essential prerequisite for plant growth (Edelmann, 1995). Plant cells principally grow by extending cell walls in response to pressure from water uptake in the vacuole (Cosgrove, 1993b). According to the author there are 3 general mechanisms which moderate water uptake: "- by relaxing wall stress to reduce cell turgor pressure; - by modifying the solute content of the cell or its surroundings and - by changing the hydraulic conductance of water uptake pathways". As well as water uptake, the new cell wall and membrane materials are also synthesized at the same time to prevent the wall to become thinner (Salisbury and Ross, 1992). To reduce wall stress and cell turgor pressure, and to allow water inflow through the cell wall, growing cells rearrange the load bearing net work in their walls. Evidence relating to the adaptability of a plant cell to a wide range of wall structures and functions suggest that cell wall extension has more than one mechanism (Cosgrove, 1993a). Cosgrove (1993b) proposed that, cell wall relaxation is achieved by a chemorheological mechanism, which in turn causes water uptake and cell expansion.

With the deposition of new microfibrils on the cell wall adjacent to the plasma membrane, the wall keeps a uniform thickness throughout its growth. The recently deposited microfibrils on the innermost layers of the wall are capable of exerting the most effect on growth. However, with the formation of new cellulose molecules, the existing microfibrils probably elongate and permit some parallel extension along their axes. The random orientation of the newly formed microfibrils tends to produce growth in all directions (e.g. leaf spongy mesophyll cells). In the case of elongating roots, stems and petioles, the microfibril orientation is mainly along one axis and favours wall growth and cell enlargement perpendicular to that axis (Salisbury and Ross, 1992). The orientation of the micro-fibrils is thought to be determined by the orientation of the microtubules. However, the orientation of these microtubules is normally influenced by growth regulators, temperature and wounding (Fujino *et al.,* 1995). Transverse cellulose microfibrils have a higher capacity for elongation. Gibberellin induces transverse orientation of the microtubules to cause elongation. Auxin also has the same effect on microtubule orientation and cell elongation. Longitudinal orientation of

the microtubules was induced by kinetin and ethylene and cell expansion was observed due to that (Katsumi and Ishida, 1991).

2.1.1.2 Shoot growth and development:

The zones of cell division in a plant are called meristems and provide a permanent embryonic tissue for growth and development. Almost all of the mitosis (nuclear division) and cytokinesis (cell division) take place in the meristems (Taiz and Zeiger, 1991). The cells in a meristem are usually isodiametric, thin walled, have prominent nuclei, rich in cytoplasm and have no central vacuole. They contain minor non-green proplastids (Sebanek, 1992).

Meristems are of 2 types namely apical and lateral (Taiz and Zeiger, 1991). Apical meristems (Figure 2.1) are the most active meristems found at the tips of stems and roots of young plant. However, the axillary buds at the nodes also have their apical meristem (Taiz and Zeiger, 1991). Apical meristems of roots and shoots are called primary meristems and are formed during embryo development. The vascular cambium, and meristematic zones of monocot nodes and grass leaves, are called secondary meristem and do not clearly appear before germination (Salisbury and Ross, 1992). Apical meristems give rise to the elongation of tips of shoots and roots and lateral appendages which are basic to the plant form and are called primary growth. The cylindrical bands of meristematic cells in woody stems and roots are called lateral meristems and give growth in girth, called secondary growth (Taiz and Zeiger, 1991).

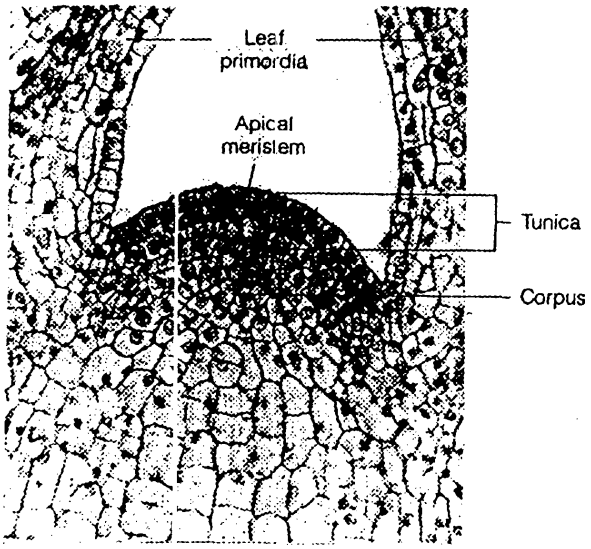


Figure 2.1 The LS of *Coleus blumei* shoot apex (From Taiz and Zeiger, 1991).

The shoot apical meristem consists of two zones. Tunica - the outer zone, where cells divide anticlinally and produce epidermal and subepidermal cells for the stems and leaves. The corpus is the inner zone, where cells divide both anticlinally and periclinally to produce the volume of the expansion. The tunica-corpus layers are often called histogenic layers as they yield progenitor cells for the stem, leaf primordia, lateral buds, and floral appendages (Taiz

and Zeiger, 1991). The growth pattern in each specific case depends probably on the co-operative and competitive activities of tunica and corpus. Once a tiny leaf is developed through this interaction, it controls subsequent developmental patterns of cells around it. Through this process neighbouring cells are transformed into leaf cells and form the whole leaf (Galston, 1994).

It is suggested that both chemical and biophysical systems work together to form the primordia at the apex (Lyndon, 1994). The leaf primordia are produced on the edge of the apical dome. The junction of a leaf primordium and the stem is known as a node and the regions between nodes are called internodes. The term phytomers is proposed to indicate one unit of shoot development, which consists of the node, the leaf initials and the subtended internode, along with the axillary buds. Experimental evidence supported the view that there were no strict boundaries confining the cells of a phytomer. The development of a specific meristematic cell was always in a revocable condition for a particular phytomer (Taiz and Zeiger, 1991). Depending on the environmental and internal factors, axillary meristems may become lateral branches or produce flowers (Meyer and Anderson, 1947).

Cleland (1969) reported the term sub apical meristem or intercalary meristem in some stems, which is situated below the apical meristem. This meristem yields enormous numbers of cells in the developing stem. Stem length mainly depends on it and dwarf plants are produced by the inactivation of this sub apical meristem. The elongation zone is situated below the sub apical meristem (Cleland, 1969), where cells increase in length and width (Taiz and Zeiger, 1991). Cell division in the sub apical meristem is normally in a transverse plane (Goodwin, 1978). The subapical meristem mainly produces longitudinal growth. Transverse expansion may occur at the same time or when elongation growth is slower (Phillips, 1971).

2.1.2 Apical dominance in shoot growth:

According to Martin (1987) apical dominance means "a) complete or nearly complete control of lateral buds by the apex, b) dominance of one growing shoot over another, and c) the apex influence on the orientation of branches and leaves." Brown *et al.*, (1967) proposed the term apical control to refer to the control by a main growing point of a perennial plant over all of its branches while apical dominance refers to bud inhibition on a single elongating shoot. Sebanek (1992) reported that the degree of apical dominance naturally varies from very strong inhibition in pea to zero inhibition in *Kochia scorpia.* Apical dominance has immense significance to agriculture through its effect on branching patterns or plant form. Manipulation of lateral bud out growth (i.e. reduced apical dominance) through pruning or training can increase food production, reduce the cost of production or can make a plant ornamentally more valuable (Cline, 1991).

The growth potential of axillary buds depends on 3 interdependent factors namely: the intrinsic growth potential of the buds, their position on the plant and the influence of other plant parts (Marcelis-van Acker and Scholten, 1995). Although the terminal bud acts as the major source of inhibition of lateral bud outgrowth, there is enough evidence that unfolded

leaves, cotyledons, portions of the stem or even roots can exert some effect (Hosokawa *et al.*, 1990). The degree of relative interference from other parts on apical dominance depends on the species and on the plant types (Cline, 1991). The relative interference of different plant parts on bud growth is known as correlative inhibition (Rubinstein and Nagao, 1976).

2.1.2.1 Mechanisms of apical dominance:

Several hypotheses or theories were put forward by different workers (Tucker and Mansfield, 1973; Hillman, 1984; Martin, 1987) to explain the mechanism of apical dominance. However, the understanding of the basic mechanism of apical dominance is still unresolved (Tamas, 1995). Most of the workers supported some common theories which includes: nutritive theory, nutritive diversion theory, direct auxin action theory, indirect auxin action theory and the hormonal balance theory to explain this complex phenomenon. Fewer other theories like vascular connection theory (Hillman 1984), oligosaccharine theory (Martin, 1987) were also proposed to explain the same mechanism. However, a brief discussion on some of these major theories or hypotheses is presented here.

i) Nutritional hypothesis: Apical dominance may be explained on the basis of the internal competition between buds for nutrients (mineral nutrients, sucrose or water). It is proposed that the availability of the nutrients near an inhibited bud is the prime requirement for its release. Shoot and root apices act as metabolic sinks because of their high growth potential and deprive the laterals of nutrients. Decapitation of the apex removes that sink so that nutrients are available to the laterals for their growth. The direct application of nutrient to the inhibited laterals did not initiate their growth, probably because of the principal demand and acropetal transport of the minerals to the shoot apex. However, further experimental evidence alternatively suggests that the cessation of auxin production through any of the restrictive treatments including decapitation, gravimorphic treatments or exposure to ethylene at shoot apex actually releases apical dominance (Cline, 1991).

In a decapitated dwarf pea plant, increased water content was correlated with the renewed cell cycle. Xylem water potential might have been affected following decapitation and ultimately many more physiological and metabolic responses were also affected. However, water was still not considered as the primary mechanism of apical dominance because some of the effects of water may be explained on the basis of ABA (Cline, 1991) or ethylene concentrations (Martin, 1987).

ii) Auxin directed nutrient transport theory: This theory is based on the idea that apically produced auxin attracts nutrients away from the laterals and inhibits their growth. This theory is criticised because of the inability of direct auxin application to the laterals to increase bud growth. Brown *et al.*, (1979) reported that in sunflower, auxin has an indirect role in apical dominance through increased sink activity with increased meristematic tissue formation at the stump apex of a decapitated sunflower plant. However, because of poor understanding of the process of phloem transport, not enough progress has been made to test this auxin directed nutrient transport theory (Cline, 1991).

iii) Hormonal balance hypothesis: At present the most widely accepted hypothesis is based on hormonal control. Martin (1987) described it as a "clue to the disputatious state of affairs." A brief role of the 5 major hormones in apical dominance is discussed below.

Auxin: The rarity of auxin transport from the main shoot axis to the apex of the laterals (Morris, 1977) and the absence of reduced auxin levels in the laterals before their growth (Hillman *et al.,* 1977) attracted a wide range of criticism of the auxin theory of apical dominance. To accommodate this criticism an indirect role of auxin on apical dominance has been proposed. But studies with transgenic plants, where endogenous auxin level is artificially increased or decreased, and also from auxin resistant mutants, still support a significant role of auxin in apical dominance (Cline, 1991). To explain some of the criticism regarding auxin transport, Bangerth (1993) proposed a new hypothesis called the "Primigen dominance hypothesis". He proposed that the dominant organs usually develop earlier and have a greater rate of auxin transport compared to inhibited organs. IAA exported by the dominant apex suppresses auxin production and export from the subordinate buds or apices through an 'auto inhibitory mechanism' and caused inhibition in the subordinate organ.

Auxin may regulate cytokinin availability by the conversion of biologically inactive cytokinin already near an inhibited bud to an active form, thereby fulfilling the cytokinin requirement of the lateral bud in a decapitated plant (King and Van Staden, 1990).

Wang *et al.,* (1994) recently proposed that IAA from the apex or when applied immediately after decapitation exerts its inhibitory effect by sustaining bound water in the buds. In this process IAA blocks the alteration of the membrane lipid and other cellular processes that otherwise lead to the release of apical dominance following water entry into the cells.

Gibberellins: Gibberellin treatments mostly cause an increase in apical dominance (Phillips, 1969; Martin, 1987; Cline, 1991) but may promote subsequent growth of buds once they are released from the apical dominance (Martin, 1987; Tamas, 1987; Cline, 1991). In peas and beans gibberellin was involved in shoot growth but did not initiate lateral bud outgrowth. Gibberellins only stimulated lateral growth after they had begun to grow (Rubinstein and Nagao, 1976). Gibberellin induced bud outgrowth was also interpreted as being indirect, through its effects on floral induction, alteration in sensitivity to cytokinin or enhanced cytokinin concentration which in turn caused bud outgrowth (Cline, 1991). Woolley and Wareing (1972) proposed that gibberellins normally antagonize cytokinin action during the initial release of lateral buds, but promote cytokinin-induced lateral shoot growth after their release.

Gibberellin induced apical dominance has also been explained in term of increased auxin transport from the apex to the tiller bud area of sorghum (Isbell and Morgan, 1982). Interaction was found between auxin and gibberellins in relation to the lateral bud release in brussels sprouts, where sub-optimal auxin activity relative to GA caused lateral bud inhibition (Thomas, 1972). A suitable auxin : GA ratio is necessary for the further growth of a lateral bud after being released from inhibition (Sebanek, 1992). Cutter (Phillips, 1975) proposed

another hypothesis for *Phaseolus coccineus*, where the interacting effects of auxins and gibberellins depend on the stage of the lateral bud development during treatment.

From all of the available evidence it is clear that, whether direct or indirect, GA_3 has a very important and specific role in apical dominance, which needs to be clarified.

Cytokinins: Irrespective of their origin (root or shoot), cytokinins initiate lateral bud growth in many species (Cline, 1991; 1994). However, in the presence of a very dominant apical bud, root derived cytokinins enhance apical dominance presumably through transportation of the cytokinins to the apical bud (Tamas, 1987). Recently Buising *et al.*, (1994) proposed that BAP induced release of soybean apical dominance was probably due to BAP's initial inhibitory effect on DNA synthesis and the resulting delaying effect on cell division followed by subsequent reprogramming of gene amplification.

In some species, after an initial cytokinin application gibberellin and/or auxin treatments were required for continued lateral bud outgrowth (Cline, 1991). "Cytokinin synthesis in or its allocation to buds" affects the amount of IAA export from different buds and hence cytokinin controls dominance relationship among the buds. Thus cytokinins mainly act as a modifiers of IAA synthesis or transport rather than IAA antagonists (Li and Bangerth, 1992). Klee and Romano (1994) proposed that the ratio of auxin to cytokinin is the major control of bud growth rather than the absolute amount of auxin and cytokinin.

ABA: In decapitated pea seedlings, apically applied ABA inhibited axillary bud growth. In *Phaseolus vulgaris* ABA alone slightly improved axillary bud growth but when applied together with IAA and kinetin it increased the inhibitory effect slightly (Phillips, 1975). Auxin induced inhibition has sometimes also been explained through a secondary effect of ABA. In tomato, ABA synthesis is enhanced through the transport of apically produced auxin in or near the lateral buds and this inhibits bud growth (Tucker, 1977). Tucker and Mansfield (1973) indicated that the endogenous cytokinin level was much higher in an inhibited lateral bud of *Xanthium strumarium* than a released bud but it was inactive in breaking apical dominance because of a higher concentration of auxin induced ABA. In tissue culture of root tuber of sweet potato the lack of cytokinin to counter ABA's action in the culture was proposed as the reason for the inability of GA to promote bud growth at high concentration (Khan, 1975). Recently Pearce *et al.*, (1995) concluded that a balance of increased IAA associated with reduced ABA content in the buds releases apical dominance in *Elytrigia repens*.

Ethylene: There is evidence that ethylene can prevent auxin transport and inhibit lateral bud out growth. Again, in a decapitated plant, cytokinin can override ethylene induced inhibitory effects on lateral bud outgrowth (Phillips, 1975). Li and Bangerth (1992) concluded that ethylene was not a secondary messenger for mediating auxin's action in apical dominance rather it might act via its effect on IAA synthesis or transport. However, Cline (1991) concluded that the specific role of ethylene in apical dominance, whether produced due to higher auxin concentrations or otherwise has not yet been clearly resolved.

To summarise the role of hormones in apical dominance, some researchers support the hormonal balance approach (Wooley and Wearing, 1972; Field and Jackson, 1974), whereas

others support a compromise approach involving hormone and nutrients (Patrick and Wareing, 1978). But it is clear, none of the mentioned theories or hypotheses are without criticism. Different hypotheses have emerged from different laboratories based on different experimental evidence obtained under specific experimental conditions. Obviously a balance of plant hormones plays a major role in controlling apical dominance but plant hormones cannot be the only factors because several other factors are also important for the control of apical dominance (Hillman, 1984; Taylor *et al.*, 1995).

2.1.2.2 Other factors affecting apical dominance:

The plant form in a particular species is a genetically inherited characteristic and Phillips (1971) reported variations within species and varieties in the expression of the degree of apical dominance. The degree of apical dominance also depends on environmental and physiological processes and on the age of the plant (Martin, 1987). Martin (1987) listed 9 chemical (IAA, GA, cytokinin, ABA ethylene, proteins, oligosaccharins, inorganic nutrients and CO_2) and 6 physical factors (temperature, radiant energy, gravity, bioelectrical fields, tension and water) that normally regulate apical dominance in plants. He added that the "environmental and physical factors can override genetic features and alter chemical events regulating apical dominance". Hillman (1984) also listed 14 different factors, including some of those environmental and chemical factors mentioned by Martin (1987) that can release apical dominance.

Soil may influence bud-burst indirectly through its effect on root growth or directly through the supply of nutrients (Williams, 1976). Gravity induced stress (horizontal or upside down orientation of the shoot) may enhance the growth of inactive buds. However, with few exceptions, the evidence still suggests asymmetric auxin transport as a mediating factor in releasing apical dominance in a gravistimulated plant (Cline, 1991).

In a field grown plant, low light increased apical dominance and high light decreased the process. The effect of light was reported to be mediated through the alteration of hormonal and carbohydrate content and also the total chemical transport features of the plant (Martin, 1987).

Lateral buds were not released if plants were exposed to increased CO_2 content for a long time but in some species CO_2 induced early lateral bud growth was also reported (Martin, 1987). In *Phaseolus*, a higher N content increased lateral bud outgrowth without decapitation. Other inorganic nutrients like P and K also increased bud release. However, their role in apical dominance release is suggested as indirect because they become available and act according to the signal expressed by the vegetative growth (Martin, 1987).

With the advent of ageing and reproduction, apical dominance is diminished (Cline, 1991). Flowering decreased the level of dominance in oats. On the contrary, axillary bud growth was severely restricted following reproductive development in *Phaseolus* through the "reproductive dominance" induced by the bean fruits (Tamas, 1987).

2.1.2.3 Events following release of apical dominance:

Cellular and subcellular: The release of apical dominance involves sharp changes in biochemical and cellular processes in the buds. Within 3-10 hr following decapitation, most herbaceous plants initiate bud elongation. In decapitated pea plants, the mitotic index was increased and cell division was enhanced 12 hr after decapitation (Cline, 1991). In *Phaseolus* decapitation reduced ABA level and in *Pisum* increased synthesis of active cytokinins in axillary buds (Tamas, 1995). Following decapitation of *Bidens pilosus* L. increased K^+ ions were measured in the nodes of the released buds (Martin, 1987). A number of other changes occurred in the cellular contents of released buds such as: increased starch content, increased protein content, increased mRNA expression of ribosomal protein gene, increased ATPase activity in plasmalemma etc. However, the initiation of mitotic activity following decapitation depends on the degree of inhibition and also on the stage of the cell cycle, at the time of initial inhibition (Cline, 1991).

Response of other lateral buds: The lateral buds on a stem respond differently to different bud breaking factors because of their different growth potential (King and van Staden, 1988) and their different degree of apical dominance (Cline, 1991). In a species with incomplete apical dominance (e.g. *Phaseolus vulgaris*) mitosis, cell expansion and bud growth proceeds slowly but in a species with complete apical dominance (e.g. *Tradescantia*) mitotic activity and growth is inhibited in the early stage of bud development (Tamas, 1995). Again, decapitation of a plant with weak apical dominance accelerated elongation of all laterals particularly those more developed than the others. But decapitation of a plant with strong or moderate apical dominance accelerated outgrowth only from the highest lateral bud, remaining after decapitation. Bud size and age were the other 2 most important determining factors for the bud outgrowth. Gould *et al.,* (1937) reported that the growth potential of a pea bud at a node was "a consequence of the number of buds and the number of primordia within a bud that are established while the terminal shoot is growing". Decapitation of a bean plant at ≥ 4 leaf stage encouraged initial growth of all laterals but subsequent growth was found only in the subterminal laterals. Competition for nutrients was suggested as the cause of this effect (Cline, 1991). King and van Staden (1990) explained the growth of the lower buds in a decapitated pea plant, on the basis of the depleted auxin concentration and increased root supplied growth regulators and nutrients.

2.1.3 Geotropism and shoot growth:

Gravitropisms or geotropisms are "growth movements of plant organs orientated in the direction of gravitational acceleration" (Mohr and Schopfer, 1995) or simply is the gravity stimulated movement of a plant organ (Sebanek, 1992). A. B. Frank in 1868 first proposed the term geotropism to described the observed geotropic curvature of a plant towards a gravity vector (Audus, 1967). Most plant organs acquire a particular symmetry angle to the gravity vector but if deflected from the stable equilibrium position, the plant changes that angle to restore their preferred orientation (Audus, 1967). The lateral stem and root branches of higher

order are generally geotropically insensitive (Sebanek, 1992). However, gravity performs a very significant role in the regulation of bud growth and also in the lateral organ orientation (Phillips, 1975; Cline, 1991).

The curvature-inducing effects of gravity may be eliminated by rotating the plant organ slowly and continuously about its main axis on a clinostat, but in practice such rotation on a clinostat sometimes does not remove all curvatures (Audus, 1967).

It was proposed earlier (Lyon, 1962) that gravity maintains an even distribution of auxins within the growth zone of the plant's axis through an effect on auxin transport. Longman (1968) proposed that, in woody stems, gravity brings about an innate variation in the hormonal metabolism and hormonal status of the apices, which might ultimately guide the differential accumulation of nutrients and promotion or inhibition of the shoot growth.

2.1.3.1 Types of geotropic responses:

Geotropic responses are classified according to the equilibrium orientation of the organ relative to the direction of the gravity force. In orthogeotropic plants the central axes of the plant array themselves parallel to the gravitational pull and they grow either towards the gravity i.e. positively geotropic (e.g. primary roots) or away from the gravity force i.e. negatively geotropic (e.g. main stem). However, a lateral organ usually attains equilibrium at an angle to the gravity vector depending on its stage and condition of development. This growth is known as plagiogeotropic (Audus, 1967) or plagiotropic (Sebanek, 1992). Sometimes a plagiogeotropic organ shows a specific growth habit by extending horizontally but strictly at right angle to the vector (e.g. rhizomes) and termed as - diageotropism (Audus, 1967).

2.1.3.2 Mechanisms of geotropic response in shoots:

Geotropism or gravitropism is expressed through 3 different components: perception of the signal, transduction of the signal, followed by an asymmetric growth response (Brock *et al.*, 1992).

Generally the perception of gravity in plant cells involves the movement of free-falling statolith, present in the gravity-sensitive plant cells called the statocytes. These statoliths are presumably starch grains, located within amyloplasts, which empower the plant to track the direction of gravity by sedimentation to the lowermost part of the cell through the cytosol. In a coleoptile, the apical region might be the principal but not the only site of graviperception (Taiz and Zeiger, 1991). In many angiosperms, amyloplasts are confined outside the vascular bundle as 1-2 layers of cells called the starch sheath. The inner layer of cortex is generally formed by these starch sheath (Salisbury and Ross, 1992).

The role of amyloplasts as gravity receptors in the shoots has not been conclusively demonstrated (Salisbury and Ross, 1992). The union of amyloplasts with the endoplasmic reticulum near the plasma membrane may redirect auxin transport in someway. The redistribution of auxin (a rapid net loss in the peripheral cells on the upper side and net gain in

the lower peripheral cells) in a ho izontally grown coleoptile could produce gravitropic curvature due to increased growth rate in the lower side and decreased growth in the upper side (Taiz and Zeiger, 1991). In a horizontally grown stem, the upper cells would not grow even though they are stretched by the elongation of bottom tissues, probably the yield threshold of the cell walls of the upper tissues were higher than the bottom tissues (Salisbury and Ross, 1992). This uneven grov/th in upper and lower cells was associated with the increased elongation of the epiderma cells on the lower surface and decreased on the upper surface during gravitropic stimulus (Meicenheimer and Nackid, 1994).

The stated auxin redistribution might involve lateral auxin transport. The unequal lateral redistribution might produce an unequal pH gradient across the shoot and ultimately cause wall loosening which is partly responsible for the differential growth (Taiz and Zeiger, 1991). Montague (1995a) reported that this differential growth response was not mediated through the participation of cell division.

Questions were raised regard ng the hyperactivity of the laterally redistributed auxin for the production of enough curvatu e (Taiz and Zeiger, 1991). However, this was explained in terms of increased (in the lower issues) and decreased (in the upper tissues) sensitivity following gravistimulation. The changed sensitivity to auxin plays very crucial role in some dicots and could happen at the same time or instead of auxin transport mechanism (Salisbury and Ross, 1992).

There are a few other theories regarding the gravity stimulated redistribution of auxins. The important one is that the pressure created by amyloplasts on the plasma membrane alters the electric potential throughout the gravistimulated cells and therefore some auxin is transported laterally. Other theories are: (a) in horizontally orientated coleoptiles, bound auxins are stimulated to produce free auxin; (b) free auxins are released from intracellular compartments; (c) there is increased auxin leakage from the stele to the cortex of the lower side (Taiz and Zeiger, 1991).

Experimentally it was proved that the gravitropic curvature did not occur if sufficient auxin is not present (Brauner and Hager, 1958). But experimentally a specific role of plant growth regulators other than auxins were also proved. Cytokinin (BAP) in soybean hypocotyl segment abolished geotropic sensitivity by increasing undifferentiated nucleic acid synthesis (Krul, 1968). Asymmetric distribution of endogenous GAs, ABA and IAA in geotropically stimulated shoots of *Ribes nigrum* was also reported (Reinhold, 1978). Kaufman and Song (1987) proposed that in conifer shoots (e.g. *Cupressus arizonica*), GA_3 and different levels of IAA, increased ethylene evolution and induced upward turning of the lateral shoots. Inhibitors of ethylene action or synthesis reduced the rate of gravitropic bending in some plants. It was proposed that ethylene in some way inhibited the growth on the top of a horizontally placed stem. However, increased ethylene level was found in the bottom tissues instead of top tissues (Salisbury and Ross, 1992).

A specific role of calcium was also proposed to explain gravitropism. Following gravistimulation, the upper side of the coleoptile accumulates calcium and those Ca^{++} ions

might increase the effect of a lateral auxin gradient on differential growth by making upper cell walls less extensible (Taiz and Zeiger, 1991). It has been reported that extracellular Ca^{++} plays an important part in the tropic response of plants and root cap applied Ca^{++} chelating agent inhibits gravitropism (Brock *et al.*, 1992).

The presented discussions are unable to provide a clear understanding of the mechanism of the gravity induced bending but Kaufman *et al.*, (1995) recently proposed a broad based approach by including several processes to explain shoot gravitropism. Their proposal involved "proton pumping, calcium redistribution, calmodulin synthesis and activation of calmodulin-binding proteins and auxin induced cell wall loosening and wall synthesis".

2.1.3.3 Other factors affecting geotropic responses:

Geotropic behaviour of an organ is not rigid and a range of circumstances can change the response (Audus, 1967). These factors may be internal or external.

Internal factors: The main shoot apex (orthogeotropic) can affect the growth of the lateral branches to produce plagiogeotropic growth. The removal of apical and axillary buds changed the growth of the plagiogeotropic rhizomes of potato into an erect shoot with leaves (Audus, 1967).

External factors: The negatively orthogeotropic stems of *Lamium* develop a plagiogeotropic habit at temperatures just above freezing. Both qualitative and quantitative changes in geotropic responses were observed with changed light conditions. Dark grown, diageotropic *Aegopodium podagraria* rhizomes become positively plagiogeotropic with red light. In *Aegopodium* the normally diageotropic rhizomes grow negatively plagiogeotropic when the surrounding atmospheric CO_2 is enriched. A horizontally positioned root grows vertically downward (orthogeotropic) in sharp sand but remains positively plagiogeotropic in moist air or in glass micro beads (Audus, 1967).

It is clear from these discussion (Section 2.1.3.2 and 2.1.3.3) that alteration of the hormonal level (specifically auxin) plays the major role in gravitropic movement (Section 2.1.3.2). Other factors perhaps affect gravitropic movement indirectly by changing the hormonal balance (?).

2.2 Physiology and biochemistry of PGRs and their effects on shoot growth.

2.2.1 Plant hormones:

"Plant hormones are a group of naturally occurring organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development" (Davies, 1987). Auxins, gibberellins, cytokinins, ABA, and ethylene are the 5 groups of plant growth hormones (Takahashi, 1986). Scientists are still not accepting jasmonic acid, polyamines, brassinosteroids and phenolic acids as plant hormones because of insufficient information regarding their distribution in the plant kingdom (Sebanek, 1992).

Plant growth regulators (PGRs) on the other hand, "form a diverse and ill defined group of compounds" (Luckwill, 1981). PGRs include naturally occurring hormones (plant hormones); ethylene releasing agents; hormone transport inhibitors; hormone mimics; hormone antagonists; growth retardants; growth inhibitors; defoliants, desiccants and ripening agents; others. By definition the PGRs or plant bio-regulators (the term reported by Schott and Walter, 1991) do not have any nutritive or phytotoxic effects at a very low concentration (Rademacher *et al.*, 1987). Plant hormones usually exist at a concentration <1μM and higher concentration are considered as supra optimal (Naqvi, 1995).

For a specific hormone, the response system should have 3 major domains: a) the proper cells must contain enough hormone; b) the hormone must be recognised and bound tightly with the target cells (e.g. receptor proteins) and c) the receptor protein must induce metabolic change towards amplification of the signal or messenger (Salisbury and Ross, 1992).

Although it is not clear how the specific genes interact with hormones to produce identical phenotypic effects (Lyndon, 1994), it is well established that plant hormones control gene activity. The activation of genes at low hormone concentration typifies a large amplification process, which involves repeated transcription of DNA into mRNA, followed by translation of those mRNA to enzymes with high catalytic activity. These finally direct a many fold increase in cellular product and expression of the phenotype of a specific species (Salisbury and Ross, 1992).

To understand hormonal effects and their interactions, now-a-days genetically engineered and transformed plants are being used. *Agrobacterium tumefaciens* is a pathogenic bacterium whose genomes (e.g. the so called T_i plasmid) contain 2 genes specifically responsible for IAA and cytokinin synthesis. The T_i plasmid can be isolated and fragments can be prepared with only the desired genes. Using this technique and by using the promoters for those genes, under certain conditions (a certain stage of plant development and in a particular organ), the plant growth mechanisms are being studied (Sebanek, 1992). However, there are fundamental differences in growth pattern following endogenous or exogenously supplied hormones through the transgenic method (Klee and Romano, 1994).

Bruinsma (1980) proposed that the exogenously applied PGRs might reach to the different sites to that of an endogenous hormone and in that way exogenously applied PGRs might express a different kind of function. The author also proposed that exogenously applied PGRs can influence biosynthesis, translocation or metabolic conversion of endogenous plant hormones or can substitute or supplement depleted endogenous hormonal levels.

Leopold and Nooden (1984) suggested that hormones generally do not work alone and they presented 4 general interaction patterns through which hormones affect plant growth and development: an equilibrium or ratio between hormones; opposing effects between hormones; changing effective concentrations of one by another hormone; and through sequential effects of different hormones. Therefore if measurable effects from an applied PGR sometimes not visible, it could be due to limiting endogenous level or other PGRs might have interactions and masks its effects (Munoz, 1995).

Little and Pharis (1995) reported that hormones are chemical signals, which probably transduce different environmental cues known to affect plant growth. Other than environmental variations (Menhenett, 1979), the observed responses to different species vary greatly with PGR application because of the differences in absorption, transport or metabolism of the compound (Sachs and Hackett, 1972). In pome fruit the degree of growth control was dependent on the dosage, relative efficacy and formulation of the materials, method of treatment, time of application, as well as the soil type, soil pH, soil organic matter content, tree age, tree vigour, rootstock, scion, and amount, frequency, and the method of irrigation (Davis and Curry, 1991). Moreover it was also found to vary even between the spray personnel (Tayama *et al.,* 1992). With so many factors involved, the practical application of PGR's is fraught with difficulty.

2.2.2 Auxins:

Went introduced the term auxin and in Greek it means 'to increase'. IAA (Appendix I), 4-chloro IAA, PAA and IBA are the common auxins found in the plants. Compounds readily oxidised *in vivo* to IAA and activated only after conversion, also have considerable auxin activity in different plants (indole acetaldehyde, indoleacetonitrile, indole ethanol). NAA, 2,4-D and MCPA are synthetic auxins. Auxins are defined as a group of compounds "similar to IAA in having a carboxyl group attached to another carbon-containing group (usually -CH_2-) that in turn is connected to an aromatic ring" (Salisbury and Ross, 1992).

2.2.2.1 Physiological effects of auxins:

Depending on some of the other factors (Section 2.2.1), auxins show a dualism in their action (Leopold, 1960). Auxins normally cause elongation growth of the stems and coleoptiles (Taiz and Zeiger, 1991). But, the growth of the pathogenically important lower plants (fungi, bacteria and virus organisms) was inhibited by auxins (Leopold, 1960). Auxin might have a possible growth regulatory effect on leaf veins. IAA is known to delay the early stage and to promote later stages of leaf abscission probably by inducing ethylene synthesis. Young leaves are less sensitive to ethylene than the older leaves (Taiz and Zeiger, 1991). Flowering and fertility of the plants were also influenced by auxins (Garbers and Simmons, 1994).

Auxin induces adventitious root development in stem cuttings of horticulturally important crops. In general, shoot supplied auxin has an effect on the root initiation. Auxin induced ethylene synthesis (with higher concentration) associated with retarded shoot and root growth has been observed in most species (Salisbury and Ross, 1992).

Auxin has a direct role in inhibiting lateral shoots. Auxin produced by an active apex can produce wider angles in the subordinate branches (Janick, 1986). A detailed discussion about the role of auxin in apical dominance is presented in section 2.1.2.

Auxin has also a specific role in maintaining and reinforcing tissue polarity and this polarity ultimately leads the differentiation of vascular tissues along auxin transport channels

(Tamas, 1995). Aloni (1995) proposed that auxin in the presence of cytokinin, stimulates early stages of vascular differentiation but later stages may occur without cytokinin.

The effects of light (phototropism) and gravity (gravitropism) on the plant growth may be mediated through the lateral redistribution of auxins. An auxin gradient and uneven growth can clearly be measured in response to unilateral light, which is presumably a result of lateral auxin transport at the tip. However, this unequal auxin is apparently not an effect due to photooxidation of auxin on illuminated side or increased synthesis on the shaded side (Taiz and Zeiger, 1991). Again the unequal lateral auxin redistribution in a gravistumulated shoot probably produces an unequal pH gradient resulting in wall loosening and differential growth responses (Section 2.1.3.2).

By influencing phloem transport auxin might enhance assimilate movement towards an auxin source (Davies, 1987). In citrus, IAA played a major role in assimilate accumulation (Kojima, 1995). In Soybean hypocotyl segments, auxin enhanced lipid incorporation into plasma membrane and stimulated phospholipid and sterol synthesis (Goodrich-Tanrikulu and Travis, 1995). On the other hand, disappearance of carbohydrate fractions and accumulation of nitrogenous materials are very common following a high auxin application (Leopold, 1960).

2.2.2.2 Mechanisms of auxin action:

The mechanism of auxin-induced growth has been studied at the tissue, subcellular and gene level. The tissue level research was mainly confined to auxin induced rapid growth kinetics and other rapid responses in tissue segments or in an intact plant. The identification and isolation of auxin receptor proteins through radioisotope-labelled auxins was the target of subcellular research and auxin induced changes in gene expression has been the concern of DNA (gene) level research (Taiz and Zeiger, 1991).

Auxin can cause a rapid and dramatic growth promotion of coleoptile or stem sections (within 10 min.) and can continue for many hours with a 5 to 10 fold increase in growth rate (Salisbury and Ross, 1992). Auxin induced cell elongation growth is the most studied mechanism, but auxin can also induce cell division in some tissues depending on other factors (Thimann, 1969).

Cell expansion induces both water absorption and permanent stretching of the cell wall and it is an irreversible process (Taiz and Zeiger, 1991). In auxin induced cell expansion, the cells maintain a more negative water potential along with greater cell wall elasticity compared to the non treated cells. They absorb more water and the cell wall yields more easily than untreated cell walls. Researchers concluded that auxins can induce wall loosening or increased plasticity of the walls in treated cells (Salisbury and Ross, 1992).

To explain cell wall loosening 3 mechanisms were considered over the last 30 yrs, but out of those, two have been rejected. The acid growth hypothesis is the most widely accepted mechanism, where auxins secrete H^+ from the receptive coleoptile or stem section cells. These H^+ ions then lower the pH and allow wall loosening and subsequent fast growth. The low pH probably also allows certain cell wal degrading enzymes that are inactive at a higher pH to

break bonds in wall polysaccharides and permit the walls to expand more freely (Salisbury and Ross, 1992).

Vanderhoef and his associates (Vanderhoef, 1979) reported that a low pH in cell walls of soybean hypocotyl sections yields rapid elongation only for 1-2 hour but the sections grew faster for about 1-2 days in auxin due to some unknown mechanisms (Salisbury and Ross, 1992). Taiz and Zeiger (1991) proposed that to achieve cell elongation auxin must effect some other important plant cell growth factors (e.g. uptake or generation of osmotic solutes; maintenance of cell wall structure and hydraulic conductivity of the cell membrane). In a recent review, after a thorough discussions of the acid growth hypothesis, Kutschera (1994) also rejected this hypothesis and concluded that IAA induced growth and cell wall loosening is independent of the proton secretion. Napier and Venis (1995) once again questioned the acid growth hypothesis and proposed that increased H^+-efflux alone is insufficient for auxin induced elongation. They also concluded that, at least in maize coleoptiles and in pea epicotyls, the cells probably respond quickly by an enormous range of responses including gene activation (through mRNA stabilization and increased transcriptional activity), H^+-efflux, hyperpolarization, secretory activity and enzyme activation by post-transcriptional processing. Recently Valero and Labrador (1995) proposed that auxin might also effect the level of the cell wall glycanhydrolytic proteins, which are related to the wall loosening process.

There are possibilities that auxin might promote the release of calcium from the cytosol into the extracellular space initially through the activation of the plasma membrane calcium pump. Auxin may also alter calcium ion efflux from the vacuole into the intracellular compartments. The changed cytosolic calcium levels in turn could effect the calcium-activated regulator protein calmodulin, which controls the activity of protein kinases. Activation of the different enzymes by phosphorylation might trigger a cascade of consequential biophysical processes (Taiz and Zeiger, 1991). Napier and Venis (1995) reported that auxin induced enhanced intracellular calcium concentration may raise the rate of constitutive secretion, which is co-ordinated with enhanced cell wall synthesizing activity and plasma membrane hyperpolarization. Felle *et al.*, (1992) questioned the role of Ca^{++} as a second messenger for the auxin action because of its low kinetics as well as a minimal observed response in the systems.

Thus it is clear from the above discussion that auxin induced elongation is a very complex matter and still a lot of contradictions remain to be resolved.

2.2.2.3 Auxin metabolism:

Plants regulate the level of auxin through its metabolism (e.g. synthesis, degradation and deactivation) and thus influence a number of physiological processes (Taiz and Zeiger, 1991).

Auxin synthesis: Although auxin was the first hormone to be discovered, its biosynthetic pathway is still not clearly understood (Nonhebel *et al.*, 1993). The apical meristems of shoots, young leaves and developing fruits are possible sites of auxin synthesis

and contain the highest concentratior of free auxin but mature leaves and root tips can also produce lower amounts of IAA (Tai: and Zeiger, 1991). Most studies indicate that IAA is synthesised from tryptophan (Figure 2.2) via 3 possible intermediates (tryptamine, indole-3-pyruvate and indole-3-acetaldoxime) but there is not enough evidence to prove that indole-3-pyruvate is the major auxin biosynthetic path way (Nonhebel *et al.*, 1993).

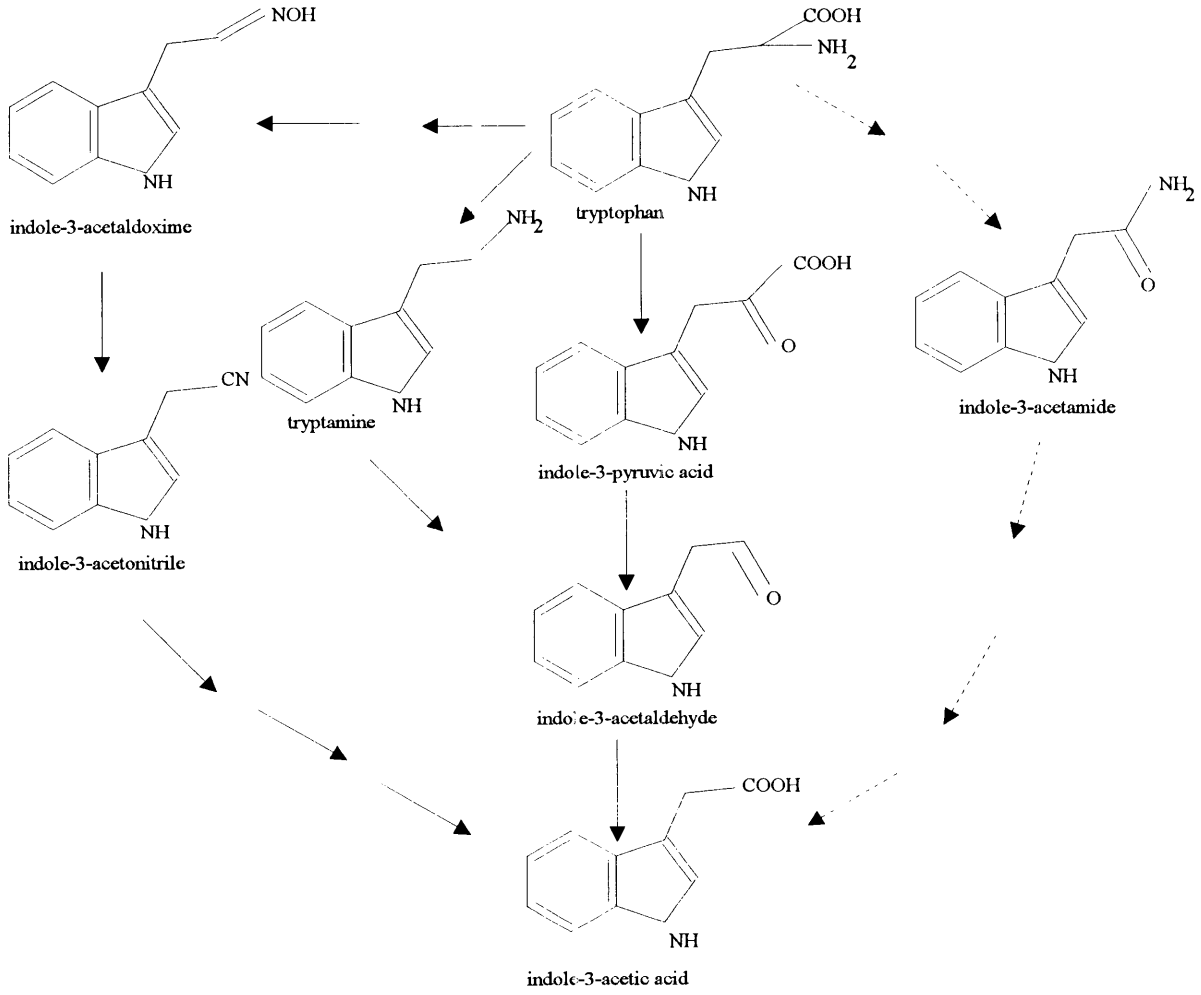


Figure 2.2 Routes for IAA biosynthesis from tryptophan. Dashed arrows indicates the pathway utilized by microbes (From Normanly *et al.*, 1995).

The work with *Lemna* and with mutant plants, showed a route without tryptophan for IAA synthesis (Balidi *et al.*, 1991) but others (Cooney and Nonhebel, 1991; Koga, 1995) still support tryptophan as a precursor and Ribnicky *et al.*, (1992) proposed both (± tryptophan) pathways for IAA biosynthesis. Bartl ng *et al.*, (1994) concluded that in *Arabidopsis thaliana* more than one pathway involving indole-3-acitonitrile for indole-3-acetic acid biosynthesis exists and these are regulated depending on plant development. Normanly *et al.*, (1995) also reported multiple IAA biosynthetic pathways in a single plant type and raised a question about the existence of mutants defective in IAA biosynthesis. They further added that as IAA biosynthesis occurs in chloroplasts as well as in cytoplasm, the lack of localization of the

amino acids to the site of IAA biosynthesis might be responsible for lack of labelled tryptophan incorporation into IAA.

It is clear from the above discussion that, depending on plant types, their stage of development and on the sites of IAA biosynthesis, the biosynthetic pathways may vary and plants may or may not use tryptophan as the major precursor for IAA biosynthesis.

GA_3 in auxin synthesis: Law (1987) reported that the final enzyme in the IAA biosynthetic pathway (indoleacetaldehyde oxidase) is enhanced by GA and kinetin but decreased by ABA. In GA_3 pre-treated stem segments of light grown pea, the total amount of labelled IAA from L-tryptophan was increased. GA_3 also produced labelled N-malonyl-D-tryptophan i.e. GA_3 increased L-tryptophan isomerization as well as IAA biosynthesis. Tryptophan racemase was capable of this transformation. His data also suggested that L-tryptophan was not converted to IAA in significant amount without being converted to D-tryptophan and then indole pyruvic acid (IPyA). This study also concluded that GA_3 might increase IAA biosynthesis at the point of tryptophan racemase and it might also decrease IAA and D-tryptophan -conjugates.

Based on the experimental evidence Law (1987) proposed a hypothetical model. In that model L-tryptophan was unable to form IAA without being converted to D-tryptophan (Figure 2.3). In that model he also advocated a role of N-malonyl-D-tryptophan as an effective auxin precursor. N-malonyl-D-tryptophan also act as an auxin precursor in soybean and tomato cells (Rekoslavskaya and Gamburg, 1984).

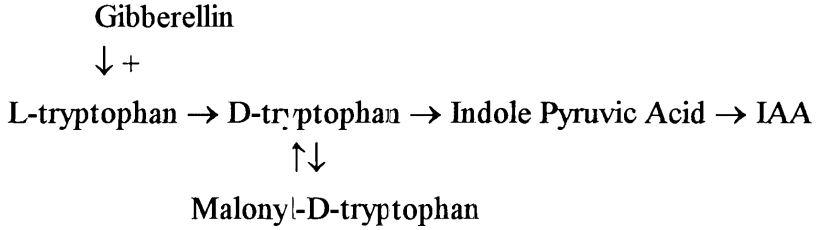


Figure 2.3 Proposed pathway of IAA biosynthesis and effect of gibberellin (From Law, 1987).

Experimental evidence from barley (Tsurusaki *et al.,* 1990) also supported the conversion of L-tryptophan to D-tryptophan via indole pyruvic acid. Tsurusaki *et al.,* (1990) also suggested D-tryptophan as the more direct IAA precursor. Their results revealed that D-cycloserine, an inhibitor of D-tryptophan aminotransferase, inhibited elongation growth of D and L-tryptophan, which suggested that D-tryptophan aminotransferase catalysed formation of indole pyruvic acid was inhibited. Again inhibition of L-tryptophan treated plants by D-cycloserine indicated that direct formation of indole pyruvic acid from L-tryptophan is relatively smaller in barley coleoptiles (Tsurusaki *et al.,* 1990).

Ludwig-Muller and Hilgenberg (1989) proposed for Chinese cabbage that L-tryptophan (not D) was the main substrate for the conversion of N-malonyltryptophan to indole-3-acetaldoxime and then to IAA (an alternate route of IAA synthesis). Normanly *et al.,*

(1995) concluded from the discussions that L-tryptophan is the only possible precursor of IAA. However, except Law (1987) none of the workers (Tsurusaki *et al.,* 1990; Ludwig-Muller and Hilgenberg, 1989; Normanly *et al.,* 1995) supported a role of GA_3 in IAA biosynthesis.

Considering these results, involvement of L-tryptophan in IAA biosynthesis is clearly observed but the role of GA_3 is in doubt. The effect of GA_3 in IAA synthesis might depend on the species and/or experimental conditions.

Auxin conjugates: The conjugates or bound auxins (the peptide; indoleacetyl aspartic acid and the esters; IAA-inositol and IAA-glucose etc.) are storage forms of IAA and the plant can deliver free auxin (IAA) by enzymic hydrolysis (Salisbury and Ross, 1992). Environmental stimuli (e.g. light and gravity) normally influence the rate of auxin conjugation and its release (Taiz and Zeiger, 1991). Lebuhn *et al.,* (1994) reported that auxin production depends on nutrient status and metabolic status of the soil microbial biomass. Frankenberger and Arshad (1991) reported that, in *Capsicum annuum,* root applied L-tryptophan was converted to the conjugated form and synthesised to auxin upon necessity. Other than their role as an endogenous auxin source, conjugates might also perform some other roles including protection against oxidative degradation and IAA transport (Kleczkowski and Schell, 1995).

2.2.2.4 Auxin transport:

Auxin (IAA) transport in higher plants can be either energy-requiring (ATP), polar transport or passive non polar transport via the phloem. Transport in coleoptiles and vegetative shoots is basipetal but in the roots it is acropetal. In both the cases polar transport predominates. Auxin synthesised in a mature leaf shows non polar movement (up or down) through the phloem with much greater velocity than that of polar transport (Taiz and Zeiger, 1991). The transportation of auxin through xylem has also been reported in some plants but is not well established (Borkovec *et al*, 1994). A strict polarity of auxin movement does not always occur in *Coleus* plants. The basipetal polarity becomes weaker with the distance from the vegetative stem apex and there is acropetal movement even at the stem tip in a flowering stem (Leopold, 1960).

Polar auxin transport occurs in all coleoptile cells or in the bundle sheath parenchyma cells adjacent to the vascular bundles. It is primarily independent of the orientation (i.e. gravity stress) of the tissue (Taiz and Zeiger, 1991). Auxin always carries an essential direction component and exerts its effect even in the shoot apices (Sachs, 1993). Polar auxin transport is controlled by its own concentration (Soumelidou *et al.,* 1994a). In a dwarfing apple rootstock, low endogenous auxin levels caused less polar transport because of slower auxin efflux from transporting cells, compared to less dwarfing rootstock. In general auxin transport has been found to depend on various factors including light, temperature, gravity, plant age, hormones and synthetic auxin transport inhibitors (Schneider and Wightman, 1978). Recently Soumelidou *et al.,* (1994b) proposed that Ca^{++} has an important role in the polar transport mechanism of different plants but their exact mode of action is not clear.

2.2.3 Gibberellins:

Gibberellins (GAs) are named on the basis of their structure (Appendix I) and not by their biological activity (Sponsel, 1987). Different oxidative states, additional functional groups and possession of varying double bonds normally lead to the modifications in GA's structure (North, 1990). All GAs are derivatives of ent-gibberellane and are acidic in nature (Salisbury and Ross, 1992). Ninety (90) different GAs are already known (Beale and Sponsel, 1993). Depending on their order of discovery, they are numbered as gibberellin A_X (or GA_X) (Taiz and Zeiger, 1991).

2.2.3.1 Physiological effects of gibberellins:

Gibberellins have diverse effects on plant growth varying from organ to organ and from plant to plant (Cleland, 1969) Although GAs are generally the controller of shoot growth, bud development, bud break and flowering of higher plants (Clemens *et al.,* 1995), several species of Pinaceae family showed little or no elongation in response to GA_3. On the other hand, a mixture of GA_4 and GA_7 produced elongation growth in those plants (Salisbury and Ross, 1992). Evans *et al.,* (1995) reported that some of the isogenic dwarf wheat lines do not respond to exogenously applied GAs due to their higher content of endogenous GAs than normal, specially GA_1 and GA_3 in vegetative leaf and stem tissues of young plants.

GA_1 was proposed as the primary gibberellin for elongation in dwarf plants and other GAs perhaps increase elongation after being converted to GA_1 (Salisbury and Ross, 1992).

In *Chrysanthemum,* sprayed GA_3 increased the number of shoots per plant and their length (Dahab *et al.,* 1987) but reduced shoot number in pea (Phillips, 1975) or had no effect in *Zantedeschia* (Funnell *et al.,* 1992). Gibberellin had no effect on leaf size, leaf count, and plant size of *Cyclamen* cultivars (Widmer *et al.,* 1974) but increased leaf enlargement and elongation was reported in many plants including *Triticum*, *Pisum* and grasses (Goodwin, 1978). In *Chrysanthemum*, chlorophyll concentration (a, b and total chlorophyll) and carotenoids were also increased by GA_3 application (Dahab *et al.,* 1987). GA delayed flowering of *Clerodendrum thomsonae* (Koranski *et al.,* 1979) but accelerated flowering in *Cyclamen* cultivars (Widmer *et al.,* 1974). GAs also have anti-senescence effects (Engvild, 1989). GAs increase uptake of K and accelerate translocation of N, P, K. GAs decrease the severity of diseases and pests (Nowak and Lawson, 1983) or the physiological rind disorder of *Citrus* (Gianfagna, 1995) and can even increase the effectiveness of some non hormonal compounds (Rappaport, 1980).

Gibberellins produce a range of other effects in plants: bolting of long day plants, modification of juvenility, induction of maleness, fruit setting and growth, enzyme production and induction of seed germination, malting of barley, increasing sugarcane yields (Taiz and Zeiger, 1991). At the cellular level, GA enhanced RNA synthesis and brought a quantitative and qualitative change in stem proteins in some plants. RNA and protein synthesis have a proven involvement in cell elongation (Cohn *et al.,* 1994).

GAs suppress lateral expansion of stem cells but facilitate longitudinal expansion. To achieve that GAs probably change the stability of the cortical microtubules and also change the characteristics of the transmembrane and/or the cross linking proteins (Shibaoka, 1993).

GA induced elongation is normally associated with increase in both cell division and cell elongation (Taiz and Zeiger, 1991). However, the inhibition of rooting in *Phaseolus* cuttings by gibberellins was due to the prevention of early cell divisions involved in the transformation of mature stem tissues to meristematic conditions (Brian *et al.*, 1960). On the other hand, in deep water rice a part of GA induced growth was credited to GA stimulated cell division (Reddy, 1995). Again, cell division rather than cell elongation was enhanced by GA_3 in tissue culture of carrot root bioassay (Krikorian, 1995).

It is clear from the above discussion that GAs effect may vary depending on the experimental conditions. Potter *et al.*, (1993) proposed that, to obtain an optimum agronomic or horticultural benefit from GA or GA inhibitor application, GA physiology of the specific crops should be clearly understood.

2.2.3.2 Gibberellin biosynthesis pathways:

To some extent, all tissues in higher plants can produce gibberellins (Graebe and Ropers, 1978) but developing fruits or seeds, elongating shoot apical regions and roots are the widely accepted sites for GA synthesis (Sponsel, 1987). The chloroplasts are probably a sub-cellular site of GA production (Graebe and Ropers, 1978).

The biosynthesis of gibberellins start from mevalonic acid (Figure 2.5). The detailed pathways of GA biosynthesis have been discussed by several authors (Graebe and Ropers, 1978; Sponsel, 1987; Taiz and Zeiger 1991; Salisbury and Ross, 1992).

In most species, the principal GA biosynthetic pathway in shoot tissues progresses through GA_{53} to GA_8 via GA_1. GA_1 can be metabolised both from GA_4 and GA_{20} (Kobayashi *et al.*, 1993; Kobayashi *et al.*, 1994). In *Phaseolus coccineus* seedlings a hypothetical "shoot -- root -- shoot" recycling system was proposed for GA_1 synthesis from GA_{19} (Crozier and Reid, 1971). The concentration of GA_1 in plant tissues is normally more than that of GA_3, which is also synthesised in plant tissues. In maize GA_3 is synthesised from GA_{20} via GA_5 (Hedden and Croker, 1992). In rice, depending on the biosynthetic pattern, the type of GA's present was very specific to the particular organ. GAs identified in the vegetative tissues carry a C-13-hydroxyl group (e.g. GA_1, GA_{19}, GA_{20}, GA_{29}) but the shoot and leaf at flowering stage showed a low level of GAs lacking the C-13-hydroxyl group (e.g. GA_4 and GA_{34}) (Takahashi and Kobayashi, 1991). The GA metabolism in potato shoots has both 13-hydroxylation and non hydroxylation pathways (Van Den Berg *et al.*, 1995).

Taiz and Zeiger (1991) reported that in peas the synthesis and level of gibberellins are under clear control of genes and Mendel's tall/dwarf alleles control GAs metabolism. Metabolic studies in *Pisum sativum* proved that the quantitative and qualitative changes of GAs occurred within an individual organ depending on genotype, developmental stages and on environmental influences (Sponsel, 1985). There are several factors affecting GA Synthesis,

including photoperiod (Zeevaart *et al.*, 1993), light (Lockhart, 1961), phytochrome (Cooke and Kendrick, 1976), temperature (Graebe and Ropers, 1978), and ammonium ion (NH_4^+) (Bruckner and Blechschmidt, 1991). GA biosynthesis can also be influenced by several GA biosynthesis inhibitors or growth retardants which are used commercially on different crops to control plant growth. A detailed discussion of plant growth retardants is presented in section 2.2.6.

2.2.3.3 Conjugation and degradation:

Although GA_3 is presumably only slowly degradable, most of the gibberellins are rapidly metabolized to inactive forms through hydroxylation during active growth. Conjugation of GAs with sugar can form inactive glycosides which may be a storage form of GA (Salisbury and Ross, 1992). However, N-containing amino acid conjugates of GA are probably not found in plants (Kleczkowski and Schell, 1995). Different factors (including enzymes), which remove active GAs or their precursor from the endogenous GA pool are also responsible for maintaining active GA levels in plants (Ross *et al.*, 1995). GAs can be leached from the plants by rain (Lang, 1970) and are degraded in the soil probably through microbial degradation (Sembdner *et al.*, 1970).

2.2.3.4 Transport of gibberellins:

Gibberellins are generally transported through both xylem and phloem (Davies, 1987). Certain gibberellins (e.g. GA_1) can move by diffusion but others (e.g. GA_5) are unable to move in this manner (Cleland, 1969). GA_{20}, GA_1, GA_8, and GA_{29} are highly mobile within plants and their metabolites move mainly towards the apex, which acts as a sink rather than a source of bio-active GAs or their precursors (Smith, 1993).

2.2.3.5 Cellular basis for GA induced elongation:

More than one site of action has been proposed to explain the diverse effects of GA. The process of stem elongation involves an increase in 3 contributing factors: cell division, cell growth and cell wall plasticity (Salisbury and Ross, 1992).

In *Samolus parviflorus*, within 24 hr after GA_3 application, the mitotic activity in the region directly below the apex was increased and the extent of the zone of cell division correlated with the growth in length of the stem. This new zone of division was considered as a virtual sub apical meristem consisting of cortical, vascular and pith tissues. Initially (72 hr after GA application) GA caused only cell division and GA induced cell divisions were predominantly (>80%) transverse (Sachs and Lang, 1961). Gibberellins stimulated the cells in the G_1 phase (Period of cell growth before DNA replication) to enter into the S phase (Period of cell growth when DNA is replicated) and simultaneously reduced the time in the S phase thus shortening and promoting cell division. However, an increase in cell number leads to more rapid stem growth following cell elongation (Salisbury and Ross, 1992).

Cell division without cell expansion does not increase the volume of growth and there is enough experimental evidence that the main effect of GA depends on cell elongation (Metraux, 1987). In the case of deepwater rice cell elongation in the intercalary meristem was the first event towards elongation growth, followed by cell division (Sauter and Kende, 1992).

GAs also increase hydrolysis of starch, fructans, and sucrose into glucose and fructose. Following respiration these hexoses may supply the energy and carbohydrate units needed for cell wall formation. These hexoses also make the cell water potential more negative increasing osmotic uptake of water into the cells to cause cell expansion (Salisbury and Ross, 1992). Similarly, in dwarf pea epicotyls GA_3 also increased the phosphorylation of endogenous protein and increased protein kinase activity (Aggrawal and Sachar, 1995).

The turgor force imposed by GA can cause irrevocable yielding of the cell wall but yielding of the cell wall involves not only a change of physical forces but also change in cell wall metabolism. Higher solute concentration in the tissue alone was unable to increase growth rate unless GA was present to change the yielding properties of the cell wall (Metraux, 1987). In the case of GA induced cell growth in *Avena* internodes, the cell wall loosening and cell wall synthesis, although interlinked, were not totally interdependent regardless of the geometric orientation of enlargement. For continuous elongation, cell wall synthesis is required but not for the initial elongation (Montague, 1995a).

It is not clear from the above discussion how GA increases cell wall plasticity and how its effect is different from that of auxin which also exerts influence on cell wall properties (Section 2.2.2.2).

2.2.3.6 Hypotheses on GAs mode of action:

There were several hypotheses put forward specifically to explain GA induced cell elongation but all of them are equivocal and none of them are totally accepted (Metraux, 1987).

Role of cell wall acidification: Attempts were made to explain rapid elongation growth induced by GA in terms of the acid growth hypothesis (Section 2.2.2.2), already proposed to explain auxin induced elongation. But in lettuce hypocotyls GA induced elongation was not accompanied by a sizeable change in medium acidification. There is also experimental evidence that GA induced growth in oat was not mediated through cell wall acidification (Metraux, 1987).

Role of calcium: Experimental evidence from lettuce hypocotyls leads to an alternate proposal to explain GA induced rapid elongation. The removal of Ca^{++} ions bound to the cell wall probably has a direct effect on wall extensibility and growth. The inhibitory effect of Ca^{++} ions seems to be a result of a non covalent ionic interactions between Ca^{++} and the cross linked cell wall polymers. In addition, the passage of Ca^{++} into the cytosol, either from higher extracellular concentrations or from intracellular compartments, activates calmodulin - a Ca-binding protein. Calmodulin activation is prerequisite for various regulatory responses of plants (Metraux, 1987).

GA induced increased Ca^{++} level was also observed by Bush (1992) in the cytosols and in the endoplasmic reticulum of barley aleurone cells. The author proposed that regulation and maintenance of the endoplasmic reticulum Ca^{++} level might effect the processing of the secreted proteins. However, Montague (1993) concluded that Ca^{++} cannot initiate GA_3 induced growth but can maintain that growth in *Avena* stem segments.

Role of cell wall metabolism: Cell wall synthesis is the most studied part of GA induced cell wall metabolism. After about an hour of exposure to GA, the cell wall of *Avena* internodes incorporate ^{14}C labelled glucose at an increased rate which corresponds with the lag period of GA induced elongation in that tissue. In GA treated *Avena* stem, sustained cell wall synthesis and continuous thickening of the meristematic cells were also noticed and rapidly dividing cells maintained their thickness throughout their extension process. GA induced cell wall synthesis was interpreted as a function to maintain the structural integrity of the cell wall during this fast elongation. GA also changed cell wall metabolism. It broke the stress bearing bonds of the cell walls allowing extension and release of cell wall polymers for subsequent growth (Metraux, 1987). In soybean and spinach, GAs control the steady supply and activity of sucrose phosphate synthase protein in leaf cytosol (Cheikh *et al.*, 1992).

Role of peroxides and phenolic cell wall components: In this hypothesis GA prevents cell wall stiffening through the inhibition of peroxidase activity. GA induced inhibition of peroxidase activity in spinach cells decreased the hydrophobicity of the cell wall and thereby exposed the cell wall to the plasticizing influence of water and the loosening action of hydrolases. However, Ca^{++} ions also affect peroxidase secretion in sugar beet. GA might have an indirect effect on peroxidase secretion through Ca^{++} (Metraux, 1987).

The effect of GA on the activity of the enzyme phenylanaline ammonia-lyase, (PAL) involved in the production of precursor for ferulic acid and lignin, which are required for lignification of the cell wall was also studied but the result was again inclusive (Metraux, 1987).

2.2.4 Cytokinins:

Cytokinins are defined as promoters of cell division in some tissues grown *in vitro* (Salisbury and Ross, 1992). McGaw (1987) defined cytokinins as substances which, in conjunction with auxin, elevate cell division and also interact with auxin in regulating the route of cell differentiation. Thirty (30) different cytokinins have been identified from a diversity of plant parts (Halmann, 1990). Zeatin (Appendix I), dihydrozeatin and isopentenyladenine (i6Ade) are the most common naturally occurring cytokinins in higher plants (Taiz and Zeiger, 1991). Kinetin and benzyladenine are the two most important and highly active synthetic cytokinins. All cytokinins have a common purine ring with a protruding nitrogen holding a side chain rich in carbon and hydrogen (Salisbury and Ross, 1992). Cytokinins can appear as ribosides and ribotides (Davies, 1987).

2.2.4.1 Physiological effects of cytokinins:

In higher plants, applied cytokinins can affect a variety of different physiological, metabolic, biochemical, and developmental processes (Taiz and Zeiger, 1991). It acts as a primary means of communication between roots and shoots (Tayama *et al.,* 1992). Cytokinin (BAP) mainly acts on immature tissues at low concentration and strongly promotes growth (Goodwin, 1978). Low cytokinin levels increased stem elongation in *Lilac* (Dragt *et al.,* 1992) and snap bean plants by playing a role in cell division and cell enlargement (El-Sayed, 1991). BAP was unable to increase plant height in some ornamental plants (Rounkova, 1985) but stimulated or inhibited plant height in *Rudbeckia* rosset plants depending on plant age and concentration (Kochankov *et al.,* 198?).

Cytokinins promoted side shoot development in different plant species (Wilkinson, 1985; Salisbury and Ross, 1992) but appropriate rates and timing of BAP applications are important for side branch stimulation (Forshey, 1991). Multiple shoot formation was initiated with higher (50-100 μM) BAP concentration while lower concentrations were recommended for subsequent development in soybean. Continuous exposure to BAP was important for additional bud production and subsequent growth (Buising *et al.,* 1994). More direct and immediate effect of cytokinin on bud growth and cultivar dependent response on shoot production of soybean was also reported (Buising *et al.,* 1994). However, the specific role of cytokinin in the process of apical dominance is discussed in section 2.1.2.3.

Cytokinins promoted (Sachs, 1977) or delayed (Heins *et al.,* 1981; Rounkova, 1985) floral initiation and development in different species. Cytokinins have a wide range of other effects including: delaying senescence, nutrient mobilization, cotyledon expansion, control of morphogenesis, maturation of chloroplasts (Taiz and Zeiger, 1991), stomatal opening (Davies, 1987), promotion of germination (Letham, 1978), induction of femenization (Champault *et al.,* 1981) fruit set and ripening (Smigocki *et al.,* 1993), reduction of virus infestation (Nowak and Lawson, 1983) and increased resistance to environmental stresses (El-Sayed, 1991). However, synthetic cytokinins have a very inconsistent effects and are active only in a few plant species (Tayama *et al.*, 1992).

2.2.4.2 Cellular basis of cytokinin induced growth:

Cell cycle: In an actively growing meristem, each cell almost doubles its cytoplasmic mass along with all of its organelles immediately after formation, to prepare for the next division. In that way, the average cell size in a dividing cell population remains constant. The G_1 phase of the cell cycle produces maximum growth for the cytoplasmic mass but S phase involves the replication of the DNA and other components of the nucleus. However, immediately before or after DNA replication, cell division may be interrupted frequently leading to production of polyploidy cells. Evidence suggests that, both cytokinins and auxins are required to maintain the cell cycle and that cytokinins control the steps towards mitosis while auxin controls the steps towards DNA replication (Taiz and Zeiger, 1991).

Cell division: Skoog and Tsui (1948) proposed that the concentration ratio of auxin to adenine is a determining factor in the formation of buds, callus, or roots. Figure 2.4 represents the relative requirement for these hormones for the growth of buds, callus or roots. Molecular genetic methods using *Agrobacterium* T_i plasmid to study tumour growth and development further confirmed the importance of auxin to cytokinin ratio in plant morphogenesis (Taiz and Zeiger, 1991). In the case of a high cytokinin to auxin ratio, meristematic cells are produced in the callus and after further division they produce new cells which develop into buds, stems or leaves. On the other hand, a low cytokinin to auxin ratio favours the formation of roots. Now a days this principle is being routinely used in tissue culture of dicotyledons plants to multiply disease resistant or other desired plant types (Salisbury and Ross, 1992).

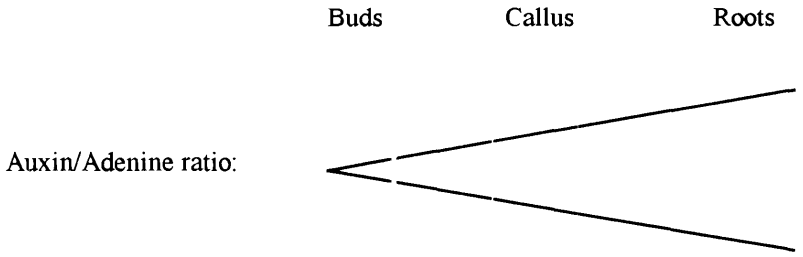


Figure 2.4 Effect of different ratios of auxin to adenine (From Leopold, 1960).

Cell enlargement: Cytokinin induced cell enlargement was clearly identified in leafy cotyledons of dicotyledons (Taiz and Zeiger, 1991). Rayle *et al.,* (1982) reported that cytokinin stimulated cucumber cotyledonary growth was linked with cytokinin induced increase in plasticity of the cell walls but had no relationship with the cell wall elasticity. The observed increased plasticity was not a result of cell wall acidification and therefore this effect is different from auxin induced growth (Ross and Rayle, 1982). Cytokinin induced growth is also different from GA induced growth because of GAs inability to expand cotyledonary cells. In young wheat coleoptiles and water melon hypocotyls elongation growth by cell expansion, with a minimum effect on cell number, was noticed with cytokinin but without the presence of exogenous GA or auxin (Taiz and Zeiger, 1991).

Chloroplast development and maturation: Cytokinin promotes chloroplast development and maturation to a greater degree only after being stimulated by light. In cytokinin treated, etiolated leaves, development of chloroplasts with ample grana is apparent after their illumination. Cytokinins also enhance the synthesis of chlorophyll and photosynthetic enzymes (for protein synthesis) and maturation of the chloroplasts after illumination (Taiz and Zeiger, 1991).

2.2.4.3 Mechanisms of cytokinin action:

Cytokinin might have different mechanisms of action in different tissues because of the variety of growth responses in plants. It is normally thought that cytokinin causes a common fundamental effect which is followed by many secondary effects depending on the

physiological status of the target cells. Evidence also suggests that it might also have a promotory effect on the formation of RNA and protein synthesis enzymes (Salisbury and Ross, 1992).

It has been proposed that a specific cytokinin receptor protein for specific cells might be responsible for their effect. Several of these cytokinin receptor proteins or binding proteins have been identified (Napier and Venis, 1990). However, except for the binding protein of barley leaves (molecular mass of 40-45 kDa), most of the other binding proteins were unable to show enough affinity for cytokinins (Romanov *et al.*, 1988).

In plant tissue culture, the rate of cell division is increased by cytokinin because it decreases the time from G_2 (Period of cell growth after DNA replication) to mitosis, perhaps through an increased rate of synthesis of enzymes or structural proteins important for mitosis (Fosket *et al.*, 1981).

Evidence suggests that cytokinin exerts specific effect on translation to increase protein synthesis. This conclusion was based on the appearance of the ribosomes grouped in long protein synthesising polysomes in cytokinin treated cells. But these mechanisms are not yet well understood. Some of the nuclear proteins were considered to be the target of cytokinins, which perhaps directly enhance cell division. It is known that the nucleus does not produce its own protein and those target nuclear proteins are synthesised in the cytosol at the time of translation. So, the main effect of cytokinin might be in the control of enhanced production of the nuclear protein (Salisbury and Ross, 1992). In soybean embryonic axes, cytokinin (BAP) caused rapid but transient inhibition of DNA synthesis which was followed by subsequent reprogramming of gene amplification. Probable mechanisms for inhibition of the DNA synthesis include "1) interference with synthesis of DNA precursors; 2) direct interference with DNA replication; 3) blockage of entry into S-phase" (Buising *et al.*, 1994).

Recently Chen *et al.*, (1987) reported that in excised pumpkin cotyledons, cytokinin (BAP) changes the type of mRNAs, increases the total quantity of some mRNAs and decreases the quantity of others. The changed mRNA levels might be because of increased or decreased transcription of the genes. It is known that the presence of a specific mRNA molecule depends partly on its rate of synthesis during transcription and partly on the rate of its degradation. So, cytokinin might influence mRNA stability or only act on mRNA transcription or on both (Salisbury and Ross, 1992).

Through an effect on transcription in the nucleus, cytokinin was found to increase 2 proteins; chlorophyll a/b binding protein and the small sub unit protein of ribulose-bisphosphate carboxylase (RuBisCo) and their mRNAs (Salisbury and Ross, 1992). Evidence is also available (with white light grown *Lemna gibba* plants) in favour of increased stability of those mRNA due to cytokinin which allows faster translation of the genetic messages into proteins (Flores and Tobin, 1987). On the other hand, the formation of phytochrome and its mRNA is reduced by cytokinin, zeatin or by red light but the exact mechanisms for these effects are still unknown (Cotton *et al.*, 1990).

Cytokinin induced, sustained and increased cell division was also observed through increased $[Ca^{++}]_i$ in mosses (Saunders, 1992). Therefore, cytokinin might act on the plasma membrane by transduction to enhance Ca-calmodulin levels (Salisbury and Ross, 1992). However, the mechanism of protein or translated enzyme induced cytokinesis and cell expansion and other effects is not clearly understood (Salisbury and Ross, 1992).

Su and Howell (1995) proposed that cytokinin can mimic some of the effects of light but it was found that cytokinin and light had independent and additive effects in *Arabidopsis thaliana* hypocotyl elongation.

It is not clear whether cytokinin has any specific effect depending on the tissue type or acts by influencing all of the processes discussed above. So, it seems, still a lot of work needs to be done before the mechanism of cytokinin action is totally understood.

2.2.4.4 Cytokinin metabolism:

Synthesis: Cytokinin levels are higher in dividing young cells of the shoot and root apical meristem (Taiz and Zeiger, 1991). Probably they are synthesised there or transported from other parts (Salisbury and Ross, 1992). Root apical meristems are the major sites for free cytokinin synthesis in whole plants (Taiz and Zeiger, 1991). The detailed biosynthetic pathways for cytokinins have been discussed by several authors (Taiz and Zeiger, 1991; Salisbury and Ross, 1992). Environmental (e.g. light, temperature) and chemical factors (including hormones) can effect cytokinin synthesis and thereby its level in plants (Letham, 1978). As for example, environmental factors interfering with root function (e.g. water stress) can reduce cytokinin content of the xylem exudate (Torrey, 1976).

Conjugation and degradation: Cellular levels of active cytokinins are dependent on their degradation and conversion probably into inactive derivatives (except nucleosides and nucleotides). Cytokinin oxidase might be responsible for maintaining a safe or desirable level within the plants, through the inactivation of the hormone (Taiz and Zeiger, 1991). Cytokinin oxidase removes the 5-carbon side chain producing free adenine or adenosine. McGaw and Burch (1995) reported that many conjugates (e.g. ribosides, ribotides, glucosides, amino acid conjugates etc.) can be formed and the most common conjugates contain glucose (glucosides) or alanine (Salisbury and Ross, 1992)

2.2.4.5 Transport of cytokinin:

Cytokinins are presumably transported passively from root through the xylem into the shoot (Taiz and Zeiger, 1991). Experimental evidence also proved some movement in phloem but cytokinins are not readily distributed in the phloem. Cytokinin transport within the shoot is rather limited except by xylem transport (Salisbury and Ross, 1992). Nucleotides might be the transportable form of cytokinin in plants. After their arrival in the leaves, these nucleotides are probably converted into the free base or to the glucosides and compartmentalized in the leaf cells having no hormonal activity in this form (Taiz and Zeiger, 1991). Polar movement of

kinetin was dependent on tissue age and IAA concentration. In *Phaseolus vulgaris,* basipetal movement of kinetin was stimulated by IAA (Letham, 1978).

2.2.5 Hormonal interactions:

Applied hormones normally express their effect through a balance with the endogenous hormones. The clearly demonstrated effects due to a single hormone application are not simply due to an effect of a single hormone (Taiz and Zeiger, 1991; Arshad *et al.,* 1995). Moreover, each of the hormones could act as a promoter or inhibitor depending on the situation. It is therefore really difficult to isolate any specific effect of an individual hormone (Naqvi, 1995)

2.2.5.1 Interaction of auxins with others:

Auxins with GAs: Dwarfism was mainly explained due to the insufficient gibberellin availability in plants, but in dwarf corn coleoptiles lower auxin content also caused dwarfism (Tsurusaki *et al.,* 1990). In excised pea internodes, gibberellin only causes growth in the presence of auxin and removal of the principal source of auxin in excised segments might be the main reason for their inability to elongate in the presence of gibberellins (Phillips, 1971). Similarly in cowpea (*Vigna unguiculata*) hypocotyls, the level of endogenous GAs seems to control the response of exogenous IAA for elongation growth (Okamoto *et al.,* 1995). They proposed that stem elongation is stimulated through the agency of both auxin and gibberellin. Again, the fibre length of *Eucalyptus* could be manipulated depending on the gradients of GA and IAA (Hasan *et al.,* 1994).

Accumulated evidence suggests that auxin is the real regulator of growth and that GA only acts via its effect on auxin synthesis. In pea stems, GA enhanced the levels of free auxin through the racemisation of L-tryptophan to D-tryptophan and stimulated the elongation of excised stem segments in a similar way to auxin (Metraux, 1987). Again in dwarf pea plants, GA induced stem elongation was associated with a marked increase in diffusible auxin production by sub apical internodes (Goodwin, 1978). Other than affecting auxin synthesis, auxin levels in plants were also raised probably due to GAs reduction of IAA-oxidase activity an "auxin-saving" mechanism (Devlin, 1969) or by the release of bound auxins (Metraux, 1987). Anti-auxins inhibited gibberellin induced elongation in *Ipomoea* petioles and in intact rice coleoptiles (Cleland, 1969) but not in lettuce hypocotyls (Metraux, 1987). In an auxin treated *Vigna unguiculata* plant Hasuike and Okamoto (1994) found that applied GA activated the proton pumping in the maturation zone. They proposed that gibberellin regulates this auxin induced activation of the proton pumping. But Miyamoto *et al.,* (1993) experimentally proved that the real mechanisms of auxin and gibberellin actions were different for pea subhook growth.

From the above discussion a specific role of GA for increasing auxin availability in plant is apparent but the effect of GA probably depends on other experimental variables (e.g. species and/or the presence of exact auxin precursor). This same idea was also proposed earlier (Section 2.2.2.3). Although auxin (Section 2.2.2.1) or GA (Section 2.2.3.1) can work

independently, under certain specific situations for optimum elongation growth, a balance of auxin to GA is very important.

Auxins with ethylene: In several systems (e.g. root growth) higher auxin concentrations were inhibitory but auxin was no longer inhibitory when ethylene synthesis was prevented or opposed or its effect was removed by various means (Davies, 1987). The IAA induced ethylene production was inhibited by CO_2 (Mathooko *et al.,* 1993) or STS (Kawa and Saniewski, 1989). These results suggested that increased ethylene production was mediated by auxin. To support this idea Peck and Kende (1995) proposed that in intact pea seedlings IAA treatment sequentially induced the enzymes of ethylene biosynthesis to increase the ethylene level.

The so called triple response of seedlings (reduced elongation, swelling of the hypocotyl and changed direction of growth) or leaf epinasty are known to occur due to auxin redistribution in response to induced ethylene (Reid, 1987). Roberts and Osborne (1981) reported that in immature tissues a correlation could be made between auxin content and ethylene production but ethylene production appears to be independent of the total endogenous auxin content in senescing or ripening tissues.

In pea stem, IAA stimulated ethylene production inhibited bud growth. Kinetin reversed this effect of IAA and ethylene (Burg and Burg, 1968). The effect of PGRs on apical dominance has been discussed in section 2.1.2.1.

Osborne (1974) proposed that ethylene has an opposite effect to auxin. He added that ethylene reduces the extensibility of the cell wall or cell enlargement, while auxin increases that. Ethylene has an inhibitory effect on auxin transport, not on IAA uptake or increased decarboxylation (Schneider and Wightman, 1978). They reported that the specific binding protein located at the plasmalemma has two receptor sites (an auxin and an ethylene receptor site). Filling either of those sites with any hormones actually prevented the binding of the other to the second site. Hence ethylene can prevent auxin transport by binding its receptor site, when auxin supply is depleted (e.g. ageing etc.). Ethylene treatment in *Prunus salicina* seeds increased auxin content, probably by inhibiting auxin transport out of the seeds. In *Coleus*, pre treatment with ethylene reduced the auxin content of the apical part of the stem from 8.3 to 4.3 µg/kg fresh weight (Schneider and Wightman, 1978). In soybean hypocotyl segments, auxin (2,4-D) induced ethylene production was inhibited by ABA (Goodwin, 1978).

Auxins with cytokinin: The auxin-cytokinin interactions have a specific role in the cell cycle and cell division (Section 2.2.4.2). In a recent review Hamill (1993) discussed auxin-cytokinin metabolism and their interactions on a genetic basis and again supported the historic findings (Skoog and Tsui, 1948) of auxin : cytokinin balance for regulating plant growth.

In *Pisum*, kinetin moves through the plant only in the absence of an intact active apex or apex replaced by IAA. The presence of IAA or an apex forced kinetin to concentrate in the shoot tip (Goodwin, 1978). Goldsmith (1969) proposed that enhanced apical dominance by auxin plus cytokinin was not only due to increased auxin transport but also due to cytokinin's role in the movement of metabolites towards their site of application.

Cytokinin can increase free IAA in plant tissue through its influence on auxin uptake and auxin conjugation. The increased auxin production may also increase auxin induced ethylene production (Goodwin, 1978). Harrison and Kaufman (1982) reported that cytokinin can overpower the inhibitory effect of auxin on lateral bud development and of ethylene on axillary growth. Specific isoenzymes of IAA-oxidase and peroxidase may also be affected by cytokinins (Schneider and Wightman, 1978).

Auxins with ABA: ABA reduced both auxin production and auxin induced growth in *Avena* coleoptiles and reduced IAA uptake and transport in epicotyl sections of *Lens* seedlings. ABA also inhibited basipetal IAA transport in *Abies balsamea* (Goodwin, 1978).

2.2.5.2 Interaction of GAs with others:

GAs with cytokinins: Gibberellins elongated *Pisum* plants, without affecting their cytokinin level (Goodwin *et al.,* 1978) but higher cytokinin levels reduced the effect of GA on stem elongation of *Cintaurea calcitrapa* (Goodwin, 1978). In some ornamental plants, a combined GA and cytokinin treatment produced taller plants with profuse branching (Rounkova, 1985). Cytokinins along with GA_3 can also control leaf formation but higher rates can cause unwanted leaf distortion (Tayama *et al.,* 1992). Cytokinins have been reported to increase GA levels in a variety of plant tissues (Goodwin *et al.,* 1978). On the other hand, CCC, a GA-biosynthesis inhibitor, also increased cytokinin synthesis in grapes (Letham, 1978). However, BAP a synthetic cytokinin, increased the rate of GA synthesis and metabolism to inhibit shoot growth through the production of less active GAs (Goodwin, 1978).

GAs with ethylene: Partially submerged or ethylene treated air grown plants both produced similar elongated internodes and GA biosynthesis inhibitors prevented that growth in all plants. It was suggested that ethylene changed the responsiveness of the tissue to endogenous GA by altering a hypothetical receptor site for GA or by modifying biochemical responses which follow the primary action of GA. But ethylene was unable to promote GAs effect in a subsequent experiment (Metraux, 1987). Recently Sauter *et al.,* (1995) reported that in deep water rice O_2 tensions increased ethylene synthesis which caused reduction of ABA and ultimately the ABA : GA balance is favourable for elongation growth.

GAs with ABA: Several GA-induced physiological responses in apples were counteracted or inhibited by ABA (Grochowska *et al.,* 1984) and conversely ABA induced dwarfness in apple shoots was also reversed by GA_3 (Goodwin, 1978). It was proposed that plant height is controlled through the balance of both GA and ABA in chillies (Yin-Sheng *et al.,* 1989) and in a deep water rice (Hoffmann and Kende, 1992). In barley aleurone layer, GA_3 stimulated Ca^{++} accumulation in isolated endoplasmic reticulum vesicles but ABA reduced Ca^{++} transport activity in the endoplasmic reticulum (Thomas, 1995). He proposed that the effect of GA_3 and ABA are expressed through the alteration of the concentration of Ca^{++} transporter in the membrane. Thus ABA generally antagonises the responses of plants to both IAA (Section 2.2.5.1) and GA.

2.2.5.3 Interaction of cytokinins with others:

Cytokinins with ABA: In tobacco leaves, the free ABA level was reduced by exogenous cytokinin because of increased conversion of free ABA to bound ABA (Goodwin *et al.*, 1978). Following different stress exposure, the whole plant or its different parts had enhanced ABA production and reduced cytokinin levels (Khan, 1975). However, Hall (1973) reported that the response of soybean callus to low level of cytokinin (0.005 mg/L) was inhibited by ABA (10 mg/L) but higher concentrations of cytokinin (approximately 0.5 mg/L) changed that antagonism of ABA to synergism. From these results and also from other discussed results Hall (1973) concluded that the ABA-cytokinin relationship is very complex and is not as simple as plus/minus interaction.

Cytokinins with ethylene: Stimulated bud break and a higher number of elongating shoots were obtained with either cytokinin or ethylene in *Plumeria*, but cytokinin produced more shoots than ethylene and ethylene also slightly retarded shoot growth (Kwon and Criley, 1991a). However, the inhibitory effect of cytokinin on *Arabidopsis* hypocotyl were largely mediated by ethylene (Su and Howell. 1995).

2.2.5.4: Multiple:

The hormonal interaction is very complex process. Each of the known hormones can alter the availability of the others. For example the transport, synthesis and metabolism of IAA depends on the level of the auxin, gibberellin, cytokinin and ethylene in the plant (Schneider and Wightman, 1978). ABA inhibited auxin induced growth of oat coleoptile sections, gibberellin induced growth of lettuce hypocotyl and cytokinin induced growth of *Xanthium* cotyledons (Leopold, 1971). Woolley and Wareing (1972) reported that cytokinins promoted both auxin (in *Avena* coleoptiles) and gibberellin (in pea seedlings) production. In etiolated peas, kinetin temporarily increased ethylene production and inhibited elongation, via auxin conjugation (Goodwin, 1978). Schneider and Wightman (1978) concluded in their review that these complex interactions could be utilised in the field of fruitful research.

2.2.6 Plant growth retardants:

Growth retardants are "organic chemicals which retard stem elongation, increase green colour of leaves, and indirectly affect flowering, without causing malformation of the plant" Cathey (1975). This reduction in stem elongation may occur without a reduction in leaf number (Dicks, 1972). The growth retardant induced reduction in sub-apical meristem activity associated with inhibited internode length (through reduced cell division and to some extent cell elongation) may be reversed by simultaneous or delayed application of appropriate gibberellic acid (Dicks, 1976). Hence, inhibition of GA biosynthesis is supposed to be the primary biochemical mechanism of growth retardants action.

Although GA biosynthesis inhibition is the primary reason for the growth retardation, applied GA could not completely reverse the growth and physiological responses of retardant treated plants (Grossman, 1992). Growth retardants must have some other non-specific effects

which cannot be reversed by GA application alone (Harvey *et al.,* 1991). In certain circumstances growth retardants also interfere with the synthesis and metabolism of cytokinins, ethylene, ABA and phytosterols (Rademacher, 1989a).

Grossman (1990) listed a series of physiological responses to plant growth retardants other than reduced stem growth including: delayed senescence along with enhanced chlorophyll, protein and mineral concentration; enhanced assimilate translocation to seeds; enhanced nutrient uptake from soil; promoted flowering and altered sex expression; reduced water use and enhanced resistance to certain environmental stress conditions (e.g. cold, heat, drought, fungal infections).

According to Sponsel (1987) the selectiveness and relative efficacy of different growth retardants are not always consistent and depend on the type of tissue treated.

Tayama *et al.,* (1992) divided growth retardants in 2 different groups depending on their solubility: a) very soluble in water (B-Nine, CCC) - which penetrates slowly into the wax layer of a wet leaf and overhead irrigation or rain within few hours of application can wash off these chemicals; b) poorly soluble in water (A-rest, Bonzi, Sumagic) - which move rapidly (within few minutes) into the wax layer and are not washed off easily so the environment has little influence on their action.

Davis and Curry (1991) also classified growth retardants in 2 broad categories, GA biosynthesis inhibitors and miscellaneous compounds and further divided these into different sub-categories depending on their chemical formulations.

2.2.6.1 Gibberellin biosynthesis inhibitors:

Paleg *et al.,* (1965) proposed 5 general mechanisms of action through which a growth retardant can inhibit GAs action. These are: i) inhibition of GA biosynthesis; ii) depressing the amount of substrate or substrates on or with which GA must act; iii) prevention of GAs reaction with the substrate; iv) destruction or inhibition of GAs; v) prevention of one of the series of reactions directly or indirectly following the GA-substrate reaction.

Now a days the term "gibberellin biosynthesis inhibitor" is being used more specifically to denote oniums along with some other growth retardants, because of their specific role in blocking gibberellins formation rather than interfering with the activity of the existing gibberellins (Davis and Curry, 1991).

Mevalonic acid is the primary precursor for GA biosynthesis (Halmann, 1990). The probable sites of interference in GA biosynthesis pathways by GA biosynthesis inhibitors are indicated in figure 2.5. Ancymidol and PBZ (a triazole) normally inhibit oxidation of ent-kaurene or later steps, whereas daminozide and CCC block earlier steps in the pathway (Harvey *et al.,* 1991). While, most of the known GA-biosynthesis inhibitors inhibit the early steps in the pathway, prohexadione calcium (BX-112), a cyclohexanetrione inhibits the later stage of 3-β-hydroxylation of GA_{20} to GA_1 *in vivo* in different plants (Kamiya *et al.,* 1992).

ABA is also capable of antagonizing GA biosynthesis or GA metabolism in higher plants (Sembdner *et al.,* 1970). Interestingly, some remotely related cytokinins were also

Mevalonic acid (MVA)

↓

Isopentenyl pyrophosphate (IPP)

↓

Dimethylallyl pyrophosphate (DMAPP)

↓

trans-Geranyl pyrophosphate (GPP)

↓

trans-Geranylgeranyl pyrophosphate (GGPP)

CCC and other Onium compounds →

↓

Copalyl pyrophosphate (CPP)

CCC and other Onium compounds →

↓

ent-kaurene

Tetcyclacis, Ancymidol, Triazoles →

↓

ent-kaurenol

Tetcyclacis, Ancymidol, Triazoles →

↓

ent-kaurenal

Tetcyclacis, Ancymidol, Triazoles →

↓

ent-kaurenoic acid

↓

ent-7a-OH kaurenoic acid

↓

GA_{12}-7-aldehyde

↓

$GA_{19} \rightarrow GA_{20} \rightarrow GA_1$

Figure 2.5 Biosynthesis of gibberellins and interference by its inhibitors (From Halmann, 1990).

reported to interfere with cell free GA biosynthesis in a similar manner to that of ancymidol (Rademacher, 1989b).

In this present research project, 5 most commonly used and available growth retardants (ancymidol, CCC, daminozide, flurprimidol and PBZ) were screened and then based on their initial performance, only PBZ was selected for subsequent experiments. The physical and chemical properties of these used 5 growth retardants are presented in appendix II. A brief discussion of different groups of GA-biosynthesis inhibitors are presented here.

i) Onium compounds: Chloromequat chloride (CCC), AMO-1618, mepiquat chloride (DPC), chlorophonium chloride, certain trimethylammonium iodides, BTS 44584, and LAB 140810 are representatives of onium compounds (Rademacher, 1989b). CCC is a choline derivative of an onium compound containing a substituted chlorine (Cl). It is highly mobile within plants but not metabolized by the plants. The most important commercial uses of CCC include lodging control in a variety of agronomic crops and height control in floricultural crops (Davis and Curry, 1991). CCC is probably species-specific and might even be cultivar specific (Tabor and Hampton, 1992) and it has a tendency to be inconsistent from crop to crop or even from year to year (Hanks and Menhenett, 1980).

ii) Pyrimidines: Ancymidol (A-Rest, Reducymol) and flurprimidol (Cutless) are the two important examples for this group (Davis and Curry, 1991). These are substituted pyrimidine compounds. Ancymidol was found to be effective on plants grown in containers in a protected environment but was not suitable for general landscape plants (Cathey, 1975). Flurprimidol was effective in retarding growth of grasses, ornamentals, and several other floriculture crops (McDaniel, 1986).

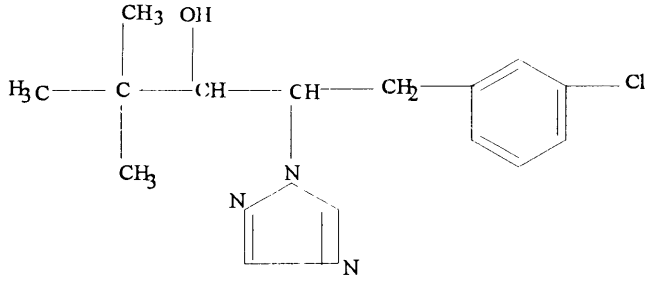


Figure 2.6 Chemical structure of PBZ, a triazole (From Taiz and Zeiger, 1991).

iii) Triazoles: PBZ (Bonzi, Cultar, PP333) and uniconazole (Sumagic or XE-1019) are important representatives of triazole compounds. The other members are tripenthenol, BAS 111, LAB 150 978. All triazoles possess a ring structure which contains 3 nitrogen atoms (Figure 2.6). The plant growth regulatory activity for this group is perhaps related to the stereo chemical configuration of the moieties on the carbon chain. PBZ consists of 2 enantiomers: 2S, 3S (responsible for the plant growth regulating activity) and 2R, 3R (responsible for fungicidal activity). Because of the asymmetric centre and tri-substituted double bond of uniconazol, it exists as 4 stereoisomers (Davis and Curry, 1991). Generally

triazoles do not cause phytotoxicity (even at a higher rates) and still retard shoot growth (Davis *et al.*, 1988).

iv) Other compounds: Tetcyclacis, prohexadione calcium, isonicotinic acid anilide derivative (Inabenfide) etc. exhibit a wide range of shoot regulatory activity. These are able to inhibit the oxidative conversion of kaurene to kaurenoic acid in the isoprenoid pathway but most of these are slower in action compared to triazoles and pyrimidines (Davis and Curry, 1991).

2.2.6.2 Miscellaneous compounds:

Daminozide (Alar, B-Nine , SADH): It is an acid and its use on edible crops has been questioned because of its possible carcinogenic effects, if ingested for an extended period (Davis and Andersen, 1989). Although recent studies cleared up all of the allegations of carcinogenic effects, still it has not been reinstated (Gianfagna, 1995). The height control of bedding plants is its primary use in commercial ornamental industry. It reduces shoot elongation perhaps via GA biosynthesis inhibition (Davis and Curry, 1991) or through reduction of translocation of GA or its precursors and may promote GA conjugation and catabolism (Rademacher, 1991).

There are several other compounds (e.g. Ethephon, Maleic hydrazide, Morphactins, Dikegulac, Cyclohexanetriones, Fatty acid derivatives etc.) belong to this category, which are discussed elsewhere (Dicks, 1976; Davis and Curry, 1991; Rademacher, 1991; Grossman, 1992).

2.2.6.3 PBZ and plant growth:

PBZ is a broad-spectrum growth retardant effective on a wide range of species (Quinlan and Richardson, 1984). Its dose dependent reduction in the internode length of terminal and lateral shoots is widely accepted (Lever, 1986). The shoot number/plant is not affected by PBZ or in some species it is reduced substantially (Davis *et al.*, 1988). PBZ normally reduces leaf area and at higher rates reduces leaf production but at lower rates leaf number is relatively unaffected. Some times PBZ treatment alters the gravitrophic behaviour of a plant (Davis *et al.*, 1988). PBZ had no effect on stem diameter of ornamental kale (Whipker *et al.*, 1994).

PBZ increases flower bud number and thereby reduces the numbers of lateral shoots (Lever, 1986). In herbs, PBZ sometimes stimulates flower initiation or significantly delays anthesis at higher concentrations (Davis *et al.*, 1988).

Root growth can be increased or decreased depending on the concentration and method of PBZ application (Rietveld, 1989). Davis *et al.*, (1988) reported increased weight, diameter, and length of fibrous roots with PBZ application in apple seedlings. It was proposed that PBZ might affect the rate of IBA metabolism during rooting and the local sink status of a mung bean cutting thereby increase the root promoting effect of IBA (Wiesman and Riov, 1994). In *Hedera helix* L. PBZ application increased adventitious root formation on aerial

shoots associated with increased shoot elongation. This result was interpreted on the basis of gibberellin production by those newly formed adventitious aerial roots (Horrell *et al.,* 1989a).

PBZ (10^{-8} to 10^{-6} M root drench) reduced stem height and internode length in safflower with a corresponding reduction in cell size and cell number (Potter *et al.,* 1993). But Dalziel and Lawrance (1984) reported that PBZ prevented increment of cell number but increased cell size in Paul's scarlet rose. However, decreased cell size and increased cell number is common in some triazole treated leaves which might be due to the reduction in GA content and a transient rise in the ABA concentration (Morrison and Andrews, 1992). In treated *Chrysanthemum* stems PBZ increased secondary xylem development with a marked reduction in the numbers of sclerenchyma bundle caps. Increased root diameter was observed because of the increase in the number of rows and diameter of cortical cells but root cortical cell length was 50-70% less than in untreated plants (Burrows *et al.,* 1992). They (Burrows *et al.,* 1992) added that the anatomical effect of PBZ might vary depending on the method of application, concentration or age of the plant materials used.

In tissue culture of *Vigna aconitifolia*, 0.5-2.0 mg/L PBZ decreased callus growth and its differentiation into roots and shoots and GA_3 reversed this effect (Davis *et al.,* 1988). However, Li and Wolyn (1995) reported that PBZ significantly increased somatic embryos and their conversion to plantlets in asparagus.

Triazoles can also influence other important physiological and biochemical processes in plants (Davis *et al.,* 1988). These include: photosynthesis (reduced photosynthetic area, delayed onset of leaf senescence, changed leaf orientation); increased chlorophyll content (through chlorophyll biosynthesis or a probable indirect effect on cytokinin biosynthesis); reduced dark respiration; changes in carbohydrate composition and transport; mineral nutrition (increase leaf content of N, P, K, Ca, Mg, Mn, B, Zn) and increased stress tolerance (to water, gaseous sulfur dioxide, high and low temperature).

2.2.6.4 Mechanisms of PBZ action:

Triazoles inhibit GA synthesis by blocking microsomal oxidation of kaurene, kaurenol, and kaurenal. This oxidation is normally catalyzed by kaurene oxidase, a cytochrome P-450 oxidase. The inhibitory effect is probably due to the interaction between the lone electron pair of the nitrogen atom located at the periphery of the heterocyclic triazole molecule with the central iron atom of cytochrome P-450. Triazoles might also alter the activity of other plant enzymes which have a slightly different cytochrome P-450 oxidase (Davis and Curry, 1991).

Lenton *et al.,* (1994) proposed that the compounds which inhibit cytochrome P-450 monooxygenases can also cause a reduction of major sterols and an accumulation of Δ^8-14α-methyl sterols. This uncommon sterol might have an adverse effect on membrane fluidity and might also cause a cessation of cell proliferation. They also proposed that interferences with GA and sterol biosynthesis might cause intense effects on different plant processes.

GA_3 completely reversed the effect of PBZ in a number of plants species (Wample and Culver, 1983; Quinlan and Richardson, 1984; Horrell *et al.,* 1989b and Cox, 1991). In

poinsettia, GA_3 (50 mg/L) applied simultaneously or 14 days after PBZ (0.5 mg/L drench or 125 mg/L spray) reversed PBZ's effect but when GA_3 was applied 28 to 42 days after, had very little or no effect on height (Cox, 1993). However, Steffens *et al.*, (1985) obtained greater reversal of apple seedling growth by delaying GA_3 application (19 or 35 days after initial PBZ application). They proposed that with simultaneous application, a part of the GA would not be properly utilised to deactivate PBZ's action in plants compared to when it was applied later to the already inhibited plants. The difference in the crop and the differences in the rate and timing of application might be the reason for these dissimilar results.

2.2.6.5 Effects of PBZ on the level of other hormones and sterols:

Davis and Curry (1991) proposed that the growth regulating effects of triazoles are not simply related to the inhibition of GA biosynthesis. Triazoles also influence the level of other endogenous hormones.

Leshem *et al.*, (1994) observed increased IAA activity following PBZ application in melon cotyledons. Again in pear shoot apices, a plentiful but transient (no more than one week) and quick increase in IAA was noticed within 2 days of PBZ application (Browning *et al.*, 1992a). The authors proposed an indirect effect of PBZ on the enzymes which are active in auxin biosynthesis or metabolism. It was also suggested that PBZ delayed petiole stump abscission in *Ligustrum vulgare* L. through activation of endogenous auxin near the base of the petiole perhaps through decreased GA and increased Auxin availability (Rauscherova and Tesfa, 1993). However, decreased endogenous auxin (IAA) by PBZ was also reported in summer soybean cultivars (Xiao *et al.*, 1990) and in *Tulipa gesneriana* (Suh and Kwack, 1990). Suh *et al.*, (1992) again proposed for the tulips that triazole (including PBZ) might have an effect on auxin metabolism.

PBZ inhibited ABA synthesis in *Cercospora rosicola,* had no effect on the endogenous ABA content of *Pennisetum purpureum* (Davis *et al.*, 1988) and had no effect on ABA production or metabolism in wheat seedlings (Lenton, 1987) but showed a gradual decrease of ABA content following application of higher PBZ concentration (1-30 μM) in wheat seedlings (Lenton *et al.*, 1994). However, in chilli, PBZ increased ABA content and the authors supported the idea of a balance between lower GA and higher ABA for that reason (Yin-Sheng *et al.*, 1989).

In pea roots, ethylene production decreased following PBZ application (Wang and Lin, 1992). In rice, PBZ increased the level of cytokinin and ABA in the grains but reduced ethylene release in the panicles (Dong *et al.*, 1990). PBZ was also found to enhance the effect of cytokinin (kinetin) on the suberisation of potato (Simko, 1993).

Lenton (1987) concluded that a part of the PBZ effect in plants possibly depends on the inhibition of sterol biosynthesis and cell division. He added that, at low concentration, cell elongation is restricted through reduced GA biosynthesis but at higher dosages PBZ probably inhibits both GA and sterol biosynthesis and finally reduces cell division and elongation. Rademacher (1989a) also reported that higher doses of growth retardants (Triazoles,

Pyrimidines etc.) interfere with sterols formation, which reduces cell division, specially in the meristematic tissues.

2.2.6.6 Transport and metabolism of PBZ:

PBZ most effectively penetrates the tissues of shoot tips, youngest leaves and the upper part of young shoots (Quinlan and Richardson, 1986) but normally does not penetrate through mature bark tissues without physical punctures (Lever, 1986).

Triazoles are primarily transported passively via xylem regardless of the application method and little or no translocation occurs via phloem (Davis and Curry, 1991). However, Browning *et al.,* (1992b) proposed that xylem was not the only pathway for PBZ transport in pear shoot. Recent evidence from hydroponically grown nectarines also suggested that PBZ can translocate basipetally (Avidan and Erez, 1995).

Once it is applied, the effect of PBZ persists for a long time. This was clearly demonstrated when an untreated apple tip was grafted on to a PBZ treated shoot. The untreated tip responded in a similar way to the treated apices (Lever, 1986). To maintain continuous GA biosynthesis inhibition, a threshold concentration of PBZ was maintained in the shoot apex by a continuous supply of PBZ into the vascular system at several points below the growing point (Lever, 1986).

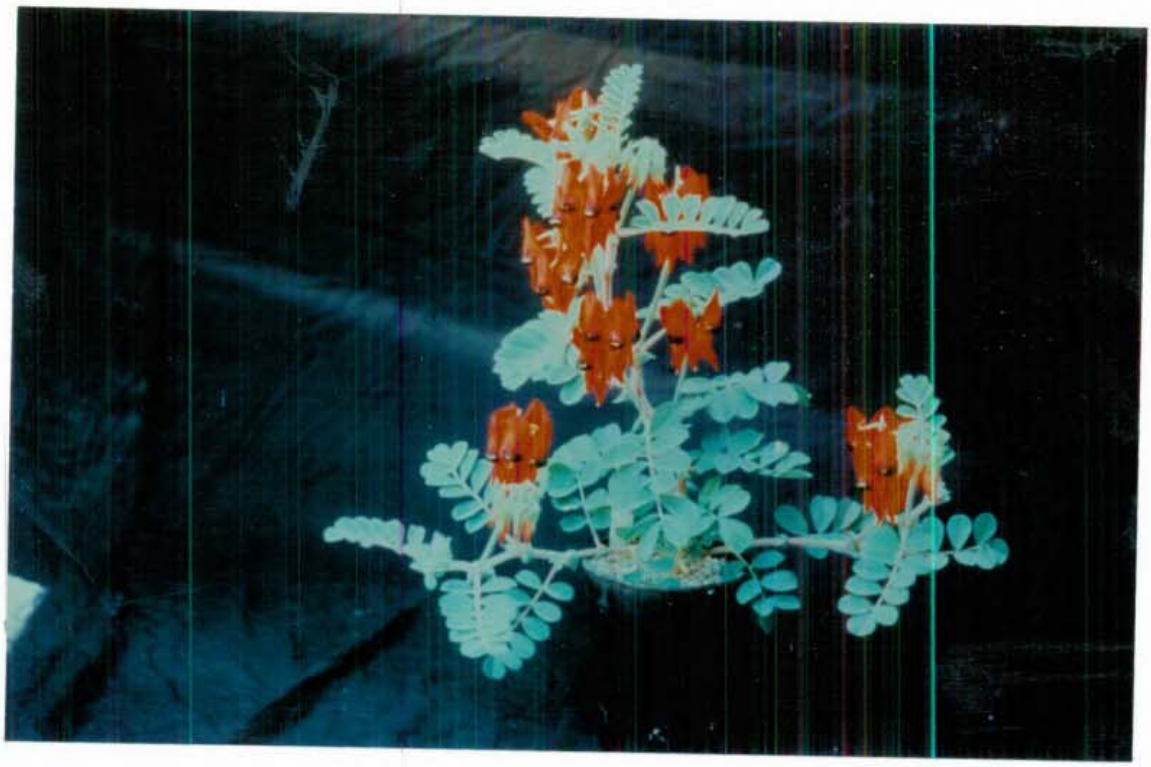
2.3 Sturt's desert pea

2.3.1 Introduction:

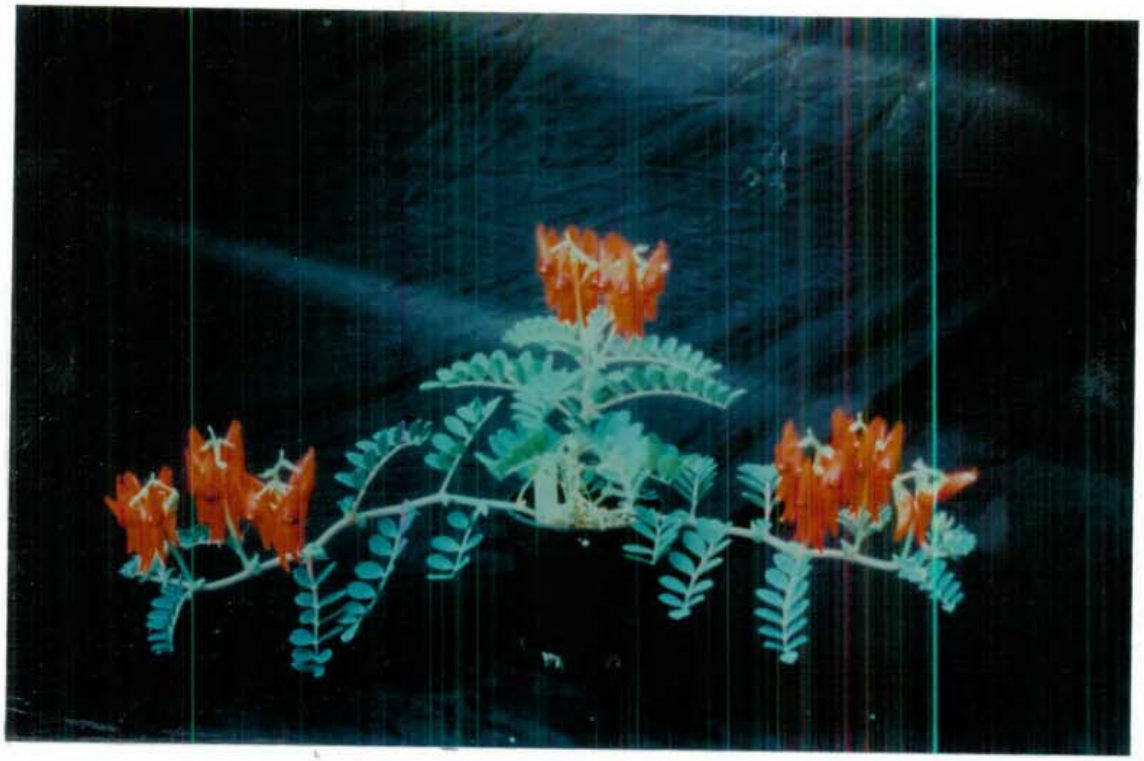
Sturt's desert pea (SDP) is one of the world's most spectacular and striking flowering plants from the Australian bush. It is the floral emblem of South Australia. Its cultivation started in 1699 when William Dampier first collected the specimen from the Dampier Archipelago in Australia. Botanist Allan Cunningham again collected the plant in 1817 and named it *Kennedya speciosa.* Six other collections were made between 1817 and 1844 and the last one was by Sturt himself. SDP has since had a series of changes, in its nomenclature. It was known as *Clianthus formosus, Clianthus speciosus* and *Clianthus dampierii* (Williams and Taji, 1991). However, very recently it has been reclassified and renamed (Thompson, 1990) as *Swainsona formosa* because of its similarity with the genus *Swainsona* (subfamily Papilionoideae; family Leguminaceae).

Swainsona formosa prefers sandy to sandy loam and flood plain soils (Jusaitis and Schmerl, 1993). It also prefers hot and dry climate (Williams and Taji, 1991) and has a high light requirement (Barth, 1990b). *S. formosa* occurs naturally throughout the arid outback regions of most mainland states in Australia (e.g. Western Australia, South Australia, Queensland, New South Wales, Northern Territory).

It has a great commercial potential as a cut flower, hanging basket or as a container grown plant both within and outside Australia. The commercial cultivation of SDP was first initiated by a German nursery, who started export of grafted stocks to the European market back in 1890 (Williams and Taji, 1991). Although its commercialization began very early in



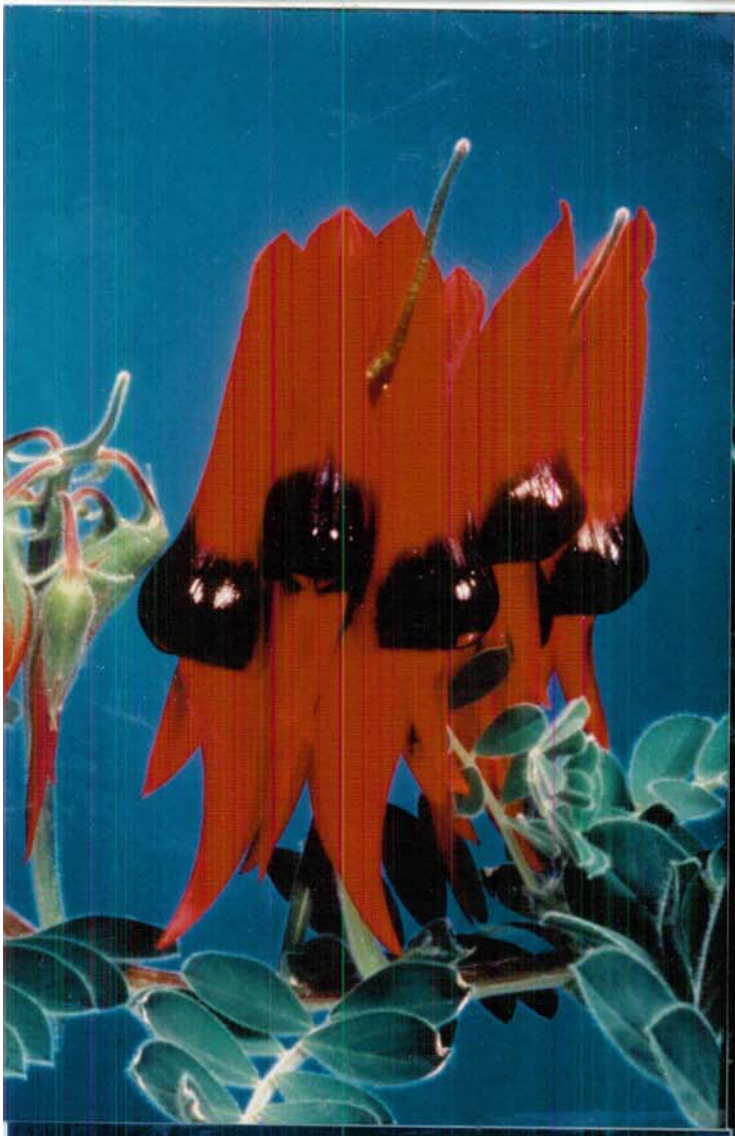
(a) Upright growth.



(b) Prostrate growth.

Plate 2.1 Variations in growth pattern of SDP plants.
(Courtesy of Prof. Williams and Dr. Taji).

Plate 2.2 Variat ons in flower colour of SDP plants.
(Cour esy of Prof. Williams and Dr. Taji).



history only recently has developmental work been done on this plant to develop it as a commercial crop. The industry is unable to meet the demand of the Japanese market for this new crop, which has a great potential to be accepted there (Barth, 1989). However, different laboratories very recently have initiated research programs to develop production and marketing packages for this beautiful plant (Kirby, 1990; Jusaitis and Schmerl, 1993).

2.3.2 Growth pattern:

SDP is an annual or biennial herb, propagated usually from seeds (Tade, 1992). Successful propagation from cuttings and tissue culture was also reported (Williams and Taji, 1987). Different scientists reported great inherent variability in SDP growth patterns (Plate 2.1) depending on the seed sources (Barth 1990a; Williams and Taji, 1991).

Barth (1989) described the growth of container grown SDP plants. She reported that an unsupported central stem grew upright (about 30 cm) but supported central stems continue to climb producing a flower at each node. These stems had a mean diameter of about 1.0 ± 0.3 cm compared with 0.7 ± 0.2 cm on lateral runners. In 105 days, stem lengths of 2 meters with up to 28 flowers were obtained on a trellised runners. Vegetative buds normally occur at the nodes on the lower 2/3 of the stem with floral development on the upper part of the main stem (Barth and Bennell, 1990). The stems are slightly coarse and often brownish or light green in colour but become woody with age. The leaves are pinnate with short petioles (Tade, 1992).

Initially two prostrate lateral branches normally develop and continue to grow but the central stem normally ceases growth after producing a flower. Additional laterals appeared at the base of the plant and these prostrate lateral runners form side branches and ultimately form the characteristic vigorous mat. Staked lateral runners grow rapidly producing flowers at each node rather than producing side branches (Barth, 1989).

The large flag-shaped flower (approximately 100 mm long) consists of an upper standard petal (i.e. flag) with a shiny black boss, and a lower keel which holds the sexual organs. Flowers are normally borne in umbels of up to six flowers suspended by a long (up to 200 mm) peduncle (Jusaitis and Schmerl, 1993). SDP is normally out crossed in the wild and shows a greater variation in colour (Plate 2.2); from shinning black boss, through shades of red and pink to soft white. The peduncle size is also variable (Williams and Taji, 1992). The flowers are ornithophilous (i.e. bird pollinated) in the wild (Jusaitis and Schmerl, 1993).

2.3.3 Problems and solutions:

In SDP, the growth habit, floral characteristics, and disease resistance are extremely variable (Jusaitis, 1994). Barth and Bennell (1990) pointed out, several constraints to the development of a successful SDP export industry. Some of the constrains are listed below:

- The individual flowering stalk (peduncle) was very short for the overseas market.
- The flowering runners were difficult to pack or transport. The transportation costs were very high. Low density packing also added more cost to the transportation.

- The product was vulnerable to the frequent handling and repacking but these were necessary to meet the overseas quarantine regulations.
- The economics of production is a limiting factor and beyond the control of the grower.
- Disease resistance is lacking.

Barth and Bennell (1990) proposed the use of strict sanitation, and total utilization of the domestic market. The utilization of the local market would provide much needed experience in handling/packaging and shipping. They have also recommended a few research priorities to tackle those above mentioned problems:

✓✓ Research programs should be developed to increase the incidence of new variants.
✓✓ The variants should be developed with a longer shelf life.
✓✓ Innovative packaging and handling systems should be considered.
✓✓ Dwarf varieties suitable for pot plant should be selected.

2.3.4 PGRs and SDP shoot growth:

Until a suitable variety is developed through plant breeding or biotechnology, the short term solution to some of the stated problems may be the use of PGRs. A brief review of PGRs research on SDP is presented here.

GA_3 (250ppm) or GA $_{4+7}$ (100-250ppm) sprays applied approximately 6 weeks after sowing doubled the internode length of SDP but caused flower abortion, distorted vegetative growth and reduced lateral shoot numbers. At lower rates (25-50ppm) GA_3 and GA_{4+7} did not interfere with the flower set and had some positive effects on flower size and appearance. An approximately 20% increase in runner length accompanied by a 16% increment in flower stalk length was reported with 25ppm GA_3. GA sprays at these lower rates (25-50ppm) were effective for about 3-4 weeks, which was sufficient for production of a flowering runner (Barth, 1989). However, Jusaitis and Schmerl (1993) although found increase in plant height but did not achieve any effect on time to flowering when GA_3 was sprayed (1, 10, 100 and 1000 mg a.i./L) at 5 weeks after sowing.

In a decapitated plant (central shoot removed at about 7 weeks) most of the laterals reached the 6th node stage by the 10th week. Neither GA nor BA (10, 50 and 100 mM) were able to increase the total number of lateral shoots or the number of potential cuttings at that stage. However, GA did increase mean shoot length. The higher GA or lower BA treatments increased the number of nodes to first flower (Williams and Taji, 1992). Barth, (1990a) obtained increased lateral shoot development at all nodes with 25-50ppm benzyladenine but Jusaitis and Schmerl (1993) did not achieve any significant interaction of BA (100 mg/L) alone or when applied with PBZ on main and lateral shoot growth and also on flowering. However, Jusaitis and Schmerl (1993) observed a reduced number of lateral runners with increased cytolin® (a mixture of GA_{4+7} and BA) concentrations (1, 10, 100 and 1000 mg a.i./L). Cytolin® also had a significant delaying effect on the days to first flower.

It is clear from the presented results that GA has the ability to influence SDP growth but the effective concentration required for effective growth control was variable. Again the effect of BA application on SDP growth was inconsistent.

Different concentrations of CCC sprays or drenches did not show any effect on SDP growth (Barth, 1990b; Jusaitis and Schmerl, 1993). Ancymidol (1, 3, 7 and 10 mg/L drench or 10, 40, 70 or 100 mg/L spray) was effective in producing a compact plant. Ancymidol drench was more effective than spraying (Jusaitis and Schmerl, 1993). However, commercially attractive compact SDP pot plants with reduced height and lateral spread were also obtained with 1-2 mL/L of Bonzi applied as a drench (Jusaitis and Schmerl 1993). Effective growth retardation in SDP (30-40%) was also found earlier with PBZ drench @ 2-4 mg a.i./pot (Barth 1990b).

The inefficiency of CCC would be due to the inappropriate concentrations or time of application. However, PBZ proved as an efficient growth retardant for SDP but again the effective concentration was variable for similar growth control.

2.3.5 Concluding remarks:

The effects of studied PGRs on SDP (Section 2.3.4) were variable and, other than seasonal variations, it could also be due to some of the other variables mentioned in section 2.2.1. Understanding the mechanisms of action of different PGRs, desired growth promotion or inhibition could be achieved and the use of PGRs could be optimised. Earlier workers did not give special emphasis to understanding the mode of action of PGRs in relation to SDP growth control. This thesis explores the mechanism of PBZ control of growth in SDP, particularly the interactions with plant hormones and the anatomical responses involved.

CHAPTER - 3: RESPONSES OF SDP TO PGRs

3.1 General introduction to chapter 3.

Little is known about the effect of growth retardants on Australian native plants in general and particularly their use on SDP (Section 1). To understand the mechanism of control of growth by growth retardants in SDP, development of certain general experimental methodology and understanding certain basic interactions of SDP with plant hormones and growth retardants are of prime importance. Accordingly several experiments were conducted with plant hormones and plant growth retardants to identify their correct type, concentration, frequency, timing of application and their mode of translocation.

This chapter presents the general experimental methodology (Section 3.2), identifies the probable stage for PGR application (Section 3.3) and also present the results of 4 experiments involving application of PGRs to SDP (Section 3.4, 3.5, 3.6 and 3.7).

3.2 General experimental methodology.

The experiments were conducted in a glass house of the Department of Agronomy and Soil Science at the University of New England, Armidale, Australia. Representative data on temperature variations of the glass house during 1993 are given in appendix III.

The SDP plants were grown from seeds of unknown origin, purchased from Arthur Yates and Co. Pty. Ltd. For germination, the seeds were immersed in 1% sodium hypochlorite solution and stirred on a electric stirrer for about 15 minutes. Immediately after this, they were rinsed 3-4 times with sterilized water, then left for 2 hours in a conical flask in sterilized water for further soaking. The seeds were germinated on filter papers soaked in a very dilute solution of Benlate® DF (DuPont Australia Ltd.) in Petrie dishes in an incubator with a constant temperature of 25°C. After germination they were placed into watered soaked compressed peat Jiffy pots ('Jiffy 7') and kept under the mister in a shaded glass house for about 3 to 6 days before transfer to normal glass house conditions. When there was a sign of roots coming through the Jiffy pots, the seedlings were transplanted into 15 cm plastic pots in the late afternoon.

The pots used in experiments were washed in water containing 1% sodium hypochlorite solution (Household bleach), rinsed in clean water and dried in the sun. The potting mixture was 3 parts sand : 1 part of peat. The potting mixture was pasteurized in a soil pasteurizer for about 12 hours at 80°C. After pasteurization of the potting mixture, 4 gm/pot of Nutricote® (Yates; N : P : K = 15 : 4.4 : 8.3) was added and thoroughly mixed with the potting mixture. Aquasol™ was applied @ 220 mg/L every week during the early stages of plant growth (up to 4 weeks) and then 440 mg/L every week during the later stages (after 4 weeks) of growth.

The hygiene of the glass house was maintained very strictly to control any insects and pests. Pirimor® (ICI) was applied @ 500 mg/L against aphid infestation whenever necessary

and Fongarid® (Ciba-Geigy) was applied @ 2 gm/L for Pythium & Phytophthora, once at the seedling stage and 6 weeks after 1st application.

Other cultural details followed standard commercial practices.

A large population of the seedlings were grown and plants of similar appearance and height were selected for the experiments to reduce the inherent variability. The required amounts of concentrated PGRs were mixed with de-ionised distilled (DD) water to make the volume to 50 mL for each treatment. A wetting agent (Agral® 600; ICI) was included at 0.1% of the spray volume. The control plants were treated with DD water plus the wetting agent. The details of the preparation of PGRs are presented in appendix IV. The PGRs were applied in the afternoon, 1 month after transplanting (i.e. 3-4 true leaf stage or approximately 30 ± 3 days after sowing). The PGRs were applied as a spray until run off then the remainder of the 50 mL was applied directly to the potting medium as a drench. Diversey manual pump dispensers were used for spraying the PGRs. Irrigation was avoided for about 8-10 hours before and after the PGR application.

Measurements were made of vegetative growth (main shoot height, number of true compound leaves, lateral shoot number, lateral length, lateral leaf number, elongated internode number and elongated internode length). When an internode reached 0.5 cm long it was counted as an elongated internode and their number was recorded as the number of elongated internodes. When an axillary bud had a length of at least 0.5 cm, it was counted as a lateral shoot. The sum of all lateral shoot growth (in length) of a plant was considered as lateral length. The total number of leaves from lateral growth was expressed as lateral leaf number. The total growth in elongation for the main plus lateral shoot was defined as the total shoot growth. The fresh weight (for roots immediately after washing) and dry weights (oven dried for 48 hr at 80°C) were also recorded in some of the experiments.

The phytotoxicity was quantified using an arbitrary scale of 1-5, where 1 represents nil or very little toxicity and 5 is for dead plants. Phytotoxicity data were normally recorded next day and 30 days after PGR application. Shoot angle was measured for some of the experiments. It was measured as degrees deviated from the main stem (90°). Vegetative data were normally recorded at 4 weeks after PGRs application (unless otherwise mentioned).

The recorded data were analysed by analysis of variance using the statistical packages NEVA (Burr, 1981) and Excel. Means were separated by Duncan Multiple Range Test (DMRT). Whenever transformation of the data was needed, they were transformed by the procedure explained for NEVA (Burr, 1981).

These general materials and methods were used for all experiments unless otherwise mentioned.

3.3 Seedling growth stage for PGR application.

3.3.1 Introduction:

A search of the literature revealed a lack of information about SDP's early growth pattern, so the first step in this project was to identify the early growth pattern of SDP

seedlings and also to identify the time required to establish plants in pots under glasshouse conditions. Accurate identification of the growth stages of the plants is critically important in making management decisions (Kaiser, 1995), including the timing of PGR applications (Section 2.2.1).

3.3.2 Materials and Methods:

Ten seedlings were used in this experiment. Leaf number, their type and phyllotaxy were recorded every 7 days and continued up to 30 days after germination. Seeds were sown on 23.01.93. and the growing procedures were followed as per the general experimental methodology described in section 3.2.

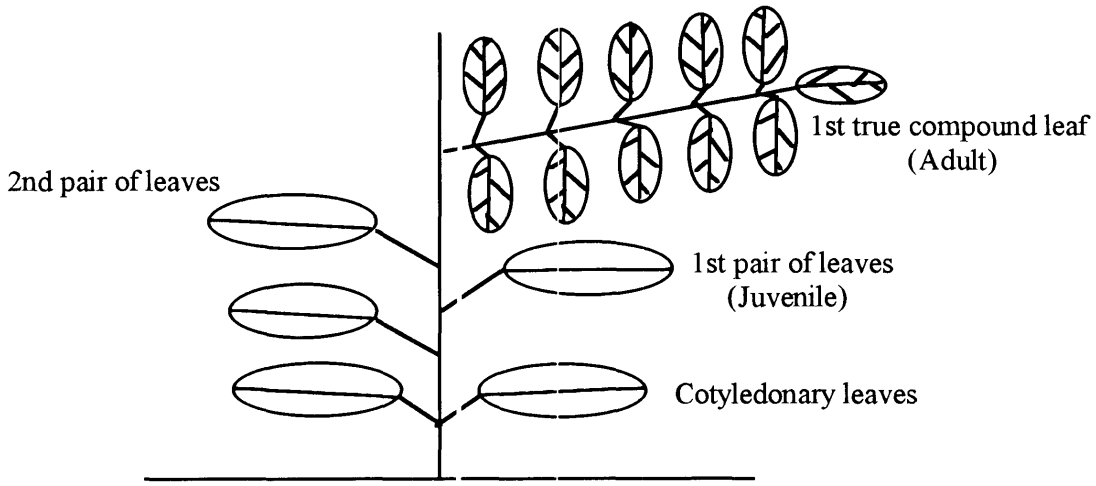


Figure 3.1 General growth pattern of SDP seedling.

3.3.3 Results:

The cotyledonary leaves appeared within 7 days of germination and they had opposite phyllotaxy (Figure 3.1). After about 14 days, the 1st pair of juvenile (simple) leaves appeared, where 80% of the plants had alternate and the rest had an opposite phyllotaxy.

By 21 days, the 2nd pair of leaves appeared and they were alternate. The 80% of the 3rd leaf was juvenile (60% simple + 20% tri or penta foliate compound leaf), while the rest had true compound leaves. The 4th leaf was a true compound leaf for all of the tested plants. By 30 days all of the plants had produced up to the 3rd to 4th true pinnately compound (adult) leaf.

3.3.4 Discussion and Conclusion:

SDP plants produced at least 3 true compound leaves (Adult leaves) within 30 days after sowing. This stage was regarded as the stage when the plant is well established in the glasshouse pots and was adopted as the probable time for application of the PGR treatments.

3.4 Interaction of growth stages and GA_3 application.

3.4.1 Introduction:

As inhibition of GA biosynthesis is the primary mode of action for the growth retardants (Grossman, 1992), understanding GA's mode of action would help to understand the mode of actions for the growth retardants.

In preliminary experiments SDP plants responded easily to a single application of GA_3 500ppm or BAP 25ppm or IAA 20 mg a.i./plant at 3rd leaf stage (4 weeks after sowing). All of these PGRs increased apical dominance of SDP and thereby reduced lateral growth. However, GA_3 enhanced elongation of the main shoot, even when it was applied 10 weeks after sowing (Data not presented). On the other hand, GA_3 reduced lateral shoot numbers but the total lateral shoot length was unaffected at the later stages of GA_3 application. It was not clear from the experiment how long an individual internode contributed towards total elongation of the plant. There was also not enough information available from that experiment about the effect of GA_3 at specific stages of the bud release and its relationship to the total lateral growth.

Accordingly the present experiment was initiated with GA_3 to determine:

a) the duration of responsiveness of a specific internode;

b) the inter-relationship of a specific internode to total plant growth and also

c) GA_3's effect on lateral shoot growth at specific growth stages.

3.4.2 Materials and Methods:

The 1st, 3rd, 5th, 7th, 9th, 11th and 13th true compound leaf stages (i.e. adult leaf stage) of plant growth (which occurred on 23.06.94; 01.07.94; 09.07.94; 17.07.94; 25.07.94; 03.08.94 and 13.08.94 respectively) were selected for this experiment. Plants were treated with GA_3 (500ppm) at each of those 7 growth stages. The treatments were arranged as a factorial design with 10 replications. Vegetative data for each treatment, including the untreated controls for each of these 7 growth stages, were recorded 4 weeks after GA_3 application. Data for the 4th internode (from the base) of the main shoot were also recorded separately 4 weeks after each GA_3 application.

The seeds were sown on 25.05.94. The rest of the procedures followed the general methodology (Section 3.2).

3.4.3 Results:

Main shoot growth: Plant height, leaf number and mean internode length increased with GA_3 application (Table 3.1a). These parameters increased with growth stage at least up to the 9th leaf stage (Table 3.1b), after which the plants ceased elongation even though leaf production continued. There was no interaction of GA_3 with growth stages on plant height (Table 3.1c).

The 4th internode was significantly increased with GA_3 (Table 3.1a) and was responsive to added GA_3 up to the 7th leaf stage of plant growth. But other internodes (upper than 4th internode) responded to GA_3 up to the end of the recorded period (Table 3.1c).

Lateral shoot growth: Irrespective of growth stages, GA_3 reduced lateral shoot and leaf number but increased lateral length (slightly) and reduced the branch angle (Table 3.2a). Plants ceased further shoot production mainly by the 7th leaf stage without GA_3 but, it continued (more slowly) up to the 9th leaf stage with GA_3 (Table 3.2c). Application of GA_3 inhibited lateral length up to the 3rd leaf stage (Table 3.2c) but at later stages the means were similar for both ± GA_3 treatments. However, GA_3 reduced lateral internode length at the 1st leaf stage of plant growth but after that it was increased compared to the untreated control plants (Table 3.2c).

Table 3.1 Effect of GA_3 (500ppm), growth stages and their interactions on main shoot growth.

(a) GA_3 (500ppm).

ppm	Plant height (cm)	Leaf number	Internode length (cm)	4th internode length (cm)
00	10.26a*	12.20a	0.82a	0.52a
GA_3 500	**28.64b**	**13.84b**	**2.02b**	**1.00b**
	t	t	t	t

(b) Growth stages.

Growth stages	Plant height (cm)	Leaf number	Internode length (cm)	4th internode length (cm)
1st true leaf	8.43a*	7.10a	1.14a	1.07c
3rd true leaf	**12.10b**	**9.75b**	1.22ab	1.10c
5th true leaf	**18.00c**	**12.30c**	**1.43bc**	**0.79b**
7th true leaf	**21.00d**	**14.05d**	**1.46cd**	**0.81b**
9th true leaf	25.08e	**14.90e**	**1.65e**	**0.54a**
11th true leaf	25.50e	**15.89e**	**1.56de**	**0.54a**
13th true leaf	26.05e	**17.15f**	**1.48cde**	**0.52a**
	t	t	t	t

(c) GA_3 (500ppm) X growth stages.

Growth stages	Plant height (cm)		Leaf number		Internode length (cm)		4th internode length (cm)	
	00	GA_3 500	00	GA_3 500	00	GA_3 500	00	GA_3 500
1st true leaf	4.75	12.10	6.20a*	**8.00b**	0.77a	**1.52c**	0.50a	**1.63b**
3rd true leaf	6.65	17.55	**9.30c**	**10.20c**	0.72a	**1.73c**	0.50a	**1.70b**
5th true leaf	8.60	27.40	**11.70d**	**12.90e**	0.73a	**2.12d**	0.52a	**1.05c**
7th true leaf	10.70	31.30	**13.40e**	**14.70f**	0.79a	**2.12d**	0.53a	**1.09c**
9th true leaf	12.95	37.20	**14.20f**	**15.60g**	**0.91b**	**2.39e**	0.53a	0.54a
11th true leaf	13.50	37.50	**14.68f**	**17.10g**	**0.93b**	**2.20de**	0.53a	0.50a
13th true leaf	14.70	37.40	**15.90g**	**18.40h**	**0.92b**	**2.04d**	0.53a	0.50a
	ns		t		t		t	

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

Lateral shoot angle: The angle of the lateral shoots (from the main stem) was reduced by GA_3 (Table 3.2a) up to 7th leaf stage (Table 3.2c) but it was the lowest when GA_3 was applied at the 3rd leaf stage. However, when GA_3 was applied after 7th leaf stage, the distal part of those laterals developed prior to this stage showed a tendency to produce insignificant but narrower angles to the main shoot.

Table 3.2 Effect of GA_3 (500ppm), growth stages and their interactions on lateral shoot growth.

(a) GA_3 (500ppm).

ppm	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle (°)
00	5.97a*	63.37a	28.69a	1.85	89.93a
GA_3 500	**4.10b**	**65.46b**	**23.00b**	**2.17**	**71.80b**
	t	t	t	ns	t

(b) Growth stages.

Growth stages	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle (°)
1st true leaf	1.55e*	3.71a	4.20a	0.54a	72.92a
3rd true leaf	**2.75d**	**11.73b**	**10.85b**	**1.00b**	67.68a
5th true leaf	**3.75c**	**29.08c**	**18.25c**	**1.56c**	**73.50b**
7th true leaf	**6.35b**	**61.68d**	**28.30d**	**2.25d**	**82.22c**
9th true leaf	**6.55ab**	**92.30de**	**33.45de**	**2.77e**	**89.75d**
11th true leaf	**7.10a**	**118.05e**	**39.27de**	**3.04e**	**90.00d**
13th true leaf	**7.20a**	**133.38e**	**46.60e**	**2.93e**	**90.00d**
	t	t	t	t	t

(c) GA_3 (500ppm) X growth stages

Growth 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 true leaf	**2.50f***	**0.60h**	**3.76b**	**3.65a**	5.80b	2.60a	0.65b	**0.42a**	90.00d	**55.83b**
3rd true leaf	**4.10e**	**1.40gh**	**15.60c**	**9.85b**	15.60cd	6.10b	0.98b	1.02bc	90.00d	**45.36a**
5th true leaf	**5.20d**	**2.30fg**	33.60de	24.55cd	23.30de	13.20c	1.46cd	1.66cd	89.50d	**57.50b**
7th true leaf	7.30ab	**5.40d**	51.40ef	71.95fg	31.20efg	25.40def	1.66cd	2.83e	90.00d	**74.44c**
9th true leaf	7.40ab	**5.70cd**	90.80fgh	93.80fgh	35.90efg	31.00efg	2.54e	3.00e	90.00d	89.50d
11th true leaf	7.60ab	6.60bc	112.30gh	123.80gh	39.33efg	39.20efg	2.87e	3.21e	90.00d	90.00d
13th true leaf	7.70a	6.70ab	136.15h	130.60gh	49.70g	43.50g	2.81e	3.06e	90.00d	90.00d
	t		t		t		t		t	

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

3.4.4 Discussion:

Main shoot growth: Although the responsiveness of the internodes to GA_3 continued up to the end of the experiment, the 4th internode finished growth by the 7th leaf stage (Table 3.1b and 3.1c), therefore the growth response to GA_3 after 7th leaf stage does not depend on

this specific internode. Irrespective of the time of GA_3 application the main shoot growth was increased 3 times their respective controls in this present experiment (Table 3.1c). The growth after 7th leaf stage following GA_3 application should come from upper internodes, as the lower internodes cease their response to GA_3 earlier (4th internode ceases by 7th leaf stage).

This result suggests that the growth response of a specific internode to GA_3 depends on its growth stages. According to Sauter *et al.*, (1995) cells that have already been displaced from the meristem before GA application are unable to respond through increased growth. Again in *Sclerocarya birrea* sp. caffra GAs were important mainly during the initial period of shoot growth (up to 14 days of GA_3 application) but by 28 to 42 days the GA_3 level had dropped considerably and was similar to that of control trees (Bertling and Bangerth, 1995).

Lateral shoot growth: In this present experiment, GA_3 extended the inhibition of lateral shoots from the 7th to 9th leaf stages (Table 3.2c) because of its reinforcement of apical dominance (Cline, 1991). The statistically similar lateral length for $\pm$ GA_3 (Table 3.2c) after 3rd leaf stage suggests that GA_3 enhanced the elongation of the already released lateral shoots. At the earlier stage of application, greater reduction of lateral growth was found. The reduction in lateral growth in earlier stages might also be because of the lower number of shoots produced and not because of their reduced elongation as evident from the statistically similar lateral internode lengths (except 1st leaf stage) for all of the treated plants (Table 3.2c) compared to the untreated plants.

Lateral shoot angle: Branch angles were reduced by GA_3 application up to the 7th leaf stage (Table 3.2c). The narrower angle was due to more vigorous and hence more upright growth of the laterals due to GA_3.

3.4.5 Conclusions:

* GA_3 tended to increase main shoot growth at all stages of plant growth.
* Growth response of a specific internode to GA_3 depends on its growth stage.
* Lower internodes of the main shoot ceased responding to added GA_3 earlier (e.g. 4th internode by the 7th leaf stage) but further elongation continued in the upper internodes.
* GA_3 delayed the release of the buds from apical dominance but enhanced further elongation of the released laterals.
* GA_3 increased apical dominance in main shoots and reinforced correlative inhibition in young buds through more vigorous growth of the released laterals.

3.5 Interaction of tryptophan (types and concentrations) with GA_3.

3.5.1 Introduction:

There are reports that direct application of IAA can cause bud out growth (Prasad and Cline, 1985). But IAA (20 mg a.i./plant) application to the whole plant caused bud inhibition in a preliminary experiment (data not presented). GA_3 was also inhibitory for the lateral bud out growth (Table 3.2c). GA induced growth is sometimes explained in terms of GA induced auxin biosynthesis where auxin is the real regulator of growth. For example, in peas GA

increased the levels of free auxin by promoting the racemisation of L-tryptophan to D-tryptophan (a direct precursor of auxin) (Metraux, 1987). Complete inhibition of lateral buds of pea by auxin plus GA, compared to auxin alone, may result from higher amounts of IAA in the immediate vicinity of the inhibited buds (Rubinstein and Nagao, 1976).

Keeping these contradictions in mind it is proposed that the absence of auxin precursor might be responsible for the inability of applied GA to cause bud out growth. Accordingly, an experiment was set up to examine the role of GA_3 in auxin synthesis and their relationship to the process of apical dominance in SDP.

3.5.2 Materials and Methods:

Fourteen (14) treatments were used in this experiment including untreated and a GA_3 (500ppm) treated control plants. The rest of the treatments consisted of different concentrations of L-tryptophan and D-tryptophan (0.3, 3.0 and 6.0 mg/kg of soil) alone or with GA_3 (500ppm). The experiment was set up in a randomised complete block design with 5 replications. The weight of the potting mixture was 1500 gm (air dried weight).

The seeds were sown on 10.11.94. Vegetative data were recorded 4 weeks after PGR application. The rest of the procedures were followed as per section 3.2.

3.5.3 Results:

Main shoot growth: L-tryptophan (0.3 mg/kg of soil) along with GA_3 gave the tallest plant. All concentrations of D-tryptophan (alone or with GA_3) had an inhibitory effect on plant height compared to their respective controls (Table 3.3).

Lateral shoot growth: All concentrations of L or D-tryptophan, when applied alone, had no effect on the number of lateral shoots. Shoot numbers were reduced with GA_3 alone or with all of its combinations with tryptophan. GA_3 or D-tryptophan (alone) or any combinations of GA_3 with tryptophan, reduced lateral length but the effect of D-tryptophan (alone) was small compared to GA_3 (Table 3.4).

Lateral shoot angle: GA_3 alone or with tryptophan produced a narrower branch angle. Tryptophan alone had no effect on the angle formation (Table 3.4).

3.5.4 Discussion:

Main shoot growth: The present results suggest that the auxin precursor L-tryptophan can produce auxin when GA_3 is supplied at the same time. Auxin produced by L-tryptophan (0.3 mg/kg of soil) along with GA_3 expressed maximum main shoot elongation of SDP probably because of a suitable auxin GA balance (Section 2.2.5.1). The concentrations of L-tryptophan higher than 0.3 mg/kg of soil along with GA_3 might be inhibitory because of supra-optimal auxin production (?).

L-tryptophan (alone) at all tested concentrations was ineffective probably because of insufficient GA_3 in the plants to interact with exogenously applied tryptophan. Alternatively, it could also be due to the lack of soil microbial biomass (Lebuhn *et al.*, 1994). Arshad *et al.*,

Table 3.3 Effect of L-tryptophan, D-tryptophan and GA_3 (500ppm) on main shoot growth in an intact plant.

mg/kg of soil	Plant height (cm)	Leaf number	Internode length (cm)
Control	**10.05bc***	11.60cd	**0.88b**
L-tryptophan (0.3)	11.60cd	12.20bcd	0.95bc
L-tryptophan (3.0)	11.00cd	12.20bcd	0.90bc
L-tryptophan (6.0)	10.60cd	11.60cd	0.91bc
L-tryptophan (0.3) + GA_3	**21.80g**	13.00ab	**1.69e**
L-tryptophan (3.0) + GA_3	16.80ef	13.40ab	1.26d
L-tryptophan (6.0) + GA_3	17.00ef	13.20ab	1.29d
D-tryptophan (0.3)	9.00ab	12.40abc	**0.73a**
D-tryptophan (3.0)	9.00ab	11.40cd	**0.79a**
D-tryptophan (6.0)	8.40a	**11.00d**	**0.77a**
D-tryptophan (0.3) + GA_3	15.20e	12.60abc	1.22d
D-tryptophan (3.0) + GA_3	16.00ef	13.00ab	1.23d
D-tryptophan (6.0) + GA_3	12.80d	12.40abc	1.04c
GA_3	**17.80f**	13.60a	1.31d
	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 3.4 Effect of L-tryptophan, D-tryptophan and GA_3 (500ppm) on lateral shoot growth in an intact plant.

mg/kg of soil	Shoot number	Lateral length (cm)	Leaf number	Internode length (cm)	Mean angle (°)
Control	5.60de*	68.90a	32.40f	2.11a	89.50d
L-tryptophan (0.3)	4.60cd	62.20ab	31.00f	2.02a	89.50d
L-tryptophan (3.0)	6.00de	72.60a	34.40f	2.12a	88.00d
L-tryptophan (6.0)	6.00de	69.00a	32.40f	2.13a	88.00d
L-tryptophan (0.3) + GA_3	**3.20bc**	**27.40cd**	18.20def	1.47bc	**41.50a**
L-tryptophan (3.0) + GA_3	**2.40ab**	**13.20def**	9.60bc	1.10c	**51.50b**
L-tryptophan (6.0) + GA_3	**2.00a**	**4.20f**	4.80a	0.60d	**57.00c**
D-tryptophan (0.3)	6.00de	**51.10b**	28.80f	1.81ab	87.00d
D-tryptophan (3.0)	8.00e	**50.00b**	34.60f	1.45bc	89.00d
D-tryptophan (6.0)	6.40de	**35.40c**	24.00ef	1.47bc	89.00d
D-tryptophan (0.3) + GA_3	**3.00bc**	**12.20def**	8.80b	1.07c	**55.00c**
D-tryptophan (3.0) + GA_3	**2.80ab**	**23.40cde**	14.80cde	1.52bc	**56.00c**
D-tryptophan (6.0) + GA_3	**2.40ab**	**8.80ef**	6.80b	1.18c	**58.50c**
GA_3	**3.00bc**	**17.50def**	13.00bcd	1.19c	**48.00b**
	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.

(1995) reported that L-tryptophan applied as a drench to cotton was converted into auxin through microbial activities. However, exogenously applied GA_3 + L-tryptophan (0.3 mg/kg

of soil) caused elongation growth in this present experiment (Table 3.3), absence of GA_3 was probably the more likely reason for the ineffectiveness of L-tryptophan (alone).

The tested concentrations of D-tryptophan might be supra optimal and probably produced enough auxin without the presence of GA_3 (Section 2.2.2.3). D-tryptophan + GA_3 treated plants might also have enough auxin from D-tryptophan and was not in a proper balance with supplied GA_3 for elongation. As a result both D-tryptophan alone or with GA_3 caused shoot growth inhibition compared to their respective controls.

In excised little marvel pea segments, D-tryptophan (5 X 10^{-4} M) mimicked exogenous IAA (5 X 10^{-5} M) application without any GA_3 application, whereas L-tryptophan (5 X 10^{-4} M) was active only if GA_3 was supplied (Law, 1987). In Alaska pea, D-tryptophan was a more effective IAA precursor than the L-stereo isomer (McQueen-Mason and Hamilton, 1989). These results again agree with the present results which suggests the ability of D-tryptophan to produce auxin without any GA_3 and also supports the idea of GA_3 involvement in the conversion of L-tryptophan to auxin.

Lateral shoot growth: GA_3 increased plant height and reduced lateral shoot growth, presumably due to enhanced apical dominance but the addition of tryptophan did not change the pattern of GA_3 response.

L-tryptophan (alone) did not affect the lateral shoots. This inactivity may be due to the lack of available GA in the plant. D-tryptophan (alone) on the other hand, inhibited lateral shoot elongation but increased lateral shoot number. Since it does not appear to require GA for its activity, D-tryptophan levels (i.e. auxin levels) may have been supra-optimal for elongation growth but within the suitable range for additional shoot production. These data (Table 3.4) suggests that there is an endogenous hormonal balance between GA and auxin and if that balance is changed, lateral bud out growth or elongation might increase or decrease.

Lateral shoot angle: The addition of tryptophan to GA did not alter the pattern of GAs response on branch angle (Table 3.4).

3.5.5 Conclusions:

* Auxin precursor L-tryptophan (0.3 ng/kg soil) yielded auxin in the presence of GA_3 and the auxin : GA balance was suitable for the maximum main shoot elongation.
* Endogenous GA and auxin must be in balance for maximum growth of main and lateral shoots.
* Added GA_3 alone or in any combination induced apical dominance in the main shoot.
* The conversion of D-tryptophan to the active auxin did not require GA_3.
* The auxin requirement was more for the lateral shoots than the main shoot.
* The tested D-tryptophan concentrations could be supra optimal for elongation growth in main and lateral shoots.

3.6 Effect of different types and concentrations of plant growth retardants.

3.6.1 Introduction:

Different types of GA biosynthesis inhibitors are available (Davis and Curry, 1991) and plants respond differently to these different types and their concentrations (Forshey, 1991). Therefore, in this present experiment an attempt was made to evaluate the effectiveness of 5 widely used and easily available growth retardants, controlling growth of SDP. Attempts were also made to understand their mechanisms of action.

3.6.2 Materials and Methods:

Six different concentrations of 5 plant growth retardants were used in 5 concurrent experiments. The treatments were: ancymidol (00, 0.1, 0.5, 1.0, 2.5 and 5.0 mg a.i./plant), chlormequat (00, 50, 200, 500, 1000 and 2000 mg a.i./plant), daminozide (00, 0.1, 0.5, 2.5, 5.0 and 10 % a.i./plant), flurprimido (00, 0.015, 0.06, 0.25, 0.5 and 1.0 mg a.i./plant) and PBZ (00, 0.1, 1.0, 10, 20 and 50 mg a.i./plant). The experiments were laid out in a randomised complete block design with 10 replications. The data were recorded and analysed separately for each of these experiments.

Seeds were sown on 11.05.93. Vegetative data were recorded at 2, 4 & 8 weeks after PGR application and flowering data whenever visible. The data for lateral and total shoot growth were not recorded at week 2. The data for lateral leaf number were only recorded at 8 weeks after PGR application. Fresh and dry weights were recorded at the end of the experiments. The rest of the procedures were followed as per Section 3.2.

3.6.3 Results:

Ancymidol: *Toxicity:* No toxicity was recorded with any of the tested ancymidol concentrations (Table 3.5a).

Main and lateral shoot growth: Apart from shorter plants and internodes at 8 weeks with the 1 or 5 mg a.i./plant of ancymidol, most of the other parameters were not significantly affected (Table 3.6a). Insignificant differences in lateral shoot growth following ancymidol application were not presented here.

Flowering: 5 mg a.i./plant delayed flowering by 7 days compared to control plants.

Fresh and dry weight: 5 mg a.i./plant increased root fresh and dry weights compared to the control plants (18.42 gm compared to 8.78 gm and 2.16 gm compared to 1.11 gm respectively).

CCC: *Toxicity:* The higher concentrations of CCC (500, 1000 & 2000 mg a.i./plant) were too toxic and caused death for all treated plants within 24 hours after application (Table 3.5a). Toxicity symptoms (e.g. leaf burning) were also visible with the lower concentrations (50 & 200 mg a.i./plant) but the plants recovered at later stages of their growth.

Main and lateral shoot growth: Except for significant reduction in main shoot leaf number with 200 mg a.i./plant at 2 weeks, the rest of the parameters were not significantly

Table 3.5 Effect of growth retardants on phytotoxicity, next day after application.

(a) Ancymidol, CCC and daminozide.

Ancymidol (a.i./plant)	Toxicity	CCC (a.i./plant)	Toxicity	Daminozide (a.i./plant)	Toxicity
00 mg	1.00	00 mg	1.00d*	00 %	1.00c
0.10 mg	1.00	50 mg	1.20d	0.10 %	1.00c
0.50 mg	1.00	200 mg	**2.00c**	0.50 %	1.50c
1.00 mg	1.00	500 mg	**4.30b**	2.50 %	1.90c
2.50 mg	1.00	1000 mg	**5.00a**	5.00 %	**4.20b**
5.00 mg	1.00	2000 mg	**5.00a**	10.00 %	**5.00a**
	ns				t

(b) Flurprimidol and PBZ.

Flurprimidol (a.i./plant)	Toxicity	PBZ (a.i./plant)	Toxicity
00 mg	1.00	00 mg	1.00b
0.015 mg	1.00	0.10 mg	1.00b
0.06 mg	1.00	1.0 mg	1.00b
0.25 mg	1.00	10 mg	1.10b
0.50 mg	1.20	20 mg	1.00b
1.00 mg	1.00	50 mg	**1.80a**
	ns		t

1 = Nil or negligible toxicity; 5 = Dead plants.
* = Means followed by the same letters are not significantly different at DMRT 5% (P = 0.05).
ns = Not significant.
t = Analysis was done on the transformed data.

Table 3.6 Effect of ancymidol and CCC on main shoot growth at 2, 4 and 8 weeks.

(a) Ancymidol.

mg a.i./plant	Plant height (cm)			Leaf number			Internode length (cm)		
	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks
0.00	2.35	3.99	7.83ab*	5.00	6.70ab	10.70	0.48	0.60	0.73ab
0.10	2.43	4.16	8.70a	5.20	6.80a	10.98	0.47	0.61	0.80a
0.50	1.91	3.07	5.92bc	4.80	5.70c	9.07	0.40	0.54	0.65ab
1.00	2.28	3.79	**5.39c**	5.00	6.10abc	10.16	0.45	0.62	0.55b
2.50	1.96	3.40	6.22abc	5.00	6.40abc	9.84	0.41	0.54	0.63ab
5.00	1.93	3.37	**4.80c**	4.80	5.90bc	9.39	0.40	0.57	0.51b
	ns	ns	t	ns		ns	ns	ns	

(b) CCC.

mg a.i./plant	Plant height (cm)			Leaf number			Internode length (cm)		
	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks
00	1.90	3.17	6.40	4.7a*	5.70	9.70	0.41	0.56	0.63
50	1.70	3.24	6.43	4.7a	6.30	8.85	0.37	0.51	0.66
200	1.67	2.81	5.30	**3.5b**	5.50	9.38	0.57	0.56	0.55
	ns	ns	ns	t	ns	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.

affected throughout the whole period of the experiment (Table 3.6b). The insignificant results from lateral shoot growth data were omitted from the presentation.

Fresh and dry weight: Fifty mg a.i./plant of CCC significantly increased shoot fresh weight (54.22 gm compared to 39.95 gm in control), dry weight (12.93 gm compared to 9.31 gm for control), root fresh weight (17.24 gm compared to 6.8 gm in control) and root dry weight (2.22 gm compared to 1.00 gm in control).

Flowering: Flower parameters were not significantly different following CCC application.

Daminozide: *Toxicity:* Toxicity of daminozide increased with the concentration. At 2.5% a.i./plant 40%, and at 5.0% or 10% a.i./plant all, of the treated plants died within 24 hours after application (Table 3.5a).

Main shoot growth: Plant height was reduced both at 2 and 4 weeks with all daminozide concentrations but by 8 week, the lower concentration (0.1% a.i./plant) was no longer effective. The main shoot leaf number was reduced with time and with the concentration of daminozide (Table 3.7a).

Lateral and total shoot growth: At 4 weeks, lateral shoot length and total shoot growth were reduced by all daminozide treatments but by 8 weeks only the higher concentrations (0.5 and 2.5 % a.i./plant) showed reduction (Table 3.7b).

Table 3.7 Effect of daminozide on main, lateral and total shoot growth at 2, 4 and 8 weeks.

(a) Main shoot growth.

% a.i./plant	Plant height (cm)			Leaf number			Internode length (cm)		
	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks
0.00	2.40a*	3.86a	8.50a	5.10a	6.50a	9.80a	0.46ab	0.61a	0.85b
0.10	**1.27b**	**2.46b**	4.87a	4.60a	6.20a	9.21ab	**0.27c**	**0.40b**	**0.54a**
0.50	**1.40b**	**2.13b**	**3.79b**	4.30a	5.90ab	**8.50b**	0.33bc	**0.36b**	**0.44a**
2.50	**1.51b**	**2.28b**	**3.52b**	**2.78b**	**5.30b**	**8.42b**	0.84a	**0.42b**	**0.43a**
	t	t	t	t		t	t	t	t

(b) Lateral and total shoot growth.

% a.i./plant	Shoot number			Lateral length (cm)		Lateral leaf number	Total shoot growth (cm)	
	2 Wks	4 Wks	8 Wks	4 Wks	8 Wks	8 Wks	4 Wks	8 Wks
0.00	2.40	3.40	3.60	5.94a*	30.05a	17.30	9.80a	38.55a
0.10	2.10	3.50	4.03	**3.46b**	28.17a	20.04	**5.92b**	33.04a
0.50	2.00	3.20	3.84	**2.91b**	21.45ab	18.05	**5.04b**	25.25ab
2.50	2.28	2.44	3.37	**1.77b**	**13.55b**	16.02	**4.05b**	**17.07b**
	ns	ns	ns			ns	t	t

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

Fresh and dry weight: The concentration 0.1% and 0.5% a.i./plant promoted root dry weight (2.52 and 2.43 gm) compared to the control (1.21 gm). Root fresh weights were also higher (24.9 and 15.32 gm compared to 10.39 gm for control).

Flowering: At the highest concentration (2.5% a.i./plant), opening of the 1st flower bud was delayed (92.01 days compared to 80.7 days for controls) and the time for 1st flowering was also delayed (116 days compared to 103 days).

Flurprimidol: *Main shoot growth:* Plant height was significantly reduced both at 4 and 8 weeks (Table 3.8a) with the higher concentrations of flurprimidol (0.5 & 1.0 mg a.i./plant). There was a corresponding and significant reduction in internode lengths only at 8 weeks.

Lateral shoot growth: Flurprimidol had no effect on shoot number through out the experiment but higher concentrations (0.5 & 1.0 mg a.i./plant) slightly reduced the lateral length at 8 weeks (Table 3.8b).

Flowering: One mg a.i. of flurprimidol per plant delayed the time to produce the 1st flower bud by 6 days but shortened the time from bud maturity to flowering by 7 days.

PBZ: *Toxicity:* The highest dose (50 mg a.i./plant) expressed a significant level of toxicity on the leaves, but they partly recovered at the later stages of growth (Table 3.5b).

Table 3.8 Effect of flurprimidol on main, lateral and total shoot growth at 2, 4 and 8 weeks.

(a) Main shoot growth.

mg. a.i./plant	Plant height (cm)			Leaf number			Internode length (cm)		
	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks
0.00	1.82ab*	3.27a	7.1[illegible]a	4.90	6.20	10.10	0.37	0.52	0.71a
0.015	2.09a	3.25a	**4.85bc**	5.10	6.50	10.17	0.40	0.49	**0.48bc**
0.06	2.09a	3.39a	6.00ab	4.90	6.50	9.80	0.42	0.52	0.61ab
0.25	1.70ab	2.82ab	**4.45bc**	4.80	5.80	9.42	0.35	0.48	**0.47bc**
0.50	1.40b	**2.34b**	**3.5[illegible]c**	4.90	6.03	9.71	0.29	0.39	**0.38c**
1.00	1.52b	**2.31b**	**3.4[illegible]c**	4.70	5.60	8.44	0.32	0.41	**0.42c**
	t			ns	ns	ns	ns	ns	

(b) Lateral and total shoot growth.

mg. a.i./plant	Shoot number			Lateral length (cm)		Lateral leaf number	Total shoot growth (cm)	
	2 Wks	4 Wks	8 Wks	4 Wks	8 Wks	8 Wks	4 Wks	8 Wks
0.00	2.50	3.60	3.70	4.72	24.15ab*	19.20	7.99	31.29a
0.015	2.70	3.80	4.03	5.64	28.66a	20.89	8.89	33.51a
0.06	3.00	3.60	3.60	5.22	28.50a	20.00	8.61	34.50a
0.25	2.20	3.40	3.47	4.15	22.17ab	16.25	6.97	26.63ab
0.50	2.20	3.07	3.13	2.86	12.56b	16.07	5.20	**16.15b**
1.00	2.80	3.60	3.82	3.38	13.02b	18.26	5.69	**16.45b**
	ns	ns	ns	ns	t	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.

Main shoot growth: Plants treated with PBZ showed a significant reduction both in plant height and internode length (except with 50 mg a.i./plant after 2 and 4 weeks) with all of the tested concentrations and throughout the whole period of the experiment (Table 3.9a). The number of leaves on the main shoot was only significantly different with 50 mg a.i. /plant at 2 and 4 weeks.

Table 3.9 Effect of PBZ on main, lateral and total shoot growth at 2, 4 and 8 weeks.

(a) Main shoot growth.

mg a.i./plant	Plant height (cm)			Leaf number			Internode length (cm)		
	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks	2 Wks	4 Wks	8 Wks
0.00	2.24a*	3.82a	8.25a	4.40a	5.90abc	10.40	0.51b	0.65a	0.79a
0.10	**1.49b**	**2.80b**	**5.25b**	4.60a	6.30a	9.40	**0.33a**	**0.45b**	**0.55b**
1.00	**1.64b**	**2.82bc**	**4.65b**	4.90a	6.00ab	9.56	**0.34a**	**0.47b**	**0.48bc**
10.00	**1.72b**	**2.60bc**	**3.30c**	4.60a	6.00ab	9.60	**0.36a**	**0.44b**	**0.34d**
20.00	**1.39b**	**2.24c**	**3.05c**	4.00a	5.20bc	9.00	**0.35a**	**0.44b**	**0.34d**
50.00	**1.66b**	**2.37bc**	**2.93c**	**2.52b**	4.75c	8.38	0.84b	0.56ab	**0.37cd**
		t				ns			

(b) Lateral and total shoot growth.

mg a.i./plant	Shoot number			Lateral length (cm)		Lateral leaf number	Total shoot growth (cm)	
	2 Wks	4 Wks	8 Wks	4 Wks	8 Wks	8 Wks	4 Wks	8 Wks
0.00	2.50	3.40b*	4.00b	4.49a	26.80a	16.30a	8.31a	35.05a
0.10	2.50	3.40b	4.10b	3.60ab	21.10ab	15.55ab	**6.40b**	26.35a
1.00	2.20	3.40b	4.30b	3.35ab	19.13ab	17.65ab	**6.17b**	23.78a
10.00	2.00	**4.50a**	**5.10a**	**2.24b**	**8.65b**	16.90a	**4.84b**	**11.95b**
20.00	1.90	3.00bc	3.00c	**0.75c**	**3.45c**	**9.80bc**	**2.99c**	**6.50c**
50.00	2.02	2.51c	2.60cb	**0.68c**	**2.20c**	**8.17c**	**3.06c**	**5.14c**
	ns		t	t	t	t	t	t

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

Table 3.10 Effect of PBZ on shoot and root weight.

mg a.i./plant	Shoot fresh weight (gm)	Shoot dry weight (gm)	Root fresh weight (gm)	Root dry weight (gm)
0.00	35.05d*	4.83b	7.70b	1.33b
0.10	48.28d	8.21a	19.33a	2.27a
1.00	43.68d	9.40a	25.63a	2.79a
10.00	**16.49c**	**2.9bc**	8.88b	1.13bc
20.00	**9.35b**	**1.44c**	7.96b	0.93bc
50.00	**4.98a**	**1.63c**	**4.25c**	**0.58c**
	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.

Lateral and total shoot growth: Both at 4 and 8 weeks, the higher doses (20 and 50 mg a.i./plant) reduced shoot numbers and 10 mg a.i./plant significantly increased the number. The lateral lengths were reduced with all of these concentrations (10, 20 and 50 mg a.i./plant) at both 4 and 8 weeks. The total number of lateral leaves were only reduced by 20 and 50 mg a.i./plant PBZ at 8 weeks data. The total shoot growth was significantly reduced for all of the concentrations at 4 weeks but only for higher concentrations at 8 weeks (Table 3.9b).

Fresh and dry weight: All of the higher concentrations (10, 20 and 50 mg a.i./plant) reduced shoot fresh and dry weights but only the highest concentration (50 mg a.i./plant) significantly reduced the root fresh and dry weight. Lower concentrations (0.10 and 1.00 mg a.i./plant) increased all weights (Table 3.10).

Flowering: Higher concentrations (10, 20 and 50 mg a.i/plant) delayed the appearance of the 1st flowering bud and the 1st flower, while 50 mg a.i./plant treated plants took maximum time to produce the 1st flower from an already open bud (Table 3.11).

Table 3.11 Effect of PBZ on flowering characteristics.

mg a.i./plant	Days to 1st. flower bud opening	Day to 1st. flowering	Days from bud to 1st flowering
0.00	78.20c*	104.30d	26.10b
0.10	82.47c	108.64d	26.18b
1.00	83.41c	108.46d	25.06b
10.00	**98.70b**	**121.20c**	22.50bc
20.00	**107.72a**	**128.05b**	20.34c
50.00	**98.64b**	**139.25a**	**40.61a**
		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.

3.6.4 Discussion:

Ancymidol: *Main and lateral shoot growth:* The tested concentrations of ancymidol had an inconsistent effects on SDP growth in this present experiment (Table 3.6a). Jusaitis and Schmerl (1993) obtained retardation of SDP with ancymidol by spraying (10, 40, 70 or 100 mg/L) or drenching (1, 3, 7 or 10 mg/L) at 6 weeks after sowing. Plant height was significantly reduced after ancymido application to Easter lilies (Bailey and Miller, 1989), *Chrysanthemum* (Bonaminio and Larson, 1978) and poinsettia (White and Holcomb, 1974) but ancymidol was ineffective when applied to *Boronia serrulata* (Lamont, 1985), or to some landscape plants (Cathey, 1975). However, the ineffectivity of the tested concentrations to reduce SDP height in this present experiment could be due to the differences in concentrations and/or due to the difference in application stages.

In a subsequent experiment (data not presented), higher ancymidol concentrations (10 and 25 mg a.i./plant) although having some toxicity problems, produced dwarf plant with shorter internodes and reduced lateral length (not shoot number). This effect is consistent with ancymidol's effect on inhibition of GA biosynthesis (Dicks *et al.*, 1974; Coolbaugh and

Hamilton, 1976; Dicks and Kawi, 1979). Cathey and Heggested (1973) also found tan specks with marginal yellowing between the veins in poinsettias with higher concentrations of ancymidol (400 ppm or more). Tayama *et al.*, (1992) also reported necrotic spots on leaves and leaf margins on flowering plants, treated with ancymidol under higher temperature.

Flowering: Flowering was delayed in this present experiment with ancymidol 5 mg a.i./plant but Jusaitis and Schmerl (1993) found no significant effect on days to flowering. Anderson and Hartley (1990) reported a delay in flowering of Satin flower with ancymidol. Early flowering was also reported in some species but was not common while slightly higher concentrations delayed flowering in many plants (Cathey, 1975).

Fresh and dry weight: Ancymidol 5 mg a.i./plant stimulated SDP root weight but had no effect on shoot weight. This ineffectivity could be due to ancymidol's inability to reduce lateral growth and less inhibition on main shoot growth.

CCC: *Toxicity:* CCC 500 mg a.i./plant or above caused severe phytotoxicity in this present experiment. Similar toxicity in SDP was reported by Williams and Taji (Personal communication) with concentrations above 2000 mg a.i./plant. Bennell and Williams (1992) also reported phytotoxic effects of CCC at higher rates (500 and 100 mg a.i./plant) on *Ptilotus exaltatus*. They suggested that CCC should be used cautiously by identifying the proper crops, stages, methods and concentrations CCC concentrations used in this present experiment might be too high when applied as a spray and drench together or the seedlings might have been too young. Drenching only might prove effective because chlorosis is a normal feature following a foliar application of CCC (Davis and Andersen, 1989). Moreover in geraniums, spray (1475ppm) was ineffective but drenching (2950ppm) was effective without any toxicity (Carpenter and Carlson, 1970).

Main and lateral shoot growth: Other than slight reduction in leaf number at 2 weeks, CCC had no significant growth retardation effect on SDP (Table 3.6b). Barth (1990b), did not get any effect of CCC on SDP by spraying or drenching. On the other hand, Bennell and Williams (1992) working with *Ptilotus exaltatus,* Roberts and Eaton (1988) with *Tibouchina* and Farthing & Ellis (1990) with several bedding plants, found significant reduction in plant height by CCC. Other results with CCC are also conflicting. Growth promotion was reported in snap dragon (Wunsche, 1969). Adedipe *et al.*, (1968) also got promotion of pea plant growth and Hildrum (1973) reported shoot elongation in *Clerondendrum thomsonae* with CCC. Initial growth retardation, followed by growth promotion in tomato was also reported earlier (Halevy and Shilo, 1970).

Fresh and dry weight: CCC at 50 mg a.i./plant increased both shoot and root weight in this present experiment. Growth promotion in tomato by CCC was also reported earlier (Halevy and Shilo, 1970).

Flowering: Hildrum (1973) reported CCC induced promotion of flowering. Flowering was not hastened in *Tibouchina* with CCC at 1000 or 2000ppm (Roberts *et al.*, 1990). Anderson and Hartley (1990) found a delay in flowering of Satin flower with CCC 2500ppm (spray) or 5000ppm (drench). The insignificant results in this present experiment might be

because of the use of ineffective co ıcentrations along with inappropriate methods of CCC application.

The phytotoxicity expressed with higher CCC concentrations (500, 1000 and 2000 mg a.i./plant) and the inability of the lower concentrations (50 and 200 mg a.i./plant) to reduce SDP height lead to testing those higher concentrations again. In a subsequent experiment (data not presented) drenching only was used to avoid any phytotoxicity associated with the CCC spray and drenching. Results indicated a trend towards reduction in all parameters with 500 and 1000 mg a.i./plant but 2000 mg a i./plant was still phytotoxic.

The results from the above experiment indicated that CCC (500 and 1000 mg a.i./plant) might be metabolised (?) or inactivated very quickly and that was why the effect was not statistically significant throughout the experiment. Tayama and Kuack (1983) found that the retarding effect on poinsettias was not measurable until 1 week after application but the effectiveness was diminished 2 weeks after application, regardless of rate and method of application of CCC. In the present experiment, data were recorded after 2, 4 and 8 weeks and by that time the effect might already have been diluted. However, Cathey and Heggestad (1973) reported that CCC retardation of internode elongation in poinsettia disappeared 3 to 4 weeks after foliar applications but soil applications were more persistent than foliar application.

Davis and Andersen (1989) stated that the effect of CCC is not persistent and it needs to be applied more than once if cropping time is long. Cathey (1964) also reported CCC has a definite dosage range beyond which it causes marginal burning and temporary chlorosis of the foliage. On many plants frequent foliar applications were required to maintain the growth control. Multiple applications at lower rates resulted in an attractive plant form in poinsettias (Tayama *et al.*, 1992).

These results collectively suggest that CCC is readily metabolised or deactivated in the plants.

Daminozide: *Toxicity:* Daminozide at concentrations more than 2.5% were toxic and could not be recommended for reducing SDP growth. Daminozide treated plants in this present experiment were less green and less compact and therefore were also less attractive. Phytotoxicity was also reported in *Osteospermum ecklonis* following 4080ppm daminozide application (Olsen and Andersen, 1995).

Main shoot growth: At the lowest concentration daminozide (0.1 % a.i./plant) was no longer effective for height control after 8 weeks (Table 3.7a). This might be due to metabolism of the lower concentration of daminozide to below the effective level for height control. Except at 2 weeks with 2.5% a.i./plant of daminozide, which produced the longest internodes, the main shoot internode length was significantly reduced all through the experiment with all daminozide concentrations. The longer internodes at 2 weeks with 2.5% a.i./plant might be due to the reduced leaf number on the main shoot. Leaf loss due to toxicity in that treatment probably lead to error in measuring internode length at 2 weeks i.e. distinction between successive internodes was not clear. Lamont (1987) observed no height reduction after

spraying daminozide (concentration not mentioned) on *Pimelea linifolia* but Von Hentig (1985) got compact plants by spraying Alar 85 (daminozide) at 0.5 % on blue Daisy.

Lateral and total shoot growth: At 8 weeks, only the highest concentration of daminozide reduced lateral shoot length. The lack of persistent effect of lower concentrations might be due to the similar reasons as explained earlier for the main shoots.

Fresh and dry weight: The increased root weight by lower concentration of daminozide suggests that daminozide probably has some promotory effect on SDP root.

Flowering: The delaying effect of daminozide on flowering might be related to the delayed vegetative maturity of the treated plants. Banko and Stefani (1988) also found delayed flowering of some bedding plants. But foliar sprays of daminozide (5000 mg/L) were ineffective in controlling flowering of *Bouvardia humboldtii* (Wilkinson and Richards, 1987) and B995 (i.e. daminozide) promoted initiation of flower buds in *Rhododendron* (Cathey, 1964).

Flurprimidol: *Main shoot growth:* Higher concentrations of flurprimidol reduced the height of SDP and the effects were more clear at the later stages of the plant growth (Table 3.8a). Flurprimidol was also effective in controlling plant height of English Ivy (Hamilton and Redo, 1985), poinsettia (McDaniel, 1986), *Chrysanthemum* (Barrett *et al.,* 1986; 1987), *Plumeria* (Kwon and Criley, 1991b) and on Bermuda grass (Johnson, 1992).

Flurprimidol treated plants were greener than control plants. Greener plants were also reported earlier in a turf grass, Tall Fescue Sward Dynamics (Spak *et al.,* 1993).

Lateral shoot growth: Lateral shoot growth was significantly less because of the reduced growth of the individual lateral shoots as flurprimidol did not reduce shoot number (Table 3.8b).

Flowering: Flurprimidol (1 mg a.i. per plant) delayed flowering in this experiment. This result is similar to the results of Kwon and Criley (1991b) with *Plumeria,* and McDaniel (1986) with poinsettia.

PBZ: *Toxicity:* Fifty mg a.i./plant was toxic for SDP plants. In *Vinca* plants PBZ (6.25 to 25ppm) applied at maturity was also phytotoxic (Barrett and Nell, 1987). However, Bausher and Yelenosky (1987) stated that relatively high concentration of PBZ (10^5ppm) in the soil showed no signs of phytotoxicity on *Citrus* foliage.

Main shoot growth: Dwarf plants with shorter internodes suggests that PBZ probably works through GA biosynthesis inhibition (Section 2.2.6) because GA has a direct role in stem elongation (Section 2.2.3.1). The reduction of plant height by PBZ in SDP was also observed by Barth (1990b) and Jusaitis and Schmerl (1993). Other scientists working with *Pimelea linfolia* (Lamont, 1987), Geraldton Wax, *Correa reflexa, Crowea exalata* and *D. trigona* (Stewart, 1991), *P. exaltatus* (Bennell and Williams, 1992), Easter lily (Jiao *et al.,* 1986) and Marigold (Keever and Cox, 1989), also obtained a significant height reduction following PBZ application. But Horrell *et al.,* (1989a) got an exceptional result; PBZ increased shoot elongation in Ivy.

The reduction in leaf number on the main shoot by PBZ at 50 mg a.i./plant (both at 2 and 4 weeks) might be due to leaf drop resulting from leaf burn. The plants recovered as they grew and had similar number of leaves after 8 weeks (Table 3.9a).

Lateral and total shoot growth: The increase in the number of shoots by 10 mg a.i./plant PBZ might also be related to the inhibition of GA synthesis followed by early release of laterals from the apical dominance. GA has been found to reinforce apical dominance in several plant species (Cline, 1991). The reduction of shoot number by the higher doses (20 and 50 mg a.i./plant of PBZ) might be due to a supra-optimal concentration of PBZ. So, 10 mg a.i./plant might be the optimum concentration for maximum lateral shoot production.

This present result is similar to that of Jusaitis and Schmerl (1993) where they got an increase in the number of laterals of SDP with PBZ (1 mL/L of Bonzi®). Maus (1987) also stimulated lateral branching of *Hibiscus* with PBZ (50, 100 and 200ppm drench). However, Stewart (1991); von Hentig and Tschirschke (1989) found a reduction in lateral shoot growth in their experiments with different PBZ concentrations on different plants.

The insignificant reduction in total shoot growth for lower doses (0.1 and 1.0 mg a.i./plant) at 8 weeks might be due to metabolism of the lower concentrations below the level required for continuous reduction of lateral growth (Table 3.9b).

Fresh and dry weights: All PBZ concentrations reduced main shoot growth but only the higher concentrations (10, 20 and 50 mg a.i./plant) reduced lateral shoot growth. Shoot weight was reduced by these higher concentrations. Again the highest concentration (50 mg a.i./plant) reduced both shoot and root weight. Therefore it is probable that SDP has different requirements for PBZ to control SDP shoot and root growth. It seems that the lateral shoots required higher concentration than the main shoot, and roots required more than main shoots and lateral shoots (Table 3.9a, 3.9b and 3.10) for controlling their growth. Banko and Stefani (1988) also got a reduction in total dry weight, when they applied PBZ to some of the selected container grown bedding plants.

Flowering: The delay in flowering caused by higher concentrations of PBZ (10, 20 and 50 mg a.i. /plant) might be related to the delayed vegetative maturity of the plants. However, Jusaitis and Schmerl (1993) with SDP, and Stewart (1991) with some other Australian native plants, found no response of flowering to PBZ. Turner (1987) used PBZ on different Kangaroo Paw varieties and observed delayed flowering (except on 1 variety). These different results might be related to the differences in species or concentrations or both.

General observation: In this present experiment PBZ treated plants were greener, more compact, more resistant against insect pests and water stress. PBZ treated plants also produced whiter and thicker fibrous roots. Several other positive side effects (such as increased tolerance to SO_2, chilling, heat etc) were also reported else where (Lee *et al.,* 1985; Kaminski, 1989; Lan and Hwa, 1990).

Interaction of PBZ and flurprimidol: PBZ (10 mg a.i./plant) and flurprimidol (1.0 mg a.i./plant) both reduced plant growth in this present experiment (Table 3.8 and 3.9) probably by inhibiting GA biosynthesis (Coleman and Estabrooks, 1992). In a subsequent experiment

the effectiveness of these 2 growth retardants was compared (Appendix V). The results indicated that 1.0 mg a.i./plant flupri nidol was less effective both on main and lateral shoots than 10 mg a.i./plant PBZ. Moreo ver, PBZ had more consistent effect throughout this experiment (at 2, 4 and 8 weeks afte PGRs application). Steffens and Wang (1986) reported that PBZ had the greatest effect on tissues which were rapidly growing and developing at the time of treatment or there after.

Reduced effectiveness for cc ntrol of elongation growth of main shoot and lateral shoots might mean that flurprimidol is a mild type of growth retardant as compared to PBZ and the concentration of flurprimidol (1.0 mg a.i./plant) was not enough to compare with PBZ (10 mg a.i./plant). Barrett *et al.*, (1987) found flurprimidol (0.1 mg/plant) to be more active for height reduction than PBZ (0.2 mg/pot) on *Chrysanthemum morifolium*. Barrett *et al.*, (1986) again found flurprimidol (25 ppm) was as effective for height control as PBZ (50 ppm) on *Exacum affine*. McDaniel (1986) found both PBZ (0.5 mg/pot drench and 25-50 mg/L for spray) and flurprimidol (0.03-0.06 mg/pot drench and 25 mg/L for spray) were equally effective for reducing the plant height in poinsettias.

The results from the floweri g data of this subsequent experiment revealed that the effects of those 2 types of the growth retardants are different (Appendix V). The delaying effect on flowering of PBZ was reversed and time to flowering was reduced when PBZ and flurprimidol were applied together. One explanation for the delayed flowering by PBZ alone could be due to its inhibition of GA b osynthesis because GAs can enhance flowering (Widmer *et al.*, 1974). McDaniel (1986) found both PBZ and flurprimidol delayed flowering in poinsettia. Kwon and Criley (1991b) found that flurprimidol delayed flowering of *Plumeria* more than PBZ. However, the early f owering due to flurprimidol, apart from its effect on GA biosynthesis, could be due to an effect on the level of other hormones (?) when applied together with PBZ.

3.6.5 Conclusions:

* PBZ at 10 mg a.i./plant was effective throughout the whole period of the experiment for producing a compact SDP pot plant with more lateral shoots, probably via GA biosynthesis inhibition. This concentration was selected for further experimental use.
* Flurprimidol (0.5 and 1 mg a.i./plant) was effective on the main shoot but had a smaller effect on lateral shoot growth.
* Lateral shoots of SDP seems to require higher concentration of PBZ and flurprimidol as compared to main shoot to control its growth.
* Repeated application of 5 mg a.i./plant ancymidol may prove effective to avoid toxicity.
* Sequential drenching with 500 mg a.i./plant CCC might give desirable growth control.
* Daminozide produced less attractive plants and had serious toxicity problems with 5 and 10% a.i./plant.
* Four weeks after the growth retardant application was acceptable for data collection.

3.7 Study on the translocation pattern of PBZ and GA_3.

3.7.1 Introduction:

Yau (1988) reported that PBZ is a xylem mobile plant growth retardant which moves upward with the transpiration stream. He also reported that following application, PBZ accumulates in the apical shoots and foliage and is not remobilized in the reverse direction. Its uptake is mainly by roots, green stems and foliage (Yau, 1988). GA_3 on the other hand, is a growth promoter and can move through both xylem and phloem (Davies, 1987; Lang, 1970). Its uptake is also through roots, stems and foliage (Graebe and Ropers, 1978).

In a preliminary experiment (data not presented) concentrations $\geq$ 250ppm GA_3 increased plant height but all tested concentrations (25, 100, 250 and 500ppm) reduced lateral shoot growth. Similarly higher PBZ or flurprimidol concentrations were effective on lateral shoot growth control (Section 3.6.5) in the preceding experiment. Were these differences in action due to the differences in mode of translocation of the PGRs to the main and the lateral shoots ? An experiment was formulated to find out the translocation pattern of these 2 PGR by localised application to different parts of SDP plants.

3.7.2 Materials and Methods:

This experiment was conducted with 6 treatments: control (water); PBZ (10 mg a.i./plant) and GA_3 (500ppm) applied either to main or lateral shoots. The experiment was laid out in a randomised complete block design with 10 replications.

The specified PGRs were applied to the whole main shoot or to the all lateral shoots including the leaves. PGRs were applied with a paint brush 5 weeks after transplanting (1 week after the normal application time of section 3.2 to allow the laterals to grow out to enable the application). Special care was taken to prevent PGR run off by placing aluminium foil over non target plant parts as well as on the potting mixture. The process was repeated for 15 successive days. The application was done either in the early morning or in the late afternoon and irrigation was accordingly avoided during the morning or afternoon of the treatment.

The seeds were sown on 16 02.94. Vegetative data were recorded 2 and 4 weeks (from the 1st day) after PGRs application. The rest of the procedures were followed as per the general methodology described in section 3.2

3.7.3 Results:

Toxicity: PBZ treatment on the main shoot almost stopped elongation of the main shoot and produced very small or deformed leaves (sometimes cup shaped). When applied to the laterals, PBZ also stopped elongation of the laterals or they grew very slowly.

GA_3 applied to the main shoot produced few abnormal leaves after repeated application but there was no toxicity when GA_3 was applied to the lateral shoots.

Main shoot growth: PBZ: Both at 2 and 4 weeks, PBZ retarded plant height when applied to the main shoot but had no effect on height when laterals were treated with PBZ

Figure 3.2 Localised PGRs application on main shoot growth at 2 weeks

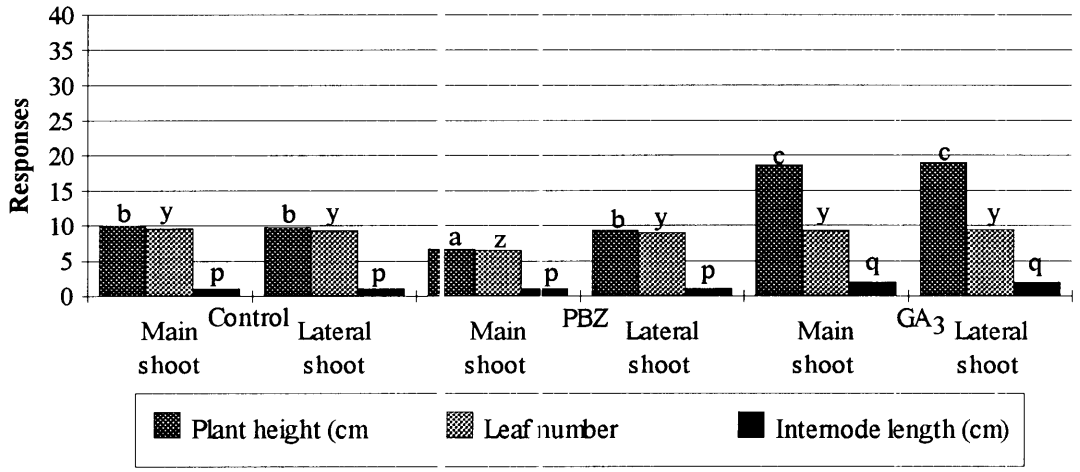
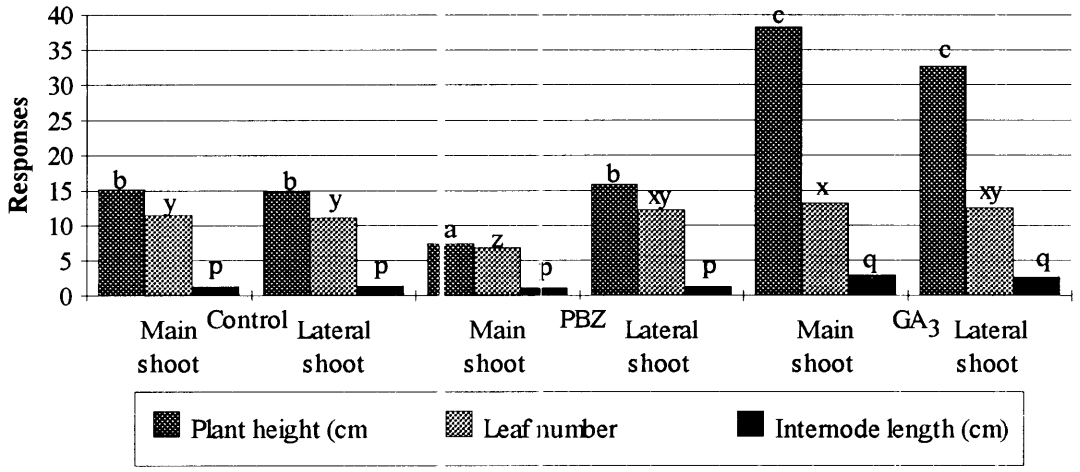


Figure 3.3 Localised PGRs application on main shoot growth at 4 weeks



Fiugre 3.4 Localised PGRs application on lateral shoot growth at 2 weeks

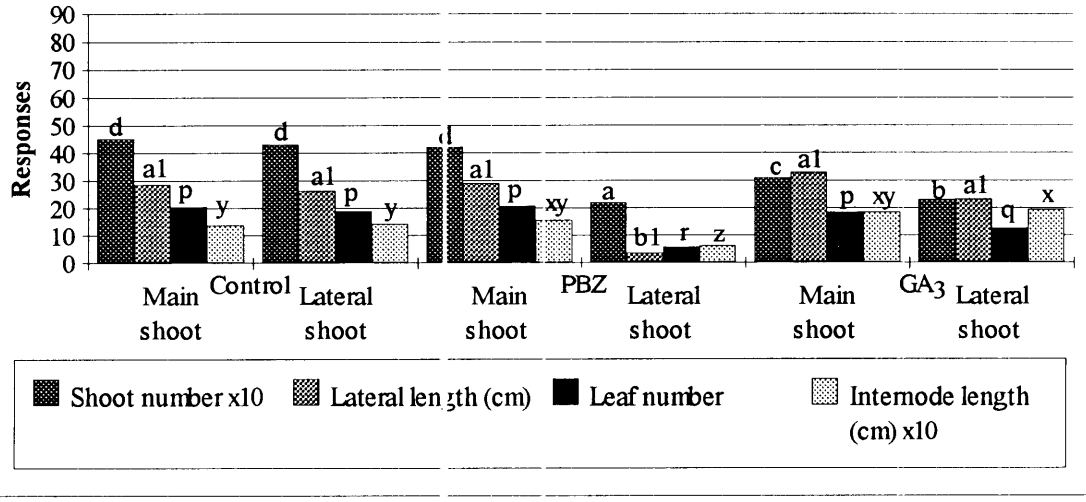
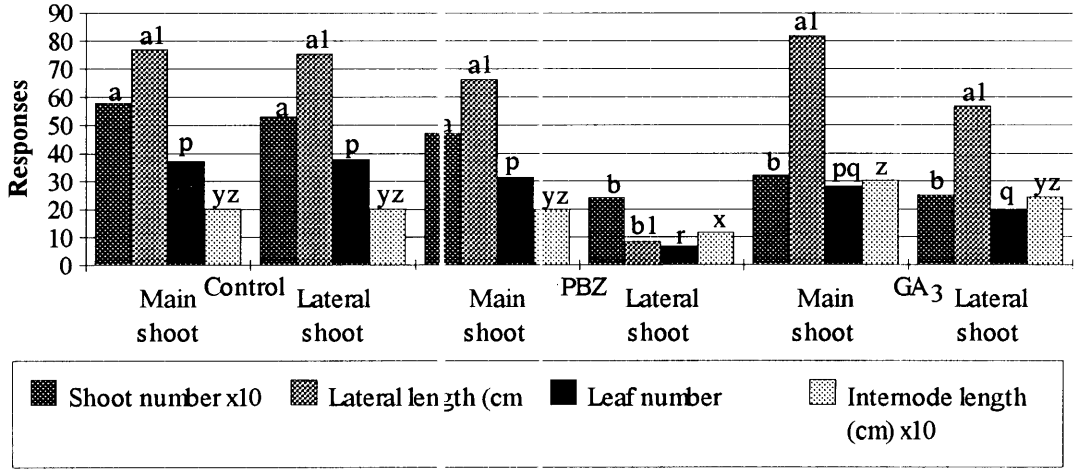


Figure 3.5 Localised PGRs application on lateral shoot growth at 4 weeks



(Figure 3.2 and 3.3). The main shoot internode length for PBZ treated plants was similar to the control.

GA_3: It increased the plant height (Figure 3.2 and 3.3) irrespective of the place of application by increasing only the length of the internodes (at 2 weeks) or by increasing both the length and the number of internodes (at 4 weeks). Either main or lateral shoots treated with GA_3 gave 11-12 elongated internodes after 4 weeks as compared to only 9 in controls.

Lateral shoot growth: PBZ: PBZ applied to the main shoot had no effect on the shoot number or lateral length but when applied directly to the laterals, the shoot number and length were reduced significantly (Figure 3.4 and 3.5).

GA_3: Irrespective of the place of application, GA_3 reduced shoot number but produced statistically similar lateral length to the control in both the weeks (Figure 3.4 and 3.5).

3.7.4 Discussion:

Toxicity: The cessation of growth at the apex or at the terminals of the laterals due to PBZ (10 mg a.i./plant) could be related to its continuous (daily) application, resulting in accumulation of excessive amounts of retardants in the plants thereby causing phytotoxicity. In other experiment the same concentration did not cause any toxicity when applied once as spray and drench (Section 3.6). Wilkinson and Padgham (1987) reported that depending on the rates of application Bonzi can terminate or suppress further growth.

Although GA_3 expressed very mild toxicity, in main shoot following repeated application, the lateral shoots did not show any toxicity perhaps they are more tolerant to repeated GA_3 application.

Main shoot growth: *PBZ:* The reduction of plant height in PBZ treated main shoots was due to the reduction in the number of internodes (i.e. leaf number) and number of elongated internodes. After 2 and 4 weeks of main and lateral shoot treatment there were 7.6

and 8.7 elongated internodes for control plants and 5.7 and 5.9 respectively for PBZ treated plants. This (reduced number of internodes and elongated internodes) might be because PBZ almost stopped further growth of the treated main shoot. Similar results were also found when tops of apple seedlings were treated with PBZ; only the growth of the upper part was inhibited (Wang *et al.*, 1986). Quinlan and Richardson (1986) also reported that PBZ had a localised effect on the function of the apical buds and terminals of the laterals. These results are consistent with the view that PBZ is translocated through the xylem only.

There was no effect on the plant height when the laterals were treated with PBZ, probably because of its immobility from the laterals to the stem or apical part of the plant. These results reinforced the idea that PBZ does not move through the phloem or if moves through phloem, the concentration translocated from the place of application to the other site of action was not enough to control the height. However, Wang *et al.*, (1986) reported that 42 days after PBZ treatment, 58.4% (93 2 µg/g fresh weight) of PBZ in the stems was located in xylem while 41.6% (66.5 µg/g fresh weight) was in the phloem (bark). They proposed this was due to lateral translocation from xylem to the phloem (bark). Rauscherova and Tesfa (1993) also stated that accumulation of PBZ may occur in the xylem or phloem close to the application site. Browning *et al.*, (1992b) confirmed that in pear shoot the xylem is not the only pathway for PBZ translocation as reported earlier.

It is worth mentioning here that, irrespective of the place of PBZ application, the leaves were smaller and greener. The greener leaves in plants with PBZ applied to the main shoot or the laterals in this present experiment might be because of little translocation by diffusion or via phloem, not enough to inhibit the lateral shoot or the main shoot growth (depending on the site of application) but enough to change the size and colour of the leaves. A reduction in leaf area with darker green leaves were also reported by Rao and Mendham (1991) following PBZ application on chinoli. Increased chlorophyll per unit leaf area might be the reason for greener leaves (Smith *et al.*, 1990).

GA_3: Since GA_3 increased plant height and internode length irrespective of the point of application (main or lateral shoot); which is consistent with the idea that GA_3 is translocated freely through the plant via both through xylem and phloem (Davies, 1987). Increased plant height along with longer internodes by GA_3 (500 mg/L) were also reported in *Boronia serrulata* (Lamont, 1985). In this present experiment most of the GA_3 treated plants had pale green leaves, but stem diameter was not reduced. Taiz and Zeiger (1991) also reported pale green leaves following GA_3 application as a general feature in many plants. However, the present result for stem diameter is similar to that of Napier *et al.*, (1986) where in hybrids of *Leucospermum conocarpodendron* X *L. cordifolium* the shoot diameter was unaffected by GA_3 applications. They again stated that similar and conflicting results have been found in other plants for stem diameter.

Lateral shoot growth: PBZ: The reduced number of shoots following direct application of PBZ to the laterals might be because of continuous application directly on the under developed shoots at a very early stage of growth, when other buds or shoots were in a

very close contact to each other. PBZ might have diffused regularly to them and arrested their further growth. It was not the same situation if PBZ was applied once as a spray and drench, where shoot numbers were increased but their elongation was reduced (Table 3.9b; Appendix V). In the literature both enhanced (Maus, 1987) or reduced (Stewart, 1991, von Hentig and Tschirschke, 1989) lateral shoot growth with PBZ spray or drench were reported.

GA_3: Presumably because of its translocation through both xylem and phloem, GA_3 produced apically dominant taller plants and thereby reduced further shoot development but elongated the already released shoots irrespective of the place of application. That was why with fewer shoots GA_3 had statistically similar lateral length with controls. Similar elongation of the released laterals were also obtained in section 3.4.5. Although insignificant, the laterals of the GA_3 treated plants had higher values in both the weeks as compared to GA_3 treated lateral shoots. Continuous supply of GA_3 at lower amount on the main shoot might have some additive effect on lateral shoot elongation.

3.7.5 Conclusions:

* PBZ reduced shoot growth only when applied locally to the specific site indicating that it was readily translocated acropetally within a shoot (via xylem) but not basipetally (via phloem) although some phloem translocation has been reported.
* GA_3 elongated the main shoot and enhanced apical dominance irrespective of the place of application suggesting it is readily translocated both through xylem and phloem.
* The translocation pattern of PGRs did not vary with the shoot types (e.g. main or lateral shoots).

3.8 General conclusions from chapter 3.

* SDP plants produced at least 3 true leaves within 30 days after sowing, which is probably the stage when the seedlings are well established in the glasshouse pots and was adopted as the probable time for application of the PGR treatments.
* Lower internodes of the main shoot ceased responding to added GA_3 earlier (e.g. 4th internode by the 7th leaf stage) but further elongation continued in the upper internodes.
* GA_3 enhanced the growth of the main stem (apical dominance) and also of the released laterals but induced correlative inhibition and therefore inhibited further growth of other lateral buds.
* Auxin precursor L-tryptophan yielded auxin in the presence of GA_3.
* The conversion of D-tryptophan to the active auxin did not require GA_3.
* An appropriate balance between endogenous auxin and GA is required for growth of SDP shoots.
* PBZ at 10 mg a.i./plant was effective throughout the whole period of the experiment for producing a compact SDP pot plant with more lateral shoots, probably via GA biosynthesis inhibition. This concentration was selected for further experimental use.

* PBZ enhanced lateral bud release from apical dominance but was unable to increase lateral shoot extension growth, probably through its role in GA biosynthesis inhibition.
* Flurprimidol (0.5 and 1 mg a.i./plant) was effective on the main shoot but had a less effect on lateral shoot growth in SDP. It may be considered as a mild growth retardant but it has an inconsistent effect compared to PBZ.
* Flurprimidol could be used to overcome the delaying effect of PBZ on SDP flowering.
* Daminozide produced less attractive SDP plants and had serious toxicity problems with 5 and 10% a.i./plant.
* For Armidale conditions; repeated application of ancymidol with 5 mg a.i./plant might produce more desirable plants. The reduction of main and lateral shoot growth by ancymidol is consistent with its proposed inhibition of GA biosynthesis.
* To get continuous and desirable growth control in SDP with CCC, sequential drench application (with 500 mg a.i./plant) might be necessary because of CCC's rapid metabolism (?) or inactivation (within 2 weeks of application) and also to avoid the phytotoxicity caused by higher concentrations.
* Higher retardant concentrations were required to reduce SDP lateral shoot growth. It seems lateral shoots may have a higher GA production or supply.
* The probable translocation patterns of PBZ and GA_3 are mainly through xylem and through both xylem and phloem respectively.
* Different growth control mechanisms for main and lateral shoots were suggested following PGR application (including auxin precursor), expressed mainly through the different effective concentrations.