CHAPTER 2
LITERATURE REVIEW

The literature review focuses on three main themes of the thesis, namely sexual reproductive growth, tuberization and paclobutrazol. Specific topics are reviewed in the relevant chapters.

2.1 SEXUAL REPRODUCTIVE GROWTH

2.1.1   Flower

The potato inflorescence is single or compound cymes, and the number of flowers per inflorescence and per cyme depends on genotype, the environment and the position of the inflorescence in the shoot system (Almekinders & Struik, 1996). Inflorescences at higher positions are characterized by fewer flowers than ones at lower positions (Almekinders & Wiersema, 1991; Almekinders & Struik, 1994).

The corolla is five lobed and can be white, yellow, blue, purple or striped according to the variety. The calyx is tubular and lobed. Five stamens are borne on the corolla tube and the pistil consists of two carpels that form a two-locule ovary with a single style and stigma, and the flower produces no nectar (Smith, 1968).

2.1.2   Pattern of flowering

A potato plant developed from a seed tuber consists of one or more aboveground shoots. In determinate cultivars the growth of each stem is terminated by an inflorescence, but stem growth

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may continue from lateral buds (Almekinders & Struik, 1994; Vos, 1995). The new branches will again terminate with an inflorescence, and this process can continues for several cycles.

2.1.3 Flowering response

It has been reported that *Solanum tuberosum ssp andigena* flowers regardless of day length, but does better under short days, while *Solanum tuberosum ssp tuberosum* usually does not flower under short days (Sadik, 1983). Long days hasten potato flower primordia initiation and development (Almekinders, 1992). Extending a 12-hour photoperiod with 4-hour incandescent light promoted flower production (Turner & Ewing, 1988).

Under natural light condition, increasing the temperature up to 28 ℃ improved flower production (Marinus & Bodlaender, 1975). Plants grown at a night temperature of 20 ℃ produced more flowers and bloomed on average eight days earlier than plants exposed to 10 ℃ night temperature (Turner & Ewing, 1988). They also reported the existence of an interaction between photoperiod and night temperature. Longer photoperiods and warmer night temperature promoted flower production, by preventing flower bud abortion.

Growing potato plants in a greenhouse where photosynthetically active ration (PAR) was reduced by about 50% inhibited flower bud development, thereby completely suppressed flower production (Turner & Ewing, 1988). Calvert (1969) observed that reducing the level of irradiance increased tomato flower bud abortion indicating that the production and availability of adequate assimilates are crucial for flower bud development.

High levels of potassium, phosphorus, and nitrogen favour flowering in potato (Bolle-Jones, 1954). High levels of nitrogen fertilizer specifically promotes flowering, according to Bamberg & Hanneman (1988).

Long photoperiods (Almekinders, 1992)**,** warm temperature (Turner & Ewing, 1988), and high nitrogen levels are inhibitory to tuberization. It has been proposed that the aforementioned factors by altering the hormonal balance delay tuber formation (Krauss, 1985; Wheeler *et al.*, 1986; Vandam *et al.*, 1996) and promote shoot growth, thereby stimulating flowering.

2.1.4   Fruit set

The berry of potato is spherical with a diameter of 1.2 to 1.9 cm and green or purplish green tinged with violet. It has two compartments and contains numerous small seeds ranging in number from 50 to 500 (Smith, 1968; CIP, 1983).

Fruit set often does not take place even when conditions are ideal for flowering (CIP, 1983). This seems to indicate that the conditions favouring flowering are not necessarily optimal for the processes of fruit development. Sadik (1983) reported that flower abscission may occur due to factors such as lack of insect pollinators, poor pollen viability, and too low temperatures for pollen germination and fertilization. He also indicated that abscission can   result from a competition between developing fruit and tubers for limiting growth factors.

Almekinders *et al*. (1995) studied berry yield and seed production as influenced by flower positions and reported that mean berry weight, number of seeds per berry and 100 seed weight

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decreased from the proximal to the distal flower position. Berries reach full development six weeks after fertilization (CIP, 1983).

2.1.5   Assimilate partitioning as affected by reproductive growth

Growth and development of different plant parts are affected by total assimilate production and partitioning among sink organs. Shoot and tuber growth are considered competing processes. Since the conventional potato propagation rely on seed tubers, less attention has been given to the effect of flowering and berry set on the growth of potato. Some researchers have studied the effects of flowering and berry formation on vegetative growth and tuber yield but the results are conflicting. Knight (1807) as quoted by Bartholdi (1940) believed that the failure of early potato cultivars to produce seed was due to tuber formation, indicating that early growth of tubers utilises materials necessary for floral and fruit development. He concluded that preventing the formation of tubers promotes the formation of numerous flowers and berries.

Abdel-Wahar & Miller (1963) impeded the downward translocation of assimilates by wire girdling, stem incision, and stolon pruning, and observed profuse flowering, indicating that assimilate availability strongly influences flowering in potato. Bartholdi (1940) using indeterminate potato varieties observed that the non-flowering plants produced the greatest weight of tops and tubers, suggesting that sexual reproductive growth reduces vegetative and tuber growth. The effect of flowering and berry formation on tuber yield in *Solanum demissum Lind.* were investigated by ProundFoot (1965). He observed that in five out of twelve fruiting plants, berry yield was higher than tuber yield, and reproductive growth significantly reduced tuber yield. Jansky & Thompson (1990) investigated the effect of flower removal on potato tuber yield. In one year, flower removal increased tuber yield of clone ND860-2 under irrigated and

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dry land conditions. In the next year, however, flower removal affect tuber yield. They concluded that, the response to flower removal appears to be dependent on the environmental conditions.

There are some reports indicating that flowering and fruiting do not affect tuber yield. Observation on reciprocal crosses between *S*. a*ndigena* and *S*. t*uberosum* clones indicated that fruiting has no effect on tuber yield (Cubelios, 1973 as quoted by Haile-Micheal, 1973). Newman & Leonial (1918) as quoted by Haile-Micheal (1973) observed a positive association between vegetative growth, tuber yield, and seed production for one cultivar grown under different conditions. Haile-Micheal (1973) working with reciprocal crosses reported that some of the highest yielding genotypes did set fruit profusely with little effect on tuber yield. This was especially true if the plants were grown under favourable environmental and cultural conditions. He concluded that fruit set did not materially contribute to the difference in tuber yield observed in reciprocal crosses.

2.2 TUBERIZATION

Potato tubers are shortened and thickened modified stems that bear scale leaves (cataphylls) each with a bud in its axil (Cutter, 1978). The usual site of tuber formation is a stolon tip. Stolons (rhizomes) are diagravitropic stems with long internodes and scale leaves. They develop as branches from underground nodes and are terminated by a curved apical portion called a hook (Peterson *et al*., 1985). According to Plaisted (1957) stolon formation starts at the most basal nodes and progresses acropetally. Wurr (1977) investigated the pattern of stolon formation in three cultivars and found that about half of the stolons were formed at the most basal node, with roughly 10% of the remaining stolons at each of the next four higher

nodes. It has been reported that stolons formed first normally grow longer, are more likely to branch, and are preferential sites for tuber formation (Lovell & Booth, 1969; Struik & Van Voorst, 1986).

The potato plant is remarkable for its plasticity in organ development (Steward *et al*., 1981; Clowes & MacDonald, 1987). Tuber formation can occur on almost every bud of the plant including axillary buds (Ewing 1985) and inflorescence (Marinus, 1993). The signal for induction to tuberization is omnipresent (can be transported to every plant part) and can express itself in all buds (Struik *et al.*, 1999).

An understanding of potato tuberization is important and the time of tuber initiation in relation to other aspects of plant development plays a vital role in determining potential yield.

2.2.1 Tuberization stimulus

The existence of tuberizing stimulus synthesized in the leaves and translocated to the site of tuber initiation was proposed by Gregory (1956). The movement of the tuberization stimulus across a graft union (from the induced scion to the underground nodes of non-induced stock) was demonstrated by Gregory (1956) and Kumar & Wareing (1973). However, reciprocal grafts did not tuberize. Studies on inter stem grafts showed that the tuberizing stimulus is transported acropetally and basipetally (Kumar & Wareing, 1973). The nature of this transmissible signal is not well known, but it is suspected to be a hormone and may have more than one component (Jackson *et al.*, 1998). The involvement of phytochrome in the production of the transmissible signal(s) was demonstrated by a grafting experiment of Jackson *et al*. (1998). Wild-type *Solanum tuberosum* ssp *andigena* induced to tuberize under

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long day by grafting on a shoot from antisense phytochrome B plants but not by grafting on another wild-type plants.

The formation of stolons and tubers takes place preferably underground although the tuberization stimulus may be present throughout the plant and affects morphological development (Ewing, 1997). Under inductive conditions, both the young and old leaves are capable of producing the stimulus (Hammes & Beyers, 1973).

2.2.2 Major changes during tuberization

Potato tuberization is a complex process involving anatomical, enzymatic, biochemical and hormonal changes leading to the differentiation of the stolon into a vegetative storage organ, the tuber (Xu *et al.*, 1998, Jackson, 1999; Fernie & Willmitzer, 2001).

Anatomical changes

It has been reported that transformation of stolon into tuber involves cell division, change in the direction and orientation of the microtubule, and cell enragement (Koda, 1997). During tuber initiation many changes have been documented to occur in stolon tips. Xu *et al.* (1998) observed cell division in the apical and subapical regions (up to approximately 5 mm from the apex) of non-swelling but elongating stolons. Upon tuber initiation, cessation of stolon growth coincides with the cessation of mitotic activity in the apical meristems (Xu *et al*., 1998). Both cell division and cell enlargement contribute to the development of tubers (Xu *et al.*, 1998).

Biochemical changes

Biochemical changes associated with tuberization have been investigated by several molecular biologists (Park 1990; Prat, *et al.*, 1990; Sanchez-Serrano & Et, 1990). Before any

sign of tuber initiation, stolon tips undergo a change that increases the accumulation of soluble carbon compounds and increase the conversion of these to insoluble compounds (Oparka & Davies, 1985). As the stolon tips begin to develop into tubers, the activity of GA-like compounds in the stolon tips decreases (Koda & Okazawa, 1983); accumulation of starch increases and concomitantly the levels of glucose and fructose decrease (Geigenberger *et al.*, 1998; Struik *et al.*, 1999); and a significant increase in the concentration of a storage protein (patatin) is observed (Hendriks *et al.*, 1991; Suh *et al.*, 1991).

In most plants fixed carbon is transported in the form of sucrose (Kühn *et al.*, 1999). It has been proposed that the ability of an organ to metabolise sucrose is one of the determining factors in regulating sink strength (Sung & Black, 1989). Carbohydrates are imported into the growing stolon and tubers via the phloem, mainly in the form of sucrose (Struik *et al.*, 1999). Elongating but non-tuberizing stolons exhibit high activity of invertase, while sucrose synthase is absent; however, upon tuber formation the activity of sucrose synthase drastically increases and the activity of invertase decreases (Ross *et al.*, 1994; Appeldoorn *et al.* 1997). The rise in sucrose synthase activity is positively associated with the onset of starch and storage protein synthesis (Obata-Sasamoto & Suzuki, 1979) and sink strength (Hajirezaei, *et al.*, 2000). A change in hexose to sucrose ratio in favour of the latter is observed in the stolon tip (Davies, 1984). This is attributed to a significant decease in hexose content, especially fructose, possibly caused by a higher fructokinase than hexokinase activity in the developing tubers (Davies & Oparka, 1985; Gardner *et al.*, 1992; Renz & Stitt, 1993). As a result, the level of fructose in the developing tubers is much lower than in stolons (Ross *et al.*, 1994; Appeldoorn *et al.*, 1997; Vreugdenhil & Sergeeva, 1999). The activity of ADPglucose pyrophosphorylase that catalyses the conversion of Glucose-1-P into ADPGlucose significantly increases upon tuberization (Visser *et al.*, 1994; Appeldoorn *et al.*, 1997).

2.2.3 Factors affecting tuberization

Genetic factors

Most wild *Solanum* species have a short day critical photoperiod for tuberization; and will become induced only if the photoperiod is less than 12 hours. This holds true for *Solanum tuberosum* ssp. *andigena*, which is adapted to the short days and cool temperatures of the Andean area (Amador *et al*., 2001). In contrast, *Solanum tuberosum* sub sp. can tuberize under longer photoperiod; it has a much longer critical photoperiod (Ewing, 1997). Genetic mapping of backcrosses between ssp *tuberosum* and wild species has revealed the presence of at least eleven genes responsible for tuberization under long photoperiods (Van den Berg *et al*., 1996). The existence of variation among genotypes with respect to photoperiod sensitivity has been reported by Ewing (1995). Cultivars differ not only as to the percentage of stolons that bear tubers, but also with respect to the pattern of tuberization at different nodes (Ewing, 1997).

Mother tuber

The size as well as the physiological condition of the mother tuber exerts a definite effect on the development of plants by affecting stolon and tuber formation (Van der Zaag & Van Loon, 1987). As the physiological age of the mother tuber increases induction to tuberize increases and its effect on the morphology of the plant resemble that of a short photoperiod. Planting physiologically older seed tubers results in smaller plants with more stems, and promotes earlier tuberization and earlier senescence (Ewing, 1997). Villafranca *et al*. (1998) from a kinetin-induced *in vitro* tuber formation study reported that early tuberization increased with physiological age of the mother tuber.

Environmental factors

The tuber forming sequence in *Solanum* species normally consists of stolon development followed by tuberization in sub apical region of the stolon (Booth, 1963). These processes are controlled by environmental factors, primarily temperature and photoperiod (Gregory, 1956; Salter, 1968).

Photoperiod and light quality

Tuberization of potato plants is strongly influenced by daylength. Induction to tuberize is promoted by short photoperiod (long dark period) and the signal is perceived in the leaves (Gregory, 1965). Interruption of the dark period with red light is more inhibitory to tuberization than other wavelengths, and the inhibitory effect of red light can be reversed by exposure to far-red radiation. This provides evidence for the involvement of a photoreceptor phytochrome in this response (Batutis & Ewing, 1982). Using an antisense approach in short day *Solanum tuberosum* sub sp *andigena,* Jackson *et al.* (1996) observed a reduced level of the expression of phytochrome B (PHYB) in transgenic plants. Consequently, transgenic plants became insensitive to photoperiodic changes and tuberized both under short day and long day conditions. This response suggested that PHYB exerts a negative control over tuberization of *andigena* under long photoperiods. Jackson *et al.* (1998) demonstrated that this photoreceptor controls the synthesis of the graft-transmissible inhibitory signal that is produced under long days, and which is absent or inactivated in the PHYB-antisense plants.

There is evidence indicating that GA is a component of an inhibitory signal and prevent tuberization under long days condition. Exogenous GA application inhibited tuber initiation (Xu *et al.*, 1998). High activity of GA-like compounds was detected in potato grown under non-inductive conditions (Vreugdenhil & Sergeeva, 1999) and reduced GA activity was

detected in leaves exposed to short days (Ewing, 1995). A dwarf mutant characterized by partial blocking of GA biosynthesis tuberized under short and long days (Van den Berg *et al*., 1995). Treating wild-type *andigena* spp plants with GA synthesis inhibitor, ancymidal, promoted tuberization under long days (Jackson & Prat, 1996). Like high temperatures, long photoperiod delays the onset of tuber growth and bulking (Vandam *et al.*, 1996). It decreases partitioning of assimilates to the tubers and increases partitioning to other parts of the plant (Wolf *et al*., 1990).

Temperature

Another important factor that exerts a major influence on tuberization is temperature. Generally, cool temperatures promote tuberization (Struik & Kerckhoffs, 1991; Vandam *et al*., 1996), and high temperatures are inhibitory for tuberization under both short and long photoperiods, albeit the degree of inhibition is greater under long days (Wheeler *et al.*, 1986). Both air and soil temperatures are important, cool air temperatures favour induction to tuberize (Gregory, 1956; Reynolds & Ewing, 1989), and high soil temperatures block the expression of the tuberization stimulus on the underground nodes (Reynolds & Ewing, 1989). There is an interaction between temperature and photoperiod. The higher the temperature the shorter the photoperiod required for a given genotype to tuberize (Snyder & Ewing, 1989).

At elevated temperatures foliage growth is promoted (Menzel, 1980), net photosynthesis decrease (Hammes & De Jager, 1990), assimilate partitioning to the tubers is reduced (Gawronska *et al.*, 1992) and dark respiration increases (Levy, 1992, Thornton *et al*., 1996). There is evidence that the inhibitory effects of high temperatures are mediated through the production of high levels of GA-like compounds known to inhibit tuber formation (Menzel,

1983). It has been suggested that high temperature exerts its influence on tuber formation by altering the balance between endogenous GA, cytokinins, and inhibitors (Menzel, 1985).

Irradiance

Similar to high temperatures and long photoperiod, low levels of irradiance during the day decrease the induction of tuberization (Bodlaender, 1963; Gregory, 1965; Demagante & Vander Zaag, 1988). Extension of the photoperiod with high level of irradiance (a mixture of fluorescent and incandescent lamps) was less inhibitory to tuberization than extending with low level of incandescent lamps only, may be due to its effect of extra assimilate production (Wheeler & Tibbitts, 1986; Lorenzen & Ewing, 1990).

Lowering the irradiance level decreases the partitioning of assimilates to the tubers (Gray & Holmes, 1970; Menzel, 1985). Shading experiment to reduce light level revealed that shading treatments had a pronounced effect in delaying tuberization, especially if applied after the onset of tuberization (Gray & Holmes, 1970; Sale 1976; Struik, 1986). Menzel (1985) reported that low irradiance increased the production of growth substances that inhibit tuber formation, and GA is the most likely candidate to play such a role.

Nitrogen nutrition

Induction to tuberize tends to decline with an increase in the level of nitrogen. Krauss (1985) demonstrated that tuberization could be manipulated by altering nitrogen supply to the plants. Continuous supply of 1 and 3 mM nitrogen completely inhibited tuber formation, while interrupting the nitrogen supply by keeping plants temporarily in a nitrogen free medium for 4 to 6 days promoted tuberization. He noted that repeated cycles of high nitrogen and nitrogen

withdrawal could result in the formation of "chain tubers", indicating that the level of nitrogen play a vital role in the control of tuber formation.

Increasing nitrogen fertilization enhanced partitioning of assimilates to the shoots rather than to the tubers (Biemond & Vos, 1992). Withholding nitrogen fertilization increased starch content of the leaves, increased the percentage export of assimilates from the leaves, and reduced the activity of sucrose phosphate synthase (Oparka *et al.*, 1987). Although how high nitrogen level inhibits tuberization is not well understood, there is a report indicating that nitrogen withdrawal affects the phytohormone balance in such a way that the level of GA decreases while increasing ABA level (Krauss, 1985). Koda & Okazawa (1983) suggested that the ratio between carbohydrate and nitrogen controls tuber formation. In an *in vitro* experiment, they observed that the inhibitory effect of higher nitrogen was observed only at 2% sucrose but not at a higher concentration.

Sucrose

There is evidence indicating high assimilate level is a contributing factor in induction besides hormonal factors. Gregory (1956) reported that for *in vitro* tuberization sucrose must be added to the growing medium. Sucrose is essential for *in vitro* tuber formation and its use is related with osmotic effect (Nawsheen, 2001). Oparka & Wright (1988) reported that starch synthesis is regulated by the osmolarity of the media. High sucrose level increases the osmotic potential of the media and enhances starch accumulation (Nawsheen, 2001). Khuri & Moorby (1995) proposed that high sucrose level provides a good carbon source that is easily assimilated and converted to starch for the microtuber growth and secures an uninterrupted synthesis of starch due to the higher osmotic potential provided by the excess sucrose. On the contrary, Perl *et*

al., (1991) pointed out that the requirement for high sucrose levels does not represent an osmotic effect or an energy demand but rather a signal for tuber formation.

Simko (1994) hypothesized that sucrose influence tuberization by altering the GA to promoter ratio in such a way that high exogenous sucrose supply causes the formation of excess UDPglucose which in turn increases conjugation of free GA. He also reported that application of glucose did not affect tuberization, because only a small amount of endogenous glucose is converted to sucrose. Cells that contain higher glucose, and lower sucrose concentration showed weak sucrose synthase activity (Sowokinos & Varns, 1992) and less UDPglucose was formed (Geigenberger & Stitt, 1993). Transgenic potato plants characterized by high level of sucrose (Müller-Röber *et al.*, 1992) and increased UDPglucose/hexose phosphate ratio (Jelitto *et al.*, 1992; Sonnewald, 1992) produced significantly higher number of tubers.

2.2.4 The role of plant hormones

Potato tuberization is a complex developmental process known to be influenced by genetic, environmental and physiological factors. Several plant hormones have been suggested to play a prominent role in the control of tuberization in potato (Vreugdenhil & Struik, 1989). Available evidence indicates that photoperiod, temperature, irradiance, nitrogen fertilization and physiological age of the mother tuber affect tuberization either directly or indirectly by mediating changes in hormone concentrations (Van der Zaag & Van Loon, 1987; Vreugdenhil & Struik, 1989; Ewing, 1990).

Gibberellin

The group of hormones most studied in relation to tuberization is the gibberellins (GA), and compelling evidences indicate that they play a vital role in tuberization. Exogenous application of GA reduced tuberization in intact plants, *in vitro* plantlets, and *in vitro* cultured excised sprouts (Menzel, 1980; Koda & Okazawa, 1983; Hussey & Stacey, 1984, Ewing, 1995). The application of GA-biosynthesis inhibitors promoted tuber initiation (Balamani & Poovaiah, 1985; Simko, 1994). Relatively high activity of GA-like compounds was detected in potato grown under non-inductive conditions, specifically under long photoperiods (Railton & Wareing, 1973), high temperature (Menzel, 1983), and high nitrogen fertilization (Krauss, 1985). On the contrary, under short day conditions GA biosynthesis is reduced (Amador *et al.*, 2001). High levels of endogenous GA promote shoot growth (Menzel, 1980) and delay or inhibit tuberization (Abdella *et al.*, 1995; Vandam *et al.*, 1996), impede starch accumulation (Booth & Lovell, 1972; Paiva *et al.*, 1983; Vreugdenhil & Sergeeva, 1999), inhibit the accumulation of patatin and other tuber specific proteins (Vreugdenhil & Sergeeva, 1999), and in combination with other inhibitors it regulates potato tuber dormancy (Hemberg, 1970).

GA inhibits tuberization and appears to play a role in the photoperiodic control of tuberization by preventing tuberization in long day (Jackson, 1999). The idea supported by enhanced tuberization of wild-type *Solanum tuberosum* sub sp. a*ndigena* treated with ancymidol, a GA biosynthesis inhibitor, under long day conditions (Jackson & Prat, 1996). A mutation that appears to block GA synthesis is associated with increased tuberization in potato (Bamberg & Hanneman, 1991, Van den Berg *et al.*, 1995). Amador *et al.* (2001) also suggested that GA is part of the inhibitory signal in potato tuberization under long days. The delaying or inhibitory effect of GA on tuberization may be partly attributed to its effect on carbohydrate metabolism especially sucrose utilization (Jackson, 1999). The involvement of GA in regulating the

pattern of assimilate partitioning was suggested by Yim *et al.*, (1997) who noted that high GA activity leads to higher carbohydrate allocation to the shoots, while low GA level resulted in more dry matter allocation to the roots. GA increases sink strength at the point of application (Mulligan & Patrick, 1979).

Cytokinins

Cytokinins belong to a class of plant hormones first noted as promoters of a cell division (Miller *et al.*, 1955). They are involved in various development processes including apical dominance, root formation, leaf senescence, stomatal behaviour, and chloroplast development (Mok, 1994). Cytokinins are necessary at the very early stage of tuber development, probably because of their vital role in stimulating cell division and radial cell growth (Ooms & Lenton, 1985; Gális *et al.*, 1995). *In vitro* induction of tuberization by exogenous application of cytokinin was reported by Palmer & Smith (1969). Menzel (1985) reported that benzyladenine treatment promoted tuberization in potato grown under high day/night temperatures (32/18 ºC). Exposing plants to inducing conditions (cool temperature and short photoperiod) temporarily increased leaf cytokinin content (Langille & Forsline, 1974). However, the concentration of cytokinin in stolon tips shows little increases until the tubers attain twice the diameter of the stolon (Koda & Okazawa, 1983). The major cytokinin isolated from potato leaves was identified as cis-zeatin riboside (Mauk & Langille, 1978). There are some indications that zeatin riboside (or other cytokinins) is at least partly involved in the tuberization stimulus (Vreugdenhil & Struik, 1989). Mauk & Langille (1978) also suspected that zeatin riboside may be the actual tuber-forming stimulus.

Recently, the involvement of cytokinins in regulating carbohydrate transport and metabolism, and in source-sink effects has drawn much attention (Roitsch & Ehneß, 2000). Kuiper (1993)

hypothesized that cytokinins are involved in regulation of the competition for assimilates and in the creation of sinks by regulating the expression of genes. Modification of the endogenous cytokinin level resulted in redistribution of assimilates in favour of the cytokinin-enriched axillary buds (Guivarc'h, *et al.*, 2002).

Auxins

The direct effect of auxins on tuberization is not yet well investigated. The available information show that auxin is involved in controlling apical dominance, and in combination with GA and cytokinins controls stolon orientation and growth (Ewing & Struik, 1992). Harmey *et al.* (1966) reported that IAA treatment promoted tuberization by inducing the formation of larger tubers at an early stage. High auxin content before tuber initiation and a subsequent decrease during tuber development was reported by Obata-Sasamoto & Suzuki (1979). The application of IAA in the tuber-inducing medium of *in vitro* plantlets led to earlier tuber initiation and produced smaller and sessile tubers (Xu *et al.*, 1998). In addition, they observed that application of IAA in a 1% sucrose medium totally blocked the growth of lateral buds of the cutting and this seems to indicate that IAA restricts elongation growth. Kumar & Wareing, (1972) speculated that IAA stimulates tuber formation by inhibiting stolon elongation and counteracting the effect of endogenous GA that promotes stolon formation and elongation.

Abscisic Acid

Conflicting results have been reported regarding the effects of abscisic acid (ABA) on tuberization. A stimulation of tuber formation in long-day-grown potato was observed in response to leaf applied ABA (El-Antably *et al.*, 1967). Wareing & Jennings (1980) reported that ABA promoted tuberization in leafless induced cuttings. Exogenous applied ABA

stimulated tuberization, reduced stolon length, increased tuber number, and induced the formation of sessile tubers (Menzel, 1980, Xu *et al.*, 1998). Furthermore, an increased ABA level under tuber-inducing conditions was reported by Krauss & Marschner (1982). On the contrary, the inhibitory effects of ABA on tuberization have been reported by Palmer & Smith (1969) and Hussey & Stacey (1984).

ABA is the most wide spread growth inhibitor in plants (Salisbury & Ross, 1992). Treatments with plant growth regulators that block endogenous GA synthesis promote tuberization in potato (Balamani & Poovaiah, 1985; Simko, 1994). This suggests that the naturally occurring tuberization stimulus contains inhibitors or antagonists of GA, and ABA is a likely candidate (Krauss & Marschner, 1982; Wareing & Jennings, 1980). Simko *et al.* (1996) reported a close association between the location of several genes controlling to tuberize under long photoperiod and genes for ABA levels.

Ethylene

The influence of ethylene on tuberization processes depends on the method of application and the type of tissue used (Stallknecht, 1985). The application of ethephon to a very old seed tuber causes a restoration of more normal sprout growth instead of the formation of sprout tubers directly at the eye. Higher GA activity was detected in the elongated sprouts than in the sprout tubers, and ethylene stimulated high GA activity that in turn inhibited tuberization (Dimalla & Van Staden, 1977).

The inhibitory effect of ethylene and promotion effect of ethylene antagonists in *in vitro* tuberization was reported by Vreugdenhil & Struik (1990). Chlorethylphosphonic acid (CEPA) inhibited tuber formation at low day/night temperatures (22/10ºC) according to

Menzel (1985). However, other studies on the effect of ethylene showed contradictory results. Garcia-Torres & Gomez-Campo (1972) reported more tubers on potato plants treated with ethrel than on untreated plants. Similarly, application of ethrel in the medium advanced tuberization and increased tuber number on excised potato sprouts cultured *in vitro* (Stallknecht & Farnsworth, 1982).

It is believed that hormones play a vital role in the communication of signals between plant organs. All classes of plant hormones have some effect on one or more aspects of the different steps leading to tuber formation (Vreugdenhil & Struik, 1989; Ewing & Struik, 1992; Ewing, 1995). The concept of a balance between hormones rather than the concentration of a single hormone as controlling mechanisms in tuber induction has received due consideration. Okazawa & Chapman (1962) suggested that the balance between inhibitory and promoting substances regulates tuber formation. Hammes & Nel (1975) also proposed that a balance between endogenous GA and tuber forming stimuli controls tuber formation; for tuberization to occur the GA must be below a threshold level.

2.3 PACLOBUTRAZOL

The use of chemical plant growth regulators to improve crop productivity has interested plant scientists for many years. Moreover, the recent development of highly active growth retardants has further enhanced the potential uses of chemical growth regulators. Among them, paclobutrazol (PBZ) is widely used. PBZ, a member of triazole plant growth regulator group, is a broad-spectrum GA biosynthesis inhibitor and used widely in agriculture (Davis & Curry, 1991).

2.3.1 Chemistry

PBZ ([(2R, 3R+2S, 3S)-1-(4-chloro-phenyl) 4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol]) has been developed as a plant growth regulator and is registered with trade names such as Bonzi, Clipper, Cultar, and Parsley. It belongs to the triazole compounds that are characterized by a ring structure containing three nitrogen atoms, chlorophenyl and carbon side chains (Fletcher *et al.*, 1986). Structurally, PBZ is a substituted triazole with two asymmetric carbon atoms (Fig. 2.1) and is produced as a mixture of 2R, 3R, and 2S, 3S enantiomers (Sugavanam, 1984, Hedden & Graebe, 1985).
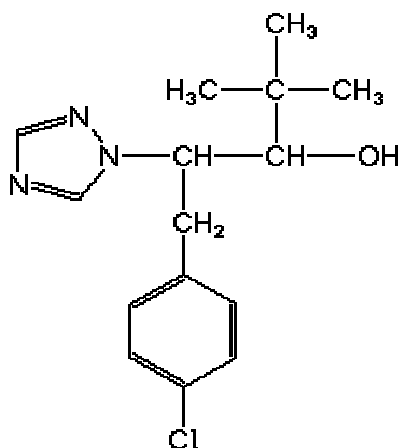

Figure 2.1 The structure of PBZ (http:/www.hclrss.demon.co.uk/ paclobutrazol.html)

2.3.2 Mode of action

Although the precise features of the molecular structure which confer plant growth regulatory activities are not well understood, it appears to be related to the stereochemical arrangement of the substituents on the carbon chain (Fletcher & Hofstra, 1988). There are indications that enantiomers having S configuration at the chiral carbon bearing the hydroxyl group are inhibitors of GA biosynthesis. In cell-free systems, the 2S and 3S enantiomers inhibited ent-kaurene oxidation more effectively than 2R and 3R forms (Hedden & Graebe, 1985). Roberts

& Mathews (1995) reported that resistance of *Chrysanthemum* plants to desiccation was associated with the activity of 2S and 3S enantiomers, presumably due to the inhibition of GA biosynthesis.

2.3.3 Translocation and chemical stability

It was previously believed that triazoles were primarily transported acropetally in the xylem (Davis *et al*., 1988). However, PBZ has been detected in xylem and phloem sap of castor bean (Witchard, 1997), pear (Browning *et al.*, 1992) and dessert pea (Hamid & Williams 1997) indicating that triazoles can be transported acropetally and basipetally. Although the metabolic fate of applied triazoles has not been investigated in detail most of them have a high chemical stability (Jung *et al*., 1986) and depending on the site of application tend to be metabolised slowly (Davis & Curry, 1991). Early & Martin (1988) observed more rapid PBZ metabolism in apple leaves than other plant parts, while Sterrett (1988) found little evidence for PBZ metabolism in apple seedlings. PBZ is comparatively more resistant to degradation than BAS 111 (Reed *et al.*, 1989).

2.3.4 Method of application

A simple, economical and efficient method of application capable of yielding consistent results is the top priority in the utilization of plant growth regulators for commercial purpose. Depending on plant species and concentration different responses have been observed for foliar and soil drenching of PBZ. PBZ spikes were more effective than drench applications in reducing shoot elongation of poinsettias (Newman & Tant, 1995). Drench application of PBZ was more effective in retarding the height of potted mussaenda than foliar spray (Cramer &

Bridgen, 1998). Foliar spray may not give uniform plant size modification if the coverage is inadequate (Barrett *et al.*, 1994). Generally, PBZ is more effective when applied to the growing media and application on the growing medium would give longer absorption time and more absorption of active ingredient than foliar spray. Moreover, drench application of PBZ may directly inhibit GA synthesis as roots synthesize large quantities of GA (Sopher *et al.*, 1999). In some cases, however, both drench and spike applications are effective in controlling plant growth with similar concentration (Barrett *et al.*, 1994).

2.3.5 Response of plants to PBZ

A. Plant hormone biosynthesis

Gibberellin

PBZ interferes with GA biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P450-dependent oxygenases (Izumi *et al.*, 1985; Graebe, 1987). However, the biosynthetic pathway from mevalonic acid to kaurene and from kaurenic acid to GA_{12} aldehyde is not affected (Izumi *et al*., 1985). The inhibitory effects of PBZ on GA biosynthesis is further supported by the fact that treated plants have lower GA concentrations (Steffens *et al.*, 1992), and some effects of PBZ could be reversed by GA application (Cox 1991; Guoping, 1997; Gilley & Fletcher, 1998).

Abscisic Acid

Triazoles interfere with the different isoforms of kaurene oxidase, a cytochrome P-450 hydroxylase and prevent abscisic acid catabolism (Zeevaart *et al.*, 1990; Rademacher, 1997). Contradictory results have been reported for the effects of PBZ on ABA levels in plants.

Increased ABA levels in response to PBZ treatment have been reported in *Actinidia* (Tafazoli & Beyl, 1993) and jack pine (Marshall *et al.*, 2000). Since increases in ABA levels have been associated with plant stress protection, it is suggested that PBZ induced stress protection could be mediated at least partly through its effects on the level of ABA (Fletcher & Hofstra, 1988). On the contrary, PBZ treatment reduced the level of ABA in rice seedling (Izumi *et al.*, 1988). The magnitude of the inhibitory effect of PBZ on ABA levels is dependent on the length of time after application (Buta & Spaulding, 1991). These differential responses may be attributed to differences in growth conditions, application methods, plant species, developmental stages, and the type and concentration of triazoles used (Grossman, 1990; Buta & Spaulding, 1991).

Cytokinin

Cytokinins are synthesized in the roots and translocated acropetally to the shoots where they regulate both plant development and senescence (Letham & Palni, 1983; Binns, 1994). They are involved in the control of various plant developmental processes such as cell division, apical dominance, stomatal behaviour, root formation, leaf senescence, and chloroplast development (Mok, 1994). The involvement of cytokinin in carbohydrate transport and metabolism has been suggested by Roitsch & Ehneß (2000).

Zhu *et al.* (2004) observed an increase in the endogenous cytokinin (Zeatin) level in xylem sap of young apple trees in response to PBZ treatment. PBZ treatment delayed the onset of senescence in grapevine (Hunter & Proctor, 1992) and blueberry (Basiouny & Sass, 1993). It has been reported that cytokinin or chemicals like thidiazuron with cytokinin-like activity stimulate chlorophyll synthesis and retard senescence (Letham & Palni, 1983; Visser *et al.*,

1992) and thus PBZ induced physiological responses may be associated with increased cytokinin synthesis or prevention of its degradation.

B. Chlorophyll synthesis

PBZ treated plants have dark green foliage. This has been associated with increased chlorophyll content of the leaf tissue (Sopher *et al.*, 1999; Berova & Zlatev, 2000; Sebastian *et al.*, 2002) and more densely packed chloroplasts per unit leaf area due to reduced leaf expansion (Khalil, 1995). The increase in chlorophyll content may be ascribed to higher cytokinin content that is known to stimulate chlorophyll biosynthesis and/or reduced chlorophyll catabolism (Berova & Zlatev, 2000). Sopher *et al.* (1999) reported that PBZ increased chlorophyll levels both on fresh weight and leaf area bases. In several plant species PBZ treated leaves were retained longer and the onset of senescence considerably delayed (Hunter & Proctor, 1992; Basiouny & Sass, 1993). The senescence delaying activity may be related to the influence of PBZ on the endogenous cytokinin content (Fletcher *et al.*, 2000).

C. Rate of Photosynthesis

Contradictory reports have been published regarding the effects of PBZ on crop photosynthetic efficiency. PBZ has little direct effect on photosynthetic efficiency; however, indirectly by reducing leaf area it may reduce photosynthetic surface area and thereby reduce the whole-plant photosynthesis (Davis *et al*., 1988). Rate of photosynthesis in rice was not affected by PBZ treatment (Yim *et al.*, 1997). Application of 250 and 500 mg PBZ per plant reduced leaf photosynthetic rate in sweet orange plants (Joseph & Yelenosky, 1992). On the contrary, there are reports indicating that PBZ enhances photosynthetic efficiency. PBZ

treatment increased productivity by enhancing photosynthesis efficiency in soybean (Sankhla *et al.*, 1985), rapeseed (Zhou & Xi, 1993), and tomato (Berova & Zlatev, 2000). Higher ribulose-1,5-biphosphate carboxylase activity and increased capacity for electron transport could be the reasons for enhanced photosynthesis after PBZ treatment (Archbold & Houtz, 1988; Joseph & Yelenosky, 1992; Van den Boogaard, 1994). Increased chlorophyll content in response to PBZ treatment may substantially contribute for enhanced photosynthetic rate because higher chlorophyll content is one of the main factors stimulating the rate of photosynthesis and biological productivity (Mojecka-Breova & Kerin, 1995; Berova & Zlatev, 2000).

D. Stress protection

PBZ increases tolerance of various plant species against several environmental stresses such as drought and temperature (Marshall *et al.*, 1991; Kraus & Fletcher, 1994; Marshall *et al.*, 2000; Zhu *et al.*, 2004). Proposed biochemical mechanisms of these protective effects include a shift in hormonal balance, decrease in endogenous GA levels and a transitory rise in ABA level (Masia *et al.*, 1994; Rademacher, 1997; Zhu *et al.*, 2004). PBZ increases the survival rate of plants under drought conditions through a number of physiological responses. A reduction in the rate of transpiration (due to reduction in leaf area), increased diffusive resistance, alleviating reduction in water potential, increased relative water content, less water use, and increased anti-oxidant activity are some of the reported responses (Marshall *et al.*, 1991; Eliasson *et al.*, 1994; Kraus & Fletcher, 1994, Zhu *et al.*, 2004). PBZ significantly decreased chilling injury in pepper fruit and cucumber seedlings (Whitaker & Wang, 1987; Lurie *et al.*, 1995), and this may be ascribed to in inhibition of chilling induced degradation of membrane lipids (Whitaker & Wang, 1987). PBZ induced chilling tolerance was also associated with change in antioxidant enzyme profiles and an increase in ABA level (Tafazoli

& Beyl, 1993; Pinhero *et al.*, 1997). PBZ protects plants from high temperature induced injuries (Kraus & Fletcher, 1994; Pinhero & Fletcher, 1994). Protection against high temperature stress is accompanied by the production of low molecular mass stress proteins (Larsen *et al.*, 1988) and the increase in the activity of antioxidant enzymes (Upadhyaya *et al.*, 1990; Kraus & Fletcher, 1994).

E. Morphological and anatomical changes

Shoot

Compared with other plant growth retardants triazoles are potent and required in small quantities to inhibit shoot growth (Davis *et al*., 1988). PBZ has been widely used to control the size of fruit trees and agronomic crops (Davis & Curry, 1991). The most noticeable effect of PBZ is internode compression resulting in compact and short plants (Berova & Zlatev, 2000; Terri & Millie, 2000; Sebastian *et al.*, 2002; Yeshitela *et al.*, 2004). Modification of shoot growth with the aid of PBZ may be helpful in maximizing return per unit land by allowing increased plant populations of the compact plants per unit land area.

Leaves

PBZ induces various leaf morphological and anatomical modifications depending on plant species, growth stage, rate and method of application. It reduces leaf area (Sebastian *et al.*, 2002; Yeshitela *et al.*, 2004), increases the thickness of the epicuticular wax layer (Jenks *et al*., 2001), increase size of a vascular bundles, epidermal, mesophyll and bundle sheath cells (Burrows *et al*., 1992; Sopher *et al.*, 1999). Depending on the species PBZ modulate leaf conductance, transpiration rate, and water use efficiency. In tomato PBZ enhanced rate of photosynthesis and slightly increased rate of transpiration along with a reduced stomatal

conductance in the third leaf while in the fifth leaf higher photosynthetic efficiency was accompanied by higher transpiration and stomatal conductance (Berova & Zlatev, 2000). Strawberry leaf diffusive conductance was increased by PBZ treatment when measured 12 months after application (Archbold & Houtz, 1988).

Stems

The reduction of plant height following PBZ treatment is accompanied by various anatomical modifications depending on species and concentration. Berova & Zlatev, (2000) observed increased radial extension in tomato stems, but stem diameter was reduced by 12-50% in citrus root stock seedlings (Yelenosky *et al.* 1995). McDaniel *et al.* (1990) reported that PBZ treatment of poinsettia resulted in weaker stems due to suppression of the thickening of cell wall of phloem fiber caps, decreased width of xylem ring, and restricting the differentiation of interfascicular supporting tissue. In *Chrysanthemum*, PBZ treatment resulted in thin stems with increased development of secondary xylem and a reduced number of sclerenchyma bundle caps (Burrows *et al.*, 1992). Aguirre & Blanco (1992) found that PBZ treatment resulted in a decreased proportion of xylem in peach shoots, with a corresponding increase in the amount of phloem and cortex. PBZ induced radial expansion in plant organs may be due to reduced endogenous GA levels. GA limits the extent of radial expansion of plant organs (Wenzel *et al.*, 2000). Barlow *et al.* (1991) observed a decreased axial growth and an increased radial expansion in GA deficient mutant tomato plants.

Roots

Depending on the plant species and the concentration applied, PBZ induces root anatomical and morphological modifications. It increased root diameter in *Chrysanthemum* by increasing the number of rows and diameter of cortical cells (Burrows *et al.*, 1992). Increased root

diameter in soybean due to an increase in the size of cortical parenchyma cells was reported by Barnes *et al.* (1989). PBZ inhibited primary root elongation of pea, while promoting radial expansion of the cells (Wang & Lin, 1992). Yim *et al.* (1997) reported that PBZ treated rice seedlings had higher root dry mass and greater ability to produce new roots. Enhanced adventitious root formation in English ivy (Geneve, 1990) and increased rooting ability of mung bean cuttings (Porlingis & Koukourikou-Petridou, 1996) have been observed in response to PBZ treatment. Improved root formation may be attributed to increased assimilate partitioning to the roots due to reduced demand in the shoot (Symons *et al.*, 1990).

By influencing shoot and root morphology PBZ alter mineral uptake, although the effects are not consistent and well investigated. Yelenosky *et al.* (1995) reported that leaves from PBZ treated citrus seedlings had higher concentrations of N, Ca, B, and Fe. In apple seedlings, PBZ increased the foliar concentration of N, P, K, Ca, Mg, Mn, B, and Zn without affecting the concentration of Fe, Si and Pb (Wang *et al.,* 1985), while Wieland & Wample (1985) found PBZ treatment did not affect the concentration of N, P, K, and Mg in apple leaves. Steffens *et al.* (1985) reported that apple fruit mineral composition was unaffected by PBZ treatment. Recently, Yeshitela *et al*. (2004) reported that PBZ increased mango leaf Mg, Cu, Zn, and Fe content without affecting the concentration of N, P, K, and Ca.

F. Assimilate partitioning

Sink regulation of photosynthesis is a well-accepted concept, possibly explaining the coordination of assimilate production and utilization (Stitt *et al.*, 1990). Assimilate partitioning to the different sinks may be controlled by environmentally regulated, hormonal balances (Almekinders & Struik, 1996). PBZ treatment increase the root-to-shoot ratio

(Pinhero & Fletcher, 1994; Yim *et al.*, 1997), increase partitioning of assimilates to economically important plant parts such as bulbs (Le Guen-Le Saos *et al.*, 2002, De Resende & De Souza, 2002) and tubers (Balamani & Poovaiah, 1985; Pelacho *et al.*, 1994; Simko, 1994). PBZ inhibits GA biosynthesis and subsequently modulates hormonal balance and thereby influences the pattern of assimilate production and allocation. The involvement of GA in regulating the pattern of assimilate partitioning was suggested by Yim *et al.* (1997). He noted that high GA level leads to a higher carbohydrate allocation to the shoots, where as low GA level resulted in more dry matter allocation to the roots.