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Review Article

Effect of Paclobutrazole (PBZ) on Fruit Production: A Review

Ashok Kumar[1]*, B.D.Bhuj[2], Shri Dhar[3], Rajkumar[4], Mohd Rizwan[4], Ravi Kumar Thapa[4], Harish Kumar[4], Jyoti[4], Vijay Kumar[4], Amit Singh[4], Vidhur Kumar[4], Anurag Rajput[4], Kuldeep Kumar[4], Vishal Kumar Misra[4]

[1]Department of Horticulture, School of Agricultural Sciences (SOAS), IIMT University, Meerut U.P, India

[2]Professor Horticulture, College of Agriculture, U.P, India

[3]Principal Scientist, Revision of Vegetable Science, New Delhi, India

[4]Assistant Professor Agriculture, School of Agricultural Sciences (SOAS), IIMT University, India

E- mail: yadavakdr@gmail.com

Abstract

Paclobutrazol (PBZ), [2RS, 3RS]-1-[4-chlorophenyl]-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol, consists of a triazole ring and a benzene ring-chloro linked to a carbon chain open. It is a plant growth regulator widely used in many crops in order to produce fruit throughout the year by inhibiting gibberellin synthesis; a hormone responsible for the vegetative plant growth. The biennial bearing is very serious problem in fruit crop production. Therefore, application of paclobutrazol is most widely studied in view of its high potential for controlling plant growth and development of fruit crops in general. It inhibits gibberellin biosynthesis at kaurene stage and has proved to be reduction of vegetative growth, promising for flower initiation in shoot bud, giving early and profuse flowering, increases fruit yield and improving quality regularly in alternate bearing cultivars. The growth regulating properties of paclobutrazol are mediated by changes in the levels of important plant hormones including the Gibberellins (GAs), Abscisic Acid (ABA) and Cytokinins (CK). Paclobutrazol (PBZ) affects the isoprenoid pathway, and alters the levels of plant hormones by inhibiting gibberellin synthesis and increasing cytokinins level. When gibberellins synthesis is inhibited, more precursors in the terpenoid pathway accumulate and that resulted to the production of abscisic acid. PBZ has been used to provide plant protection against numerous abiotic stresses such as chilling, water deficit stress, flooding and salinity. The main aim of this review is to focus upon contemporary information about paclobutrazol in fruit production. Its agronomic management includes it as an emerging technology to reduce vigour, promote flower induction and flower development in fruit trees with increased economic returns. Its use is banned in some countries because of concerns about residues that can cause harmful effects on the environment. Therefore, the aim of this article was to collect, analyse and summarise relevant information on the use of PBZ in fruit tree production and its possible risks to the environment. Usually it is applied as a soil application in the month of September-November in case of mango.

Keywords: Environment, Flower induction, Gibberellin biosynthesis, Growth bioregulator, Vegetative growth.

INTRODUCTION

Plant growth retardants are being used widely in chemical manipulation of growth anddevelopment by modifying associated biochemical and physiological processes. Among them,paclobutrazol is considered as one of the most versatile plant growth retardant which restricts vegetative growth and induce flowering in many fruit crops like apple

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and pear (Williams and Edgerton, 1983) , peach (Erez, 1984), citrus (Aron et al., 1985) and mango (Sarkarand Rahim, 2012). It restricts induced tree vigour and flowering responses which have beenreported as the consequences of modifications in physiological activities as well as changes incellular metabolites (Upreti et al., 2014). Among the cellular metabolites, accumulation ofphenols in vegetative organs and altered biochemical balance are important in restriction ofvigour in mango (Murti et al., 2000) and also induction of flowering (Patil et al., 1992).

Paclobutrazol (PBZ) is a triazole derivative with the empirical formula [(2RS, 3Rs) -1 - (4-chlorophenyl) 4.4-dimethyl-2-(1H-1, 2, 4-triazole-1-yl) pentan-3-ol], which plays animportant role in regulating excessive vegetative growth, enhancing and advancing flowering,inducing early bearing, managing biennial bearing tendency, establishing a high density plantation. The application of paclobutrazol to soil promotes flowering and increasing yield inmany fruit crops. Besides reducing gibberellins level, paclobutrazol increases cytokinincontents, root activity and C: N ratio, whereas its influence on nutrient uptake lacksconsistency. It also affects microbial population and dehydrogenase activity in soil.

PBZ has been characterized as an environmentally stable compound in soil and waterenvironments with a half-life of more than a year under both aerobic and anaerobic conditions.However, when it is applied in optimized rate the residual concentration detected will not be above quantifiable level (0.01 ppm) in soils and fruits Globally, agriculture is one of the most important economic activities, given its contribution to food production. Population growth has a direct impact on the need to increase crop yields, and consumers expect high quality, nutritious, clean, healthy and safe products. The integration of emerging technologies in the different production systems must be in an environment of safety and sustainability.Therefore, the Food and Agriculture Organization of the United Nations (FAO) coined the concept of food security as “physical and economic access to sufficient, safe and nutritious food to meet one’s dietary needs and food preferences for an active and healthy life”. The application of nutrients and growth bioregulators increases crop productivity.

The latter can be classified according to the physiological processes with which they are associated and their response to application. Previous reports indicate that low concentrations of these compounds facilitate agronomic management, and their effect can be linked to inhibition, slowing and induction of sprouting, flowering and fruit ripening. Among these growth bioregulators is paclobutrazol (PBZ) (growth inhibitor), which reduces vigour, and promotes flower induction and developmen. In the last decade, among the common agronomic management practices for fruit tree production is the use of PBZ, which promotes various effects. For example, in India it is used to increase fruit quality in litchi (Litchi chinensis Sonn) “China” by inhibiting vegetative growth , pear “Clapp’s” (Pyrus pyrifolia L.) and Indian walnut “Ullal-3” (Anacardium occidentale L.). In China, it is used to reduce vegetative growth in the cultivation of “Western Schley” and “Mahan” pecan [Carya illinoinensis (Wang.) K. Koch]. In Brazil, it is used to increase the size and shape of the fruit of the “Hass” avocado (Persea americana L.). In Mexico it is used to promote flowering and increase production in guava (Psidium guajaba L.) “Calvillo”, “Hidrosac” and “Caxcana”, and in mango (Mangifera indica L.) “Tommy Atkins”, “Ataulfo” and “Manila”. The use of PBZ contributes to increase production value (yield and fruit quality), however, there is evidence of its residual effects and its negative effect on consumers’ health. In addition, there is information that it can cause environmental pollution (groundwater and soil). The aim of this paper was to collect, analyse and summarise information on the use of PBZ in the production of some economically important fruit trees and their possible risks to the environment.

MODE OF ACTION

Paclobutrazol inhibits gibberellins biosynthesis by blocking the conversion of kaurene and kaurenoic acid, which inhibits cell elongation and internode extension and ultimately retards plant growth. Gibberellins stimulate cell elongation. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. That result in the production of shoots with the same numbers of leaves and internodes compressed into a shorter length. Even reduction in the diameter of the trunk is noticed. Paclobutrazol treated trees shows increased production of the hormone abscisic acid and the chlorophyll component phytol, which are beneficial to tree growth and health. It also induce morphological modifications of leaves, such as smaller stomatal pores, increased number and size of surface appendages, thicker leaves, and increased root density that may provide improved environmental stress tolerance and disease resistance and it also has some fungicidal activity due to its capacity as a triazole to inhibit sterol biosynthesis (Chaney, 2005).

Translocation of PBZ in Plant

PBZ is applied as a soil drench (application to roots, more popular and convenient) through trunk injection (directly to the vascular system of the stem using pressure). Through xylem it translocate to other parts of plant, however a few research evidences have been provided to support this assumption. Gas chromatography-mass spectrometry confirmed that PBZ was taken up by roots and transported primarily through xylem to stems and accumulated in leaves. Effects of PBZ on various tree attributes In tropical fruit orchards, it is desirable to control the vegetative growth and to reduce the canopy size since small trees capture and convert the sunlight into fruit biomass in a better way than larger trees because of more surface area. Increase in production with enhanced fruit quality can be achieved by managing the tree canopy. Manipulation in tree physiology with the use chemical growth retardants has been considered as an important determinant of productivity enhancement in many fruit crops. Application of paclobutrazol in the soil has been commercialized for early and enhanced flowering in some of the fruit crops. Effect on vegetative growth many investigations have revealed the beneficial effects of PBZ in restricting vegetative growth and successful induction of flowering in apple, mango, grape etc. The application of paclobutrazol (1500 to 3000 ppm) at full bloom and 21 days after full bloom resulted in the reduction of shoot growth in 'Golden Delicious' apple (Greene, 1982). Quinlan and Richardson (1984) inferred that application of paclobutrazol at 500 ppm alone was effective in reducing the shoot length (9.5 cm) and the combination with GA3 was not effective in apple seedlings. Five-year-old MM.106 (Malus domestica Borkh.) trees growing under a high-density (10000 trees/ha) planting system treated with paclobutrazol at 250 mg per tree in August by Khurshid et al., 1997 showed reduced number of total shoots and buds. This showed that PBZ can be used to manipulate apple tree growth in a high- density apple production system. Paclobutrzol when applied during early summer has been observed as an effective suppressant of stem growth in sweet cherry (Quinlan and Webster, 1982). Similarly, Webster et al. (1986) reported that application of paclobutrazol at 1.6 g a.i. tree-1 and followed by 0.8 g a.i. in next year inhibited extension of growth in young cherry trees on either colt or FB22 rootstocks. 2-year-old nashi trees treated with paclobutrazol as soil drench and foliar sprays (Klinac et al.,1991). The cultivars treated were 'Hosui', 'Kosui', 'Nijisseiki', and 'Shinsui'. All cultivars showed a significant reduction in vegetative growth within the first season and for up to 4 years after initial application. Most reduction in growth was obtained from soil applications. Least reduction in growth was from a foliar application at the lower rate of 125ppm. Application of paclobutrazol on 'Redhaven' cultivar of peach reduced terminal growth and advanced leaf fall (Young, 1983) . Similarly, the vigour of mango was consistently reduced with paclobutrazol application in a range of Indian cultivars (Kulkarni, 1988). The soil drenching with paclobutrazol at the rates of 12, 10, and 8 g a.i. suppressed the vegetative growth, canopy volume, and flush length of reproductive shoots, fruit setting, panicle length as compared to control in mango (Nafeez et al., 2010). Similar results were observed by Teferi et al. (2010) in Tommy Atkins mango with maximum effect at 8.25 g a.i. per tree. Soil application of paclobutrazol recorded significant reduction in canopy volume by noticeable reduction in number of shoots per terminal and also checked the growth of new shoots (Tandel and Patel, 2011). Similarly, the growth inhibitory response of PBZ reported in different varieties of mango (Sarkar and Rahim, 2012) could be the consequences of modification in photosynthesis rate (Gonzalez and Blaikie, 2003) and carbohydrates (Upreti et al., 2014) besides reductions in gibberellins (Upreti et al., 2013.

PBZ Effect on Flowering parameters and Yield

The fruit set was increased in paclobutrazol treated tress @ 1500 and 3000 ppm due to an increase in initial fruit set in delicious apple (Greene, 1986) similar results was observed by Elfying et al. (1990) in McInthosh apples that the foliar application of paclobutrazol reduced pre harvest drop when applied within 5 weeks after full bloom. Stan et al. (1989) reported that foliar and soil application of paclobutrazol enhanced the flower bud formation and fruit set in high density planting of sweet cherry. In avocado, paclobutrazol enhanced the fruit set by increasing the portioning of dry matter to fruits (Wolstenholme et al., 1990). Jindal and Chandel (1996) applied paclobutrazol in 'Santa Rosa' plum at 125, 250 and 500 ppm once at full bloom and again at pit hardening stage and reported maximum fruit weight of 24.33 g and fruit volume of 21.6 cc in fruits treated with 500 ppm paclobutrazol. Ratna and Bist (1997) reported that application of 0.15 g a.i. paclobutrazol cm-1 trunk diameter increased fruit yield of 'Gala' pear and during the next year, yield was significantly increased with the same application. They also noticed that paclobutrazol 0.3 g a.i. cm-1 trunk diameter increased the yield by more than 1.35 times during both the years. Arzani et al. (2000) reported that paclobutrazol application advanced flowering of five year old vigorous 'Sundrop' apricot trees by 2-4 days and also increased the fruit set, final fruit number, crop density and yield efficiency. Selva strawberry cultivar using paclobutrazol (0,100 mgl^{-1}) and other nutrient combination indicated that vegetative growth was reduced with application of paclobutrazol and highest vitamin C was obtained at concentration of0-100 mg l^{-1} PP333 (Abdollahi et al., 2010). Kulkarni (1988) observed that there was a significant increase in yield of mango per tree by the soil application of paclobutrazol (10 g a.i./tree). In terms of fruit size and quality for at least two years in five years old bearing trees. Effect of PBZ on promotion of flowering in citrus was studied by Fuentes et al. (2013) and result revealed that PBZ significantly increased the percentage of sprouted buds and leafless floral shoots (both single flowered shoots and inflorescence) and reduced the number of vegetative shoots. The application of paclobutrazol at 1 g a.i./m of canopy diameter increased the female inflorescence production (18.10%) without negative effect on fruit set (90.68%) in 'Eviarc Sweet' cv. of jackfruit. Female inflorescences were produced in the offseason (August and September) which was not observed in untreated trees. (Lina and Protacio, 2015). Among the chemicals suggested, paclobutrazol is considered as one of the most versatile plant growth retardant which restricted vegetative growth and induced flowering in many fruit crops like apple and pear (Williams and Edgerton, 1983), peach (Erez, 1984), citrus (Aron et al., 1985) and mango (Sarkar and Rahim, 2012).

Early and intense flowering induced by PBZ may be the consequence of early shoot maturity and increased photosynthesis rate (Singh and Singh 2003), carbohydrate accumulation (Abdel Rahim et al., 2011) and decline in flowering reducing hormone, gibberellins (Upreti et al., 2013). The effects of PBZ on different flowering parameters such as regular, profuse and early flowering (Kulkarni, 1988; Nartvaranant et al., 2000; Jogande and Choudary, 2001; Karki and Dhakal, 2003; Yeshitela et al., 2004; Blaikie et al., 2004; Singh & Ranganath, 2006; Reddy and Kurian, 2008; Hussen et al., 2012; Reddy et al., 2014), reduced panicle length (Vijayalaksmi and Srinivasan, 2000; Hoda et al.,2001; Nafeez et al., 2010; Sarkar & Rahim, 2012), increased the number of perfect flowers and fruit set (Burondkar & Gunjate, 1993; Kurian and Iyer, 1993; Hoda et al., 2001; Singh et al., 2000) were reported in various fruit crops. All the available evidences opined that carbohydrate reserves played an important role in flower bud differentiation and they provide conditions favorable for the synthesis of substances which are required for flower bud differentiation (Suryanarayana, 1987; Pongomboon et al., 1997). The high C: N ratio during flower bud differentiation was ascribed to the increased carbohydrate availability (Ito et al., 2004) and is considered as an important factor in regulation of flowering in fruit crops (Jyothi et al., 2000; Palanichamy et al., 2012).

Paclobutrazol is known to decrease vegetative growth rate through early cessation of growth, which results in the accumulation of carbohydrates in trees and slightly decreasing the total nitrogen in the terminal shoots, which favours flowering by maintaining high C: N ratio in the shoots. The C: N ratio differs with growth of shoots in the varieties revealing its dependence on environmental conditions and prevailing metabolic balance. The paclobutrazol induced enhancement in C: N ratio has been reported in mango (Subhadrabandhu et al., 1997; Protacio et al., 2000; Abdel Rahim et al., 2011, Rakshe and Nigade, 2013; Upreti et al., 2013; Upreti et al., 2014) and in pummelo (Phadung et al., 2011). Distinct differences in carbohydrate pattern are seen in vegetatively growing shoots and flowering shoots. Shoots that are going to differentiate into flower buds are the growing sinks and the actively dividing cells of induced flower buds require high energy (Davenport, 2007). Apparent increase in sugar levels during floral induction period has been reported in mango by several researchers (Jyothi et al.,2000; Abdel Rahim et al., 2008; Palanichamy et al., 2012). Consistently higher production of total sugars and reducing sugars with peak availability at bud burst in apical buds of paclobutrazol treated trees is reported in mango (Shivu Prasad et al., 2014: Upreti et al., 2014). Paclobutrazol induced increase in soluble sugars at flowering has also been reported in mango (Abdel Rahim et al., 2011).

Among the cellular metabolites, accumulation of phenols in vegetative organs has been depicted as one of the important in imparting of vigour restriction effects in mango (Murti et al., 2001; Murti and Upreti, 2003) and also for induction in flowering (Patil et al., 1992). The possible mechanism by which phenols exert its effects on tree vigour and regulation of flowering in mango are less understood. However, steady increase in phenol content with advancement of flower bud differentiation has been reported in mango by Palanichamy et al. (2012) and Kumar et al. (2014). The paclobutrazol induced tree vigour restriction and flowering responses have been reported as the consequences of changes in cellular metabolites (Abdel Rahim et al., 2011; Upreti et al., 2013). High phenol content in terminal buds due to paclobutrazol application restricted the vigour and enhanced the flowering has also been reported by Kurian and Iyer (1993).

PBZ Effect on Fruit Quality

Fruit quality improvement with respect to pulp content, TSS,TSS to acid ratio, total sugars and reducing sugars in response to PBZ application can be related to the assimilate partitioning in plant. The greater suppression of vegetative growth causes assimilates demand in unidirectional manner to the developing fruit, resulting in high quality fruits from PBZ treated plants. Application of paclobutrazol @ 0.33, 0.50, 0.66 and 1.32 g a.i. as soil application to 'Flavorest' peach hastened the fruit colour than control (Martin et al., 1987). Similarly, application of 500 mg l-1 paclobutrazol sprayed within 5 weeks after full bloom to 'McIntosh' apples gave high percentage of fruit with acceptable red color at harvest (Elfying et al., 1990). Singh and Dillon (1992) reported that soil application of PBZ to Dashehari mango recorded higher fruit yield and high TSS: acid ratio compared to foliar application, while fruit weight: stone and pulp: stone ratio did not differ significantly. Vijaylaxmi and Srinivasan (2000) in an experiment with 10 years old Alphonso mango trees treated with paclobutrazol (10 ml), KNO3 (1%), urea (1%), ethrel (200 ppm), NAA (20 ppm) or mepiquat chloride (500 ppm) found that among all the treatments, paclobutrazol (10 ml) resulted in increased ascorbic acid content, total sugars and reducing sugars, TSS, acidity and sugar: acid ratio in harvested fruits. A significant improvement in the fruit quality of cv. Langra in terms of total soluble solids (TSS), total acidity, total chlorophyll, total carotenoids and α-amylase activity due to paclobutrazol @ 6 g a.i./tree in comparison to control was reported by Singh and Saini (2001). Further they evaluated the efficacy of soil applied paclobutrazol (2, 4, 6 and 8 g a.i./tree) on Langra cultivar of mango for three consecutive years at Lucknow and reported a significant increase in fruit set, fruit retention per panicle and yield per tree due to PBZ 6 g a.i./tree. Saxena et al. (2013) reported that paclobutrazol, a flower inducing chemical, enhanced the catalase and peroxidase activities over the untreated control and maximum enhancement was recorded at 8 g a.i. The decreasing trend of protein with paclobutrazol treatment was recorded in adjacent leaves of flower buds. The results implicated the possible role of catalase and peroxidase and other associated biochemical changes in paclobutrazol induced flowering in mango. The soil drenching of paclobutrazol at 3.0 ml m-1 canopy diameter to the mango cv. Totapuri was done in order to study the role of carbohydrates in the paclobutrazol induced floral initiation by Upreti et al. (2014).The results indicated that paclobutrazol induced flowering was accompanied by an increase in starch in leaf concomitant with increased insoluble sugars like sucrose, glucose and fructose in apical buds as well as inhibition in the amylase activity in association with increase in the activities of acid invertase, sucrose phosphate synthase and sucrose synthase in the apical buds. Similarly in CO 2 papaya (dioecious) there was increase in amino acids, total carotenoids, TSS, sugars, ascorbic acid and sugar-acid ratio as compared to control, the response being linear with the increasing concentrations PP333 as soil drench at two levels viz., 25 and 50 mg a.i./plant (Auxcilia et al., 2010). The improvement in fruit quality parameters such as high edible portion, longer shelf life, higher TSS, increased vitamin C, lower titrable acidity, high dry matter content and high reducing and total sugars with PBZ was reported by Vijaylakshmi and Srinivasan (2000), Hoda et al. (2001), Bamini et al. (2009), Sarkar and Rahim (2012) and Reddy et al. (2014) in different varieties of mango. An increase in the contents of ascorbic acid and carotenoids which are documented as potential antioxidants with PBZ application has also been reported in mango (Reddy et al., 2014), papaya (Auxcilia et al., 2010), guava (Jain & Dashora, 2011). However, non-significant effect on fruit quality with PBZ application was reported by Tandel and Patel (2011) and Upreti et al. (2013). Effects on physiological attributes.

Effect on Leaf Water Potential (Ψw)

The PBZ induced increase in Ψw is speculated as the result of increased root hydraulic conductivity, reduced transpiration and increased ABA levels. Increased ABA reduces the transpirational losses by inducing stomatal closure (Hauser et al., 1990). As ABA is known to induce stomatal closure, and is expected to reduce the water loss through transpiration. The increased water levels due increased ABA are expected to induce bud dormancy which could be of relevance to flower bud differentiation in mango (Abdel Rahim et al., 2011; Upreti et al., 2013; Murti et al., 2001).

Influences the Mechanism of Nutrient Uptake

Werner (1993) reported that, cultar treated mango trees showed an increase of N, Ca, Mn, Zn and B contents and decreased contents of P, K and Cu. On the other hand, the significant increase in the root activity towards the trunk and close to soil surface and sparser root activity in the subsoil zone and in drip line area in paclobutrazol treated mango plants was observed by Kotur (2006). Paclobutrazol alsopromotes the avoidance of salt stress in mango by increasing the levels of photosynthetic pigments, water content, K+ uptake and uptake of harmful Na+ and Cl– ions (Kishor et al., 2015).

Garcia (2014) opined that the efficacy of cultar in terms of shoot growth and production efficiency depends on the time of pruning. Ram et al., (2005) observed reduction in tree height, shoot length, shoot girth and internodal length when paclobutrazol (12 and 16 ml) applied with pruning (4 or 5 m height) of mango cv. Dashehari trees. Singh et al., (2005) reported that paclobutrazol as soil drenched reduced tree height, shoot length, tree spread and panicle size in mango cv Dashehari. Soil application of paclobutrazol at 5 g/tree was most effective to induce more number of flowering shoots in mango cv. Gulab Khas (Singh and Singh, 2006) . Similar reports were obtained by Bagel et al., (2004) in 10-year-old mango cv. Langra trees. Soil application of Cultar promoted flowering, along with cauliflower and axillary flowering (Singh et al., 2000). Reddy and Kurian (2008) also observed residual influence of PBZ in soil if applied continuously for three consecutive years and suggested discontinuation of application or to taper down its dose. Singh (2005) also detected paclobutrazol residue below permissible limit (0.4898-1.0005 μg/g) in the rhizosphere after two years of application.

Growth Bioregulators

Among the production costs associated with the application of products to improve and increase the yield and quality of harvested fruit, the application of growth bioregulators constitute a minimal portion. However, in recent years, their application has increased due to the fact that they help to improve the productivity of fruit trees. Plants synthesise various compounds, including growth bioregulators, which promote and regulate their physiological processes (vegetative growth, flower induction, fruit set and fruit size increase). In addition, they influence the response to biotic and abiotic stresses. Traditional bioregulators include auxins, gibberellins, cytokinins, and abscisic acid. Research also considers brassinosteroids, salicylic acid, triazoles, jasmonates, polyamines and recently strigolactones as bioregulators. Growth bioregulators can be classified according to their mode of action. It is reported that they can enhance plant defence mechanisms, promote cell division and growth, processes that help to increase yield and quality fruit. They can also delay or inhibit growth. They also play an important role in responses to stress and adaptation such as to water deficit, high temperatures, salinity and flooding. Recent studies report that growth bioregulators, when applied in small concentrations, are easily uptaken and mobilised through the xylem, with the purpose of modifying the physiological behaviour of the plant. There are commercial products that are used as part of the agronomic management of plant growth and development, synthetic analogues of natural bioregulators, including PBZ.

Paclobutrazol (PBZ)

PBZ was first announced in 1986 as a new bioregulator, which was introduced to the market by ICI Agrochemicals (now part of Syngenta). It is a synthetic compound [(2 RS, 3 RS) -1- (4-chlorophenyl) - 4,4-dimethyl-2- (1 H-1,2,4-triazol-1-yl) -pentan-3 ol] that inhibits vegetative growth, belonging to the triazole group. Chemical properties of PBZ include: molecular weight 293.8, molecular formula C15H20ClN3O, melting point 165°C–166°C, density 1.22 g ml–1 and water solubility 35 mg L–1. PBZ is a hydrophobic and slightly polar molecule, with hydrophilic parts. It has two chiral centres (two asymmetric carbons), hence the existence of two pairs of enantiomers [(2R, 3R)- and (2S, 3S)-] and [(2S, 3R)- and (2R, 3S)-]. However, among the stereoisomers, 2S and 3S show higher inhibition efficiency in gibberellin biosynthesis, but 2R and 3R are more easily degraded. PBZ is found as an active ingredient in several commercial products such as: “Cultar 25 SC” and “Bonzi” (Syngenta, USA), “Regalis Plus” (BASF, USA) and “AuStar” (Chemicals Direct Pty, Ltd., Australia). It is a non-polar compound with a broad-spectrum nature that is mainly translocated via xylem. However, it will depend on the application route, as it can also be transported via phloem.

The mode of action of PBZ is framed as part of the terpene pathway. This is, it inhibits the biosynthesis of gibberellins by inactivating the enzyme ent-kaurene oxidase, which catalyses their oxidation to ent-kaurenoic acid. This favours the activation of the enzymes geranylgeranyl reductase and phytoene synthase for chlorophyll and abscisic acid biosynthesis, respectively (Figure 1). As a result, it decreases vigour and promotes floral induction and development.

The Curse of Healthcare and its People and Financial Resources Requirement

Plant growth and development is associated with cell division and expansion induced by gibberellin activity. PBZ applications inhibit its synthesis; consequently, cell elongation does not occur. In the tree you can see a greater number of leaves, shoots and shorter internodes. Likewise, it increases the thickness of the leaves and reduces the size of stomatal pores.’

The largest group of plant growth retardants consists of chemicals antagonistic to gibberellins (GA), the hormone that is responsible for plant growth. Commercially used inhibitors of GA biosynthesis are: (a) onium-type compounds, (b) compounds with a N-heterocycle (triazole-type), (c) structural mimics of 2-oxoglutaric acid, and (d) 16, 17-dihydroGAs. PBZ, a member of triazole plant growth regulator group, is used widely in agriculture. It is a cell elongation and internode extension inhibitor that retards plant growth by inhibition of gibberellins biosynthesis. Gibberellins stimulate cell elongation. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same numbers of leaves and internodes compressed into a shorter length. Reduced growth in the diameter of the trunk and branches has also been observed. Another response of trees to treatment with PBZ is increased production of

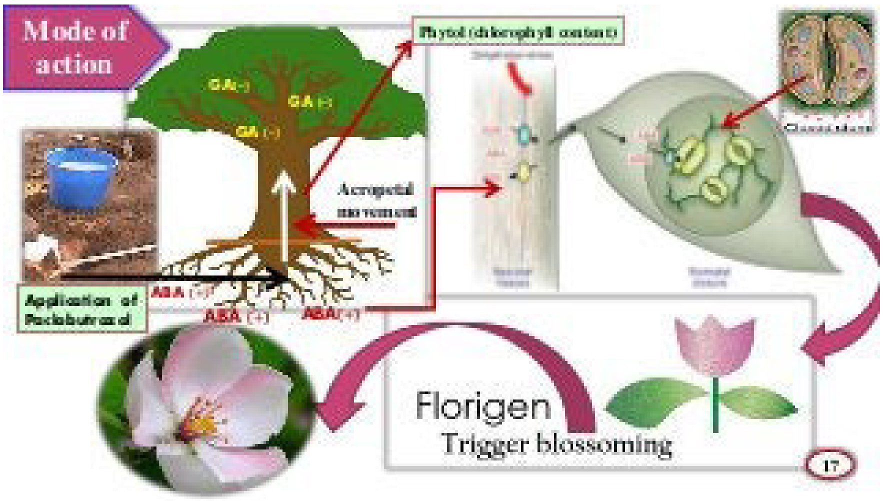

Figure 1. Floregin triggering blossoming.

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the hormone abscisic acid and the chlorophyll component phytol, both beneficial to tree growth and health. PBZ may also induce morphological modifications of leaves, such as smaller stomatal pores, thicker leaves, and increased number and size of surface appendages, and increased root density that may provide improved environmental stress tolerance and disease resistance . PBZ also has some fungicidal activity due to its capacity as a triazole to inhibit sterol biosynthesis.

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. Structurally, PBZ is a substituted triazole with two asymmetric carbon atoms and is produced as a mixture of 2R, 3R, and 2R, 3R, and 2S, 3S enantiomers .

Triazole compounds are systemic fungicides having plant growth regulating properties and are called as stress protectants, because of their innate ability to induce abiotic stress tolerance by increasing antioxidant enzymes and molecules in stress-affected plants. The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including Gibberellic Acid (GA), Abscisic Acid (ABA) and Cytokinins. They induce a variety of morphological and biochemical responses in plants; inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses. PBZ (Bonzi), a triazole family having growth regulating property, is an extremely active chemical and affects almost all plant species, whether applied as a spray or a soil drench. PBZ inhibits GA biosynthesis by blocking the oxidation of ent-kaurene.

It is applied to plants in the floricultural industry to control their size and quality. It is applied to perennials and other pot crops at rates of 1–90 mg $L-1$. When applied as a foliar spray, PBZ is absorbed by petioles and stems and is translocated through the xylem to the growing tip. When applied as a soil drench, it is taken up through the roots and then translocated through the xylem to the apical meristems. Soil drenches with PBZ may be more effective than the foliar sprays due to increase activity and less probability of stunting and flowering delay, due to no direct contact with flowers or flower buds. Depending on plant species, PBZ can delay or promote flowering. PBZ half-life in the soil varies between 6 and 12 months depending upon the soil type and environmental conditions. Effectiveness of drenches is reduced if the crop is grown in a bark medium, because the chemical will adsorb to the bark and less will be available in the medium solution for the plant to absorb. Phytotoxicity symptoms are not common when applied to perennials, but care must be taken with those species that are known to be sensitive. Therefore, the aim of this review is to summarize the evidence on the biochemical and physiological responses of PBZ as a growth regulator and as a stress protectant.

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. There are indications that enantiomers having S configuration at the chiral carbon bearing the hydroxyl group are inhibitors of GA biosynthesis. One of the inhibitor of GA biosynthesis, paclobutrazol, is mainly used as growth retardant and stress protectant. This retardation of growth is due to the interference of PBZ with gibberellin biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kauronoic acid through inactivating cytochrome P450-dependent oxygenase. In addition, it tends to be much more effective than various other plant growth regulators at relatively low rate of applications.

PBZ is also known to affect the synthesis of the hormone abscisic acid and phytol. Abscisic acid is also synthesized via the terpenoid pathway (Figure 2). When gibberellins synthesis is blocked, more precursors in the terpenoid pathway are accumulated and shunted to promote the genesis of abscisic acid. It has also been reported to inhibit normal catabolism of ABA. The effect of PBZ on both the synthesis and catabolism processes leads to enhanced concentrations of ABA in leaves. One of the major roles of ABA is to cause closing of stomatal aperture and decreasing loss of water from leaves through transpiration. Improvement of water relations in treated plants takes place because of enhancement in ABA content that decreases stomatal aperture, decreases shoot growth and causing less surface area for transpiration, more roots for uptake of water, and anatomical alterations in leaves that impart barriers to water loss. Terpenoid pathway for biosynthesis of gibberellins, abscisic acid, phytol, and steroids, and path for degradation of abscisic acid. Steps blocked by paclobutrazol indicated with Geranyl Diphosphate Synthase (GPS), Farnesyl Diphosphate Synthase (FPS), Geranyl Geranyl Diphosphate Synthase (GGPS), Ent-Copalyl-Diphosphate Synthase (CPS), ent-Kaurene Synthase (KS), ent-Kaurene Oxidase (KO), ent-Kaurenoic Acid Oxidase (KAO), Geranyl Geranyl Reductase (GGRS), Chlorophyll synthase (CHL) and Phytoene synthase (PSY) are the enzymes involved in the terpenoid pathway. ABA 8′-hydroxylase (ABA 8′OH) involved in the enzymatic degradation of ABA into Phaseic

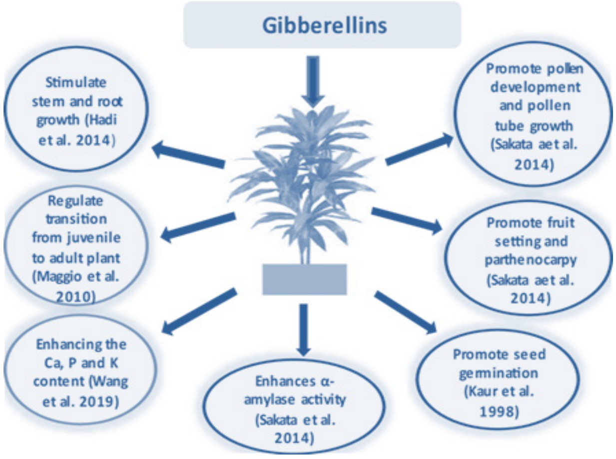

Figure 2. Diagrammatic representation of various roles governed by gibberellins in plants.

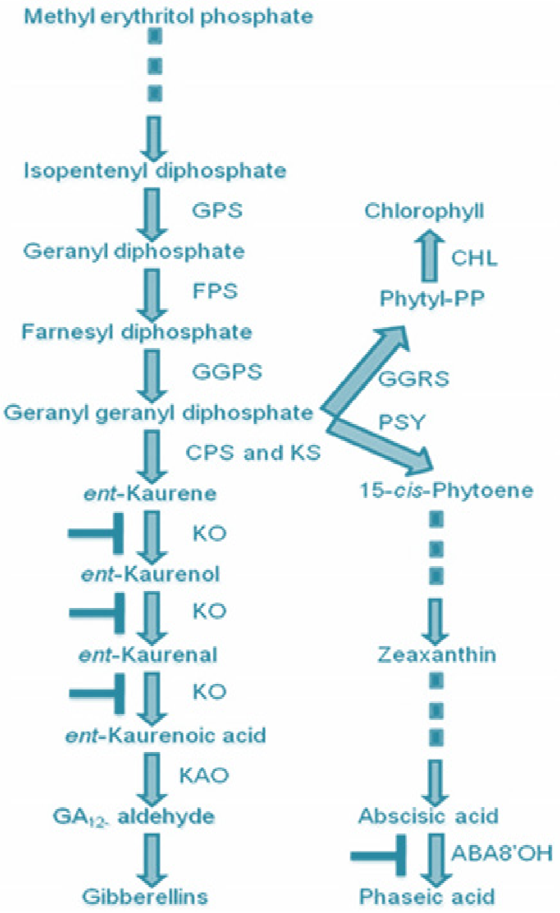

Figure 3. Simplified pathway of terpenes involved in gibberellin biosynthesis, and inhibition sites for abscisic acid and chlorophyll formation.

acid. KO, KAO and ABA 8′OH are the enzymes inhibited upon PBZ application (Figure 3).

It was previously believed that triazoles were primarily transported acropetally in the xylem. However, PBZ has been detected in xylem and phloem sap of castor bean and pear indicating that triazoles can be transported acropetally and basipetally. Although the metabolic fate of applied has not been investigated in detail most of them have a high chemical stability and depending on the site of application tend to be metabolized slowly. Early and Martin observed more rapid PBZ metabolism in apple leaves than other plant parts, while Sterrett found little evidence for PBZ metabolism in apple seedlings. PBZ is comparatively more resistant to degradation than BAS 111.

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PBZ is considered a phloem immobile chemical, though some direct and indirect evidence exists that it is partially mobile in phloem. Studies indicate that PBZ and uniconazole-P move in plants acropetally via the xylem, accumulate in leaves, and have very low mobility in phloem. This results in a low level of PBZ residues in seeds and fruits as they are supplied with nutrients via the phloem. However, low phloem mobility of PBZ further reduces the effectiveness of foliar spraying, since PBZ action on plant growth would be restricted to the site of application.

A lot has been done to identify the best application rate of PBZ in different places. Factors like age of the trees, extent of vegetative growth and method of application should be considered when determining the rate of PBZ to be applied. The rates also affect the different tree parameters variously. In general, the amount of PBZ required to promote flowering and fruiting in fruit crops is very low. The rate of soil application is a function of tree size and cultivar. The rate is determined by multiplying the diameter of tree canopy in meters by 1–1.5 g of active ingredients of PBZ. They indicated that other factors including soil type, irrigation system, etc. may affect PBZ activity and, thus, may be necessary to improve the effectiveness of the chemical. As to them, overdose may cause undesirable effects such as restricted growth, panicle malformation (too compact), and shoot deformity. They also asserted that to insure uniform flowering and reduce the detrimental side effects, the search for better application methods were investigated and one approach is to apply high volume of low PBZ concentration to improve better coverage.

Optimizing PBZ dose is a prerequisite for any yield improvement programmes. Severe and undesirable loss in seed and oil yield of *Camelina* was observed when the plants were treated with higher PBZ concentration (125 mg L−1), while PBZ dose between 75 mg L−1 and 100 mg L−1 can effectively improve the economic traits, including higher seed and oil yields in *Camelina*. Severe retardation of *Camelina* growth was also reflected in plant height, branch and canopy size when the plants were sprayed with higher PBZ concentration (125 mg L−1). He also reported that *Camelina* seed yield increased by 74.23% when compared to the control with the applications of 100 g L−1. Similarly, reduced yields were recorded in peanut and Jatropha associated with higher PBZ concentrations. Kamran et al. described that soaking of seeds under 300 mg L−1 PBZ increased the average maize grain yield by 61.3% as compared to the control. Patil and Talathi also reported that application of 5 g of PBZ through soil enabled to induce early and regular fruiting with 2.8 times increase in yield in mango var. Alphonso. In addition, PBZ at a rate of 150 mg L−1 in bottle gourd, 100 mg L−1 in bitter gourd, 150 mg L−1 in French bean, 125 mg L−1 in cucumber and 40 mg L−1 in tomato increased the yield and quality of fruits.

RESPONSE OF PLANTS TO PBZ

Plant Hormone Biosynthesis

Gibberellin: Gibberellins (GAs) are a large family of tetracyclic diterpenoid plant growth regulators. Since its original discovery, > 130 GAs have been identified in plants, fungi and bacteria, although only a few GAs have biological activit; many non-bioactive GAs exist in plants, and these act as precursors for the bioactive forms or are de-activated metabolites. Gibberellins (GAs) are plant hormones that are essential for many developmental processes in plants, including seed germination, stem elongation, leaf expansion, trichome development, pollen maturation and the induction of flowering. The major bioactive GAs, which includes GA1, GA3, GA4 and GA7, are derived from a basic diterpenoid carboxylic acid skeleton, and commonly has a C3 hydroxyl group (Figure 4 & 5).

Triazole compounds are antagonistic to gibberellins and auxins, reducing cell elongation and cell division by inhibiting GA3 biosynthesis. They exhibit varying degrees of both plant growth and fungicidal activity. The intensity of their biological activity is dependent on their isomeric form. The growth retarding property of PBZ is largely attributed to interference with gibberellins biosynthesis. Gibberellins are synthesized from mevalonic acid via the isoprenoid pathway, and the PBZ specifically inhibits the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P-450-dependent oxygenases. Furthermore, PBZ-induced growth inhibition can be reversed by exogenous application of gibberellins. These observations support the hypothesis that growth inhibition due to PBZ is primarily due to reduced gibberellins biosynthesis.

Abscisic acid: The effect of PBZ on ABA is of interest because ABA, like the gibberellins, is synthesized via the isoprenoid pathway, and the two compounds often exhibit opposing physiological activities. The action of PBZ on ABA could be the source of stress protection that has been observed with PBZ. ABA is a natural plant growth regulator that has been implicated in plant acclimation and protection against environmental stress. Exogenous application of ABA has been shown to increase plant resistance to salinity, ozone, heat, chilling and freezing.

Mackay et al. demonstrated that PBZ induced stress resistance and it also increased the endogenous concentrations of ABA in snap beans. Hauser et al. also demonstrated that PBZ considerably increased endogenous ABA levels in detached leaves and hydroponically grown seedlings of oilseed rape. ABA accumulated in proportion to PBZ concentration. Mackay et al. also hypothesized that stress protection inferred by PBZ may in part be the result of their effect on endogenous concentrations of ABA. However, both experiments showed that increases in ABA were short lived

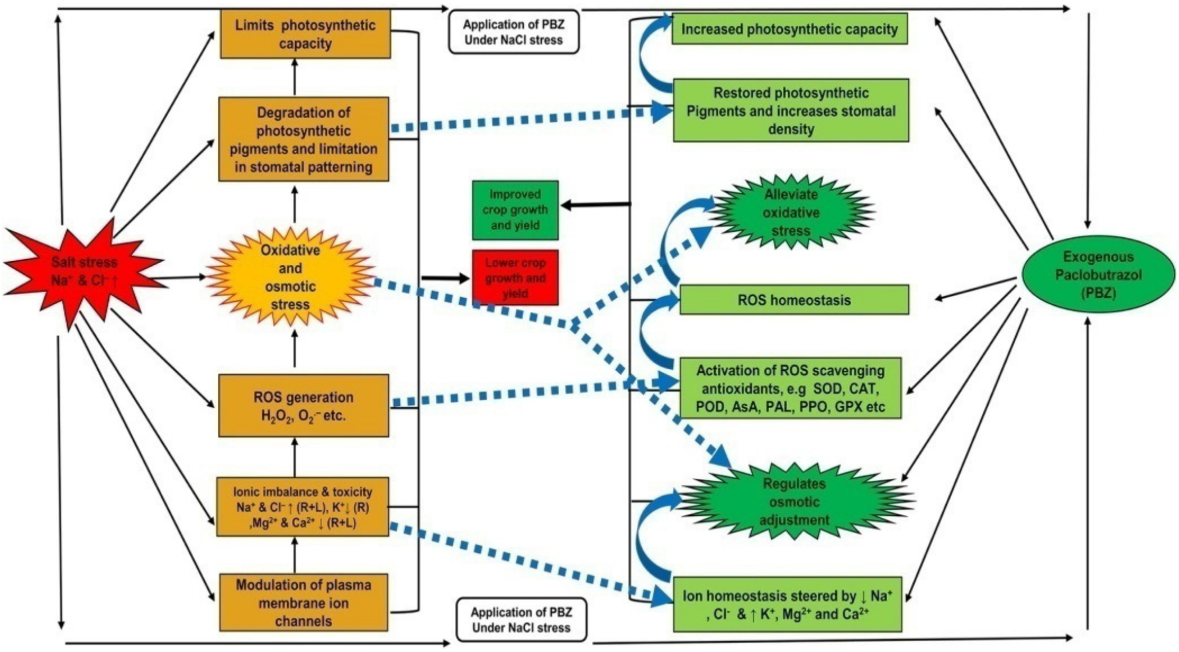

Figure 4: Soil drenching of paclobutrazol (PBZ; 20 mg l^{-1}) was used to understand the ionic relations, gaseous exchange characteristics, oxidative defense system and yield under saline conditions (400 mM NaCl) including normal (0 mM NaCl) and no PBZ (0 mg l^{-1}) as controls.

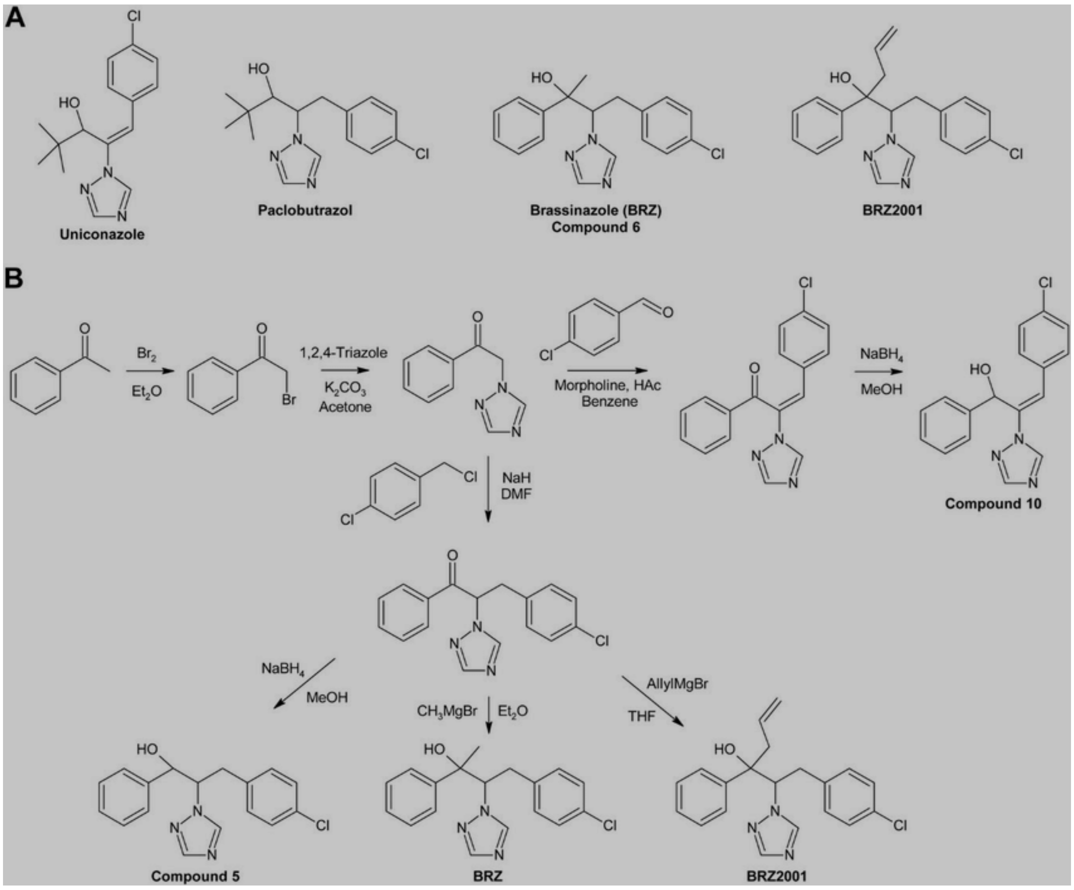

Figure 5: BRZ and BRZ2001. (A) Structures of uniconazole, paclobutrazol, brassinazole (BRZ) and BRZ2001. (B) Synthesis of BRZ, BRZ2001 and compounds 5 and 10. Abbreviations: DMF, N, Ndimethylformamide; Et2O, diethylether; HAc, acetic acid; MeOH, methanol; THF, tetrahydrofurane.

and eventually decreased to normal or below control levels. Hauser et al. hypothesized that this may be due to stimulated ABA catabolism and/or by an inhibition of its biosynthesis Ghosh et al., (2010). Therefore, providing a continuous supply, over the growing season, of the PBZ could help to maintain higher levels of endogenous ABA and thereby prolong its stress-protecting effects Hartmann et al., (2011). In addition, Aly and Latif also reported that PBZ increased the endogenous level of ABA in wheat Häuser et al., (1990).

Cytokinin: Cytokinins are synthesized in the roots and translocated acropetally to the shoots where they regulate both plant development and senescence Fernandez et al., (2006). They are involved in the control of various plant developmental processes such as cell division, apical dominance, stomatal behavior, root formation, leaf senescence, and chloroplast development Chartzoulakis et al., (2002).

Zhu et al. observed an increase in the endogenous cytokinin (Zeatin) level in xylem sap of young apple trees in response to PBZ treatment Izhaki et al., (2001). PBZ treatment delayed the onset of senescence in grapevine and blueberry Fengling et al., (2000). It has been reported that cytokinin or chemicals like thidiazuron with cytokinin-like activity stimulate chlorophyll synthesis and retard senescence and thus, PBZ-induced physiological responses may be associated with increased cytokinin synthesis or prevention of its degradation Buchenauer et al., (1984).

Fletcher et al. also proposed that triazoles stimulate cytokinin synthesis and that enhance chloroplast differentiation, chlorophyll biosynthesis and prevent chlorophyll degradation. An increased level of cytokinins and polyamines over the senescence-promoting hormones ABA and ethylene was reported in plants treated with PBZ. PBZ delayed senescence and extended period of 'stay-green' in *Camelina* sativa by enhancing endogenous levels of cytokinins and that promoted chlorophyll formation and increased activity of certain antioxidant enzymes Kianmehr et al., (2012). A longer 'staygreen' character simultaneously increased the period of leaf photosynthesis in PBZ-applied plants by keeping the leaves photosynthetically efficient for a longer time which in turn enhanced the plant productivity of *Camelina* Abod & Jeng (1993); Basiouny, (1993).

Stress protection: Biochemical effects of the triazole include detoxification of active oxygen species, increased contents of antioxidants and chlorophyll (Chl). More recently, it was found that triazole compounds have been reported to protect plants from various environmental stresses, including chilling, drought, heat, waterlogging, air pollutants, and heavy metals Papafotiou & Chronopoulos (2004). The triazole-mediated stress protection is often explained in terms of hormonal changes such as an increase in cytokinins, a transient rise in ABA and a decrease in ethylene. Enhanced chilling tolerance in triazole-treated tomato was associated with increased antioxidant enzyme concentrations. In treated tomatoes, apart from the increase in the antioxidants a-tocopherol and ascorbate, free fatty acids were higher and there was a reduction in the loss of membrane phospholipids, as compared to the untreated controls. PBZ prevents the decline in total chlorophyll content in corn plants after exposure to chilling temperatures, PBZ-induced tolerance to low temperature stress has been associated with increased levels of endogenous ABA, which has been reported to trigger the genetic processes for hardening. In field studies, winter survival of peas and cereal crops and resistance to frost damage in corn were enhanced by PBZ. PBZ increases the survival rate of plants under drought conditions through a number of physiological responses Pressman & Shaked (1988).

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. PBZ also protects plants from high-temperature-induced injuries. Protection against high temperature stress is accompanied by the production of low molecular mass stress proteins and the increase in the activity of antioxidant enzymes. It has also been reported that several environmental factors such as drought, low and high temperature can cause an excess of toxic oxygen-free radicals. Some of the free radical scavenging enzymes are reported to increase in wheat and corn plants after PBZ treatments and their activities are conserved even after exposure to extreme temperature. The triazole compounds enhance the free radical scavenging capacity of treated plants including the levels of carotenoids, ascorbate, superoxide dismutase and ascorbate peroxidase. Berova et al. suggested that the protection caused by PBZ was due to a similar mechanism of enhanced free-radical scavenging systems.

Assimilate partitioning: Assimilate partitioning to the different sinks may be controlled by environmentally regulated, hormonal balances. PBZ treatment increased the root-to-shoot ratio, increased partitioning of assimilates to economically important plant parts such as bulbs, potato tubers, carrot root and rice grain yield. The mechanism of tubers to act as a dominant sink during assimilate partitioning might be associated with PBZ stimulated low GA level in the tuber tissue that increases tuber sink activity. Setia et al. also reported that the application of PBZ resulted in an overall increase in dry weight per plant and better partitioning of assimilates (percent ratio of siliqua dry weight to plant dry matter) in *Brassica juncea* and *Brassica carinata*. Similarly, Kumar et al. reported that PBZ treatment enhanced seed yield in *Camelina* sativa and this enhancement of yield was correlated with improvement in CO2 assimilation physiology, sink activity partitioning of assimilates and rooting.

In addition, Yeshitela et al. reported that the higher PBZ rates suppressed vegetative growth of mango and the assimilate that was to be expended for vegetative growth was diverted to intensifying flowering. This was proved by a higher total non-structural carbohydrate level of the shoots of the treated trees before flowering. Similarly, the reduction in vegetative growth of grape by altering relative sink strengths within the plant had an indirect consequence of allowing a greater partition of the assimilates to reproductive growth, to flower bud formation, fruit formation and fruit growth of treated plants.

Alters growth regulation: Paclobutrazol regulates growth of plants by mediating changes in the balance of important plant hormones including the gibberellins, ABA and cytokinins (Fletcher & Hofstra 1990). It has been shown to decrease plant growth by reducing plant height, internode elongation and leaf area effectively. Paclobutrazol has been reported to reduce plant growth, height, internodal distance with thicker and darker leaves in pepper and tomato (Rahman et al. 1989a, b). Reduced height is a consequence of paclobutrazol induced gibberellin inhibition exemplified by reduced internodal elongation (Fletcher et al. 2000). Paclobutrazol has also been reported to suppress elonga- tion growth in plants by interfering cell division and enlargement processes due to its antigibberellin activity (Grossman 1988). Plants treated with PBZ were found shorter; more branched and produce more seeds in rape (Zhou and Xi 1993) and chinese potato (Kumar et al. 2006) (Figure 6 & 7).

PBZ application was observed to improve the growth potential of *Catharanthus roseus* plants grown under saline conditions (Jaleel et al. 2007a). Internal carbon dioxide concentration and leaf thickness was found to be high in PBZ treated *Catharanthus roseus* (Jaleel et al. 2007c). In oilseed brassicas, PBZ was reported to reduce plant height, increased resistance to lodging, and enhanced branching and improved yield (Setia et al. 1996). The reduction of shoot growth in cucumber plants treated with paclobutrazol might be attributed to decrease of IAA and GA3, revealed by pre- vious study on triazoles (Wang and Chen 1997). PBZ was also reported to increase root growth in American elm seedlings (Watson 2001).

Improves plant water relation: PBZ applications have been observed to improve leaf water potential under water deficit stress in american elm seedlings (Watson 2001) and reduce the transpiration rate in tomato seedlings (Still and Pill 2004). PBZ applications have been reported to increase the water use efficiency (WUE) in Pseudotsuga menziesisi and Pinus cornata seedlings (Van den Driessche 1996). Paclobutrazol induces partial closure of stomata and increases inter cellular CO2 concentration and it may be the reason for enhanced WUE in PBZ applied plants. The increase in leaf moisture content was attributed to the ability of PBZ to partially close the stomata by increasing ABA content and consequently reducing transpiration and water loss from plant leaves (Sreethar 1991). The reduction in water consumption after PBZ treatment has been ascribed to decrease leaf area (Asamoah and Atkinson 1985; Parvin et al. 2015) and alteration in plant anatomical character- istics (Wample and Culver 1983). Furthermore, an anti- transpirant action of PBZ was reported in bean (Asare- Boamah et al. 1986) and peach (George and Nissen 1992) (Figure 8).

Relative Water Content (RWC) has been reported as an important indicator of water stress in leaves (Merah 2001). Stress exposed plants were observed to reduce RWC of their

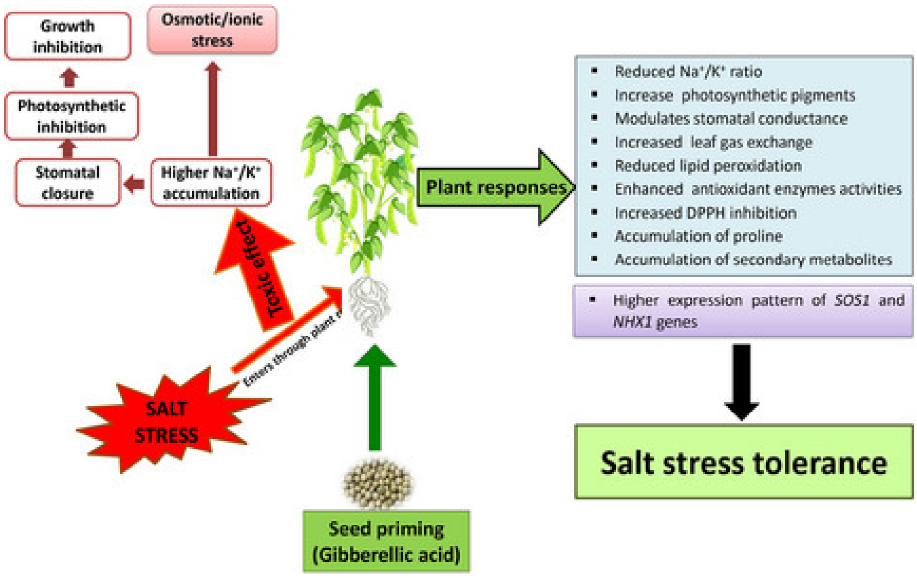

Figure 6: Seed priming with gibberellic acid induces high salinity tolerance in *Pisum sativum* through antioxidants, secondary metabolites and up-regulation of antiporter genes.

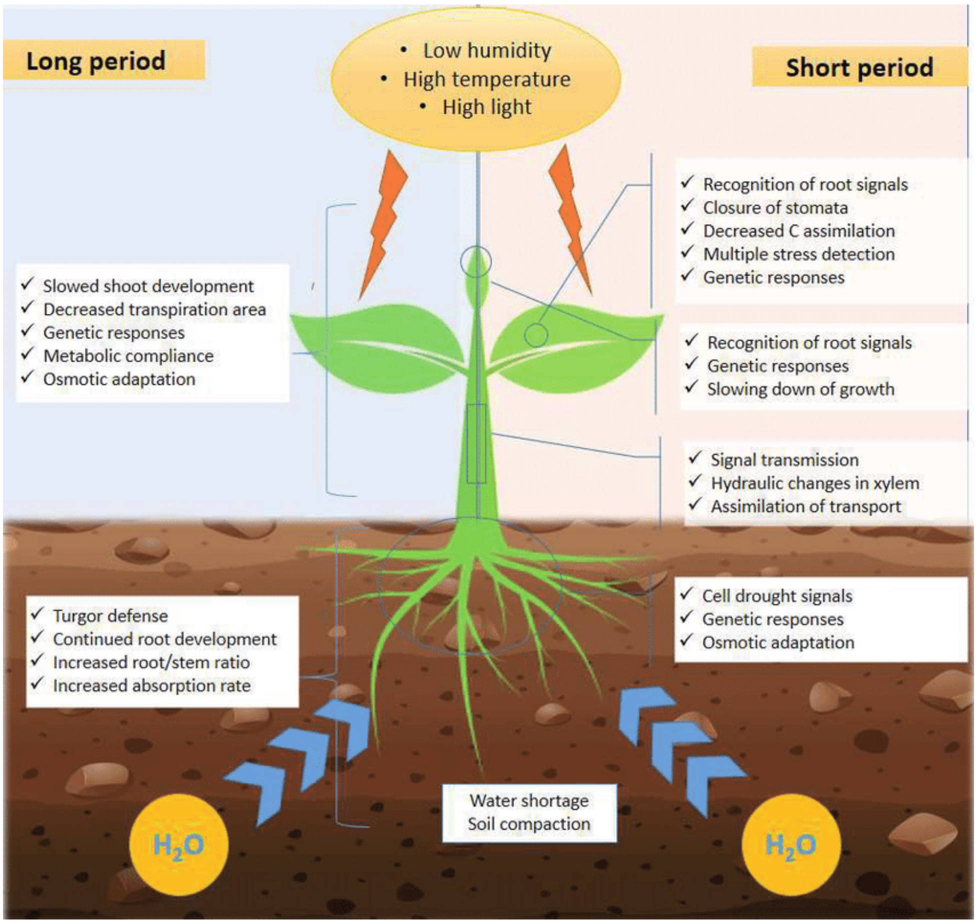

Figure 7: Long and short term responses of plants to drought stress.

leaves (Grover et al. 2004). Paclobutrazol has been found to accelerate stomatal response, enhanced water retention and increased survival under drought in jack pine (Marshall et al. 1991), oak (Percival and Albalushi 2007). PBZ reduced transpiration rates, increased relative water content and thus significantly improved yield in crop plants under water deficit stress (Sairam et al. 1995; Gadi et al. 2001; Aly and Latif 2011). Further, reduced transpiration rate increased the moisture content as reported in the *Amorphophallus campanulatus* plants (Manivannan et al. 2007).

Paclobutrazol application significantly reduced water consumption as estimated in *Arbutus unedo* (Navarro et al. 2007) and strawberry plants (Parvin et al. 2015). The lower water consumption in paclobutrazol treated plants was the result of combination of means which reduced transpiration loss (Navarro et al. 2007) and increased resistance (Asare-Boamah et al. 1986). Paclobutrazol treated plants were found to show higher value of relative water content in *Curcuma alis- matifolia* (Jungklang and Saengnil 2012), triticale (Berova and Zlatev 2003) and cucumber seedlings (Baninasab & Ghobadi 2011). Agarie et al. 1995 used the cell membrane stability as a abiotic stress (mainly water deficit) tolerance test. It was observed that electrolyte leakage showed an increase with increasing water deficit. Paclobutrazol has protective effect on cell membrane and inhibits membrane damage under saline stress conditions as reported in *Mangifera indica* (Srivastava et al. 2010). PBZ application was reported to decrease electrolyte leakage in carrot (Gopi et al. 2007). The inhibition of electrolyte leakage was correlated with the proved ability of PBZ in maintaining the membrane integrity (Fletcher et al. 2000) of plants. PBZ was reported to alter the sterol synthesis and modify the constitution of sterol in the plasma membrane (Burden et al. 1987). This change in sterol constitution might cause changes in plant cell membrane that might be reflected in enhanced membrane stability, adaptation and frost hardiness as observed in white spruce (Sailerova and Zwiazek 1997). PBZ treatment was observed to reduce electrolyte leakage in wheat seedlings (Aly and Latif 2011), horse chestnut (Percival and Noviss 2008) and strawberry plants (Parvin et al. 2015) during water deficit. Seedlings of winter rape and tomato treated with PBZ have much less electrolyte leakage than the control seedlings during high temperature stress (Zhou and Leul 1999; Still and Pill 2004).

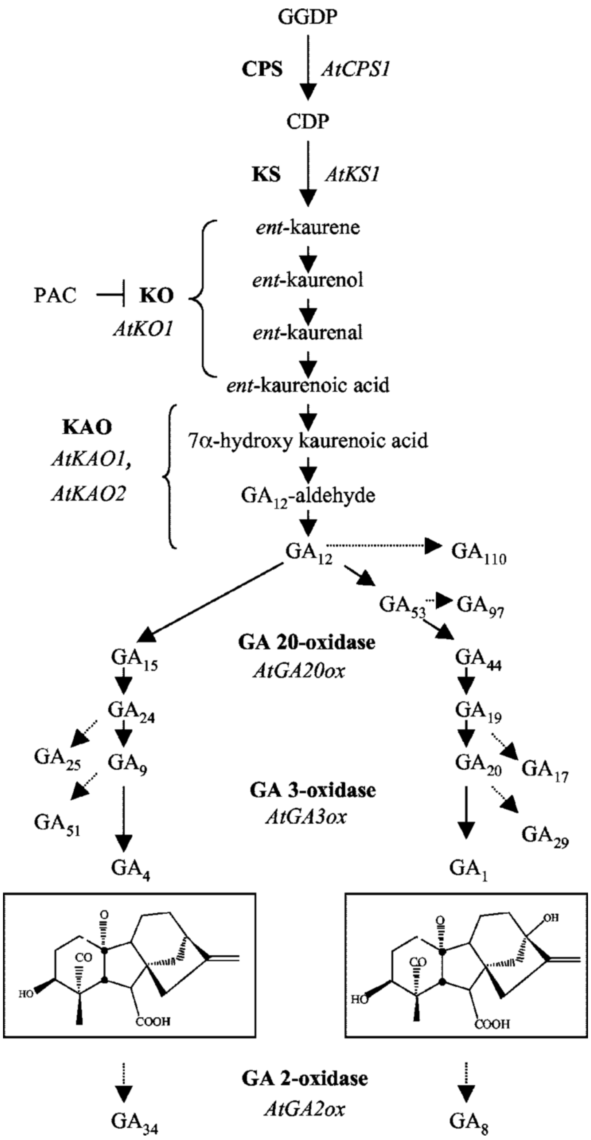

Figure 8: GA biosynthesis in Arabidopsis. Solid arrows, Metabolic steps in GA biosynthesis. Dashed-line arrows, Inactivation.

Alters anatomical characteristics of plant: PBZ has been reported to increase the thickness of epicuticular wax layer in rose cultivars (Jenks et al. 2001) and maize (Sopher et al. 1999). Cells number in palisade (mesophyll) tissue per unit area have been reported to enhance to a larger extent in PBZ treated *Catharanthus roseus* (Jaleel et al. 2007c) and chrysanthemum (Burrows et al. 1992). PBZ has been reported to increase the palisade cell length and width and exhibited dense and spongy mesophyll tissue in potato plants (Tekalign et al. 2005). Furthermore, PBZ was observed to increase diameter and length of fibrous roots, enhances lateral root formation, reduced the diameter of xyelm vessels, however, phloem sieve tubes had shown an increased diameter in PBZ treated *Catharanthus roseus* plants (Jaleel et al. 2007c). PBZ reduced the fraction of xylem and increased that of phloem and cortex and increased xylem density in maize seedlings (Sopher et al. 1999). Increased root length in PBZ treated plants was found associated with larger parenchyma cells and promotes cell expansion radially (Fletcher et al. 2000). Paclobutrazol stimulated radial elongation of cells and it increased siliqua diameter in brassica (Setia et al. 1996). It also increased radial elongation of peach root parenchyma cells (Williamson and Coston 1986) and leaf thickness in rape plant (Zhou and Xi 1993) and wheat leaves (Sopher et al. 1999) due to elongated palisade cells.

Protects ultra structure of chloroplast: The major damage to the chloroplast caused by abiotic stresses consists of structural changes resulting from excessive swelling, distribution of thylakoids and the appearance of lipid droplets (Ristic and Cass 1991). Changes in chloroplast ultrastructure induced by water stress share many common features with development of senescence manifested as

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appearance of large plastoglobuli, thylakoid swelling and dilation (Kolodziejek et al. 2003). Under imposed water deficit condition, destruction of thylakoid, disappearance of starch inclusions, changes in the lamellar structure, number of grana with loose type of thylakoid, large enhancement in osmiophillic granules and reduction of amount of starch in the bundle sheath chloroplast have also been reported earlier (Utrillas and Alegre 1997). However, PBZ applications maintain the integrity of the chloroplast structure and its grana with compact thylakoid under water deficit stress in chickpea (Soumya 2014). PBZ applications maintain chloroplast structure integrity under water deficit by enhancing the activity of antioxidant enzymes and restricting lipid peroxidation (Soumya 2014) (Figure 9).

Enhances plant photosynthetic pigments: Exposure to various abiotic stresses including water stress reduces the level of total chlorophylls due to disintegration of thylakoid membranes (Synkova and Rulcova 2000) and destruction of chloroplasts is caused by different reactive oxygen species (Smirnoff 1995). PBZ application has been reported to increase the level of photosynthetic pigments in oak (Percival and Albalushi 2007), *Setaria italica* (Bisht et al. 2007), *Amorphophallus campanulatus* (Gopi and Jaleel 2009), cucumber seedling (Baninasab & Ghobadi 2011) and *Stevia rebaudiana* (Hajihashemi and Ehsanpour 2013). PBZ application has also been found to improve chlorophyll fluorescence, total foliar chlorophyll, carotenoids concentrations in horse chestnut (Percival and Noviss 2008). The increase in chlorophyll content with PBZ treatment is attributed to the ability of PBZ to increase the cytokinin content and thereby the enhancement of chlorophyll biosynthesis (Fletcher et al. 2000). Paclobutrazol application was observed to enhance chlorophyll, carotenoid and soluble protein in *Triticum aestivum* (Nouriyani et al. 2012) and photosynthetic pigment per unit leaf area in *Zea mays* (Khalil and Rahman 1995). Its treatment also increased the content of photosynthetic pigment under water stress in *Festuca arundinacea* and *Lolium perenne* (Shahrokhi et al. 2011). Paclobutrazol significantly enhanced chlorophyll a, b and carotenoids in wheat cultivars (Aly and Latif 2011). Furthermore, paclobutrazol application was found to increase carotenoid content in *Catharanthus roseus* (Jaleel et al. 2006), *Raphanus sativus* (Sankari et al. 2006), *Sesamum indicum* (Abraham et al. 2008) and *Amorphophallus campanulatus* (Gopi and Jaleel 2009). Paclobutrazol application was observed to induce physiological adaptations for tolerating drought by increasing total leaf content of carotenoids (Lutein, betacarotein, alpha carotein, neoxanthin) and xanthophylls (antheraxanthin, zeaxanthin, violaxanthin) and chlorophylls in evergreen oak (Percival and Albalushi 2007). Plants treated with PBZ treatments showed more dense photosynthetic pigments particularly carotenoids belonging to xanthophyll cycle under normal condition and also maintained their profile under water deficit stress condition which in turn indicated that the PBZ applications stimulated the xanthophyll cycle and thus contributed in the protection of the photosynthetic machinery in chickpea (Soumya 2014). PBZ treatment increased chlorophyll and carotenoids which helped in resistance of black spruce to high light and thermal stress (Mahoney et al. 1998). Paclobutrazol has been reported to enhance the total chlorophyll content of the siliqua wall and seed in brassica (Setia et al. 1996). Paclobutrazol treated plants have more total chlorophyll, lesser Chl a/b ratio and higher hill's reaction activity of chloroplasts in wheat (Fedtke 1973, 1974) and brassica (Setia et al. 1996).

Abiotic stresses are known to inhibit photosynthetic reaction in plants due to disparity between light absorption and its usage (Foyer and Noctor 2001). Stomatal limitation is generally assumed to be the major reason for decrease in photosynthesis under abiotic stress conditions (Cornic 2000). This has been ascribed to decrease in both photosynthetic rate and internal CO2 concentration which ultimately inhibits photosynthetic metabolism. Paclobutrazol application has been observed to increase photo-

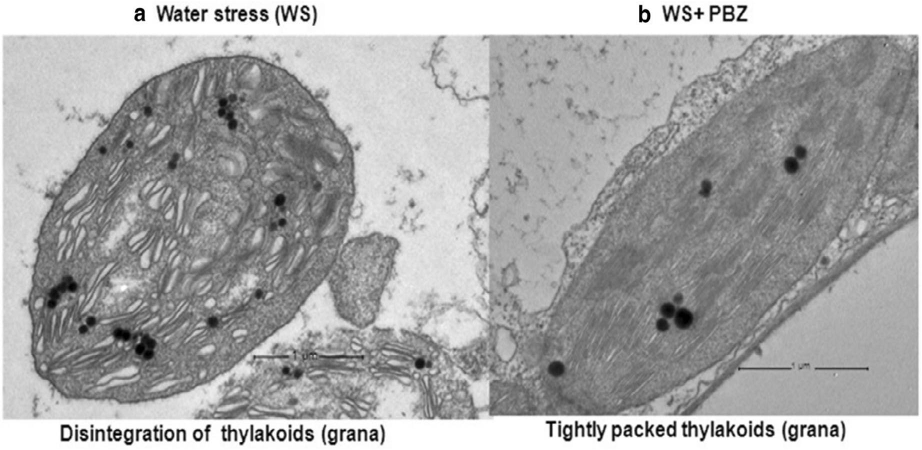

Figure 9: Effect of PBZ application on chloroplast ultrastructure (a Water stress; b water stress? PBZ).

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synthetic rate, prolonged leaf longevity and increased green pod area in mustard (Zhou and Xi 1993). Its treatment was also reported to enhance net photosynthetic rate and reduce stomatal conductance in Setaria italica grown under field condition (Bisht et al. 2007). In horse chestnut, chlorophyll fluorescence (Fv/Fm) and photo- synthetic rate have been reported higher in trees applied with paclobutrazol (Percival and Noviss 2008). PBZ treatment reduced the chilling induced photoinhibition by protecting PS II in as reported in water melon seedlings (Baninasab 2009). Plants treated with PBZ maintain rel- atively higher fluorescence ratio (Fv/Fm) under low temperature stress (Pinhero & Fletcher 1994). Paclobu- trazol also enhanced the activity of rubisco and rate of photosynthesis in peanut (Yan and Pan 1992). Foliar and drenching applications of PBZ in chickpea were found to maintain higher rates of photosynthesis, Fv/Fm ratio, water use efficiency under water deficit condition and faster recovery after water stress termination (Soumya 2014). Treatment of PBZ significantly increased the Fv/ Fm ratio in cucumber leaves (Baninasab & Ghobadi 2011). The increase in intercellular CO2 concentration and alteration in stomatal conductance was assessed the reasons for higher photosynthesis in PBZ treated Amor- phophallus campanulatus (Manivannan et al. 2007; Gopi & Jaleel 2009) and potted red fire spike (Rezazadeh et al. 2016).

Alters level of plant growth hormones: Plants respond to various abiotic stresses through several physiological and biochemical modifications (Emam & Seghatoeslami 2005) involving alterations in endogenous phytohormones levels such as abscisic acid (ABA), gib- berellin (GA3), indole acetic acid (IAA), zeatin (ZT), or cytokinin (CKs) (Wang et al. 1987). Triazoles have been reported to affect the isoprenoid pathway and alter the contents of certain plant hormones by blocking gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Fletcher et al. 2000). Paclobutrazol was found to restrict gibberellin biosynthesis in plant by inhibiting activity of ent-kaurene oxidase and cytochrome P450 oxidase, thus restricting the oxidation of ent-kaurene to ent-kaurenoic acid (Dalziel & Lawrence 1984). Its treatment has also been reported in apple and wheat to reduce endogenous abscisic acid level by about one-third and prevent the marked accumulation of water stress induced abscisic acid that occurred in untreated seedlings (Wang et al. 1987, Buta and Spaulding 1991). It has also been shown that paclobutrazol stimulate accumulation of ABA in the leaves (Asare-Boamah et al. 1986). Fletcher et al. 2000 proposed that triazoles stimulate cytokinin synthesis and that enhances chloroplast differentiation, chlorophyll biosynthesis and prevent chlorophyll degrada- tion. Further, paclobutrazol was reported to significantly enhance zeatin and zeatin riboside (Zhu et al. 2004). In wheat, paclobutrazol altered the endogenous level of phy- tohormones i.e. level of GA3, IAA was decreased and ABA was increased (Aly & Latif 2011). PBZ considerably delayed the onset of senescence in grape vines and treated plants retained photosynthetically active leaves for longer period (Hunter and Proctor 1994). An increased level of cytokinins and polyamines over the senescence promoting hormones ABA and ethylene were reported in plants treated with PBZ. PBZ delayed senescence and extended period of 'stay-green' in *Camelina* sativa (Kumar et al. 2012) by enhancing endogenous levels of cytokinins and that promoted chlorophyll formation and increased activity of certain antioxidant enzymes. A longer 'staygreen' character simultaneously increased the period of leaf pho- tosynthesis in PBZ applied plants by keeping the leaves photosynthetically efficient for a longer time which in turn enhanced plant productivity of *Camelina* (Kumar et al. 2012).

INDUCES ANTIOXIDANT ACTIVITIES

Enzymatic Antioxidants

Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), Ascorbate Peroxidase (APX), peroxidase (POX). SOD activates the dismutation of superoxide anion radicals (O2-) with great efficiency resulting in the production of H2O2 and O2 (Smironoff 1993) which reduces the accumulation of destructive radicals under abiotic stresses. Catalase enzyme is an important antioxidant system that catalyzes hydrogen peroxide, which is a precursor of reactive oxidants (Larson et al. 1988) and acts along with H2O2 directly to form water and oxygen (Smironoff 1993;). POX is one of the major systems for the enzymatic removal of H2O2 in plants. PBZ application has been reported to significantly enhance antioxidant enzymes (SOD, CAT, POX, APX) activity in various plant species e.g. oak (Percival and Albalushi 2007), *Arachis hypogaea* (Sankar et al. 2007), *Daucus carota* (Gopi et al. 2007), *Dioscorea rotundata* (Jaleel et al. 2007b), *Sesamum indicum* (Somasundaram et al. 2009), *horse chestnut* (Percival and Noviss 2008), *Festuca arundinacea and Lolium* perenne (shahrokhi et al. 2011), tomato (Mohamed et al. 2011), rice (Pan et al. 2013) and wheat (Abbadi et al. 2015). Similarly other triazoles like hexaconazole and triadimefon application were noted to enhance antioxidant enzymes activity in Manihot esculenta (Gomathinayagam et al. 2009), and wheat (Sairam et al. 1991, 1995; Sairam 1994; Aly and Latif 2011). Enzymatic antioxidant activity induced by PBZ was reported to protect wheat leaves from damage caused by free radical generated in wheat (Gilley & Fletcher 1997). PBZ minimized the negative effects of salinity with evidence of less membrane damage by up-regulating the endogenous production of antioxidant enzymes like SOD, CAT, and POX (Srivastava et al. 2010) in *Vigna unguiculata* (Manivannan et al. 2007). Lowering of lipid peroxidation in paclobutrazol treated *Curcuma alismatifolia* plants was due to increase in antioxidant potential system which reduced the membrane damage (Jungklang & Saengnil 2012).

Non enzymatic antioxidants: The non-enzymatic antioxidants play major role in main- taining the equilibrium between free-radicals production and eradication (Jaleel et al. 2007c). A~-tocopherol, ascorbic acid and reduced glutathione (GSH) are major non-enzy- matic antioxidants in crop plants (Foyer 1993). These non- enzymatic antioxidants are concentrated in the chloroplasts and the cytosol and guard the photosynthetic machinery under abiotic stress conditions by scavenging excess reactive oxygen species (ROS) (Smirnoff 1995). Ascorbic acid has the ability to scavenge various ROS including singlet oxygen, superoxide and hydroxyl radicals (Foyer 1993). Reduced glutathione (GSH) plays an important role in tolerance response of plants to various abiotic stresses due to the production of active oxygen species (Szalai et al. 2009). Improved a~-tocopherol and ascorbic acid content was observed in Vigna unguiculata plants treated with PBZ under water deficit condition (Manivannan et al. 2007). PBZ application increased ascorbic acid and reduced glu- tathione and a~-tocopherol contents in lemon (Jain et al. 2002) and *Dioscorea rotundata* (Jaleel et al. 2007b). Both PBZ and hexaconazole treatment have been reported to increase the GSH content to an appreciable level in carrot (Gopi et al. 2007) and C. *roseus* (Jaleel et al. 2006). An enhancement in ascorbic acid and reduction in glutathione level due to PBZ application is the core of the abiotic stress protection capacity of PBZ (Fletcher et al. 2000).

Proline plays a pivotal role in cytoplasmic tolerance under several abiotic stresses faced by plants (Bandurska 2000). Proline is one of the most important compatible osmolytes in water deficit stressed plants. Proline may act as a non-toxic osmolyte in the cytoplasm and sustains the composition of macromolecules and organelles. Its accumulation supports to maintain turgor and stimulates continued growth under water stress. Proline content of carrot leaves and tubers was found to increase in PBZ treated plants (Gopi et al. 2007). PBZ induced a transient rise in ABA content (Davis et al. 1988) and that enhanced proline and amino acid content in carrot plants (Gopi et al. 2007). The increased proline in *Curcuma alismatifolia* (Jungklang and Saengnil 2012), *Stevia rebaudiana* (Hajihashemi and Ehsanpour 2013) and strawberry plants (Parvin et al. 2015) is one of the mechanisms for water deficit stress amelio- ration. Higher amounts of proline in the treated plants imply that paclobutrazol induced water deficit stress tol- erance in plants (Jungklang and Saengnil 2012; Turkan et al. 2005; Aly and Latif 2011). Enhancement in proline due to PBZ treatment resulted reduction in membrane damage of *Mangifera indica* leaves (Srivastava et al. 2010), *Festuca arundinacea* and *Lolium perenne* (Shahro- khi et al. 2011) and horse chestnut (Percival and Noviss 2008) thus improved tolerance.

Lipid peroxidation is a major indicator of oxidative damage under several abiotic stress conditions. Significant enhancement in the concentration of lipid peroxide was reported in water deficit stressed rice seedlings (Sharma and Dubey 2005). Water deficit stress enhanced the malonaldehyde content, an end product of lipid peroxidation, in green bean (Yaser et al. 2008) and wheat (Aly and Latif 2011; Abbadi et al. 2015). Reduction in lipid peroxidation in PBZ treated plants was interlinked with the capacity of PBZ in maintaining the membrane integrity by enhancing the potential antioxidant enzymes activity and hence improved tolerance (Fletcher et al. 2000). To summarize whole review on plant response to PBZ application under abiotic stress. This model depicts that PBZ applica- tion alters methyl erythritol phosphate (MEP) pathway in chloroplasts by inhibiting the activity of ent-kaurene oxi- dase that causes restriction of oxidation of ent-kaurene to ent-kaurenoic acid results repression of gibberellin syn- thesis and accumulation of substrates (Farnesyl Diphos- Phate (FPP) and Geranyl Geranyl Diphosphate (GGPP) which reverts and enhances the synthesis of ABA and chlorophyll. PBZ induced level of ABA reduces stomatal conductance and transpiration and consequently improves the relative water content and water use efficiency. PBZ also enhances activity of enzymatic and non-enzymatic antioxidants and restricts lipid peroxidation and thus improves membrane stability and chloroplast integrity. These alterations improve the performance of photosyn- thetic machinery and ultimately enhances grain yield under stress conditions.

Mineral uptake: By influencing shoot and root morphology, PBZ alters mineral uptake. Rieger working in hydroponics on ‘Nemaguard’ peach rootstocks found that PBZ treatment induced decreases in N, P, K, Fe and Mo, whereas levels of Ca, Mg, B and Mn were increased by PBZ. This author stated that the magnitude of changes in foliar nutrition was proportional to the degree of growth suppression. In the case of Fuji apple trees, Huang et al. found that the differences in the total dry matter accumulated per kg of leaves were negligible. On the other hand, Wang et al. observed that the PBZ treatments increase the content of N, P, K, Ca, Mg, B, and Zn in leaves of pear tree. Rieger and Scalabrelli demonstrated in peach tree that the foliar concentrations of N, P, K, and Fe decrease slightly, while increase those of Ca, Mg, B and Mn. Recently, Yeshitela et al. also reported that PBZ increased mango leaf Mg, Cu, Zn, and Fe content without affecting the concentration of N, P, K, and Ca. In addition, this author indicated that the higher concentration of PBZ (8.25 g a.i./tree) resulted in a decreased Cu concentration, while the increase in PBZ concentration (2.75-8.25 g a.i./tree) did not show an increment of the concentration of Zn.

Effects of PBZ on the Environment

Recent studies have demonstrated that the residual nature of PBZ affects soil microbial life (reduction in the number of fungi, bacteria, actinomycetes and earthworms), which impairs plant growth and development. In addition, leaching and contamination of aquifers can occur. On the other hand, the behaviour of PBZ in soil has a residual effect of 450 to 950 days and can be maintained at a depth of 60 to 120 cm. Other authors reported that PBZ has an easy uptake by the crop roots, and can have a persistence in soil from 43 to 618 days, with concentrations ranging from 1.1 to 50 mg kg–1. Another important effect is the inhibition of root growth and development of plantations in soils with previous PBZ applications. On the other hand, in mango, Lin et al. report a reduction in the density of beneficial soil microorganisms, in particular the number of bacteria, fungi and actinomycetes by 58%, 28% and 28%, respectively, when evaluating PBZ applications. The life of PBZ in water is shorter (i.e., no longer than 3 weeks), but it can cause potential damage to the flora and fauna. The presence of PBZ in water can cause various physiological effects in fish (survival rate, embryonic hatching, failure in the development of the skeleton of the head and eyes). Such is the case of Daphnia magna (water flea) which showed physical deformities when exposed to 240 µg L–1 of PBZ in the embryonic stage. When evaluating the survival and deleterious effect of PBZ (0.34, 3,4, 17 µm) applied to zebrafish (Danio rerio) embryos at 24, 36, 48, 60, 72 and 96 hours after fertilisation, Wang et al. report the development of pericardial oedema, cardiovascular dysfunction and skeletal malformations. Osuna-García et al. found traces of PBZ in fruit samples when applying doses of 5 and 2.5 mL of PBZ per tree to "Tommy Atkins" mango; however, the authors indicate that these concentrations are not harmful to human health. In some countries it is prohibited and/or restricted the use of PBZ due to the residual effects on fruits that can be harmful to human health. PBZ residues can affect human health and health through direct contact, inhalation, contamination of water bodies, consumption of contaminated fish and fruits containing residues. Adverse health effects can occur after years of minimal exposure in the environment, food and water. There are few studies on the harmful effects caused to human health by ingestion or contact with PBZ. Experiments have been carried out on pregnant rats were fed with doses of 1.0 mg kg–1 PBZ and the results have been extrapolated to humans: no reproductive effects were observed, but malformations (cleft palate) were determined. These malformations were attributed to a toxic effect of PBZ. Further studies were recommended to determine whether PBZ is the cause of this effect.

Future Perspectives

Paclobutrazol is a growth inhibitor and also belongs to the triazol group. The use of this product on fruit trees (mango, lime, apple and guava) inhibits the biosynthesis of gibberellins; cell division occurs, cell elongation and expansion do not occur. This allows a greater production of shoots, number of leaves and internodes, but they will be shorter. PBZ induces flowering with a consequent increase in fruit yield, weight, size, and it improves the organoleptic properties of fruit. On the other hand, in some countries it is prohibited and/or restricted by the documentary evidence of its residual and harmful effects on the environment (soil and groundwater) and human health (LMR in fruits). However, fruit production is associated with an extensive use of PBZ in Latin America. There is little evidence of a legal framework that allows users to implement the optimal use of this product, to mitigate possible effects on the environment and human health. For this reason, the agronomic management of PBZ must have protocols that seek its regulation with a rational and sustainable approach.

CONCLUSION

Paclobutrazol is a growth inhibitor and also belong to triazol group. It inhibit the biosynthesis of GA3 at kaurene stage and it is most commonly used for the induction of flowering in off season, control tree vigour for HDP (canopy management), increase fruit set and yield, improve fruit quality when applied to the soil. Studies aiming to adjust the amount of application dose of paclobutrazol to each crops will allow the formulation of recommendations for more efficient applications, which can not only provide quality fruit production throughout the year but also reduce the risk of residues in orchard soil, tree, fruit and environment. Application of PBZ enhances the level of ABA and chlorophyll by suppressing GA biosynthesis. Augmented ABA level improves RWC (%) and WUE of crop plants by reducing stomatal conductance and transpiration rates. PBZ improves membrane stability and maintains integrity of photosynthetic machinery under various abiotic stress conditions by increasing the activity of antioxidants and restricting lipid peroxidation. It also improves the density of zeaxanthin in photosynthetic pigment profile which indicates that the PBZ application induces the operation of xanthophylls pigments cycle and thus contributes in the protection of the photosynthetic machinery. Consequently PBZ application enhances grain yield by enhancing photosynthetic gas exchange, higher chlorophyll content and photosynthetic activity for a longer period that facilitates higher photoassimilation. Therefore, for the development of sustainable agricul- tural practice under water stress, salinity, high temperature stress, low temperature stress and climate change condi- tions, PBZ application induced physiological activities may be utilized for enhancing the yield of various crops. However, this all depends on gaining the focus of agri- cultural scientists and confidence of the farmers towards this novel compound in future. In addition to this, more research is needed to unveil PBZ induced mechanism of multi-stress protection, particularly from the point of its interaction, interrelation and crosstalk with other phyto- hormones as well as stress with responsive genes.

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