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Chemistry of Paclobutrazol (PBZ) and its Function in Agriculture: A Review

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Abstract

The plant growth retardant paclobutrazol, (PP333) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol, inhibits specifically the three steps in the oxidation of the gibberellin-precursorent-kaurene toent-kaurenoic acid in a cell-free system from Cucurbita maxima endosperm. Paclobutrazol (PBZ) is a plant growth retardant and triazole fungicide. It is a known antagonist of the plant hormone gibberellin. 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. It acts by inhibiting gibberellin biosynthesis, reducing internodial growth to give stouter stems, increasing root growth, causing early fruit set and increasing seedset in plants.). 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. Paclobutrazol is an inhibitor of enzymes which use cytochrome P450 as a co-factor. Their active site contains a heme center which activates oxygen from the air to oxidise their substrates. The (2S,3S) isomer inhibits the enzyme ent-kaurene oxidase which is on the main biosynthetic pathway to gibberellins, which are important plant hormones.

Keywords: Triazoles; Strigolactone Inhibitors; Phytohormones; MOFs

Introduction

Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan-3-ol] belongs to the triazole family. Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan-3-ol] belongs to the triazole family. This compound regulates plant growth by influencing the isoprenoid pathway, inhibiting GA synthesis, decreasing ethylene production, and enhancing the content of both CKs and ABA. Coolbaugh et al. showed that ancymidol blocks with high specificity the oxidative steps leading from ent-kaurene to ent-kaurenoic acid in the pathway of GA' biosynthesis. The same oxidative steps are thought to be inhibited by the active triazol derivatives. Paclobutrazol has been reported to inhibit GA biosynthesis in plants by inhibiting kaurene oxidase, a Cyt P-450 oxidase, thus, blocking the oxidation of kaurene to kaurenoic acid. The objectives of this study were to determine 'Abbreviations: GA, gibberellin; El, electron impact; TMSi, trimethylsilyl ether; amu, atomic mass unit, the translocation and distribution pattern of paclobutrazol from root system of apple seedlings at various time intervals by GC and to confirm the presence of paclobutrazol in apple seedling tissues by GC-MS. Plant growth retardants are compounds which are used to reduce plant growth without changing developmental patterns or being phytotoxic [**1**]. 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 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 of Paclobutrazol

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. Paclobutrazol (PBZ) is a plant growth retardant and triazole fungicide [**2**]. It is a known antagonist of the plant hormone gibberellin. It acts by inhibiting gibberellin biosynthesis, reducing internodial growth to give stouter stems, increasing root growth, causing early fruitset and increasing seedset in plants such as tomato and pepper. PBZ has also been shown to reduce frost sensitivity in plants.

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The structure of Paclobutrazol (Table 1)

Chemistry of 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 $C_{15}H_{20}ClN3O$, 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 a higher inhibition efficiency in gibberellin biosynthesis, but 2R and 3R are more easily degraded.

Paclobutrazol (**PBZ**) is the ISO common name for an organic compound that is used as a plant growth retardant and triazole fungicide. It is a known antagonist of the plant hormone gibberellin, acting by inhibiting gibberellin biosynthesis, reducing internodal growth to give stouter stems, increasing root growth, causing early fruitset and increasing seedset in plants such as tomato and pepper. PBZ has also been shown to reduce frost sensitivity in plants. Moreover, paclobutrazol can be used as a chemical approach for reducing the risk of lodging in cereal crops. PBZ has been used by arborists to reduce shoot growth and shown to have additional positive effects on trees and shrubs. Among those are improved resistance to drought stress, darker green leaves, higher resistance against fungi and bacteria, and enhanced development of roots. Cambial growth, as well as shoot growth, has been shown to be reduced in some tree species.

Structure and synthesis

The first synthesis of paclobutrazol was disclosed in patents filed by an ICI group working at Jealott's Hill. 4-Chlorobenzaldehyde and pinacolone are combined in an aldol condensation to form a chalcone which is hydrogenated using Raney nickel as catalyst to give a substituted ketone. This material is brominated and the resulting compound treated with the sodium salt of 1, 2, 4-triazole in a nucleophilic substitution reaction. The final reduction reaction uses sodium borohydride, which in cold methanol gives almost exclusively the diastereomer pair having the absolute configuration (2*R*,3*R*) and its enantiomer (2*S*,3*S*), with only about 2% of the alternative (2*R*,3*S*) and (2*S*,3*R*) isomers. However, this pair of isomers can be produced when the reduction is carried out using butylmagnesium bromide.

In a 1984 study, ICI workers separated the individual enantiomers by chiral resolution and were able to demonstrate that only the (2*R*,3*R*) isomer displays substantial fungicidal activity, whereas the (2*S*,3*S*) isomer is responsible for the growth regulating properties [**3**]. However, the commercial product (developed under the code number PP333) was the racemic material, since separation of the isomers was unnecessary when both components had utility in agriculture.

Mechanism of action

Paclobutrazol is an inhibitor of enzymes which use cytochrome P450 as a co-factor. Their active site contains a heme center which activates oxygen from the air to oxidise their substrates. The (2*S*,3*S*) isomer inhibits the enzyme ent-kaurene oxidase which is on the main biosynthetic pathway to gibberellins, which are important plant hormones. A secondary effect arising from the inhibition of ent-kaurene oxidase is that its precursor, geranylgeranyl pyrophosphate accumulates in the plant and some of this is diverted into additional production of the phytol group of chlorophyll and the hormone abscisic acid. The latter is responsible for controlling transpiration of water through the leaves and hence PBZ treatment can lead to better tolerance of drought conditions. The (2*R*,3*R*) isomer is a better fit to the active site of the fungal cytochrome P450 14α-demethylase. This inhibits the conversion of lanosterol to ergosterol, a component of the fungal cell membrane, which is lethal for many species Many other azole derivatives including propiconazole and tebuconazole show this type of activity, so the main commercial opportunity for paclobutrazol was as a plant growth retardant and it was first marketed by ICI in 1985 under the trade names Bonzi, Clipper, Cultar and Parlay. 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.

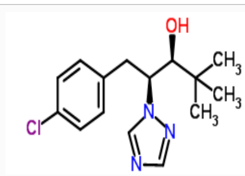
The mode of action of PBZ is framed as part of the terpene pathway [**4**]. This is, it inhibits the biosynthesis of gibberellins by inactivating the enzyme ent-kaurene oxidase, which catalyses their oxidation to ent-kaurenoic acid. This favours the activation of the enzymes geranylgeranyl reductase and phytoene synthase for chlorophyll and abscisic acid biosynthesis, respectively. As a result, it decreases vigour and promotes floral induction and development.

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 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.

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

Table 1: Structure of Paclobutrazol.

Common Name	PACLOBUTRAZOL			
CAS Number	76738-62-0	Molecular Weight	293.792	
Density	1.2±0.1 g/cm³	Boiling Point	460.9±55.0 °C at 760 mmHg	
Molecular Formula	C15H20ClN3O	Melting Point	165-166°C	

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 [**5**]. 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. 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 acid. KO, KAO and ABA 8′OH are the enzymes inhibited upon PBZ application.

Plant growth regulators are widely used in contemporary agriculture to promote plant growth, yield and grain quality. Both beneficial and adverse effects of plant growth regulators on growth and development as well as plant metabolism have been documented. The term growth retardants is used for all chemicals that retard cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects. Paclobutrazol is a member of the triazole family of plant growth regulators and has been found to protect several crops from various environmental stresses, including drought, chilling, heat and UV radiation.

Paclobutrazol (PBZ) is a triazole derivative that inhibits sterol and gibberellin biosynthesis. This compound can markedly affect plant growth and development by altering the photosynthetic rate and modifying the phytohormone levels. Paclobutrazol inhibits the activity of ent-kaurene oxidase, which is an enzyme in the GA biosynthetic pathway that catalyzes the oxidation of ent-kaurene to ent-kaurenoic acid. PBZ application has reduced plant height, improved stem diameter and leaf number, altered root architecture directly contributed to yield increase, and indirectly reduced the event of lodging. It was also reported that application of paclobutrazol effectively reduced vegetative growth of rice plants and increased chlorophyll content. Rice seedlings treated with paclobutrazol allocated less photosynthates for vegetative growth; allocated more photosynthates for seed development compared to control plants or those plants treated with gibberellin. In corn (*Zea mays* L.) under drought stress, application of 50 ppm paclobutrazol increased yield and average weight of 1,000 seeds [**5**]. Moreover, the possible hypotheses on drought tolerance regulation by PBZ have been proposed, which state that it maintains the endogenous cytokinin levels and stabilizes leaf water potential and causing increased leaf and epidermal thickness. Alternatively, regulation of free proline and glycine betaine as major osmoprotectants and promotion of enzymatic and non-enzymatic antioxidant activities, reduce the toxicity derived from drought stress.In a view of this, the objective of this article is to review the effect of paclobutrazol on morphological, biochemical, yield and stress responses of crop.

Paclobutrazol induced responses in plants

Morphological response

Paclobutrazol is used in high input crop management to shorten the stem, thereby reducing the risk of lodging. There are several reports describing the various effects of paclobutrazol on plant morphology of crops. For example, reported PBZ application significantly decreased plant height of *Camelina sativa* when compared to control and induced dwarfing effect and with highest concentration of PBZ in which maximum reduction (47.5% decrease) in plant height with respect to control was obtained. Similarly, paclobutrazol concentrations of 200 mg/L to 600 mg/L decreased gibberellin content in the leaves compared to that of control when applied to rice plant during preanthesis]. Paclobutrazol application reduced plant height and the greater concentration of paclobutrazol caused severe dwarfism as indicated in (Figure 1). Reduction in plant height is considered as the most imperative morphological outcome of paclobutrazol application. According to Tesfahun and Menzir 2018, plant height reduction strongly associated with reduced elongation of the internodes, rather than lowering the number of internodes and they found uppermost internodes to be shortened under paclobutrazol application [**6**]. reported that foliar application of paclobutrazol at 12.5 g a.i ha^{-1}, under a single-application scheme reduced plant height of sunflowers without adverse effects on achene and oil yields, thus providing a basis for reducing the risk of plant lodging.

Yield response

The positive effects of paclobutrazol on yield components such as greater fertile tillers, spike, fertile panicle or spikelet and in some cases

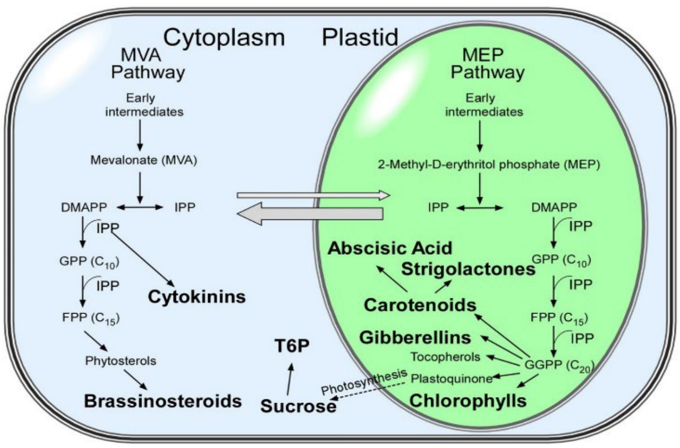


Figure 1: Isoprenoid biosynthetic pathways in plants. Metabolites discussed in this review are shown in bold. The mevalonate (MVA) and methylerythritol phosphate (MEP) pathways both generate isopentenyl diphosphate (IPP) in parallel and contribute to particular isoprenoids (Swiezewska and Danikiewicz, 2005). Thick grey arrows show the exchange of intermediates between the MVA and MEP pathways. Abbreviations: DMAPP: dimethylallyl diphosphate; FPP: farnesyl diphosphate; GPP: geranyl diphosphate; GGPP: geranylgeranyl diphosphate; IPP: isopentenyl diphosphate.

mean grain weight has been shown in studies evaluating the production potential of cereals; however, numerous studies have revealed that the increased fertile tiller, altered phenology and better canopy have been the main important components that significantly associated with enhanced grain yield in response to paclobutrazol application. One of the possible increments in grain yield is (i) the change in canopy coverage, in which the plant developed broader canopy this in turn facilitated improved light interception for better photosynthesis in leaves and stems of PBZ treated plants. Further, (ii) the leaves in PBZ treated plants were closely packed, dark green and remained on plants for a larger period than controls. This may explain increased dry matter accumulation in stem and root and simultaneous yield increments despite reduced plant height due to PBZ treatments. linked the grain yield increment (iii) with slow senescence in leaves which prolong the phase of seed development and maturation and as a consequence, the yield can be increased, but the harvest time delayed. The other possible grain yield increment is closely related to (iv) the spread of roots, which determines the uptake and utilization of water and nutrients. In similar way, reported that greater root biomass is significantly and positively correlated with ear characteristics and enhanced biomass and grain yields. The increased in the grain yield is attributed partly to (v) decreased investment in above ground parts, due to a relatively stouter canopy of paclobutrazol treated plants, (vi) as well as enhanced grain filling in the treated plants due to the improved rooting system, which possibly increased the nutrients and water uptake.

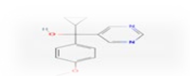
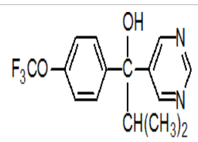
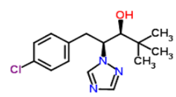
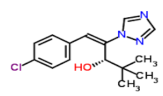
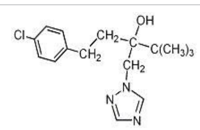
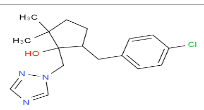
Physiological response

Chlorophyllis a critical component of the primary photosynthetic reaction has a dual function in photosynthesis. It captures light, and also serves as a medium for the light-driven charge separation and transport of electrons. The biosynthesis of chloroplast pigments was significantly affected by paclobutrazol as indicated in (Table 2). Several studies on tef and camilena showed that chlorophyll was higher on plants treated with paclobutrazol compared to control. The increased chlorophyll content treated with paclobutrazol might be from minimized damage caused by reactive oxygen and changes in the levels of carotenoids, ascorbate and the ascorbate peroxidase. The report of Nivedithadevi, Somasundaram and Pannerselvam 2015 also showed that plants treated with paclobutrazol synthesized more cytokinin, which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis, and prevented chlorophyll degradation [7]. Furthermore, paclobutrazol appears to have delayed the onset of senescence, represented by the rate of chlorophyll degradation in attached mung bean leaves, which was probably due to the enhanced endogenous level of cytokinins through their secondary effect on plants. Paclobutrazol application in *Camelina sativa* L. Crantz also increased chlorophyll content which led to greater rate in photosynthesis and higher yield. The results of showed that black rice plants treated with either 25 or 50 ppm paclobutrazol have greener leaves compared to control and the leaves also experienced late senescence. This could be due to an increase in the activity of oxidative enzymes that prevented cell maturation.

Stress response

Since early migration from aquatic to terrestrial environments, plants have had to cope with periodic and unpredictable environmental stresses, such as drought and salinity. Crop production in arid or semi-arid regions is usually restricted by soil moisture deficit as well as soil salinity. Water deficit coupled with salinity in irrigation water is the

Table 2: Growth retardents.

S.No.	Chemical Structure	Structural Formula
1.	**Ancymidol** = (*RS*)-α-cyclopropyl-4-methoxy-α-(pyrimidin-5-yl)benzyl alcohol	
2.	**Flurprimidol** = (*RS*)-2-methyl-1-pyrimidin-5-yl-1-(4-trifluoromethoxyphenyl) propan-1-ol,	
3.	**Paclobutrazol** = (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pentan-3-ol	
4.	**Uniconazole** = (*E*)-(*S*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl) pent-1-en-3-ol,	
5.	**Tebuconazole** = (*RS*)-1-p-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol	
6.	**Metconazole** = (1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol.	

major limiting factor in most regions where cereals are subjected to extreme water deficit during dry seasons. Enhanced stress tolerance in cereals can be achieved by exogenous application of some plant growth regulators, including paclobutrazol. Exogenous application of paclobutrazol can reduce some of the harmful effects of drought and salt stress and in some cases, compensate losses or damages caused by these stresses. Paclobutrazol increased stress tolerance of plants through the following methods.

Increasing root activity

Paclobutrazol are often referred as multi-stress protectants due to their innate potential of mitigating the negative effects of abiotic stresses had on plant growth and development, by regulating hormones level, enzymatic and non-enzymatic antioxidants and osmolytes. The 2-year results showed that root activity and root-bleeding sap flow were significantly higher in paclobutrazol treatments than compared to control. As root-bleeding sap is the indicator of root pressure, therefore, the improved root-bleeding sap is attributed to higher root growth and root vigor in response to the paclobutrazol application. Also the study of Morita, Okamoto, Abe and Yamagishi 2008 showed the presence of a close relationship between the bleeding rate and the root traits in maize. The rate of root bleeding sap is correlated to active water absorption of the root system and reflects the physiological root activity. Yan et al. 2013 also observed that uniconazole, a triazole with a function similar to paclobutrazol promoted root activity, root bleeding sap and improved root growth in soybean. Previously, Zhao, Fang and Gao 2006 also observed a higher root activity in rice and wheat treated with plant growth regulators. Thus the application of paclobutrazol may improve plant performance under stressful condition through stimulating root activity of the plant [**8**].

Submergence tolerance

Also paclobutrazol has a role on submergence stress. The long-time submergence is also detrimental to rice crop, and where this cannot be avoided some corrective measures are to be taken to exploit yield potential of rice crop. Under submerged conditions, 200 ppm paclobutrazol spray to rice seedlings resulted in 50% increase in percent survival over control. The increased seedling survival is presumably due to low energy use in elongation, while, the same was available for maintenance processes, for synthesis of anaerobic proteins and maintenance of membrane integrity essential for submergence tolerance.

Increasing antioxidant enzyme

Increased the levels of antioxidant enzyme activities in plants under stress conditions are natural responses, which can help plants better tolerate the stress. Exogenous application of paclobutrazol enlarged these traits and enhanced stress tolerance in plants. Additionally, the enhanced antioxidant enzyme activities in response to paclobutrazol application may also protect their photosynthetic machineries against damages caused by Reactive oxygen species during water-deficit conditions.

Among these SOD and CAT are well-known antioxidative enzymes in cells, which can catalyze the poorly reactive oxygen species converting them to non-toxic substances. SOD constitutes the first line of defence against active oxygen species (AOS). This enzyme removes O_2^- by catalyzing its dismutation, wherein one O_2^-Mis reduced to hydrogenperoxide (H_2O_2) and another is oxidized to oxygen. CAT is an enzyme that can convert H_2O_2 directly into water and oxygen. This enzyme is present in every cell and in particular on peroxisome. SOD and CAT plays a significant role in defending against oxidative stress induced by abiotic stress in plant tissues. Similarly, Rady and Gaballah 2012 also found that the application of paclobutrazol on barley crop had a significant role in increasing CAT and SOD concentration. This compound reduced damage in plants grown under water stress conditions by enhancing the activity of these antioxidative enzymes. A number of studies showed that paclobutrazol minimizes the adverse effects of water-deficit stress by increasing the levels of the activities of antioxidative enzymes in many plants such as groundnuts, sesame seeds, mangos and tomatoes.

Proline content

Proline is well-known as an osmotic regulator that can reduce osmotic damage. It was reported that under non-water-stressed condition paclobutrazol does not have any significant effect; however, under water stress conditions, paclobutrazol (40 mg l^{-1}) treatment resulted in a significant increase in proline content of barley plant as indicated in. Recent studies showed that paclobutrazol has effect in increasing free proline content of crops to protect from drought stress. However, the effect of paclobutrazol on proline content is still unclear. Supporting this idea Mohamed et al. 2011 reported that free proline content in 50 mg L^{-1} paclobutrazol-treated tomato plants grown under 60% field capacity peaked at 54.56 mg g^{-1}, which is 1.52-fold compared to control. In contrast, free proline content in 10 mgL^{-1}paclobutrazol pretreated peanut under water deficit conditions (1.04-folds over control) was lower than non-treated plants (1.49-folds over control) [**9**]. The accumulation of proline in leaves could possibly play a protection role apart from osmoregulation during drought stress. In sight of this sense we understand that paclobutrazol might act as a stress ameliorating agent crops, as this plant does not need to accumulate the proline content in the leaves. Previous studies have proved that proline accumulation was lower in tolerant plants when compared to sensitive plants during periods of drought stress. However, further study is needed in order to reach conclusive agreement on the effect of paclobutrazol on free proline content of crop leaves.

Translocation and chemical stability

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.

Methods of application

The most common application methods of PBZ are foliar sprays and media drench. PBZ shows good results for both methods; however, drenches act longer and provide uniform control of plant height with lower doses. When PBZ is applied by foliar spray, the compound is poorly soluble in water and consequently little translocated in the phloem. Thus, when applied by spray to the plant canopy, its action is restricted to the wet contact area. On the other hand, the application of PBZ by drench is uniform and increases the product efficiency in lower concentrations compared to foliar spray. Moreover, drench application of PBZ may directly inhibit GA synthesis as roots synthesize large quantities of GA. Similarly, Banon et al. and AlKhassawneh et al.

demonstrated that drench applications were more effective, allowing to use lower quantity of PBZ, which is desirable for both ecological and economic reasons. This effectiveness may be directly related to its high persistence in the soil drench and in plant organs. Gent and McAvoy also indicated that PBZ persists in annuals, herbaceous perennials and, especially, woody ornamentals. PBZ is considered a phloem immobile chemical 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.

Application rates

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.

Morphological and physio-biochemical responses of plants to PBZ

Effect of PBZ on relative water content

Relative water content (RWC), directly related to the content of soil water is a significant indicator of water stress in leaves. Plant exposure to water stress results in an immediate reduction of RWC. PBZ accelerated the stomatal closure, improved water retention, and increased drought tolerance in jack pine and oak. PBZ-treated plants maintained higher RWC than the non-treated ones', stated that the application of PBZ (30 mg/l) in wheat under control and water-stressed plants resulted in an increase of 5% and 11% respectively in the mean RWC. The reduced rate of evapotranspiration helps plants maintain a higher RWC, and overcome stress, and developed tolerance to various environmental stresses. RWC increased in PBZ-treated triticale (*Triticale hexaploide*) plants during water stress, Under water stress, PBZ treatment assists plants in retaining water for 30-40 days (, observed that application of PBZ (90 mg/l) under drought in rice genotypes was responsible for about a 15% increase in RWC as compared to drought without PBZ treatment., found that in *Curcuma alismatifolia* leaves, PBZ (1500 mg/l) increased RWC by 5% under drought., reported that in okra (*Abelmoschus esculentu*) cultivar Nutec, application of PBZ (80 mg/l) along with drought increased RWC (60.1%) compared to drought without PBZ treatment (57.2%) although the result was not statistically significant. Similarly, in Safflower (*Carthamus tinctorius L.*) application of PBZ under drought enhances the RWC. Overall PBZ enhances the RWC of plants under drought conditions by a reduction in evapotranspiration.

Effect of PBZ on membrane stability index

Membrane stability is a common criterion for determining drought tolerance because water deficit induces water loss from plant tissues, which severely impairs membrane structure and function. The stability of the cell membrane was used as a drought tolerance indicator and leakage of electrolytes showed an increase in water deficit, reported that PBZ (90 mg/l) in rice genotypes led to an 11% increase in mean MSI as compared to drought-stressed plants without PBZ treatment. PBZ (20 mg/l) minimized the leakage of electrolytes in carrots Reported that the application of PBZ (30 mg/l) in wheat under control and water-stressed plants resulted in an increase of 1-2% and 4-5% respectively in the mean MSI. Similarly, reported that PBZ (1500 mg/l) decreased electrolyte leakage by 60% under water deficit stress in *Curcuma alismatifolia*., observed that the application of PBZ (150 mg/l) in mungbean under drought decreased electrolyte leakage from 52.6% (drought without PBZ) to 47.1%. Similarly, in Safflower (*Carthamus tinctorius L.*) application of PBZ under drought enhances the cell membrane stability. Collectively, these findings suggest that PBZ improves MSI by minimizing electrolyte and ion leakage under stress conditions.

Effect of PBZ on plant growth

The most striking growth response observed in PBZ-treated plants is a reduction in shoot growth. This response is mainly attributed to internode length reduction., reported that canola plant height was reduced by 27% when PBZ was applied at 10 cm stalk height as compared to without PBZ., reported that red firespike plants treated with PBZ (.24 mg/pot) under drought were 11 cm taller than untreated plants. Under water deficit stress, found that applying PBZ (1500 mg/l) decreased the plant height of *Curcuma alismatifolia* by 50% relative to non-treated plants. In *Amorpha fruticosa*, found that PBZ treatment (150 mg/l) under extreme drought (RWC 35-40%) resulted in a 61% increase in height relative growth rate compared to drought without PBZ, observed that in Patumma after 40 days of withholding water, the plant height was 1.2 times lower in PBZ (1500 mg/l) treated plants compared to water-stressed without PBZ. When PBZ (3750 mg/L) was applied to Patumma, shoot height was reduced by 48.93% relative to untreated plants. In comparison to non-treated plants, soil drenching with PBZ (1500 mg/l) under water stress for 20- and 30-days periods-maintained shoot length. However, in sunflower and zinnia shoot height was reduced by 26.3 and 42.1%, respectively, after soil drenching with PBZ (2.0 mg/pot), *Syzygium myrtifolium* (Roxb.) Walp Plant height was reduced by 19.93% when treated with PBZ (3750 mg/L). According to PBZ (500ppm) increased panicle number, resulting in higher grain yield while reducing water demand, hence increasing rice water use efficiency under drought conditions.

Reported that PBZ (50 mg/l) increased wheat seedling length, fresh and dry weight of shoots, under low-temperature stress as compared to control (low-temperature stress without PBZ) PBZ has been shown to increase both the fresh and dry weight of shoots and roots in cucumber seedlings that have been exposed to high temperatures., reported that seed soaking of maize with PBZ (300 mg/l) under a semi-arid region increased root dry weight by 102.1% at the seventh leaf stage, 65.1% at the ninth leaf stage, 47.9% at the twelfth leaf stage, compared to drought without PBZ treatment., reported that in peanut plants at 80 days after sowing (DAS) application of PBZ (10 mg/l) under drought increased root length from 18.17 to 28.15 cm/plant, total leaf area from 96.38 to 117.31 cm^2/plant, whole plant fresh weight from 33.72 to 39.16 g/plant, whole plant dry weight from 3.49 to 4.12 g/plant as compared to drought-stressed plants without PBZ treatment. A similar pattern of results was also obtained by in *Sesamum indicum* by application of PBZ (5 mg/l) during drought. observed that in sweet potatoes, PBZ (34 μM) under drought increased vine fresh weight, root fresh weight, vine dry weight, and root dry weight by 40.10, 65.47, 66.91, and 67.86% respectively, compared to water-stressed plants.

After PBZ (500 mg/l) application, the root dry weight of *Aesculus hippocastanum* was improved (18.4% reduction) after water deficit stress. Under drought conditions, the dry weight of PBZ (60 mg/l) treated tomato shoots (37.17% reduction) and root dry weight (13.04% reduction) were higher as compared to the control. Similarly, the dry weight of PBZ (50 mg/l) treated plants decreased by 20.45%, compared to 36.77% for non-treated plants. In turf grass, shoot dry weight was extremely responsive to water deficit conditions (25% FC), resulting in 95 to 97% reduction, respectively, while treatment with PBZ (30 mg/l) reduced the shoot dry weight by 3.14% only. The leaf area of *P. angustifolia* plants treated with PBZ (30 mg/l) and grown under well-watered conditions was reduced by 83.25%. However, when exposed to mild water deficit conditions, the growth of PBZ-treated plants improved but declined when exposed to severe water deficit stress. When exposed to drought, shoot height, leaf area, and root length of PBZ (10 mg/l) pre-treated peanut plants improved compared to the control, reported that the diameter of *Vetiveria Zizanioides* increased in stressed plants due to 12% PBZ application. According to, PBZ (1.6 mg/l) reduced leaf area (LA) in tomato plants by 24% under water deficit conditions. Overall, PBZ enhanced plant development under stressful circumstances by increasing shoot and root biomass. Although some research implies that PBZ reduces plant height, others report that PBZ increases plant height, hence a greater knowledge of the influence of PBZ application on plant development is required before future application.

Effect of PBZ on photosynthetic pigments

Water stress alters the total chlorophyll content and stability within thylakoid membrane protein-pigment complexes which are the first structures to be weakened under stress conditions. Chlorophyll reduction under water deficit stress is mainly due to chloroplast damage caused by ROS. PBZ (3 g a.i./tree) increased Chlorophyll a (27.35%), Chlorophyll b (54.54%), total chlorophyll (30.98%) and carotenoids (13.55%) compared to control without PBZ in cashew. According to applying PBZ (30 mg/l) to wheat plants under water deficit stress resulted in a 25.7% increase in chlorophyll content as compared to stressed plants without PBZ., reported that in maize PBZ (300 mg/l) increased the chlorophyll content by 48.2%, 54.3%, 51.2%, and 79.0%, at 0, 15, 30, and 45 DAS respectively Similarly carotenoid contents increased by 15.7%, 17.3%, 27.9% and 36.7% at 0, 15, 30 and 45 DAS in water deficit stress as compared to control (drought without PBZ application) observed that PBZ treatment was 15–18% more effective than the control at preventing chlorophyll loss in wheat during low-temperature stress. PBZ (10 mg/l) increased total chlorophyll, carotenoid, xanthophyll, and anthocyanin content in 80 days old *Arachis hypogaea* by 120.22%, 112.66%, 116.48%, 111.26%, 114.44%, and 112.24% respectively over control under drought reported that PBZ (2 mg/l) increased chlorophyll content by 62% as compared to control in maize., observed that treatment with 25 or 50 mg/l PBZ in black rice plants had greener leaves and encountered late senescence than control plants. Similarly, in Safflower (*Carthamus tinctorius L.*) application of PBZ under drought enhances the photosynthetic pigments., reported that net photosynthesis was 51% higher in red firespike plants treated with PBZ (0.24 mg/pot) under drought than in those without PBZ. In *Zoysia japonica*, PBZ (50 mg/l) during water deficit stress increased leaf chlorophyll content by 0.6 mg/g FW compared to water-stressed without PBZ. Similarly, , recorded that PBZ in both irrigated and deficit-irrigated plants increased Chlorophyll content as compared to control plants (without PBZ). PBZ increased the photosynthetic pigment content in *Festuca arundinacea* and *Lolium perenne* under water stress. Under water deficit stress, PBZ significantly increased chlorophyll a, chlorophyll b, and carotenoids in wheat cultivars reported that PBZ (150 mg/l) treatment in mungbean under drought increased SPAD value from 34 (drought without PBZ) to 37.7. All prior investigations have concluded that PBZ improves photosynthesis by increasing chlorophyll and other photosynthetic pigments under stressful circumstances.

Effect of PBZ on grain yield and dry matter partitioning

Drought primarily affects production by reducing the number of seeds by either influencing the quantity of dry matter produced at the time of flowering or by directly affecting pollen or ovules, leading to a decrease in seed collection. PBZ has been shown to modify sink efficiency, prompting assimilates to be redistributed to meristematic regions other than shoot apices and improving assimilate flow to reproductive structures in plants. Under drought, the use of PBZ (50 mg/l) increased the average weight of 1,000 seeds and yield in maize (*Zea mays* L.). According, average maize grain yields increased by 61.3% after seed soaking with 300 mg/l PBZ, while seed dressing with PBZ at 2.5 g/kg increased yield by 33.3% compared to control without PBZ in semi-arid regions.

Under water stress, wheat genotypes treated with PBZ increased grain yield per plant by 6-7%, grain numbers per panicle by 24-33%, 1,000-grain mass by 3-6%, and harvest index by 2-4%. According to , under water stress, yield per plant was reduced. Stress effects, on the other hand, were found to be reduced when PBZ was applied (40 mg/l). reported that the application of PBZ (150 mg/l) in mungbean under drought increased seed yield from 622 (drought without PBZ) to 1921 kg/ha. Drought impaired flowering in red firespike plants, but PBZ treatment (0.24 mg/plant) promoted flowering and maintained the same number of flowers (6 flowers/plant) as the control. Tomato plants treated with PBZ (50 mg/l) produced 1.37 times more fruit than non-treated plants. The yield of pre-treated plants was reduced by 4.79% when they were subjected to drought at 60% field capacity. observed that PBZ (30 mg/l) pre-treated tomato plants retained their fruit yield (3.89 kg/plant) and fruits per plant (31 fruits/plant) when exposed to water deficit stress. Overall, past research indicates that the use of PBZ boosted grain yield/

fruit set under drought by improving sink efficiency. PBZ hampered the gibberellin biosynthesis. GAs are growth regulators which fall under a large family of tetracyclic diterpenoids. GAs are plant hormones that are required for a variety of developmental activities in plants such as pollen maturation, stem elongation, leaf expansion, trichome creation, seed germination, and flowering induction. Furthermore, the exogenous application of gibberellins can reverse PBZ-induced growth inhibition. These findings support the theory that PBZ-induced growth inhibition is due to a reduction in gibberellin biosynthesis. studied the effect of PBZ (200 mg/l) in rice varieties under submergence stress and found that gibberellic acid content was decreased by the application of PBZ compared to submergence stress without PBZ. found that PBZ (150 mg/l) under severe drought (RWC 35-40%) decreased GA content more than drought without PBZ in *Amorpha fruticosa*. PBZ-induced abscisic acid biosynthesiAbscisic acid (ABA) is classified as a stress phytohormone because it accumulates quickly in response to stress and mediates many stress responses that help plants survive. The effect of PBZ on ABA is of significant importance because ABA is synthesized through the isoprenoid pathway. Reported that PBZ (150 mg/l) under severe drought (RWC 35-40%) increased ABA (27.1%) than without PBZ in *Amorpha fruticosa* Similarly, , recorded that treatment with PBZ in wheat cultivars did not significantly affect ABA content, however, mean ABA content was significantly enhanced by 25% under water deficit stress., showed that DI (Deficit irrigated) + PBZ treated plants significantly increased ABA accumulation compared to DI control plants. PBZ application increased ABA and decreased gibberellins during the reproductive stage in the shoot of mango plants. Compared to untreated seedlings, PBZ treatment has been shown to minimize endogenous ABA by about one-third caused by water stress in apples and wheat, found that PBZ-induced stress tolerance in snap beans was due to increased endogenous ABA content. PBZ substantially enhanced endogenous ABA levels in hydroponically grown seedlings and detached leaves of oilseed rape, according to. According to, PBZ enhanced the endogenous level of ABA in wheat under water deficit stress., observed that PBZ (200 mg/l) increased ABA content in rice varieties under submergence stress compared to submergence stress without PBZ application. The effect of PBZ on ABA may be the source of stress defense.

PBZ elevated antioxidant enzymes activity

PBZ enhances the detoxification of ROS, antioxidant, and chlorophyll (Chl) content. As photosystem II (PSII) operation is reduced, an imbalance between electron generation and usage occurs, causing quantum yield shifts. These changes in chloroplastic photochemistry cause excess light energy to be dissipated in the PSII core and antenna under drought, resulting in the development of potentially harmful active oxygen species (O_2^{-1}, $1O_2$, H_2O_2, OH). ROS detoxification pathways can be found in all plant species and are classified as enzymatic which include ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and non-enzymatic which include reduced glutathione (GSH), ascorbic acid and tocopherol.

showed that PBZ (5 mg/l) application to *Sesamum indicum* resulted in 464.74%, 267.49%, and 359.08% increase in SOD, APX, and POX activity respectively in leaf tissue under drought conditions as compared to without PBZ. Different PBZ treatments increased SOD activity in maize grown in the semi-arid environment to varying degrees. From 0 to 15 days after silking (DAS), SOD activity increased, then decreased until it reached 45 DAS. The APX activity of PBZ-treated ryegrasses was found to be 25% higher than that of untreated under drought. No considerable difference in CAT activity was observed in PBZ-treated plants under drought. PBZ increased POX activity considerably under drought.

PBZ enhanced proline content

Proline is a key amino acid in protein and membrane structures, as well as a ROS scavenger under drought. PBZ treatment enhanced proline content and improved drought tolerance. However, further research is needed to determine the actual molecular mechanism underlying the effect of PBZ on mobile proline concentration in plants. PBZ treatment (75 mg/L) significantly reduced proline content (0.030 µmol/g FW) in pomegranate leaves by 59.22% to control (0.067 µmol/g FW) found that free proline concentration increased by 54.56 mg g^{-1} in PBZ (50 mg/l) treated tomato plants grown at 60% field capacity, which was 1.52-fold greater than the control. However, in water-stressed conditions, the free proline level in PBZ (10 mg/l) in pre-treated peanuts was lower (1.04-fold over control) than in untreated plants (1.49-fold over control), showed that the wheat plants treated with PBZ under water stress had a 40% decrease in proline content as compared to the stressed plants without PBZ. These findings suggested that the wheat genotypes experienced less stress (as indicated by the proline content) and improved drought tolerance as a result of PBZ application. Another study showed a considerable increase in free proline content after Mannitol+PBZ treatment in wheat cultivar Sakha 8 (3.342 mg g^{-1} f.w) as compared to control (without PBZ+Mannitol) and the same pattern was observed in all the wheat cultivars. Endogenous proline level increased by 17% in mango leaves treated with PBZ (1500 mg/L) under salt stress when compared to salinized plants without PBZ treatment, showed a significant increase in proline content in drought-sensitive and drought tolerant rice genotypes after priming with PBZ under drought as compared to their unprimed samples. reported that the application of PBZ (150 mg/l) in mungbean under drought increased proline content from 7.28 (drought without PBZ) to 7.87 µmol/g f.wt. Similarly, in Safflower (*Carthamus tinctorius L.*) application of PBZ under drought enhances the proline content.

PBZ reduced malondialdehyde content

Usually, membrane lipid peroxidation in plants is detected by measuring malondialdehyde (MDA). MDA is a widely used marker of oxidative lipid injury caused by environmental stress., showed that the MDA content was significantly lower in the PBZ-treated maize plants over the control under drought. PBZ treatment under drought considerably reduced the MDA content in maize leaf by 31.5% at 0 DAS, 31.4% at 15 DAS, 32.2% at 30 DAS, and 20.2% at 45 DAS compared with drought without PBZ. Other studies carried out on PBZ-primed rice samples indicated that PBZ showed insignificant change in MDA content in the sensitive genotype under drought while a 55% decrease in MDA content was found in the tolerant genotype as compared to PBZ treated under control conditions. Similar findings were documented by, who observed that plants raised from PBZ-primed seeds had lower MDA levels under control and drought conditions than plants raised from unprimed seeds. The amount of MDA decreased as the amount of PBZ increased. PBZ (80 mg/l) decreased MDA content (51.15 mol/g f.wt.) under water deficit stress relative to drought alone (61.92 mol/g f.wt.) reported that PBZ (300 mg/l) in the semi-arid region reduced MDA content by 44.1%, 50.4%, 66.3%, 40.5%, at 0, 15, 30, and 45 DAS respectively compared with the water-stressed plants without PBZ treatment.

PBZ influence on protein content

The protein content in plants decreases with the onset of water deficiency. PBZ treatment increased the protein content of the leaves and tubers in carrots. From 0 to 15 DAS, the soluble protein content of maize increased slightly, then steadily decreased from 15 to 45 DAS. Plants treated with a high concentration of PBZ under drought retained higher protein content from 0 to 15 DAS, but protein content was significantly inhibited from 30 to 45 DAS. Wheat seeds primed with PBZ had increased protein content. Also, there are other similar reports which showed that PBZ priming increased the protein content under abiotic stress and non-stress conditions. According to when PBZ was applied under drought to the okra cultivar Nutec, total soluble proteins increased as the amount of PBZ was increased. Total soluble proteins were 11.04, 11.29, 10.75, and 11.76 mg/g f.wt. at four different PBZ treatments of 0, 20, 40, and 80 mg/l, respectively under water stress conditions.

PBZ influence on sugar content

During drought, the accumulation of compatible solutes such as carbohydrates is claimed to be an effective stress tolerance mechanism. Sugar resulting from transitory starch degradation was noticed in PBZ-pretreated plants, which retains the leaf water potential under water deficit stress conditions. PBZ treatment in mango increased total sugar, sugar: acid ratio, reducing sugar, and titratable acidity reduction. In drought-stressed ryegrass, PBZ application significantly increased soluble sugar content compared to untreated plants. The impact of PBZ was mainly pronounced on 30 and 45 days of drought treatment in Iranian perennial ryegrass. According to, PBZ (150 mg/l) under extreme drought (RWC 35-40%) had 119% higher soluble sugar content than drought without PBZ in *Amorpha fruticosa*. In untreated and PBZ-treated (50 mg/l) tomato plants total soluble sugars increased by 1.16 and 1.52 times under water deficit (60% FC), respectively. Sugar content increased by 2 mg/l after foliar application of PBZ under 6% PEG-induced water deficit stress in *S. rebaudiana Bertoni* as compared to stressed plants. Total soluble sugar enrichment in PBZ-treated sweet potatoes may be required for cellular osmotic adjustment under water deficit stress situations.

Molecular responses of plants to PBZ

PBZ inhibits GA biosynthesis by inactivating cytochrome P 450-dependent oxygenase, which inhibits the oxidation of ent-kaurene to ent-kauronoic acid. PBZ inhibits ABA degradation into phaseic acid, resulting in ABA accumulation. In drought-stressed tomato plants, PBZ increased the expression of ABA biosynthesis genes (SlZEP, SlNCED, and SlAAO1). To gain a better understanding of the dwarfism mechanism, , analyzed gene transcripts of Lily leaves after PBZ treatment. 2704 genes were found to be differentially expressed by comparing PBZ-treated samples to untreated samples. PBZ increased the expression of nine genes encoding GA biosynthesis enzymes (one KAO and eight GA20ox genes) while decreasing the expression of a gene involved in GA deactivation (GA2ox gene). reported that the expression of ent-kaurene oxidase (ZmKO1-2), ent-kaurene synthase (ZmKS1,2,4), and ent-copalyl diphosphate synthase (ZmCPS) decreased, whereas the expression of GA 3-oxidase (*ZmGA3ox1*), GA20-oxidase (*ZmGA20ox1,5*) and ent-kaurenoic acid oxidase (ZmKAO) increased in maize seedlings treated with PBZ. PBZ has been shown to increase SLGA20ox-3 and SLGA3ox2 expression in tomato plants through feedback regulation. Upregulation of SLGA20ox-3 and SLGA3ox2 transcript accumulation was observed in response to PBZ-induced ent-kaurene oxidase inhibition, which was thought to be a feedback upregulation of GA biosynthesis in response to lower GA content.

Another study examined the expression profiles of GA biosynthesis genes (ent-kaurene oxidase; *KO*, gibberellin 20-oxidase1; *GA20ox1* and *gibberellin 3-oxidase*; *GA3ox*) and floral transcription factor genes (*UFO*, *WUSCHEL*; *WUS*, and *LFY*) in response to 1,250 mg/l of PBZ treatment of *Jatropha* floral buds. Then, samples were selected at the different time points of 14 days (no sex organs observed), and 20 days after treatment (blooming and sex organs observed). The results showed that PBZ significantly reduced the expression level of *GA20ox1*, *GA3ox*, and *LFY* as compared to the control ($P<0.05$) at 14 days. On the other hand, the expression level of *UFO* and *WUS1* were significantly higher than the control. At 20 days, there was no difference in the expression level of GA biosynthesis genes between the control and treatment. At the same time blooming time of PBZ-treated flowers was delayed which might be due to low expression levels of *GA20ox1*, *GA3ox*, and *LFY* in treated floral buds.

PBZ (200 mg/l) inhibited the GAs content in rice varieties under submergence stress compared to submergence stress without PBZ. QRT-PCR was used to analyze the expression of GAs biosynthetic genes such as OsCPS1, OsKS1, and OsGA2ox1. *OsCPS1* mRNA was repressed in PBZ treatment, which was consistent with the GA content in leaves. PBZ application increased ABA content regardless of rice genotypes due to the upregulation of 9-cis-epoxycarotenoid dioxygenase (NCED), the main enzyme in ABA biosynthesis, encoded by OsNCED. In contrast to plants not treated with PBZ, Rubisco-small subunit expression was higher at the anthesis and post-anthesis stages in all wheat cultivars with PBZ. At the anthesis and post-anthesis stages of wheat growth, the PBZ-treated water-stressed plants showed downregulation of the stress marker pyrroline-5-carboxylate synthase (P5CS) expression in all genotypes studied. At various growth stages after the formation of the basal second internode of wheat, the complex changes in the activities of enzymes involved in lignin biosynthesis, such as phenylalanine ammonia-lyase (PAL) and 4-coumarate: CoA ligase (4CL), were assessed in response to PBZ (200 mg/l) application. The activity of PAL and *4CL* were higher by 42% and 35.6% respectively as compared to the control.

PBZ (PBZ) at 0.8 and 1.6 mg/l significantly increased aquaporin (gene and protein) expression in tomato plants compared to controls, implying a coordinated increase in ABA and aquaporin levels in response to water stress. Treatment with PBZ during deficit irrigation increased SlTIP2 expression by 5.3-fold above the control and resulted in greater PIP2-7 protein levels (compared to PBZ-irrigated). The increased expression of PIP2-7 in response to PBZ treatment during deficit irrigation shows that it enhances water intake and management by encouraging *de novo* synthesis of aquaporin (AP) channels. Under deficit irrigation, PBZ (0.8 and 1.6 mg/l) administration raised citrate content 2.18 and 1.64-fold, respectively, compared to PBZ-treated irrigated plants (control). This was due to the up-regulation of Sl Citrate synthase (SlCS) by 1.28 and 1.73-folds, respectively. Application of PBZ under irrigated conditions and PBZ-treated deficit irrigated plants increased Sl Succinyl-CoA ligase, SlSCoAL1, and SCoAL2 expression by 1.66 and 2.01-fold, 1.21, and 3.66-fold, respectively, resulting in substantially increased succinate abundance (1.63-fold). PBZ-treated irrigated and deficit irrigated plants produced more GABA than control plants. When PBZ-treated irrigated and deficit irrigated plants were compared to their respective control plants, increased expression of glutamate decarboxylase, SlGAD, was connected to better GABA buildup. GABA production was boosted by increasing the expression of SlGAD, an enzyme necessary for glutamate to GABA conversion. DNA methylation plays an important role in plant growth and development. Recent research findings have shown that the imposition of various biotic and abiotic stresses on the plant contributes to increased methylation of the genome and thus leads to genome activity degeneration, found that the application of PBZ under water deficit stress leads to hypermethylation which was predominant in the drought susceptible genotype as compared to drought tolerant genotypes

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 activity [Yamaguchi S.,2008]; 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 [,2009]. The major bioactive GAs, which includes GA_1, GA_3, GA_4 and GA_7, are derived from a basic diterpenoid carboxylic acid skeleton, and commonly has a C_3 hydroxyl group [Yamaguchi S., 2008].

Triazole compounds are antagonistic to gibberellins and auxins, reducing cell elongation and cell division by inhibiting GA_3 biosynthesis [Hartmann A, Senning M, Hedden P, Sonnewald U, Sonnewald S., 2011]. They exhibit varying degrees of both plant growth and fungicidal activity. The intensity of their biological activity is dependent on their isomeric form [Fletcher RA, Hofstra G, Gao J., 1986]. 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 [Hedden P, Graebe JE., 1985]. Furthermore, PBZ-induced growth inhibition can be reversed by exogenous application of gibberellins [Lever BG., 1086]. 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 [Fletcher RA, Hofstra G., 1988]. 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 [Aly A, Latif H., 2011]. Mackay et al. [Aly A, Latif H., 2011] demonstrated that PBZ induced stress resistance and it also increased the endogenous concentrations of ABA in snap beans. [Hauser et al. Hauser C, Kwiatkowski J, Rademacher W, Grossmann K.,1990] 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. [Aly A, Latif H., 2011] 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 and eventually decreased to normal or below control levels. Hauser et al. [Hauser C, Kwiatkowski J, Rademacher W, Grossmann K., 1990] hypothesized that this may be due to stimulated ABA catabolism and/or by an inhibition of its biosynthesis. 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. In addition, Aly and Latif [Aly A, Latif H., 2011] also reported that PBZ increased the endogenous level of ABA in wheat.

Cytokinin

Cytokinins are synthesized in the roots and translocated acropetally to the shoots where they regulate both plant development and senescence [Binns AN., 1994]. 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 [Mok MC., 1994]. Zhu L, Van De Peppel A, Li X., 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 DM, Proctor JTA., 1992] and blueberry [Basiouny FM, Sass P., 1993]. It has been reported that cytokinin or chemicals like thidiazuron with cytokinin-like activity stimulate chlorophyll synthesis and retard senescence Visser C, Fletcher RA, Saxena PK., 1992 and thus, PBZ-induced physiological responses may be associated with increased cytokinin synthesis or prevention of its degradation.

Fletcher R, Gilley A, Sankhla N, Davis T., 2000. 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* [Kumar S, Ghatty S, Satyanarayana J, Guha A, Chaitanya BSK, Reddy A.,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 photosynthesis in PBZ-applied plants by keeping the leaves photosynthetically efficient for a longer time which in turn enhanced the plant productivity of *Camelina* [Kumar S, Ghatty S, Satyanarayana J, Guha A, Chaitanya BSK, Reddy A., 2012].

Stress protection

Biochemical effects of the triazole include detoxification of active oxygen species, increased contents of antioxidants and chlorophyll (Chl) [Rady M, Gaballah S., 2012]. 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 Fletcher R, Gilley A, Sankhla N, Davis T.,2000],[Zhang M, Duan L, Tian X, He Z, Li J, Wang B, Li Z.,2007]. 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 [Aly A, Latif H., 2011]. Enhanced chilling tolerance in triazole-treated tomato [Pinhero RG, Fletcher RA., 1994.] 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 [Pinhero RG, Fletcher RA., 1994.]. PBZ-induced tolerance to low temperature stress has been associated with increased levels of endogenous ABA [Fletcher R, Gilley A, Sankhla N, Davis T., 2000], which has been reported to trigger the genetic processes for hardening. In field studies, winter survival of peas and cereal crops [Davis TD, Steffens GL, Sankhla N., 1988] 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. 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 [Zhu L, Van De Peppel A, Li X.,2004] [Pinhero RG, Fletcher RA.,1994] . PBZ also protects plants from high-temperature-induced injuries [Pinhero RG, Fletcher RA., 1994] [Pinhero RG, Fletcher RA., 1994]. Protection against high temperature stress is accompanied by the production of low molecular mass stress proteins [Larsen MH, Davis TD, Evans RP., 1988] and the increase in the activity of antioxidant enzymes [Pinhero RG, Fletcher RA., 1994].

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 [Scandalios J., 1993]. Some of the free radical scavenging enzymes are reported to increase in wheat [Kraus TE, Fletcher RA., 1994] and corn [Pinhero RG, Fletcher RA.,1994] 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 [Senaratna T, Mackay C, McKersie B, Fletcher R.,1988]. Berova M, Zlatev Z, Stoeva N., 2002, 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 [Almekinders CJM, Struik PC., 1967]. PBZ treatment increased the root-to-shoot ratio [Pinhero RG, Fletcher RA.,1994] increased partitioning of assimilates to economically important plant parts such as bulbs [Le Guen-Le Saos F, Hourmant A, Esnault F, Chauvin JE.,2002.] [De Resende GM, De Souza RJ.,2002], potato tubers [Tekalign T, Hammes PS.,2005] [Mabvongwe O, Manenji BT, Gwazane M, Chandiposha M.,2005], carrot root [Gopi R, Jaleel C, Sairam R, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R., 2007] and rice grain yield [Pan S, Rasul F, Li W, Tian H, Mo Z, Duan M.,2013]. 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 [Tekalign T, Hammes PS., 2005]. Setia RC, Kaur P, Setia N..1996 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. [Senoo S, Isoda A., 2003] reported that PBZ treatment enhanced seed yield in *Camelina sativa* and this enhancement of yield was correlated with improvement in CO_2 assimilation physiology, sink activity partitioning of assimilates and rooting.

In addition, Yeshitela T, Robbertse PJ, Stassen PJC.1995, 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 { Christov C, Tsvetkov I, Kovachev V.,1995]

Mineral uptake

By influencing shoot and root morphology, PBZ alters mineral uptake. Rieger M., 1990. 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. [Huang WD, Shen T, Han ZH, Liu S.,1995] found that the differences in the total dry matter accumulated per kg of leaves were negligible. On the other hand, Wang et al. [Wang SY, Byun JK, Steffens GL., 1985] 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 Rieger M, Scalabrelli G.1990] 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 T, Robbertse PJ, Stassen PJC..1995. 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.

Plant growth, yield and quality

Germination and seedling development

Common problems found when treating seeds with growth regulators are reduction or absence of germination and delay in seedling emergence. Apple seeds (*Pyrus malus* Mill.) imbibed in 7 mg L^{-1} PBZ solution had 35% inhibition of germination and a germination delay by 2 days [Mage F, Powell L.,1990]. Similarly, Almond (*Prunus dulcis* L.) seeds soaked in 4000 or 8000 mg L^{-1} PBZ solutions during 15 min failed to germinate [Koukourikou-Petridou MA., 1996].

Germination percentage of tomato seeds that were soaked in 500 or 1000 mg L^{-1} PBZ for 6, 16, or 24 h was lower than that of water-soaked seeds [Pasian CC, Bennett MA., 2001]. They further found that seedling height suppression at 36 days after sowing was > 30% for seeds that had been soaked for 16 h in 500 or 1000 mg L^{-1} PBZ compared to those soaked in water. Pill WG, Gunter JA., 2001, also found that exposing *Cosmos bipinnatus* seeds to 1000 mg L^{-1} PBZ during soaking or priming reduced seedling height and also reduced seedling emergence with the responses being greater with longer exposure during priming than during soaking. Similarly, Pasian and Bennett[Pasian CC, Bennett MA.,2001] noted that 500 or 1000 mg L^{-1} PBZ reduced and delayed germination of tomato, geranium, and marigold seeds. These could be due to PBZ that adheres to the seed coat of treated seeds and then diffuses into the growth medium where it can be taken up by the seedling roots [Pasian CC, Bennett MA., 1999]. However, PBZ may penetrate the seed coat and exert a direct toxic effect on the embryo. The seed coats of tomato have a semipermeable layer [Beresniewicz MM, Taylor AG, Goffinet MC, Terhune BT., 1995] that may prevent the PBZ from entering the endosperm and embryo thereby lessening negative effects of PBZ on germination. In addition, Kar and Gupta [Kar C, Gupta K.,1991] described that treating sunflower (*Helianthus annus* L.) and safflower (*Carthamus tinctorius* L.) seeds with PBZ diminished the rate of germination and reduced seedling growth. Similarly, treating seeds with PBZ (at 250 mg per 1 kg seeds) retarded elongation of primary leaves in wheat (*Triticum durum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), and rye (*Secale cereale* L.) [Buchenauer H, Kutzner B, Koths T., 1988]. The first true leaves of seedlings from triazole-treated seeds had a disturbed morphology. In these seedlings, root elongation was less severely retarded than shoot growth; roots were thicker and had higher water content [Kar C, Gupta K., 1991]. For celery seeds, which require light for germination, the action of PBZ on GA biosynthesis was also light dependent. PBZ had a low effect on seed germination in the light, which might be due to increase in seed sensitivity to GA and, respectively, lower GA requirement for germination [Pressman E, Shaked R., 1988]. Other data also suggested that germination of PBZ-treated seeds is dependent on seed GA levels influenced by light intensity [Li FL, Chen JC, Zhao YJ., 2000].

Addition of GA or other chemicals (ethephon) to seeds may overcome the influence of growth regulators on seed germination. Only 5% of petunia (*Petunia hybrida* L.) seeds germinated after imbibing them for 14 days in an agar medium containing 5 mg L^{-1} PBZ. The addition of 10 μM GA solution to the PBZ-treated substrate improved petunia germination up to 65% [Izhaki A, Swain SM, Tseng TS, Borochov A, Olszewski NE, Weiss D., 2001]. Soaking in PBZ solutions inhibited germination of amaranth (*Amaranthus* sp.) seeds, while further soaking seeds in gibberellin, ethephon, or 1-aminocyclopropane-1-carboxylic acid reversed the inhibitory effect of PBZ [Kerczynski J, Kerczynska E, Knypl JC.,1988].

Shoot growth

Triazole treatments normally decreased the shoot length and increased thickness of the young plant stem, as well as the accelerated root formation is a significant advantage of the paclobutrazol treatment in *Lycopersicon esculentum* [Berova M, Zlatev Z.,2000]. Triazole treatments have more pronounced effect of reducing height in wheat plants and appeared greener [Hajihashemi S, Kiarostami K, Saboora A, Enteshari S., 2007]. The most striking growth response observed in different species treated with PBZ is shoot growth reduction [Hua S, Zhang Y, Yu H, Lin B, Ding H, Zhang D, Ren Y, Fang Z., 2014]. This response could be attributed primarily due to decreased internode length. PBZ was also found to effectively inhibit plant height, leaf expansion and alter the stem in *Syzygium campanulatum* [Ahmad Nazarudin MR, Mohd Fauzi R, Tsan FY., 2007]. Similarly, plant height was significantly reduced by PBZ application in canola [Hua S, Zhang Y, Yu H, Lin B, Ding H, Zhang D, Ren Y, Fang Z.,2014], *Vigna radiata* [Bekheta MA, Talaal IM.,2009], *Epidendrum radicans* [Pateli P, Papafotiou M, Chronopoulos J.,2004.], mango [Murti GSR, Upreti KK.,2005], wheat [Berova M, Zlatev Z, Stoeva N.,2002], *Dianthus caryophyllus* [Banon S, Gonzalez A, Cano EA, Franco JA, Fernandez JA.,2002], *Sesamum indicum* [Abraham SS, Jaleel CA, Chang-Xing Z, Somasundaram R, Azooz MM, Manivannan P, Panneerselvam R.,2008], and *Ocimum sanctum* [Divya Nair V, Jaleel CA, Gopi R, Panneerselvam R.,2009]

The PBZ effectively suppresses growth in a wide range of plant species, where treated plants tend to be smaller and more compact in appearance and have darker green leaves [Esmaielpour B, Hokmalipour S, Jalilvand P, Salimi G., 2011] [Brito CLL, Matsumoto SN, Santos JL, Goncalves DN, Ribeiro AF., 2016] [Rahman MN, Shaharuddin HA, Wahab NA, Wahab PEM, Abdullah MO, Parveez GKA., 2016]. Terri and Millie { Terri WS, Millie SW.,2000] and Sebastian et al. [Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF.,2002] also reported that PBZ-treated plants tend to be dark green, shorter and more compact in appearance. Similarly, treating Chrysanthemum plants with PBZ as a soil drench resulted in thicker leaves, reduced stem diameter, and roots with an increased diameter [Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF., 2002]. 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.

Leaf growth

Leaf area: Triazole treatments significantly reduced the leaf area and the reduction might be due to the reduction in leaf size [Sheena A, Sheela VL.,2010]. Gomathinayagam M, Jaleel CA, Alagu Lakshmanan GM, Panneerselvam R.,2007., also reported that triazole treatments reduced the leaf area in *Manihot esculenta*. PBZ induces various morphological modifications depending on plant species, growth stage, rate and method of application [Yeshitela T, Robbertse PJ, Stassen PJC..1995] [Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF.,2002]. Vijayalakshim and Srinivasan [Vijayalakshmi D, Srinivasan PS.,1999.] found that application of PBZ in mango found to be significantly superior in increasing the leaf area compared to other treatments like potassium nitrate, urea and ethrel recording an average area of 94.89 cm^2, whereas the control was only 63.65 cm^2. According to these authors, the increase in leaf area has overcome the limitation of depletion for reserve food materials. As the reserve food materials were then plenty, the breaking up of alternate bearing cycle in the cultivars chosen has been achieved. However, this was found to be contradictory to the finding of Fernandez JA, Balenzategui L, Banon S, Franco JA., 2006. who reported a decrease in leaf area with PBZ in *Phillyrea angustifolia*. Similarly, Paclobutrazol treatment also reduced the leaf area in *Solanum tuberosum* [Tekalign T, Hammes PS.,2005], *Ocimum sanctum* [Divya Nair V, Jaleel CA, Gopi R, Panneerselvam R.,2009], *Hordeum vulgare* [Sunitha S, Perras MR, Falk DE, Zhang R, Pharis RP, Fletcher RA.,2004], *Catharanthus roseus* [Jaleel CA, Gopi R, Chang-xing Z, Azooz MM, Panneerselvam R.,2008] and zinnia plants [Asgarian H, Nabigol A, Taheri M.,2013]. Although PBZ decreased the surface area of the plants, it improved the durability of leaves; therefore, the decrease in the surface area of leaves was compensated by the lack of leaf falling and by the leaf durability [Tekalign T, Hammes PS., 2005].

Chlorophyll synthesis

Several studies demonstrated increase in chlorophyll content in triazole-treated plants [Fletcher R, Gilley A, Sankhla N, Davis T., 2000] [Berova M, Zlatev Z., 2000]. The greening effect caused by plant treatments with growth regulators can be explained by an increase in chlorophyll content and/or more densely packed chloroplasts per unit leaf area due to a reduction in leaf area [Khalil IA, Rahman H., 1995]. A similar explanation is suggested for the increased chlorophyll a and b contents in potato leaves [Tekalign T, Hammes PS., 2005]. Dewi et al. [Dewi K, Agustina RZ, Nurmalika F., 2016] reported that black rice plants treated with either 25 or 50 ppm PBZ have greener leaves compared to control and the leaves also experienced late senescence. This could be due to an increase in the activity of oxidative enzymes that prevented cell maturation. Similarly, studies on Jatropha [Ghosh A, Chikara J, Chaudhary DR, Prakash AR, Boricha G, Zala A.,2010], tef [Tekalign T. Growth, 2007] and *Camelina* [Sumit K, Ghatty S, Satyanarayana J, Guha A, Chaitanya BSK, Reddy AR.,2012] showed that chlorophyll was higher on plants treated with PBZ compared to control. The increased chlorophyll content treated with PBZ might be from minimized damage caused by reactive oxygen and changes in the levels of carotenoids, ascorbate and the ascorbate peroxidase. The report of Nivedithadevi et al. [Nivedithadevi D, Somasundaram R, Pannerselvam R., 2015.] showed that plants treated with PBZ synthesized more cytokinin, which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis, and prevented chlorophyll degradation. Berova and Zlatev [Berova M, Zlatev Z.,2000] also reported that the increase in chlorophyll content may be ascribed to higher cytokinin content that is known to stimulate chlorophyll biosynthesis and/or reduced chlorophyll catabolism. Furthermore, PBZ appears to have delayed the onset of senescence, represented by the rate of chlorophyll degradation in attached mung bean leaves, which was probably due to the enhanced endogenous level of cytokinins through their secondary effect on plants [Fletcher R, Gilley A, Sankhla N, Davis T.,2000]. In several plant species, PBZ-treated leaves were retained longer and the onset of senescence considerably delayed [Hunter DM, Proctor JTA., 1992][Basiouny FM, Sass P.,1993.]. The senescence delaying activity may be related to the influence of PBZ on the endogenous cytokinin **content [Fletcher R, Gilley A, Sankhla N, Davis T., 2000].**

Rate of photosynthesis and transpiration rate

Contradictory reports have been published regarding the effects of PBZ on crop photosynthetic efficiency; however, indirectly by reducing leaf area it may reduce photosynthetic surface area and thereby reduce the whole-plant photosynthesis [Davis TD, Steffens GL, Sankhla N., 1988]. DeJong and Doyle [DeJong T, Doyle JF.,1984] noted no apparent effect of PBZ on photosynthetic rate of well exposed nectarine leaves during most of the growing season. Davis et al. [Davis TD, Steffens GL, Sankhla N., 1988] reported that photosynthetic rate decreased as a result of inhibition of leaf expansion. Leaf is an important plant part as it contains mesophyll cells that are specialized as photosynthetic tissues. Gaussoin et al. [Gaussoin RE, Branham BE, Flore JA., 1997] also reported that plants treated with plant growth retardants often had a moderate restraining effect on carbon dioxide exchange rate, thus possibly reducing the photosynthetic rate.

Ahmad Nazarudin MR, Tsan FY, Mohd FR., 2012, described that photosynthetic rate and transpiration rate of *Syzygium myrtifolium* were reduced after treatment with PBZ. This might be due to the reduction in photosynthetic rate that would also imply reduction in the transpiration rate as both processes are associated with the opening and closing of stomata [Salisbury FB, Ross CW., 1992]. The decline in transpiration rate would then reduce the percentage of water released through stomata. Olsen and Andersen [Olsen WW, Andersen AS., 1995] reported that reduction in transpiration rate would protect the plant against abiotic stress due to water restriction or drought period. In addition, PBZ enhanced stress tolerance of plants by increasing the xylem pressure potential of the treated plants and thereby enhancing plant moisture status during drought period. According to Abod and Jeng [Abod SA, Jeng LT., 1993], the reduction in photosynthetic rate and transpiration rate was influenced by the stomatal activity and leaf area. However, Ahmad Nazarudin et al. [Ahmad Nazarudin MR, Tsan FY, Mohd FR., 2012] reported that stomatal activity was not the main factor in the reduction of photosynthetic and transpiration rate in *Syzygium myrtifolium*. The possible reason could be the reduction of leaf area, which further contributed to the reduction of the total leaf surface that absorbs the sunlight.

On the contrary, PBZ increased the rate of net leaf photosynthesis [Tekalign T, Hammes PS., 2005]. This could be attributed to the higher chlorophyll content and earlier tuberization in response to the PBZ treatment. The increased net photosynthesis in response to PBZ has also been reported in *Setaria italic* [Bisht R, Singariya P, Bohra SP, Mathur N.,2007] and horse chestnut [Percival C, Noviss K.,2008]. Similarly, Soumya [Soumya PR., 2014] reported that foliar and drench applications of PBZ in chickpea were found to maintain higher rates of photosynthesis under water deficit condition and faster recovery after water stress termination. The increase in intercellular CO_2 concentration and alteration in stomatal conductance was assessed the reasons for higher photosynthesis in PBZ-treated *Amorphophallus campanulatus* [Gopi R, Jaleel C.,2009] [Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Sridharan R.,2007] and potted red fire spike [Rezazadeh A, Harkess RL, Guihong B.,2016]. Leaf thickness: The higher epicuticular wax deposition on treated leaves may be related to the increase in endogenous ABA levels in response to PBZ treatment [Rademacher W., 1997]. An increase in ABA stimulates the synthesis of lipid transfer proteins in barley that play an important role in the formation of epicuticular waxes, a process that affects the water relation of the leaves [Hollenbach B, Schreiber L, Hartungs W, Dietz KJ.,1997.]. PBZ treatments caused an increase of 10% in total wax load and change the proportion of certain wax constitutes in potted rose cultivars within 11 days of application [Jenks MA, Andersen L, Teusink RS, Williams MH., 2001]. The development of a thicker epicuticular wax layer provides better protection against some plant pathogens and minor mechanical damage [Kolattukudy PE., 1987].

Tekalign and Hammes [Tekalign T, Hammes PS.,2005] observed that potato plants cv. Zemen treated with PBZ at the dosage of 67.5 mg a.i. per plant increased total leaf thickness from 215 to 267 µm bringing about 24% increase higher than the control. These authors also indicated that the increase in leaf thickness is attributed to an increase in epidermal cell diameter, palisade cell length and spongy mesophyll depth. Sopher et al. [Sopher CR, Krol M, Huner NPA, Moore AE, Fletcher RS.,1999] also reported that in maize, PBZ-treated leaves showed more epicuticular wax deposition and were thicker and broader owing to enlarged vascular elements, epidermal, mesophyll, and bundle sheath cells. In peanut (*Arachis hypogaea* L.), leaves treated with PBZ exhibited well-differentiated palisade and spongy mesophyll layers with longer and longer cells [Sankar B, Karthishwaran R, Somasundaram BSK.,2016]. Similarly, Kishorekumar et al. [Kishorekumar A, Jaleel CA, Manivannan P, Sankar B, Sridharan R, Somasundaram R, Panneerselvam R., 2006.] indicated that Chinese potato leaves (*Solenostemon rotundifolius*) treated with PBZ showed an increased thickness of the upper and lower epidermis, as well as the length of the palisade and spongy cells. In addition, Jaleel et al. [Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Sankari S, Panneerselvam R., 2007] reported that cells number in palisade (mesophyll) tissue per unit area have been reported to enhance to a larger extent in PBZ-treated *Catharanthus roseus*.

Stem growth

Suppression of plant height by PBZ occurs because the compound blocks three separate steps in the terpenoid pathway for the production of gibberellins (GAs). GA enhances internode elongation of intact stems [Salisbury FB, Ross CW., 1992]. Liu and Loy [Liu PBW, Loy JB.,1967] showed that GA promotes cell division by stimulating cells in the G_1 phase to enter the S phase and by shortening the duration of S phase. They concluded that increased cell numbers lead to more rapid stem growth. But treating plants with PBZ resulted in stems with the same numbers of leaves and internodes compressed into a shorter length [Taiz L, Zeiger E., 2006]. Similarly, reduction in internode length was indicated in tomato in response to PBZ treatment [Rahman H, Khan M, Khokhar M., 1989]. He further noticed that the application of PBZ at a rate of 400 ppm resulted in a decreased internode length as compared to 200 ppm PBZ application. PBZ can be effective for obtaining sturdy plant and reducing plant height in several species without decreasing flowering quality [Mansuroglu S, Karaguzel O, Ortacesme V, Sayan MS.,2009] [Currey CJ, Lopez RG.,2010]. Webster and Quinlan [Webster AD, Quinlan JD..1984] also reported that PBZ has great efficacy in reducing height growth of many temperate fruit species and cultivars. Similar reductions in plant height were reported in potato [Tekalign T, Hammes PS.,2005.], Scaevola [Terri WS, Millie SW.,2000] and *Dianthus caryophyllus* [Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF.,2002], *Syzygium myrtifolium* [Ahmad Nazarudin MR, Tsan FY, Mohd FR.,2012] and *Mangifera indica* [Yeshitela T, Robbertse PJ, Stassen PJC..1995] in response to PBZ treatment.

Ahmad Nazarudin et al. [Ahmad Nazarudin MR, Tsan FY, Mohd FR., 2012] reported that PBZ application showed a stunted growth of *Syzygium myrtifolium* during the 5-month period after application, while the increased PBZ concentration (1.25–3.75 g L^{-1}) did not further reduced the plant height. Similarly, Tekalign and Hammes [Tekalign T, Hammes PS., 2005] indicated that both foliar and soil drench application of PBZ reduced the height of potato, though foliar application of PBZ is more effective than soil drench application. These authors further indicated that the increase PBZ concentration (45–90 mg a.i./plant) resulted in a reduced plant height. In addition, Yeshitela et al. [Yeshitela T, Robbertse PJ, Stassen PJC., 1995] also described that the height of mango trees and length of new shoots shortened due to both foliar and soil drench application of PBZ. PBZ treatment increased cortex thickness, size of the vascular bundles, and pith diameter and resulted in thicker stems [Tekalign T, Hammes PS., 2005]. This modification may be attributed to radial expansion of cells due to reduced endogenous GA activities in response to the treatment. Wenzel et al. [Wenzel C, Williamson RE, Wasteneys GO., 2000] reported that GA limits the extent of radial expansion of plant organs. In dicot stems, cell shape alterations are apparently caused by a more longitudinal orientation of cellulose microfibrils being deposited in the cell walls, preventing expansion parallel to the these microfibrils but allowing expansion perpendicular to them [Elsinger W.,1983]. The non-uniform distribution and arrangement of the vascular elements in the potato stems resulted in irregularity in the shape of the stems. Various authors reported different results in various plant species with respect to PBZ-induced stem anatomy modifications. PBZ induced both cell number and length in safflower stem [Potter TI, Zanewich KP, Rood SB., 1993]. Burrows et al. [Burrows GE, Boag TS, Stewart WP., 1992] reported that PBZ treatment brought about a 50% reduction in chrysanthemum stem diameter because of an enhanced development of secondary xylem and a marked reduction in the number of sclerenchyma bundle caps. In peach shoots, PBZ reduced the proportion of xylem and increased that of phloem and cortex, and increased xylem density [Aguirre R, Blanco A., 1992].

Root growth

Triazole treatments induced the root growth in cucumber, which was associated with increased the endogenous cytokinin levels [Feng Z, Guo A, Feng Z.,2003]. PBZ treatment increased the root length and enhanced the lateral roots in tomato plants [Pasian CC, Bennett MA.,2001], *Vigna unguiculata* [Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Panneerselvam R.,2008] and *Festuca* plant [Mahsa S, Ali T, Haniye H, Yahya S.,2011]. PBZ induced the root growth in both maize and wheat [Nayyar H, Gupta D.,2006], mango [Tahir FM, Ibrahim M, Hamid K.,2003], avocado [Chartzoulakis K, Patakas A, Kofidis G, Bosabalidis A, Nastou A.,2002], *Abelmoschus esculentus* [Sankar B, Jaleel CA, Manivannan P, Kishorekumar A, Somasundaram R, Panneeelvam R.,2007] and *Ocimum sanctum* [Divya Nair V, Jaleel CA, Gopi R, Panneerselvam R.,2009]. Swietlik and Miller [Swietlik D, Miller SS.,1983] reported that root length was stimulated by PBZ applications at low to moderate concentrations. Higher concentrations, however, may reduce root growth.

PBZ increased root diameter by increasing the width of cortex and by favoring the formation of more secondary xylem vessels. This modification may be attributed to radical expansion of cells due to reduced endogenous GA activities in response to the treatment [Tekalign T, Hammes PS.,2005]. These authors further indicated that untreated plants had more, thinner and longer roots compared to the treated plants. Increased root diameter has been correlated with larger cortical parenchyma cells in soybean and maize [Barnes AM, Walser RH, Davis TD.,1989]. Increasing root diameter in chrysanthemum was due to an increase number of rows and diameter of cortical cells [Burrows GE, Boag TS, Stewart WP.,1992]. PBZ was observed to increase diameter and length of fibrous roots, enhances lateral root formation, reduced the diameter of xylem vessels; however, phloem sieve tubes had shown an increased diameter in PBZ-treated *Catharanthus roseus* plants [Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Sankari S, Panneerselvam R.,2007]. The increased root length in PBZ-treated plants was found to be associated with larger parenchyma cells and promotes cell expansion radially [Fletcher R, Gilley A, Sankhla N, Davis T.,2000].

Flower enhancing

PBZ is effective not only in flower induction but also in early and off season flower induction in mango [Christov C, Tsvetkov I, Kovachev V.,1995; Protacio CM, Bugante RD, Quinto J, Molinyawe G, Paelmo G., 2000; Blaikie SJ, Kulkarni VJ, Muller WJ.,2004. ; Nafees M, Faqeer M, Ahmad S, Alam KM, Jamil M, Naveed A.,2010; Burondkar MM, Rajan S, Upreti KK, Reddy YTN, Singh VK, Sabale SN, Naik MM, Ngade PM, Saxena P.,2013]. PBZ, a gibberellin inhibitor, reduces vegetative promoter level and thereby increases florigenic promoter/ vegetative promoter ratio which stimulates flowering shoots in weakly inductive shoots of fruit crops [Yeshitela T, Robbertse PJ, Stassen PJC..1995; Voon CH, Pitakpaivan C, Tan SJ.,1991; Iglesias DJ, Cercos M, Olmenero-Flores JM, Naranjo MA, Rios G, Carrera E, Ruiz-Rivero O, Lliso I, Morillon R, Tadeo FR, Talon M. Physiology of citrus fruiting. Braz J Plant Physiol. 2007; Adil OS, Rahim A, Elamin OM, Bangerth FK.,2011].

Exogenous application of GA as well as endogenous high levels of gibberellins have proved a major hindrance in the way of flower bud differentiation in a number of temperate as well as tropical fruits [Tomer E.,1984]. PBZ, owing to its anti-gibberellin activity could induce or intensify flowering by blocking the conversion of kaurene into kaurenoic acid. The latter is a precursor of gibberellins. PBZ can considerably enhance the total phenolic content of terminal buds and alter the phloem to xylem ratio of the stem [Kurian RM, Iyer CPA.,1992]. Such alterations could be important in restricting vegetative growth and enhancing flowering by altering assimilates partitioning and patterns of nutrient supply for new growth. The application of PBZ before flower bud differentiation or 3 months earlier than anticipated flowering has been effective in inducing flowering in mango without accompanying reduction in shoot length. However, higher concentration leads to canopy and panicle compaction. The response to PBZ varied with cultivar and crop load. The effectiveness of PBZ in promoting flowering in Citrus sp. depends on the crop load as the heavy fruit load trees scarcely flowered. In medium to low fruit load trees, PBZ significantly increased the percentage of sprouted buds and floral shoots and reduced the number of vegetative shoots.

Fruit and tuber yield

Fruit yield Foliar application of PBZ (200 ppm) was effective in increasing yield and minimizing fruit drop and fruit cracking in ber. The effectiveness of PBZ was dependent on stage of development as the application of PBZ at bud bursting and 2 weeks before anthesis of grape increased the yield significantly [Christov C, Tsvetkov I, Kovachev V.,1995]. Soil application around the tree trunk (collar drench) was more efficacious than foliar application as it ensures proper uptake in inducing fruiting [Kulkarni VJ, Hamilthon D, Mc Mahon G.,2006.]. On the other hand, Yeshitela [Christov C, Tsvetkov I, Kovachev V.,1995] reported that application of PBZ both as a soil drench and foliar application was effective in suppressing vegetative growth and enhancing yield in mango. Souza-Machado et al. [Souza-Machado V, Pitblado R, Ali A, May P, Bieche BJ.,1999] reported that significant earliness in harvest maturity was recorded in PBZ-treated tomato plants but no significant total yield differences were recorded between the PBZ and control plants. However, Giovinazzo R, Souza-Machado V, Hartz TK.,2001 found significant yield increases of 13% due to the PBZ treatments together with earlier harvest maturity by 6%. Similarly, Berova and Zlatev [Berova M, Zlatev Z.,2000] reported that application of PBZ increased both early fruit yield and index of economic earliness in tomato. In addition, Mohamed et al. [Mohamed GF, Agamy RA, Rady MM.,2011] also reported the remarkable improvement in fruit yield and water use efficiency of tomato. There is usually a yield increase in mango associated with PBZ treatments, but Voon et al. [Voon CH, Pitakpaivan C, Tan SJ.,1991] emphasized the importance of supplying adequate nutrients, irrigation and generally good tree maintenance to maintain these high yields. In the experiments of Medonca et al. [Medonca V, Araujo Neto SE, Ramos JD, Pio R, Souza PA.,2002] also, PBZ increased the productivity of 'Tommy Atkins'. Similar increase in productivity of 'Tommy Atkins' mango (increased total fruit number and total fruit weight per tree) was indicated in response to PBZ treatment [Medonca V, Araujo Neto SE, Ramos JD, Pio R, Souza PA.,2002]. He also indicated that soil application of PBZ increased total fruit number per tree and total fruit weight per tree as compared to foliar spray and the increased application of PBZ (2.78–8.25 g a.i./tree) also resulted in the higher total fruit number per tree and total fruit weight per tree.

Tuber yield In the trials of Balamani and Poovaiah [Balamani V, Poovaiah BW.,1985], PBZ application resulted in increased tuber yield per plant. However, it is not clear whether the reported yield increments were a consequence of an increase in tuber size or number. On the contrary, Bandara and Tanino [Bandara PMS, Tanino KK.,1995] reported that PBZ nearly doubled the number of tubers per plant without affecting the total fresh weight of the tubers. This discrepancy may probably be explained by the cooler growing conditions in their experiment. Tekalign and Hammes [Tekalign T, Hammes PS.,2005] showed that PBZ application resulted in decreased tuber number per plant, which could be linked to the decline in stolon number as a result of a decrease in GA activity that may be associated with stolon initiation, and a strong negative correlation between tuber fresh mass and number signifying that the substantial increase in individual tuber size was responsible for the yield increment. This may be due to the interplay of early tuberization, increased chlorophyll content, enhanced rate of photosynthesis, and retaining photosynthetically active leaves longer in response to the treatment. In addition, these authors described that PBZ application increased tuber fresh mass, dry matter content and specific gravity. They further indicated that low concentration of PBZ increased tuber fresh and dry weight, while the increase in PBZ concentration (67.5–90.0 mg a.i./plant) did not increase the value of these parameters.

Similarly, the application of PBZ reduced the number of potato tubers [Mabvongwe O, Manenji BT, Gwazane M, Chandiposha M.,2005, Esmaielpour B, Hokmalipour S, Jalilvand P, Salimi G.,2011], the number of mini tubers [Kianmehr B, Otroshy M, Parsa M, Mohallati MN, Moradi K.,2012.] and the number of cassava tuber [Medina R, Burgos A, Difranco V, Mroginski L, Cenoz P.,2012]. On the contrary to the increased fresh and dry weight of potato tuber [Esmaielpour B, Hokmalipour S, Jalilvand P, Salimi G.,2011.; Samy MM, El Aal AA, Khalil MM.,2014], potato mini tuber [Kianmehr B, Otroshy M, Parsa M, Mohallati MN, Moradi K.,2012] and elephant foot yam [Gopi R, Sridharan R, Somasundaram R, Lakshmanan GMA, Panneerselvam R.,2005], the fresh weight of cassava tuber was reduced in response to PBZ treatment [Medina R, Burgos A, Difranco V, Mroginski L, Cenoz P.,2012].

Fruit and tuber quality

Fruit quality Fruit quality of mango and lemon (TSS and acid content) increased with PBZ application. Similarly, Vijayalakshmi and and Yeshitela et al. reported that applying PBZ in mango had the greatest effect in increasing total sugar, reducing sugar, TSS, sugar: acid ratio and a decrease in titratable acidity. On the other hand, PBZ had shown no improvement in fruit quality of grapes, strawberries and peach.

Foliar application of 2500–3000 ppm PBZ three weeks after full bloom increased TSS and reduced acidity in peach and cherries. Yeshitela also reported that the application of 8.25 g a.i./tree PBZ resulted in increased TSS. Even though, PBZ increased the quality of fruits, it was ascertained that the accumulation of PBZ residues on the surface or inside mango fruit (especially due applications of higher rates) is unfriendly to human health [.

Tuber quality: The PBZ application to cassava plant significantly increased starch content was reported by Yang and Cao and Medina et al.. Similarly, in potato, the application of PBZ decreased the assimilate partitioning to stem, leaves, root and stolon but increased the partitioning of dry mass production to tubers. An increase in specific gravity and dry matter content of the tubers in response to PBZ may be attributed to reduced GA activity in the tuber tissue that in turn increased sink strength to attract more assimilates and enhance starch synthesis. Under favorable conditions for tuberization (GA content below threshold level due to PBZ), the activities of enzymes involved in potato tuber starch biosynthesis such as ADPG-pyrophosphorylase, starch phosphorylase and starch synthase increased.

PBZ also help to increase the production of antioxidant such as carotenoid content in plants to fight against oxidative stress. Similar results were reported on tuber crop species that utilize low concentrations of triazole compound derivatives as treatments with concentrations ranging from 10 to 30 ppm to increase carotenoid content in the tuber of white yam.

Response of Paclobutrazol

Morphological response

Paclobutrazol is used in high input crop management to shorten the stem, thereby reducing the risk of lodging. There are several reports describing the various effects of paclobutrazol on plant morphology of crops. For example, reported PBZ application significantly decreased plant height of *Camelina sativa* when compared to control and induced dwarfing effect and with highest concentration of PBZ in which maximum reduction (47.5% decrease) in plant height with respect to control was obtained. Similarly, paclobutrazol concentrations of 200 mg/L to 600 mg/L decreased gibberellin content in the leaves compared to that of control when applied to rice plant during preanthesis. Paclobutrazol application reduced plant height and the greater concentration of paclobutrazol caused severe dwarfism as indicated in

(Figure 2). Reduction in plant height is considered as the most imperative morphological outcome of paclobutrazol application. According to Tesfahun and Menzir, plant height reduction strongly associated with reduced elongation of the internodes, rather than lowering the number of internodes and they found uppermost internodes to be shortened under paclobutrazol application. Correspondingly, reported that foliar application of paclobutrazol at 12.5 g a.i ha^{-1}, under a single-application scheme reduced plant height of sunflowers without adverse effects on achene and oil yields, thus providing a basis for reducing the risk of plant lodging.

On Yield response

The positive effects of paclobutrazol on yield components such as greater fertile tillers, spike, fertile panicle or spikelet and in some cases mean grain weight has been shown in studies evaluating the production potential of cereals; however, numerous studies have revealed that the increased fertile tiller, altered phenology and better canopy have been the main important components that significantly associated with enhanced grain yield in response to paclobutrazol application. One of the possible increments in grain yield is (i) the change in canopy coverage, in which the plant developed broader canopy this in turn facilitated improved light interception for better photosynthesis in leaves and stems of PBZ treated plants. Further, (ii) the leaves in PBZ treated plants were closely packed, dark green and remained on plants for a larger period than controls. This may explain increased dry matter accumulation in stem and root and simultaneous yield increments despite reduced plant height due to PBZ treatments. linked the grain yield increment (iii) with slow senescence in leaves which prolong the phase of seed development and maturation and as a consequence, the yield can be increased, but the harvest time delayed. The other possible

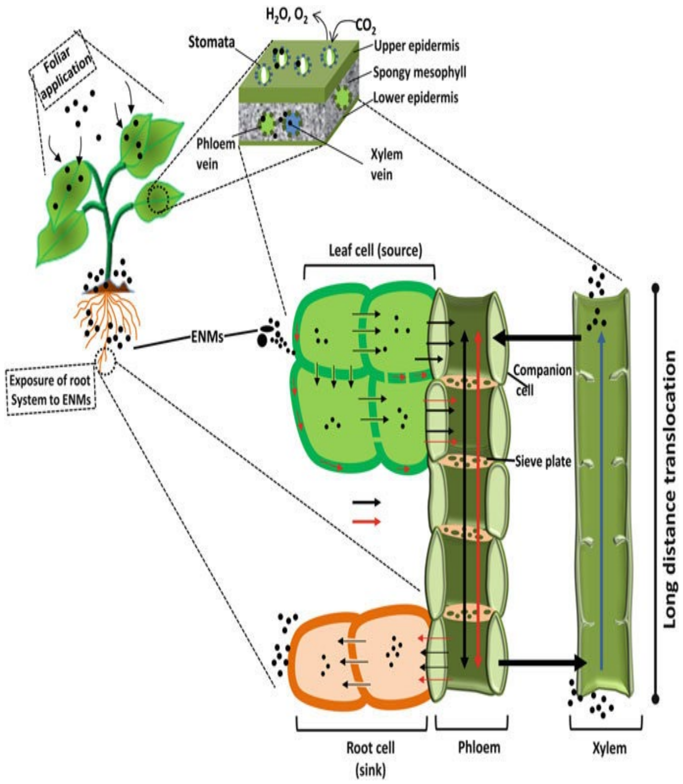


Figure 2: plant system and their translocation to different parts of plant. Red arrows indicate the apoplastic movement, while black arrows indicated symplastic movement of Engineered Nanomaterials (ENMs) and Edible Plants.

grain yield increment is closely related to (iv) the spread of roots, which determines the uptake and utilization of water and nutrients reported that greater root biomass is significantly and positively correlated with ear characteristics and enhanced biomass and grain yields. The increased in the grain yield is attributed partly to (v) decreased investment in above ground parts, due to a relatively stouter canopy of paclobutrazol treated plants, (vi) as well as enhanced grain filling in the treated plants due to the improved rooting system, which possibly increased the nutrients and water uptake.

On the Physiological response

Chlorophyllis a critical component of the primary photosynthetic reaction has a dual function in photosynthesis. It captures light, and also serves as a medium for the light-driven charge separation and transport of electrons. The biosynthesis of chloroplast pigments was significantly affected by paclobutrazol as indicated in Table 2. Several studies on tef and camilena showed that chlorophyll was higher on plants treated with paclobutrazol compared to control. The increased chlorophyll content treated with paclobutrazol might be from minimized damage caused by reactive oxygen and changes in the levels of carotenoids, ascorbate and the ascorbate peroxidase. The report of Nivedithadevi, Somasundaram and Pannerselvam also showed that plants treated with paclobutrazol synthesized more cytokinin, which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis, and prevented chlorophyll degradation. Furthermore, paclobutrazol appears to have delayed the onset of senescence, represented by the rate of chlorophyll degradation in attached mung bean leaves, which was probably due to the enhanced endogenous level of cytokinins through their secondary effect on plants. Paclobutrazol application in *Camelina sativa* L. Crantz also increased chlorophyll content which led to greater rate in photosynthesis and higher yield. The results of Dewi showed that black rice plants treated with either 25 or 50 ppm paclobutrazol have greener leaves compared to control and the leaves also experienced late senescence. This could be due to an increase in the activity of oxidative enzymes that prevented cell maturation.

On the Stress response

Since early migration from aquatic to terrestrial environments, plants have had to cope with periodic and unpredictable environmental stresses, such as drought and salinity. Crop production in arid or semi-arid regions is usually restricted by soil moisture deficit as well as soil salinity. Water deficit coupled with salinity in irrigation water is the major limiting factor in most regions where cereals are subjected to extreme water deficit during dry seasons. Enhanced stress tolerance in cereals can be achieved by exogenous application of some plant growth regulators, including paclobutrazol. Exogenous application of paclobutrazol can reduce some of the harmful effects of drought and salt stress and in some cases, compensate losses or damages caused by these stresses. Paclobutrazol increased stress tolerance of plants through the following methods.

Increasing root activity

Paclobutrazol are often referred as multi-stress protectants due to their innate potential of mitigating the negative effects of abiotic stresses had on plant growth and development, by regulating hormones level, enzymatic and non-enzymatic antioxidants and osmolytes. The 2-year results of Kamran showed that root activity and root-bleeding sap flow were significantly higher in paclobutrazol treatments than compared to control. As root-bleeding sap is the indicator of root pressure, therefore, the improved root-bleeding sap is attributed to higher root growth and root vigor in response to the paclobutrazol application. Also the study of Morita, Okamoto, Abe and Yamagishi showed the presence of a close relationship between the bleeding rate and the root traits in maize. The rate of root bleeding sap is correlated to active water absorption of the root system and reflects the physiological root activity. Yan et al. also observed that uniconazole, a triazole with a function similar to paclobutrazol promoted root activity, root bleeding sap and improved root growth in soybean. Previously, Zhao, Fang and Gao also observed a higher root activity in rice and wheat treated with plant growth regulators. Thus the application of paclobutrazol may improve plant performance under stressful condition through stimulating root activity of the plant.

Submergence tolerance

Also paclobutrazol has a role on submergence stress. The long-time submergence is also detrimental to rice crop, and where this cannot be avoided some corrective measures are to be taken to exploit yield potential of rice crop. Under submerged conditions, 200 ppm paclobutrazol spray to rice seedlings resulted in 50% increase in percent survival over control. The increased seedling survival is presumably due to low energy use in elongation, while, the same was available for maintenance processes, for synthesis of anaerobic proteins and maintenance of membrane integrity essential for submergence tolerance.

Increasing antioxidant enzyme

Increased the levels of antioxidant enzyme activities in plants under stress conditions are natural responses, which can help plants better tolerate the stress. Exogenous application of paclobutrazol enlarged these traits and enhanced stress tolerance in plants. Additionally, the enhanced antioxidant enzyme activities in response to paclobutrazol application may also protect their photosynthetic machineries against damages caused by Reactive oxygen species during water-deficit conditions.

Among these SOD and CAT are well-known antioxidative enzymes in cells, which can catalyze the poorly reactive oxygen species converting them to non-toxic substances. SOD constitutes the first line of defence against active oxygen species (AOS). This enzyme removes O_2^- by catalyzing its dismutation, wherein one O_2-Mis reduced to hydrogenperoxide (H_2O_2) and another is oxidized to oxygen. CAT is an enzyme that can convert H_2O_2 directly into water and oxygen. This enzyme is present in every cell and in particular on peroxisome. SOD and CAT plays a significant role in defending against oxidative stress induced by abiotic stress in plant tissues. Similarly, Rady and Gaballah also found that the application of paclobutrazol on barley crop had a significant role in increasing CAT and SOD concentration (Table 2). This compound reduced damage in plants grown under water stress conditions by enhancing the activity of these antioxidative enzymes. A number of studies showed that paclobutrazol minimizes the adverse effects of water-deficit stress by increasing the levels of the activities of antioxidative enzymes in many plants such as groundnuts, sesame seeds, mangos and tomatoes.

Proline content

Proline is well-known as an osmotic regulator that can reduce osmotic damage. It was reported that under non-water-stressed condition paclobutrazol does not have any significant effect; however, under water stress conditions, paclobutrazol (40 mg l^{-1}) treatment resulted in a significant increase in proline content of barley plant as indicated in Table. Recent studies showed that paclobutrazol has effect in increasing free proline content of crops to protect from drought stress. However, the effect of paclobutrazol on proline content is still unclear. Supporting this idea Mohamed reported that free proline content in 50 mg L^{-1} paclobutrazol-treated tomato plants grown under 60% field capacity peaked at 54.56 mg g^{-1}, which is 1.52-fold compared to control. In contrast, free proline content in 10 mgL^{-1}paclobutrazol pretreated peanut under water deficit conditions (1.04-folds over control) was lower than non-treated plants (1.49-folds over control). The accumulation of proline in leaves could possibly play a protection role apart from osmoregulation during drought stress. In sight of this sense we understand that paclobutrazol might act as a stress ameliorating agent crops, as this plant does not need to accumulate the proline content in the leaves. Previous studies have proved that proline accumulation was lower in tolerant plants when compared to sensitive plants during periods of drought stress. However, further study is needed in order to reach conclusive agreement on the effect of paclobutrazol on free proline content of crop leaves.

PBZ antagonist again Gibberellins

The main hormonal functions of GAs in higher plants are the promotion of longitudinal growth, the induction of hydrolytic enzymes in germinating seeds, the induction of bolting in long-day plants, and the promotion of fruit setting and development. Accordingly, a number of uses of GAs in crop production have become general practice. In addition, there is also considerable use to accelerate the process of malting for beer-making. In the early 1950s, when the work of Japanese scientists on GAs from the fungus *Gibberella fujikuroi* became known in other countries, companies in the UK and in the USA started to work on both GA production by fermentation and their application on crop plants. Work at ICI's Akers Laboratories in the UK on fermentation and chemical identification yielded the first production patents in 1954. Parallel, agricultural uses for GAs were investigated at ICI's Jealott's Hill research facility. By late 1957, ICI had granted manufacturing and use licenses to Abbott Laboratories, Merck & Co., Inc., Eli Lilly & Co. and Pfizer, Inc. in the USA and to Takeda Chemical Industries in Japan. Syngenta (into which ICI has merged) is still a major distributor of GA preparations. Valent BioSciences Corporation emerged from Abbott Laboratories and is now part of Sumitomo Chemical Co., Ltd. In addition to selling GA-containing products, Valent BioSciences is still actively engaged in finding new uses and improved formulations of GAs. Currently, Valent BioSciences lists several dozens of uses in plant production for its different GA preparations. Fermentations of *G. fujikuroi* are used to produce GA_3 and a mixture of GA_4 and GA_7 on a commercial scale.

A chemical synthesis of GAs is possible. However, it is highly complex and much too expensive for any commercial exploitation. Likewise, testing other GA-producing fungi did not result in competitive alternatives. Production of pure GA_4 might have been achieved, for instance, with the fungus *Sphaceloma manihoticola*, which causes the superelongation disease of cassava. However, attempts made at ICI, Norsk Hydro and BASF failed because of difficulties to establish competitive large-scale fermentations (Rademacher, unpublished). The phthalimide-type compound AC 94,377 [1-(4-chloro-1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)cyclohexane-1-carboxami-de], a relatively simple chemical structure with approximately 5 to 10% of the activity of GA_3 , was discovered and developed in the agricultural branch of former American Cyanamid Company (now part of BASF). It was sold under the trade name Surestem for a short time for the elongation of stems of roses, but for commercial reasons, it is no longer available. Currently, the majority of GAs sold globally originates from China.

Several Chinese companies indicate on their websites huge supply capacities for GA_3. Prices around US$ 200.00 per kilogram for bulk quantities (>90% purity) of GA_3 are typical. Total global annual use of GA_3 is in the range of 100 tons with approximately three quarters of this used in plant production, the rest in the beer-brewing industry. GA_3 is among the most widely used PGRs. It has found many applications in viticulture, horticulture and agriculture, e.g. in the production of seedless table grapes, in berry thinning of wine grapes, in improving citrus fruit quality, in increasing fruit size in pears and sweet cherries, and in accelerating seed germination. GA_4 is less persistent than GA_3 and GA_7 and is, therefore, better suited where too long-lasting effects are unwanted. However, due to the close chemical similarity of GA_4 with GA_7, their separation in fermentation extracts from *G. fujikuroi* is very difficult. As a result, the content of (mostly unwanted) GA_7 in different commercially available preparations varies between approximately 40% and insignificant amounts. The main use of $GA_{4/7}$ is to reduce fruit russetting in apple. The structures of the commercially available GAs are shown in (Figure 3).

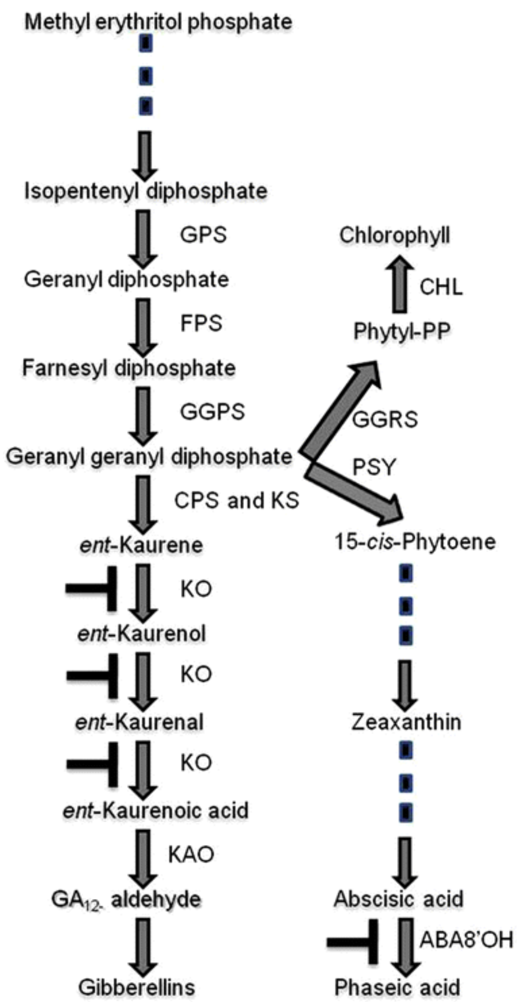


Figure 3: Terpenoid pathway for biosynthesis of gibberellins, abscisic acid, phytol, and steroids, and path for degradation of abscisic acid. Steps blocked by paclobutrazol. Geranyl diphosphate synthase (GPS), Farnesyl diphosphate synthase (FPS), Geranyl geranyl diphosphate synthase (GGPS), *ent*-copalyl-diphosphate 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 acid. KO, KAO and ABA 8'OH are the enzymes inhibited upon PBZ application.

(a) gibberellin A_3 = gibberellic acid = GA_3 = (3*S*,3a*R*,4*S*,4a*S*,7*S*,9a*R*,9b*R*,12*S*)-7,12-dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-9b,3-propeno[1,2-*b*]furan-4-carboxylic acid,

(b) gibberellin A_4 = GA_4 = (3*S*,3a*R*,4*S*,4a*R*,7*R*,9a*R*,9b*R*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-3,9b-propanoazuleno[1,2-*b*]furan-4-carboxylic acid and

(c) gibberellin A_7 = GA_7 = (3*S*,3a*R*,4*S*,4a*R*,7*R*,9a*R*,9b*R*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-9b,3-propenoazuleno[1,2-*b*]furan-4-carboxylic acid.

Inhibitors of Gibberellin Biosynthesis

The biosynthesis of GAs in higher plants is relatively well understood. A rough outline of the steps involved and the points of inhibition by the different growth retardants dealt with in this chapter is shown in (Figure 4). GA formation can be separated into three stages, according to the nature of the enzymes involved and the corresponding localisation in the cell: (1) terpene cyclases catalyse the formation of *ent*-kaurene via *trans*-geranylgeranyl diphosphate and *ent*-copalyl diphosphate (CDP) in proplastids, (2) cytochrome P_{450}-dependent mono-oxygenases associated with membranes of the endoplasmic reticulum are involved in the steps leading from *ent*-kaurene to GA_{12}, (3) dioxygenases, which are located in the cytosol and which require 2-oxoglutarate as a cosubstrate are required for the subsequent hydroxylations into different GAs. More details on GA biosynthesis can be found in Hedden and Kamiya, Yamaguchi, Sponsel and Hedden, Hedden, Hedden and Thomas, and in Chapter

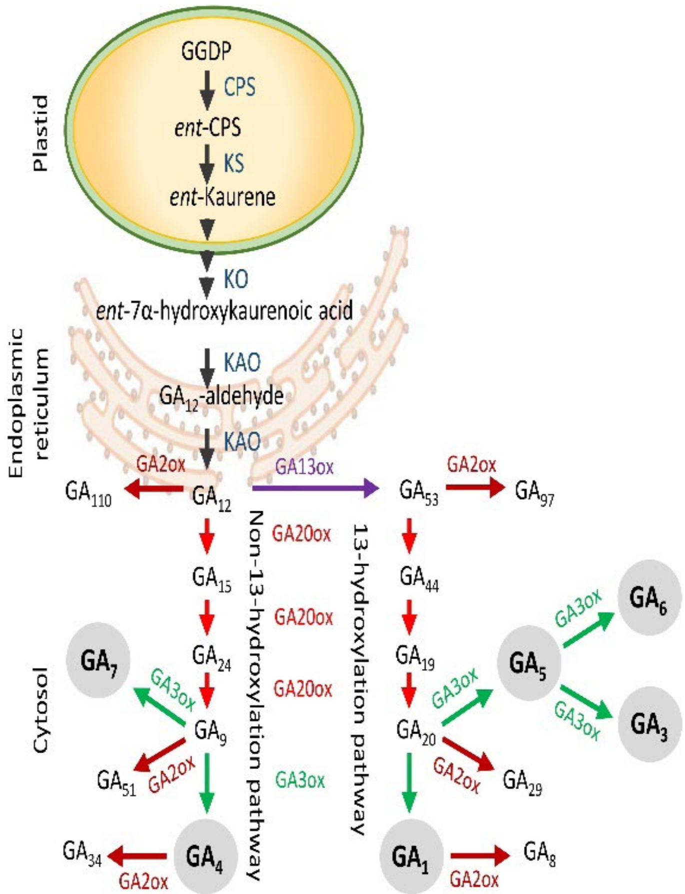


Figure 4: The mode of action of PBZ.

Gibberellin Biosynthesis in Higher Plants of this volume. Inhibitors of GA biosynthesis lead to less cell elongation and cell division, thereby making plants more compact, which may have a range of benefits in crop production. Such compounds are often referred to as 'growth retardants'. Several inhibitors of GA biosynthesis are known, some of which are used in crop production. These compounds represent the most important group of PGRs, both in terms of commercial value and of treated area. The site of interaction of growth retardants with distinct steps in the biosynthetic sequence of GA formation has primarily been elucidated by using cell-free enzyme systems prepared, for instance, from *G. fujikuroi* or from immature pumpkin or pea seeds. Analysing the spectrum of GAs and their precursors from fungal cultures or intact plants treated with inhibitors has also been helpful for this purpose. Overviews on the biochemical mode of action of growth retardants have been given by Hedden and Rademacher. Since that time, little additional information has become available on this subject.

Quaternary Ammonium Compounds

Several compounds that possess a positively charged ammonium, phosphonium or sulfonium group inhibit cyclases involved in early stages of GA biosynthesis, thereby blocking the formation of *ent*-kaurene. Out of these, the quaternary ammonium compounds chlormequat chloride and mepiquat chloride are of practical relevance. For more 'onium-type' representatives see Rademacher. Chlormequat chloride and related compounds inhibit CDP-synthase, both in the GA-producing fungus *G. fujikuroi* and in cell-free preparations of this fungus and of higher plants. *ent*-Kaurene synthase is also inhibited, but mostly at a lower degree of activity. To obtain any significant effects in cell-free preparations, relatively high concentrations of chlormequat chloride have to be used and, in some cases, the compound is even inactive. The same is true of mepiquat chloride: in an enzyme system derived from pumpkin (*Cucurbita maxima*) endosperm, concentrations as high as 10^{-3} M of this compound, as well as of chlormequat chloride, did not affect the spectrum of GAs and GA precursors . A possible explanation for this lack of activity could be the fact that these compounds are almost inactive in intact pumpkin plants and this may also be expected for corresponding cell-free preparations. Consequently, chlormequat chloride has been tested with enzymes derived from germinating wheat seedlings, where it gave pronounced effects. Chlormequat chloride lowered the levels of GA_1 in both the shoots and grains of *Triticum aestivum* . Likewise, it led to a dose-dependent reduction of all GAs (GA_{12}, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, GA_8) present in two cultivars of *Sorghum bicolor* .In *Eucalyptus nitens*, it caused a reduction of GA_{20} and GA_1 .

Chlormequat Chloride

Chlormequat chloride was first described in 1960 by N.E. Tolbert from Michigan State University at East Lansing in the USA to reduce shoot length in several plant species. The commercial rights were held at that time by American Cyanamid Company. The significance of using chlormequat chloride as an anti-lodging agent in intense European wheat production was soon recognised. It was introduced under license as Cycocel® in Germany by BASF in 1965. After 50 years, this growth retardant is still the most widely used PGR in cereal production, particularly in wheat, rye, triticale and oats. Together with uses in other cultivated plants, it is, in terms of treated area, the number one PGR on a global scale.

Mepiquat Chloride

After having success with chlormequat chloride, BASF developed another quaternary ammonium compound: mepiquat chloride. After its introduction in 1979, mepiquat chloride became a very successful PGR, particularly in cotton. Alone or in combination with other PGRs, it is also used in other crops. Mepiquat has been detected in samples of processed plant material, such as roasted coffee beans, roasted barley seeds, crust of bread and alfalfa pellets, even if any previous use of mepiquat chloride or other sources of contamination could be ruled out. Recent work involving coffee beans and barley seeds indicates that such findings result from a Maillard-driven degradation of lysine under dry thermal conditions and in the presence of naturally occurring trigonellin. Pipecolatebetain, which is structurally close to mepiquat and which occurs at relatively high concentrations in most vascular plants may also serve as a source of mepiquat under such processing conditions.

1. Compounds with a Nitrogen-Containing Heterocycle

Distinct pyrimidines, 4-pyridines, norbornanodiazetins, imidazoles and triazoles inhibit GA formation. Out of these, ancymidol, flurprimidol, paclobutrazol, uniconazole and its (*E*,3*S*) isomer uniconazole-P (Figure 5-7) are of practical relevance. The triazole-type fungicides tebuconazole and metconazole induce a clear growth-retarding effect particularly in oilseed rape and are used both as fungicides and PGRs in this crop. These growth retardants act as inhibitors of cytochrome P450-dependent mono-oxygenases, which catalyse the oxidative steps from *ent*-kaurene to *ent*-kaurenoic acid and which are primarily located in the endoplasmic reticulum. Steps lying after *ent*-kaurenoic acid, which may still involve mono-oxygenases, do not seem to be affected. The structural feature common to all these inhibitors of *ent*-kaurene oxidation is a lone electron pair on the sp^2-hybridised nitrogen of their heterocyclic ring. In each case, this electron

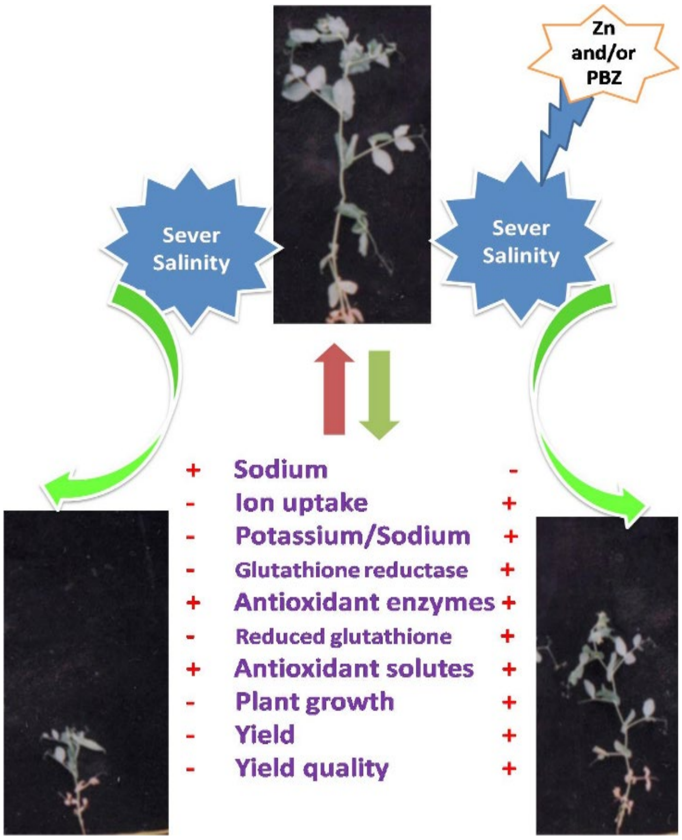


Figure 5: Zinc sulfate (Zn) and paclobutrazol (PBZ) as a cost-effective agent, has multiple biochemical functions in plant productivity. Meanwhile, their synergistic effects on inducing salt tolerance are indecisive in Plants.

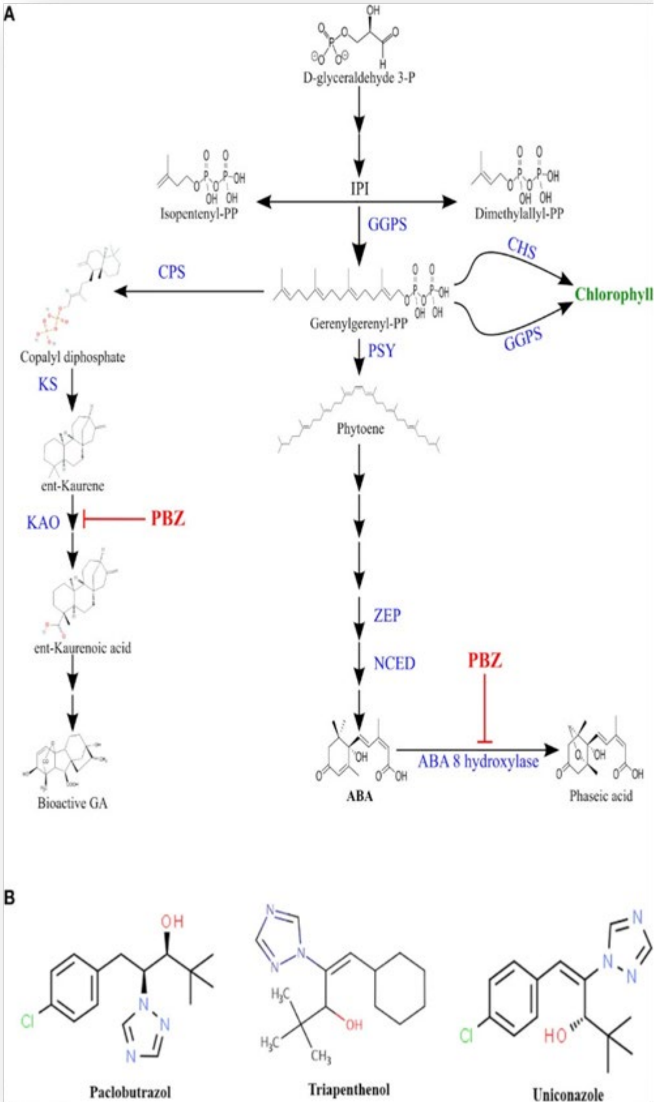


Figure 6: (A) Terpenoid pathway. Paclobutrazol inhibition is indicated by . (*CPS*), Copalyl diphosphate synthase; (*KAO*), ent-kaurenoic acid oxidase; (*KS*), ent-kaurene synthase; (*GGPPS*), geranylgeranyl pyrophosphate synthase; (*PSY*), phytoene synthase; (*ZEP)*, zeaxanthin epoxidase; (*NCED*), 9-*cis*epoxycarotenoid dioxygenase; (*CHS*), chlorophyll Synthase; (*GGRS*), geranylgeranyl reductase. (B) Chemical structure of some triazoles.

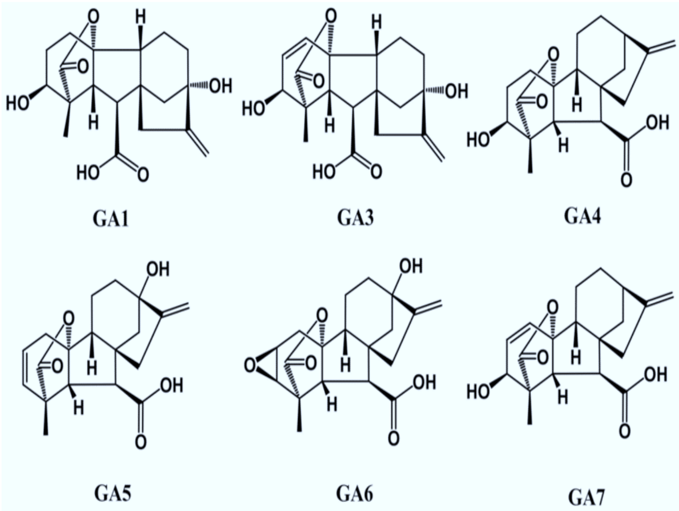


Figure 7: Chemical structures.

pair is located at the periphery of the molecule and it appears likely that it displaces oxygen from its binding site at the protoheme iron. Evidence for such a type of interaction has been presented for ancymidol in microsome preparations of *Marah macrocarpus* and for BAS 111.W (an experimental triazole-type PGR), using microsomal membranes isolated from immature pumpkin endosperm.

Chemical structures Growth Retardents (Table 2)

Depending on the presence or absence of a double bond, uniconazole-P and paclobutrazol possess one or two asymmetric carbon atoms, respectively. Since commercial paclobutrazol consists mainly of the *2RS, 3RS* diastereoisomer, this structure allows virtually only two enantiomers, as does uniconazole-P. Detailed experiments carried out with the optical enantiomers of paclobutrazol have shown that the *2S,3S* form exhibits more pronounced plant growth-regulatory activity and blocks GA biosynthesis more specifically, while the *2R,3R* enantiomer is more active in inhibiting sterol biosynthesis. Fungicidal side activities of paclobutrazol are attributed to its effect on fungal ergosterol formation. It has been demonstrated that the *2S,3S* enantiomer is structurally similar to *ent*-kaurene, whereas the *2R,3R* form is closely related to lanosterol, the respective intermediates of GA and ergosterol biosynthesis. Similar chiralic specificities have been found for uniconazole-P and further related compounds, such as triapenthenol: in all cases, the *S* enantiomer was more inhibitory to *ent*-kaurene oxidation than the respective *R* counterpart. Using computer-assisted molecular modelling methods, clear structural similarities between the norbornanodiazetin tetcyclacis and the growth-retarding forms of paclobutrazol and uniconazole-P with *ent*-kaurene and *ent*-kaurenol could be demonstrated. This indicates that, within limits, distinct structural features are required to bind to and thereby block the active site of the enzyme. One may assume that the structures of the other growth retardants possessing an N-containing heterocycle also fit into this scheme. Clear evidence is available that reduction of shoot growth caused by pyrimidines, 4-pyridines, norbornanodiazetins, imidazoles and triazoles is due to a lowered content of biologically active GAs. Reduced levels of such GAs have, for instance, been analysed by modern techniques under the influence of ancymidol in beans (, paclobutrazol in barley and wheat and in *Eucalyptus nitens* and uniconazole-P in rice and *Sorghum bicolor*.

2. Ancymidol and Flurprimidol

Ancymidol and flurprimidol are closely related in structure. These pyrimidines were introduced by Elanco Products (now part of Dow AgroSciences) in 1971 and 1989, respectively. SePRO Corporation has, meanwhile, become a major distributor. The compounds are used to decrease the rate of growth in a wide range of mono- and dicotyledonous species, including perennial turf grasses, ornamental cover species, herbaceous and woody ornamentals, and deciduous and coniferous trees grown in gardens and parks. There are no registrations in plants used for human or animal nutrition.

3. Paclobutrazol, Uniconazole and Uniconazole-P

Paclobutrazol was first reported as a new and very potent PGR by Lever . Market introduction by ICI Agrochemicals (now part of Syngenta) was in 1986. The first international publication on uniconazole was by Izumi . The closely related paclobutrazol, uniconazole and uniconazole-P are very persistent, with an average half-life of approximately six months, both in plants and in the soil. They are used particularly in countries with warmer climates to control vegetative growth of fruit trees such as avocados, mangos or litchis. Lodging control in rice and the production of more compact ornamentals are further uses of these compounds.

4. Tebuconazole and Metconazole

In addition to blocking ergosterol biosynthesis in fungi, some triazole-type fungicides may also block GA biosynthesis in distinct plant species. This is of practical interest for using tebuconazole and metconazole to control shoot growth in oilseed rape. In Germany, metconazole is additionally in use as a PGR for ornamentals. Kuck and Berg from Bayer AG reported first on tebuconazole, which was commercialised as a fungicide starting in 1988. Metconazole was discovered by Kureha Chemical Industry Co., Ltd. in 1986 with the first international report by Sampson . The compound was jointly developed with Shell International and, later, American Cyanamid Co. and BASF AG. Market introduction (first as a fungicide, then, additionally, as a PGR) was by Cyanamid Agro in France in 1994.

5. Structural Mimics of 2-Oxoglutaric Acid

This group is represented by the acylcyclohexanediones prohexadione-calcium and trinexapac-ethyl. Also daminozide, a succinic acid derivative, falls into this category (Figure 8).

The free acids prohexadione and trinexapac represent the active forms of the respective calcium salt and ethyl ester. Prohexadione is formed immediately from its calcium salt upon dissolving in water. In contrast, trinexapac-ethyl has to be saponified via biochemical processes, which may lead to delays in the onset of action, particularly when weather conditions are unfavourable. Prohexadione and trinexapac block soluble 2-oxoglutarate-dependent dioxygenases involved in late steps of GA biosynthesis. Studies with cell-free preparations have revealed that most steps after GA_{12} are inhibited by prohexadione and other acylcyclohexanediones. Enzyme kinetic data indicate that the retardants act competitively with respect to 2-oxoglutarate, a cosubstrate for these enzymes. GA 3-oxidase, which catalyses hydroxylations at position 3β (e.g. the formation of GA_1 from GA_{20}) and also GA 2-oxidase (hydroxylating at position 2β – e.g. the conversion of GA_1 into GA_8) appear to be the primary targets of acylcyclohexanediones. These findings are supported by analytical data, generally showing that growth reduction is accompanied by lowered levels of biologically active GAs (e.g. GA_1) and their inactive metabolites (e.g. GA_8), but increased concentrations of the inactive immediate (e.g. GA_{20}) and earlier precursors. In selected cases, compounds like prohexadione-calcium and trinexapac-ethyl may, paradoxically, lead to increases in shoot growth, most likely by protecting endogenous active GAs from being metabolically inactivated by GA 2-oxidase. Likewise, the inactivation of exogenously applied GA_1 by 2β-hydroxylation can be inhibited by simultaneous treatment with an acylcyclohexanedione, resulting in increased GA activity see also (Figure 10). 2-Oxoglutarate-dependent dioxygenases catalyse many different reactions in plant metabolism (Farrow and Facchini). Accordingly, some important side activities of prohexadione-calcium and trinexapac-ethyl have been detected: high dosages of these and other acylcyclohexanediones inhibit the formation of anthocyanins in flowers and other plant organs. It has been suggested that 2-oxoglutarate-dependent dioxygenases, in particular flavanone 3-hydroxylase, which is involved in the biosynthesis of anthocyanidins are targets for these growth retardants. This hypothesis has been confirmed by the finding that young shoots of apple are unable to convert eriodictyol by 3-hydroxylation into flavonoids such as catechin after treatment with prohexadione-calcium. Instead, eriodictyol accumulates and large amounts of luteoliflavan, which does not normally occur in apple tissue, can be found. This shift in flavonoid metabolism is seen as the major underlying reason for reduced susceptibility of treated pome fruit trees to bacterial and fungal diseases: luteoforol, the highly reactive and unstable precursor of luteoliflavan, shows clear *in vitro* biocidal activity against a number of bacterial and fungal pathogens, including *Erwinia amylovora* and *Venturia inaequalis*, the causal agents of fire blight and apple scab, respectively. Apigeninidin, luteolinidin and their derivatives,

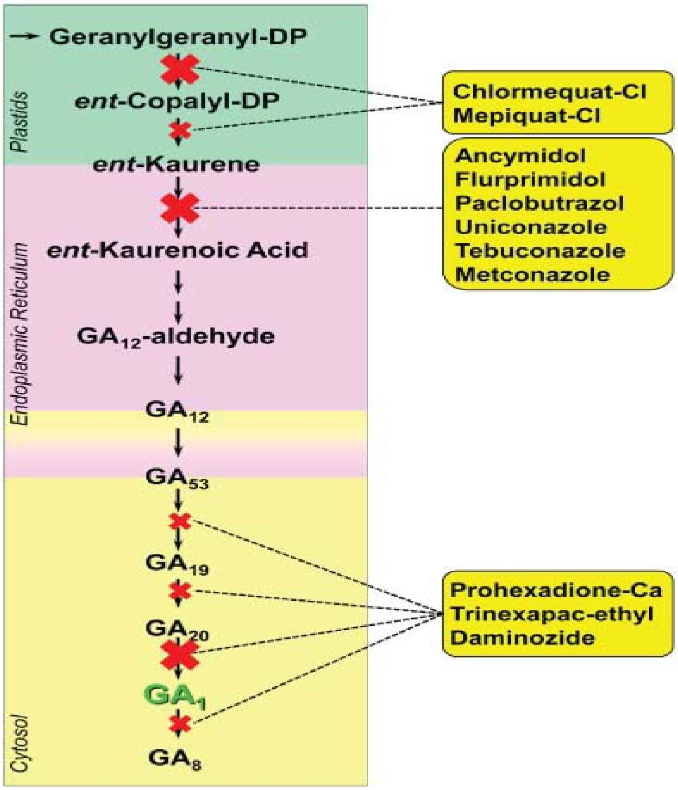


Figure 8: Main steps of gibberellin biosynthesis leading to biologically active GA1 and points of inhibition by plant growth retardants. The cellular locations of the reactions is indicated by different greyscales. (The con]version of GA12 into GA53 can be located in both the endop\lasmic reticulum or the cytosol.)

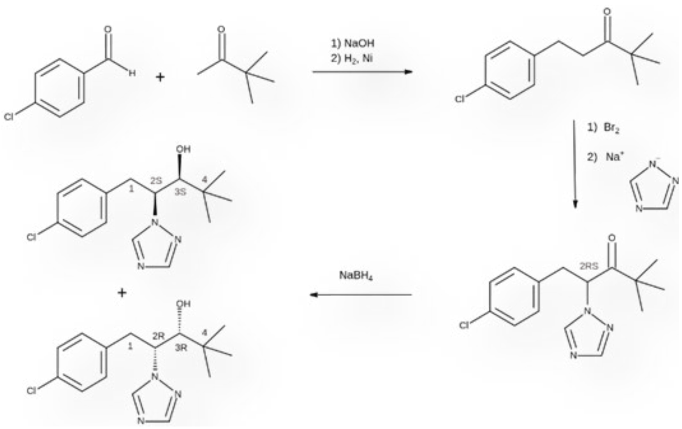


Figure 9: The first synthesis of paclobutrazol was disclosed in patents filed by an ICI group working at Jealott's Hill. 4-Chlorobenzaldehyde and pinacolone are combined in an aldol condensation to form a chalcone which is hydrogenated using Raney nickel as catalyst to give a substituted ketone.

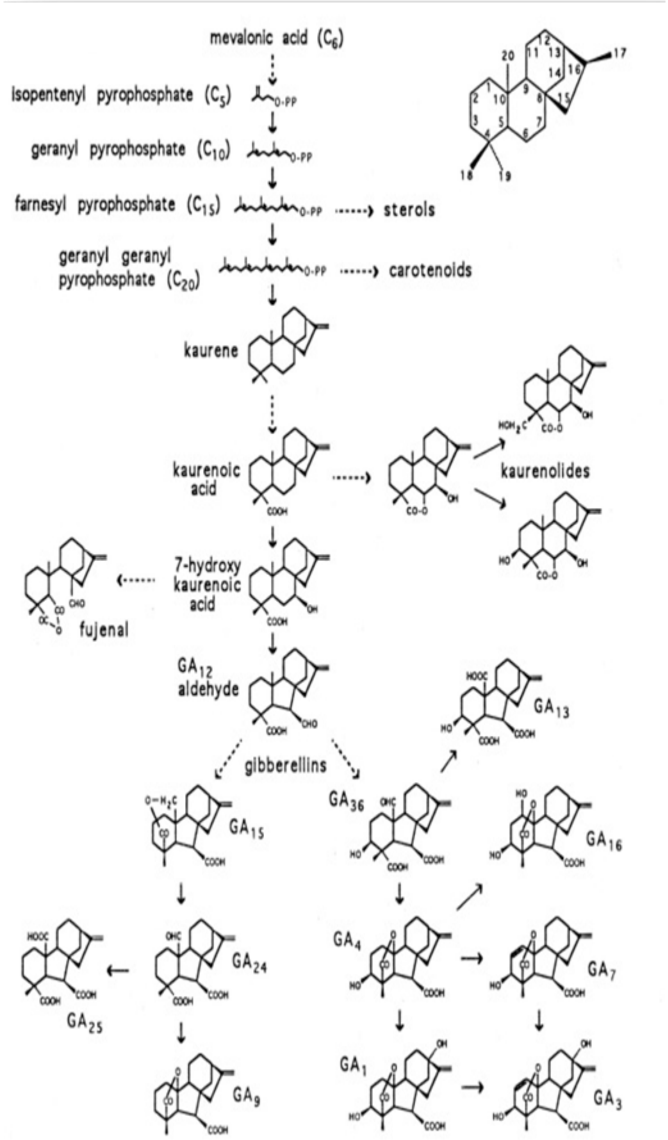


Figure 10: Main steps of gibberellin biosynthesis leading to biologically active GA_1 and points of inhibition by plant growth retardants. The cellular locations of the reactions is indicated by different greyscales. (The conversion of GA_{12} into GA_{53} can be located in both the endoplasmic reticulum and the cytosol.)

which are also 3-deoxy flavonoids, act as phytoalexins in *Sorghum bicolor*. For more information see Römmelt, Halbwirth, Rademacher and Halbwirth. Prohexadione-calcium and trinexapac-ethyl reduce ethylene formation in sunflower cell suspensions and in leaf disks of wheat. Ethylene is generated from aminocyclopropanecarboxylic acid (ACC) in a reaction catalysed by ACC oxidase. This is a dioxygenase that requires ascorbic acid as a cosubstrate, whereas 2-oxoglutaric acid and similar compounds inhibit its activity. Employing an enzyme system prepared from ripe pear fruits, it could be shown that prohexadione-calcium also inhibits ACC oxidase, presumably by displacing ascorbate from the enzyme's active site.

Long after its introduction, it could also be demonstrated that daminozide interferes with GA biosynthesis. Considering structural similarities between daminozide and 2-oxoglutaric acid and newly interpreting older results from the literature, it was proposed that daminozide, like acylcyclohexanediones, would block GA formation as an inhibitor of 2-oxoglutarate-dependent dioxygenases. This hypothesis was later proved by working with an enzyme preparation derived from cotyledons of *Phaseolus coccineus* and by analyzing the GAs of treated peanut plants.

6. Daminozide

The plant growth-regulatory activity of daminozide was first reported by Riddell and its market introduction by Uniroyal Chemical Co., Inc. followed in 1963. Daminozide was used in several fruit tree species, particularly in apple, to reduce vegetative growth and improve fruit colouration and firmness (trade name: Alar®). Treatment of peanut plants led to more compact plants, allowing for a more efficient harvesting (trade name: Kylar®). However, due to toxicological concerns, its use on food crops was banned in 1989. The compound is still used as B-Nine® in ornamentals, with Chemtura Corp. a major supplier.

Trinexapac-Ethyl and Prohexadione-Calcium

Trinexapac-ethyl was introduced to the scientific community by Kerber and Adams . Ciba-Geigy AG (now Syngenta) started with sales in 1992. Prohexadione-calcium originates from Kumiai Chemical Industry Co., Ltd. and Ihara Chemical Industry Co., both of Japan. The first international publication on its properties as a growth retardant was by Nakayama. In 1991, BASF obtained a license to develop this compound in Europe, North America and several other countries. Kumiai launched prohexadione-calcium in Japan as an anti-lodging agent in rice, while BASF started commercialisation of the compound in combination with mepiquat chloride as a stem stabiliser in cereals in 1998 and solo for use in pome fruit trees in 2000.

7. Dihydro-Gibberellins

17-Dihydro-GAs represent the most recent group of growth retardants. A number of different structures of this type, mostly GA_5 derivatives, have been shown to reduce shoot elongation in *Lolium temulentum* and other grasses. Evidence is available that their growth-retarding activity is due to an inhibition of dioxygenases, which catalyse the late stages of GA metabolism, particularly GA 3-hydroxylation. Treating plants with 16,17-dihydro-GA_5 results, indeed, in changes of GA levels similar to the ones caused by acylcyclohexanediones: In *Lolium temulentum* and in *Sorghum bicolor* the levels of GA_1 declined, whereas GA_{20} accumulated significantly.

With a view to find new anti-lodging compounds for small grains, several 16, 17-dihydro-GA_5 derivatives have been systematically tested in suitable formulations. As a result of these investigations, *exo*-16, 17-dihydro-GA_5-13-acetate emerged as the most active growth retardant ever known for graminaceous plants (Figure 11). Under greenhouse conditions effects with as little as 500 mg per hectare can be monitored in wheat and barley. However, in order to reduce the risk of lodging under practical conditions, rates in the range of 20 g per hectare have to be used. In order to explain the high biological activity it can be assumed that *exo*-16,17-dihydro-GA_5-13-acetate and related structures compete very effectively in grasses with the natural GA substrates, e.g. GA_{20}, for the respective enzymatic sites. In contrast to graminaceous plants, *exo*-16, 17-dihydro-GA_5-13-acetate and related structures are virtually inactive in reducing shoot growth in any other plant species tested. In spite of the promising results obtained with *exo*-16, 17-dihydro-GA_5-13-acetate, its synthesis from GA_3 in bulk quantities proved to be too expensive for commercialisation (Rademacher, unpublished).

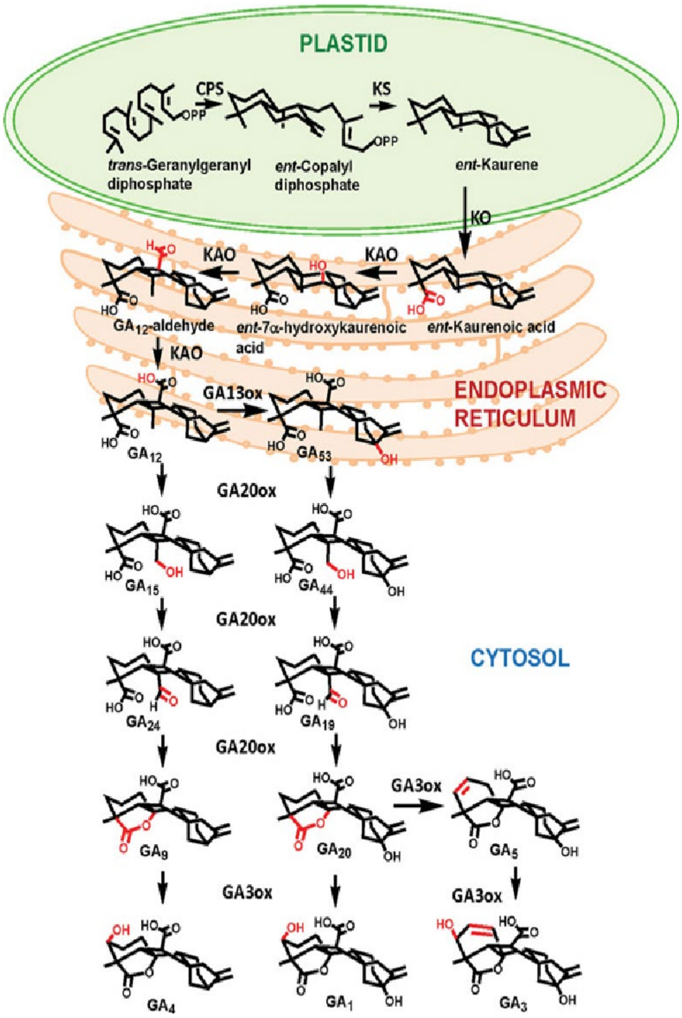


Figure 11: The GA-biosynthetic pathway from trans-geranylgeranyl diphosphate to GA1, GA3 and GA4.

8. Uses for Gibberellins and Inhibitors of Gibberellin Biosynthesis in Crop Production

Space limitation does not permit coverage of all practical uses that have been established for the different GAs and inhibitors of GA formation. Therefore, only the major uses and some recent additions are referred to. Detailed information on how to use a given PGR in a distinct crop can be found in the respective labels, which are provided by the distributing companies via the internet.

9. Wheat, Barley, Rye, Oats and Other Small-Grain Cereals

The production of wheat and other small grains has undergone drastic changes since the introduction of science-based agricultural methods. This development is particularly obvious in West Europe with its maritime climate, long days at the time of grain filling and other growing conditions favourable for winter wheat. Productivity data are almost continuously available for Germany since 1878. Starting at yield levels of some 1.3 tonnes per hectare, just above 2.0 t/ha was reached prior to World War I and, after a post-war dip, again in the 1930s. However, enormous increases in yield levels could be achieved since the beginning of the 1950s: within six decades, productivity was almost quadrupled from approximately 2.0 to 7.5 t/ha. Similar degrees of intensification were reached in countries with comparable production conditions such as France and the United Kingdom (UK) (FAOSTAT; Rademacher. Likewise, seed yield per unit of land could also be raised significantly in other small-grain species such as barley, rye, triticale, oats and spelt. It is estimated by several authors that the increases in productivity have mainly resulted from increased and better-targeted fertilisation (40–45%), followed by breeding (25–30%) and crop protection plus soil management (25–30%). These factors for success are closely interconnected: dispensing, for instance, with fungicides treatments could certainly lead to yield reductions of much more than 30% under adverse production conditions. It must also be noted that the mentioned achievements have been a major prerequisite for creating modern and wealthy societies in industrialised countries with limited area available for agriculture.

With rising production intensity in Germany, UK, France and other countries, lodging became increasingly a problem in cereal cultivation in the 1950s and 1960s: heavy ears could no longer be kept upright by long stems, particularly when their leverage was increased by wind and rain. Lodging occurs mainly during the two months preceding harvest and may drastically reduce profitability through reduced yield and quality and increased costs for harvesting and grain drying. If lodging occurs early (e.g. shortly after anthesis), its impact on seed yield and quality will be more intense as compared with lodging close to harvesting. Under UK growing conditions, severe lodging in cereals may be expected in one out of three to four years. It is likely that the situation is similar in other countries with high intensities of production. Assuming an average yield of 7.5 t/ha and a producer price of 180.00 €/t for wheat, a reduction in yield of 20% due to lodging is equivalent to 270.00 €/ha. Additional financial losses are likely to result from inferior grain quality and increased costs for harvesting and grain drying. The use of anti-lodging products in wheat has been banned in Sweden in 1987 in order to reduce production intensity and, thereby, lower the negative impacts agriculture may have on the environment. Not least due to this, a reduction of wheat productivity by approximately 25% has resulted in comparison to countries with similar production conditions such as Denmark, Germany or the UK (data from FAOSTAT). A few years ago, the ban on PGR use in Swedish wheat production was lifted.

Two forms of lodging can be differentiated: (1) stem lodging occurs when heavy wind and rainfall exert a force that breaks the stem base. Often, stem lodging is found after a severe thunderstorm. Eyespot, caused by *Pseudocercosporella herpotrichoides*, and other foot rot diseases may intensify the risk of stem lodging. (2) Root lodging is typically observed when, after several days of rainfall, the plant's root system is unable to keep the stem, with its heavy, water-soaked ear, upright. The risk of both forms of lodging is strongly influenced by cultivar and husbandry factors, including sowing date, seed rate, drilling depth and rate of nitrogen application. In spite of this knowledge, the use of anti-lodging products has become an integral part of the production system in order to secure seed yield and quality. These products reduce stem length, thereby lowering the leverage of the ear and other upper plant parts. Increased stability results also from histological changes caused in the stems. Evidence is also available that anti-lodging agents lead to increases in root growth, thereby providing better anchorage against falling over and enabling plants to absorb water and nutrients more effectively. Breeding for short-strawed varieties has only partly contributed to stem stabilisation under production conditions targeted for high yield and quality. It is suggested that the optimum mature height of winter wheat in the UK is close to 80 cm. Shorter stems would have a negative impact on light interception, encourage leaf diseases and make harvesting more difficult. Consequently, breeders are relying to a considerable extent on stem shortening 'when needed' by means of

PGRs. This is also reflected by the fact that on approximately 34% of the land in Germany devoted to seed propagation in 2015 for wheat, barley, rye, triticale, oats or spelt varieties with a lodging susceptibility rated from 'medium' to 'very high' (grades 5 to 9) were grown. Even grade 4 varieties ('low to medium'), which are propagated on approximately 42% of the area (Anonymous,), are candidates for anti-lodging treatments, particularly when grown at high intensity. This situation is similar in the other major cereal-producing countries in Europe.

After its market introduction in 1965, chlormequat chloride was the first PGR to be used on a large scale as an anti-lodging agent in cereal production. Meanwhile, additional PGRs have been introduced for stem stabilisation and lodging control in small grains. Combinations of chlormequat chloride or mepiquat chloride with trinexapac-ethyl or prohexadione-calcium, either by tank-mixing or by using ready-mix formulations, currently represent the best technical solutions for lodging control: chlormequat chloride and mepiquat chloride act at relatively low temperatures and may be used early in the season. Their onset of activity is relatively slow, but long-lasting. Complementing these compounds, trinexapac-ethyl and prohexadione-calcium act comparatively quickly, but are relatively short-lived. Furthermore, they require somewhat higher temperatures for activity. When chlormequat chloride is applied at early stages of tillering, it increases the number of fertile tillers in addition to reducing stem length. This can be of special interest after winter losses of plants. Barley is less responsive to chlormequat chloride and mepiquat chloride than wheat, rye, triticale or oats. Therefore, products containing trinexapac-ethyl and prohexadione-calcium or the ethylene-releasing ethephon are preferentially used to reduce the risk of lodging in this crop. Additional information on lodging in small grains and on the use of anti-lodging agents in cereal production can be found in Easson . Area- and value-wise, stem shortening in small grains (and in intense rice production in Japan and South Korea) to reduce the risk of lodging is the main application of PGRs worldwide. Indeed some 25% of the global PGR sales (equalling approximately € 270 million) are represented by stem stabilisers. The usage of such products is general practice in countries with intense production of wheat, barley, rye, triticale and oats, such as France, Germany and the UK. For instance, 89% of the winter wheat, 76% of the winter barley, 73% of the oats and 100% of the winter rye acreage were treated with anti-lodging products in the UK in 2012. Chlormequat chloride and trinexapac-ethyl rank third and seventh, respectively, in terms of treated area of all crop protectants in all field crops in this country. Anti-lodging products for small-grain cereals play only a minor role in countries where these crops are generally grown at relatively low levels of intensity. In the USA, Canada and Australia, climatic factors, in particular unfavourable temperatures and shortage of moisture, represent the main restrictions. Since sufficient arable land is available, significant surpluses for export can still be produced on a national level. However, the use of PGRs is indispensable when wheat or other small grains are grown more intensively in areas where this is possible. To date, chlormequat chloride and ethephon represent only niche markets in Canada. Ethephon has recently been withdrawn, whereas trinexapac-ethyl has just been registered as an anti-lodging agent in the USA. A survey with agronomists working for major rural supply companies across Australian grain-growing regions has recently been conducted: only 20% of the participants recommended the use of PGRs for crop management in wheat. Chlormequat chloride and ethephon are available for cereal lodging control in Australia. The registration of trinexapac-ethyl is expected for 2015.

1. Rice

In direct-seeded rice, application of GA_3 as a seed dressing is relatively common. This treatment significantly improves germination percentage, seedling emergence and seedling height and is especially important at sub-optimal temperatures. As in small grains, lodging can also be a severe problem in intense rice production. However, modern semi-dwarf cultivars produce relatively high yields, while being largely lodging-resistant. In spite of this option, many farmers still prefer to grow tall but lodging-susceptible varieties, which are tastier and achieve a higher price. For instance, Japanese consumers prefer rice from the traditional long-strawed cultivar 'Koshihikari', which is grown on approximately 35% of the Japanese rice-producing area. Without treatment, 'Koshihikari' typically reaches a final shoot length around 110 cm and is very prone to lodging. As with cereal grains, several husbandry methods can be employed to minimise lodging in rice. Reducing overgrowth of stem and leaves by applying PGRs at mid-tillering stage reduces lodging incidence and gives a generally improved plant stature. In 1988 some 12% of the Japanese rice-growing area was treated with stem stabilisers. One may assume that this percentage has at least remained constant. Whereas stem stabilisers are applied to cereal plants by spraying the leaves, granules for throwing into the paddy field are preferred in rice production, particularly in Japan and South Korea. In order to be absorbed via the roots, such stem stabilisers have to be relatively persistent. Accordingly, preparations based on long-lived uniconazole-P and paclobutrazol are the main active ingredients used as anti-lodging agents in this crop. Prohexadione-calcium, which has to be spray-applied, is only of minor importance.

2. Sugarcane

Ethephon, glyphosate or other herbicides are often used as chemical ripeners in sugarcane production. They have to be applied via aircraft or ground-operated booms. By rapidly reducing the sink demand of young and growing plant parts, sucrose storage within the stalk is accelerated leading to high harvest yields. Even better effects can be achieved with trinexapac-ethyl. Meanwhile, trinexapac-ethyl is registered in Brazil, Australia, the USA and other countries for use in this crop.

3. Pasture and Turf Grasses

GA_3 has found some use in the USA and other countries to stimulate shoot growth in pasture grasses. However, much more interest is directed towards reducing shoot elongation. Here, inhibitors of GA biosynthesis are important in high-intensity fine turf, particularly on golf courses. A main reason is to reduce vertical leaf growth, which leads to smoother and more uniform playing surfaces. Darker leaf colour, intensified root growth, reduced water consumption, seed head suppression of unwanted annual bluegrass *(Poa annua)*, and, not least, less need for mowing are additional benefits. Trinexapac-ethyl, paclobutrazol, flurprimidol and different combinations of these retardants are the main PGRs used for this purpose in the USA. Similar products for growth regulation in fine turf grasses are available in several other countries. Prohexadione-calcium is available as a PGR for use on turf grasses in Germany. A recent survey of growth regulators in turfgrasses is available from March. As in cereal grain production, lodging may also be a problem when grasses are grown for seed production. Trinexapac-ethyl and prohexadione-calcium are the main active ingredients used to reduce this risk, particularly in the US state of Oregon, where grass grown for seed is a major business. Prohexadione-calcium has a small advantage in performance: most likely due to its more immediate action after application.

4. Oilseed Rape

Winter oilseed rape (*Brassica napus, ssp. napus*) has become an important oilseed crop in many European countries and elsewhere.

It can be kept from too intensive growth in late autumn, thereby making it less vulnerable to freezing and desiccation in winter. Later in its development, yield losses due to lodging may occur, which can also be reduced by stem-shortening agents. The leading compounds used are the triazoles tebuconazole and metconazole, which are marketed for this purpose in France, the UK, Germany and several other European countries. Recent introductions are the combination of metconazole with mepiquat chloride and paclobutrazol with the fungicide difenoconazole. Tebuconazole and metconazole are primarily used as fungicides in a number of crop plants including oilseed rape. Their shoot growth-reducing activity is restricted to oilseed rape and a few other species.

5. Cotton

Cotton is a perennial plant, which, however, is cultivated in most countries in an annual cycle. In its native habitat, cotton plants do not die in the autumn, but continue to grow until environmental conditions become too restrictive. Another growth characteristic associated with its perennial nature is its indeterminate fruiting habit. Rather than flowering during a distinct period following vegetative growth, cotton plants simultaneously produce vegetative and fruiting structures. In order to enable high yield and quality formation and to allow efficient mechanical harvesting, intense usage of PGRs has become a standard practice in many cotton-producing countries. The regime typically comprises control of vegetative growth by mepiquat-containing products, defoliation by thidiazuron and boll-opening by ethephon. The growth-retardant mepiquat chloride was commercially introduced for vegetative growth control in cotton in the USA in 1980 and has since become a cornerstone of modern cotton production. Instead of or in addition to mepiquat chloride, chlormequat chloride is used for the same purpose in some countries, for instance in Australia. Another variant is mepiquat pentaborate, which was brought to the US market in 2003. Due to its more rapid uptake (Rademacher, unpublished) and the nutritive value of the contained boron, the pentaborate form of mepiquat leads to better plant performance under distinct growing conditions as compared to its chloride salt (e.g. Norton and Borrego). Mepiquat chloride is also available in combination with kinetin or cyclanilide. However, in the majority of cases the different mepiquat-containing products on the market give comparable results Treatment of cotton plants with mepiquat starting at the beginning of flowering reduces the intensity of new growth and, thereby, improves the sink strength of the first six to ten fruiting branches. This is of great importance because the bolls retained in this part of the plant will give the highest and earliest yields. The shifting of assimilates into the older fruiting structures is at the expense of younger fruits, which the plant is continuously forming, even late in the season and which are unlikely to contribute to yield at mechanical harvesting. Plants treated with mepiquat produce higher yields and can, typically, be harvested three to ten days earlier than untreated plants. Earliness is of great importance because harvesting can often be performed prior to periods of rainfall. This would also reduce the incidence of fungal diseases. Further benefits from short-season production may result from savings in late-season irrigation and insecticide costs. Finally, decreases in quantity and quality of the lint due to weathering are reduced in the oldest, first-opened cotton bolls. Valuable contributions on different aspects of using mepiquat chloride in cotton have been presented by Cathey and Meredith.

6. Peanuts

The foliage of peanut plants is still green at harvesting such that excessive vine growth may reduce digging efficiency. Prohexadione-calcium, which is registered for this use in the USA, retards vegetative growth and improves the visibility of rows, resulting in improved harvesting efficiency. Pod yield and kernel quality may also be improved.

7. Opium Poppy

Lodging is also a problem in poppy cultivation. Trials in Tasmania, Australia, where a major portion of the global legal opium poppy production is located, were therefore conducted with trinexapac-ethyl and prohexadione-calcium to overcome this problem. Surprisingly, it was found that treatments with these compounds did not only improve lodging resistance, but also changed the alkaloid spectrum in the harvested plant material in a desirable way: more thebain, which is of higher value, is formed at the expense of lower-valued oripavine. It has been postulated that a 2-oxoglutarate-dependent dioxygenase, which catalyses the conversion of thebain into oripavine is blocked. Trinexapac-ethyl is now registered in Australia for use in opium poppy.

8. **Fruit Trees:** Growing in Temperate ClimateGibberellins and growth retardants have been used for many years in the cultivation of pome and stone fruit trees such as apples, pears, peaches, plums and cherries, which are typically grown in temperate climates. It is much more difficult to apply PGRs in such perennial fruit crops than in annual arable crops. Mistakes made in one year may often lead to problems in the years following. On the other hand and in contrast to field crops, fruits, typically, represent a higher-value crop and, hence, allow the use of more elaborate and expensive products. Recent overviews on different PGR uses in fruit production have been presented by Petracek *et*, Rademacher and Brahm and Looney and Jackson. Table gives an overview of uses for GAs and inhibitors of GA biosynthesis in fruit trees.

Gibberellins

In pears, parthenocarpic fruit formation can be achieved with GA_3. This may lead to an increased fruit set and is particularly important when the generative part of the flower has been damaged by frost or when there has been poor pollination. Some varieties, e.g. 'Williams' and 'Abate Fetel', are more responsive than others. GA_3 may also be used in sweet cherries to produce brighter-coloured, firmer fruits with increased size. The mixture of GA_4 and GA_7 is often used by apple growers to reduce fruit russetting, a superficial disorder in which the fruit surface is interrupted by raised corky outgrowths. Evidence is available that the GA_4 component is the primary active ingredient for the control of russetting, whereas GA_7 rather inhibits flowering and reduces return bloom (Carlson and Crovetti, . Hence, preparations low in GA_7 may have an advantage for this use. Combined with the cytokinin benzyladenine, $GA_{4/7}$ is also used to improve size and shape of apples fruits. Fruit elongation and development of more prominent calyx lobes in Red Delicious apples are of special interest in North America.

Inhibitors of Gibberellin biosynthesis

Proper tree growth management is of major concern in commercial pome fruit production: avoiding excessive shoot growth will induce earlier flowering and fruiting in young trees. Older trees have to be restricted to their allocated space, thereby reducing crowding and shading. The crowns of fruit trees should be sufficiently open to allow good light penetration to the inner parts of the canopy, thereby improving photoproductivity and fruit colouration. Additionally, efficient crop protection is significantly facilitated in such trees. Since the beginning of professional apple and pear production, several techniques have been employed to avoid excessive shoot growth: different types of dwarfing rootstocks and scions have become available, particularly for apple. Different cultivars may also show significant differences in shoot vigour. Dormant and summer pruning are the main cultural practices for shoot control in addition to regulation of fruit set. Other methods include: root pruning, root restriction, stem girdling or stem sawing, limb bending, breaking or wounding, and restrictive fertilisation and irrigation. However, each of these methods is cost-intensive and/or bears a high risk of failure. Furthermore, part of the trees' assimilates or potential assimilates are lost.

Chemical regulation of shoot growth has been practiced over many years by using distinct inhibitors of GA biosynthesis. However, health concerns about daminozide have led to the ban of this compound in edible crops. Additional negative attitudes towards using PGRs for shoot growth regulation resulted from unacceptably high residues of chlormequat in pear fruits, due to excessive use in this crop. In essence, only paclobutrazol remained registered in some countries at the end of the 1990s as a regulator of shoot growth in pome and stone fruits. However, this compound is extremely persistent. Its half-life in the plant and in the soil is in the range of six months. Application is via spraying or as a soil drench. In order to avoid effects on following crops, the label for the UK recommends to with hold using paclobutrazol for up to seven years if an orchard is due for grubbing. Consequently, paclobutrazol is currently registered for use in fruit trees in the UK and Spain as the only European countries. Likewise, there is no legal use of this compound in fruit trees in the US or in Canada.

A new option for controlling growth of fruit trees became available with the introduction of prohexadione-calcium, which has a half-life in plants of approximately 10 to 14 days and of less than one day in microbially active soil. Due to its simultaneous effects on the formation of GAs, ethylene and flavonoids, treated pome fruit trees benefit in several ways: Figure 12 demonstrates that GA_{19} and GA_{20}, inactive precursors of GA_1, accumulate in apple shoots after application of prohexadione-calcium. In contrast, levels of GA_1, the main active GA in apple shoots, decline. The result is reduced shoot elongation. The labour needed for dormant and summer pruning in treated trees is typically reduced by approximately 30%. Furthermore, a more open canopy allows better light penetration into the central parts of the crown leading to improved fruit quality. Spray application of crop protectants is also made more efficient.

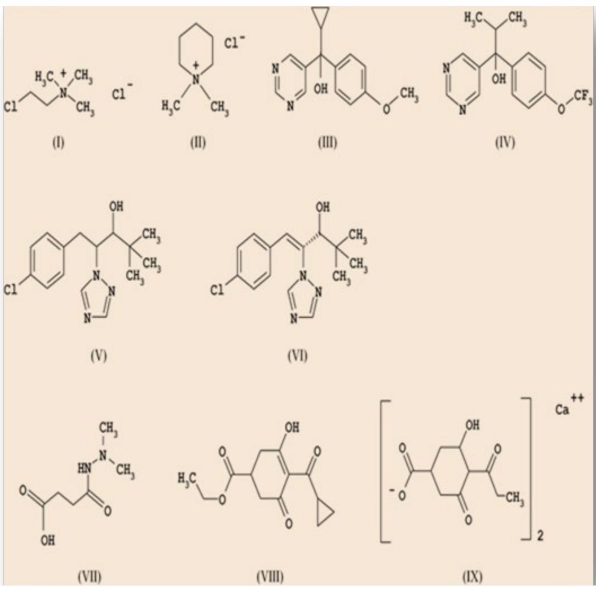


Figure 12: Chemical structures of Structures of inhibitors of GA biosynthesis: (I) Chlormequat chloride = chlorocholine chloride (CCC) = (2-chloroethyl)-trimethylammonium chloride, (II) mepiquat chloride = 1,1-dimethylpiperidinium chloride, (III) ancymidol = cyclopropyl-(4-methoxyphenyl)-5-pyrimidinylmethanol, (IV) flurprimidol = 2-methyl-1-(5-pyrimidinyl)-1-[4-(trifluoromethoxy)phenyl]-1-propanol, V paclobutrazol = (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol, (VI) uniconazole-P = (1E,3S)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-1-penten-3-ol, (VII) daminozide = N-(dimethylamino) succinamic acid, (VIII) trinexapac-ethyl = ethyl (RS)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate, and IX prohexadione-calcium = calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate.

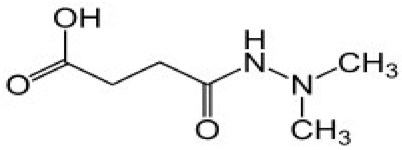
The inhibition of ethylene formation may be employed to increase fruit set. Since assimilates no longer needed for shoot growth are available, fruit size and internal quality, as well as return bloom do not suffer, provided that over-cropping is avoided by appropriate dosaging and timing. Blocking flavanone 3-hydroxylase activity in pome fruit shoots leads to a *de novo* formation of 3-deoxyflavonoids, such as luteoforol and luteoliflavan. Luteoforol shows biocidal activity against several bacterial and fungal pathogens, including *Erwinia amylovora* and *Venturia inaequalis*, the causal agents of fire blight and apple scab, respectively. The triggering of such phytoalexin-like compounds explains why treated pome fruit trees are significantly less affected by a number of diseases. This induction of defence is of particular value to control shoot fire blight, a disease caused by the bacterium *Erwinia amylovora*, which is primarily controlled by using antibiotics, a treatment that is highly controversial. The action of fungicides is promoted by this enhanced resistance, but also by the more open canopy resulting from reduced levels of active GAs. Apple and pear trees treated with prohexadione-calcium are also less affected by insect pests, such as aphids, psyllids and leafhoppers, which synergises the action of insecticides. The underlying biochemical mode of action could also be changes in the spectrum of flavonoids, which may, for instance, repel sucking and chewing insects. However, paclobutrazol-treated trees are also less affected by insect pests. This indicates that a thicker epidermis or thicker cell walls resulting from reduced elongation growth may also be the reason for less insect attack.

Fruit and nut trees growing in subtropical and tropical climates

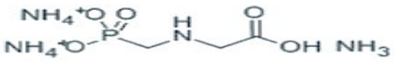
Uses of GA_3 and growth retardants in fruit and nut trees growing in warm climates are listed in Table **12.7**. GA_3 is of major interest in most citrus-growing countries for use with a variety of citrus species and varieties. It is mainly applied to increase fruit set, delay harvesting and improve fruit quality. In Navel oranges, rind aging may be delayed. Growers can spray part of their groves to allow sequential harvesting after picking fruits from non-treated blocks early. A delayed harvest may also be used to 'store' citrus fruits (which are non-climacteric) on the tree until a better market window opens (Figure 13). Fruits from treated trees will also display less rind disorders (e.g. rind staining, water spotting, puffy rind, sticky rind). 'Creasing' or 'puff and crease' is an important rind disorder, which is of particular concern in Navel and Valencia oranges and in Satsuma mandarins. The disorder occurs when the rind tissue (orange-coloured flavedo layer plus epidermis) continues to stretch when the albedo layer (white tissue under the rind) has stopped growing. As a result, some parts of the fruit surface appear inflated ('puffy'), whereas other areas are indented (creased). Puffiness occurs also in grapefruits. Application of GA_3, together with certain cultural practices, is employed to reduce the incidence of this disorder. GA_3 may also be used to increase fruit set and yield in Navel and Valencia oranges, as well as in clementines, tangelos and tangerines.

Grapevines

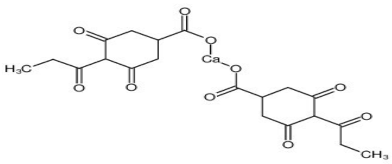
Testing of GA_3 on wine and table grapes started in the late 1950s



(a) Daminozide =4-(2,2-Dimethylhydrazin-1-yl)-4-oxobutanoic acid



(b) Trinexapac-ethyl = ethyl (RS)-4-cyclopropyl (hydroxy) methylene-3,5-dioxocyclohexanecarboxylate



(c) Prohexadione-calcium = calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate

Figure 13: Chemical structures of (a) daminozide = *N*-(dimethylamino) succinamic acid, (b) trinexapac-ethyl = ethyl (*RS*)-4-cyclopropyl (hydroxy) methylene-3,5-dioxocyclohexanecarboxylate and (c) prohexadione-calcium = calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate.

same day.

	GA_1 Produced
	% of control
GA_{20} alone	100[a]
GA_{20} + GA_5	52.4 ± 1.2
GA_{20} + *exo*-16,17-dihydro GA_5	51.4 ± 5.7
GA_{20} + *endo*-16,17-dihydro GA_5	51.2 ± 0.9
GA_{20} + 16,17-dichloromethano-dihydro GA_5	27.5 ±1.9
GA_{20} + 16,17-methano-dihydro GA_5	30.3 ± 6.0
GA_{20} + *exo*-16,17-dihydro GA_{20}	21.1 ± 2.6
GA_{20} + *endo*-16,17-dihydro GA_{20}	21.9 ± 0.4
GA_{20} + *exo*-16,17-dihydro GA_5-acetate	3.6 ± 0.1
GA_{20} + *endo*-16,17-dihydro GA_5 acetate	6.0 ± 0.3
GA_{20} + *exo*-16,17-dihydro GA_{20}-acetate	0.26 ± 0.01

[a]90 ng of GA_1 was produced in 5 min.

Figure 14: Table I. Inhibitory effects of GA5 and a range of ring D-modified GA5 and GA20 derivatives on 3b-hydroxylation of GA20 by recombinant AtGA3ox1.

and led to the first major practical uses of this new plant hormone. As far as can be judged, the main use of GA_3 on a global scale is still in grapevines, particularly in seedless table grapes, where its use has become a standard practice. Table grapes without seeds are attractive to consumers. However, their small size represents a problem for commercialisation. GA_3, applied at the right time and dosage for a given variety, is used to overcome this problem. In general, treatment at approximately 20 mm of cluster length may be used to 'stretch' the rachis, application at 30% to 80% cap (calyptra) fall reduces berry set and later treatments increase berry size. Under advanced production conditions (e.g. in California, Italy or Chile), seedless varieties may be grown on more than 80% of the area where they are virtually all treated with GA_3. For additional reading see Dokoozlian and Peacock and Casanova. Several varieties of (seeded) wine grapes tend to form very dense clusters. At veraison, the pressure exerted by the ripening and expanding adjacent berries causes wounding and leakage of juice, which may lead to bunch rot (caused by *Botrytis cinerea*) and also to sour rot (caused by different bacteria and yeasts) (Figure 14) . This is of particular concern to vintners, when rainfall keeps the clusters moist, thereby facilitating the spreading of diseases. GA_3 is used to elongate the rachis and reduce berry set. The window for application lies between a cluster length of approximately 7.5 cm and the end of flower opening. Earlier treatments lead primarily to a stretching of the inflorescence, whereas later timings cause berry thinning or the formation of non-seeded shot berries. Berry abortion may result from too high GA levels, a combination of endogenous hormone produced by the developing seeds and that applied externally. Many varieties, for instance of the Pinot family and Chardonnay, respond relatively well to treatment with GA_3. However, Riesling, Sauvignon Blanc and other varieties suffer from poor induction of shoot and flower buds for the next season and, therefore, are not suitable for GA_3 treatment. Recently, an alternative strategy was developed through the application of prohexadione-calcium. Trials at BASF in Germany with a view to controlling excessive shoot growth were without success since too high dosages were required. However, in the course of these investigations, an interesting effect on berry-thinning was observed after applying moderate dosages early in the season. This phenomenon was systematically pursued and prohexadione-calcium is now available for this use in Germany and Austria. Independently, Lo Giudice at Virginia State University also observed that prohexadione-calcium reduced berry set and suggested that this could be of practical interest. However, there was no follow-up in the USA. Mid flower opening, when the caps on 20% to 80% of the flowers have abscised, is recommended as an ideal timing for prohexadione-calcium application, which overlaps with the time window for GA_3. At first glance, it may appear paradoxical that an inhibitor of GA biosynthesis is giving effects equivalent to those of an active GA. First attempts to find out the underlying mechanisms remained inconclusive. However, it appears likely that prohexadione-calcium blocks the inactivation of active GAs present at the time of treatment. Its continued activity is likely to be the cause of berry thinning. Different from applying the relatively persistent GA_3, this effect will be relatively short-lived and will not cause negative effects for the following season. This explanation corresponds well with the detailed analytical data on the presence of different GAs in developing grape berries: GA_1 and GA_4, which are both biologically active, give a clear peak at anthesis and decline sharply thereafter. Prohexadione-calcium is likely to inhibit the hydroxylation of these GAs via GA 2-oxidase into inactive GA_8 and GA_{34}, respectively. GA-like effects resulting from treatment with prohexadione-calcium has been reported for *Matthiola incana*. The authors suggest that inactivation of existing GAs by GA 2-oxidase is blocked by prohexadione-calcium as the underlying mechanism.

Ornamentals

GA_3 has found some use in the production of ornamentals, when longer stems or peduncles are desirable. Increased flowering is also induced by GA_3 in certain species. However, reducing shoot elongation and promoting lateral branching and flowering in ornamental and bedding plants is of much greater relevance: compact and dense plants require less space in a greenhouse, they need less water for irrigation, they have an increased shelf life, but, above all, they sell better because of their dark green leaves, which is generally associated with better quality. A wide assortment of growth retardants is currently available to ornamental growers in the USA. These products are based on chlormequat chloride, daminozide, ancymidol, flurprimidol, paclobutrazol and uniconazole-P. Detailed use recommendations for a large number of ornamental species and bedding plants raised under greenhouse conditions are given by Whipker. The spectrum of active ingredients available in EU member countries consists primarily of chlormequat chloride, paclobutrazol and daminozide. In Germany metconazole and prohexadione-calcium are additionally allowed for use in ornamentals. The latter compound should not be used in plants with red or blue flowers because of its interference with anthocyanin formation. Inhibitors of GA biosynthesis, in particular flurprimidol and paclobutrazol, are also being used in the USA and several other countries to reduce the growth of woody and non-woody ornamentals in gardens or parks. Paclobutrazol and flurprimidol often serve as tools to arborists to limit the size and growth of trees and shrubs in power line and utility rights-of-way corridors. Tree growth regulation is regularly applied in high visibility locations such as parks, historic downtowns, residential areas and other places, where trees have a cultural value and where pruning and trimming is difficult to conduct or unwanted. Whereas paclobutrazol is applied via soil injection or soil drenching, flurprimidol is typically administered via stem injection.

Hybrid seed production

Hybrid cultivars have become common in maize and several other species. However, no cost-efficient hybrid seed production systems exist so far for a number of other crop plants, including wheat. A prerequisite for hybrid breeding is tight pollination control, which avoids self-fertilisation and provides viable pollen from the 'male' plant at the right time and at the right place to fertilise the 'female' plant. GA_3 is used by breeders in several plant species to coordinate the development of the 'male' (fertile) and the 'female' (male sterile) plant for crossing. For instance in rice, GA_3 increases the emergence of the 'female' panicles from the leaf sheath, thereby improving the ability to accept pollen from the 'male' plant.

Summary

GAs and inhibitors of GA biosynthesis are well established in agriculture, horticulture and viticulture. For the time being, the existing products are suitable for the applications for which they were developed. Therefore and because of the enormous costs involved, it will be difficult to introduce new active ingredients. However, it is likely that additional markets or additional uses for the known PGRs will be found. For instance, climate changes in several European countries have significantly raised the risk of over-growth in winter cereals in late autumn. Therefore, it appears overdue that autumn applications of anti-lodging products are registered in order to avoid winter damage. Prohexadione-calcium is well suited to assist in the production of high-quality strawberry transplants. In the same crop, it may also be used to reduce runner formation under long-day conditions, thereby enhancing flower induction and berry yield in the following year. Introducing new combinations of registered PGRs may also offer new and improved solutions. For instance, prohexadione-calcium plus trinexapac-ethyl combine immediate and longer-lasting actions, respectively, in graminaceous species. Also, active ingredients may lose registration due to toxicological concerns, in which case it is likely that substitutes will be needed. Furthermore, it is possible that a competitive synthesis can be found for *exo*-16,17-dihydro-GA_5-13-acetate. Because of its high specificity, this compound might represent a compound with 'ultra-safe' toxicological features.

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.

Future line of work

PBZ, a triazole, is an extremely active chemical and affects almost all plant species, whether applied as a spray or a soil drench. It 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. It inhibits GA biosynthesis by blocking the oxidation of ent-kaurene. PBZ has been used to provide plant protection against numerous abiotic stresses such as chilling, water deficit stress, flooding and salinity. PBZ depressed the vegetative growth components, but GA induced vegetative growth components through total shoot length and total bud number increases. PBZ induced the increase in tuber yield, specific gravity and dry matter yield, fruit number and yield, TSS, TSS/TA, reducing sugar and total sugar, and a decrease in TA. This review has compiled and discussed the nature of PBZ, the role of PBZ as a protection against numerous abiotic stresses such as chilling, water deficit stress and heat stress, the effects of PBZ on the vegetative growth, yield and quality of crops. This review will be useful for the professionals and researchers working on plant growth regulators to improve crop production through the use of PBZ. The first use of plant growth retardants such as paclobutrazol was for reduction in plant height to prevent stem lodging. However, nowadays, the effect of paclobutrazol on stem length reduction seems to be less important, due to the release of dwarf and semi dwarf cultivars. Irrespective of reduced lodging, the practice of paclobutrazol in crops with the aim of chemical regulation of growth and development to achieve higher grain yield needs advance research. It seems that the importance of paclobutrazol will be greater under stressful conditions, which draws the attention of researchers to paclobutrazol induced stress tolerance. Several studies only focused on physiological, morphological and biochemical response of crops to paclobutrazol. However, there is limited study in soil residual activity for succeeding crops. Thus, area needs further investigation because paclobutrazol is relatively immobile in soil and bound mainly by organic matter. A number of studies documented that chlorophyll content of crops increased due to paclobutrazol application but still there is doubt regarding to the mechanism of paclobutrazol effect on chlorophyll content. The effects of paclobutrazol in free proline content under water stress condition is still unclear, few study showed the proline content is raised while other literature showed the level of proline is decreased due to the role of paclobutrazol acting as a stress-ameliorating agent in plant, as the plant does not need to accumulate the proline content in the leaves. Therefore, the aforementioned gap shows still further study is need to increase our understanding about the effect of paclobutrazol on the mechanism of plant physiology, stress condition and soil residual activity for succeeding crops.

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Conflicts of Interest

The authors declare that they have no conflict of interest to report in this paper.

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Ethics declarations

Ethics approval and consent to participate

It is not applicable.

Consent for publication

The authors confirm that the content of the manuscript has not been published, or submitted for publication elsewhere.

Competing interests

There are no competing interests between authors.

Conclusion

PBZ, a triazole, is an extremely active chemical and affects almost all plant species, whether applied as a spray or a soil drench. It 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. It inhibits GA biosynthesis by blocking the oxidation of ent-kaurene. PBZ has been used to provide plant protection against numerous abiotic stresses such as chilling, water deficit stress, flooding and salinity. PBZ depressed the vegetative growth components, but GA induced vegetative growth components through total shoot length and total bud number increases. PBZ induced the increase in tuber yield, specific gravity and dry matter yield, fruit number and yield, TSS, TSS/TA, reducing sugar and total sugar, and a decrease in TA. This review has compiled and discussed the nature of PBZ, the role of PBZ as a protection against numerous abiotic stresses such as chilling, water deficit stress and heat stress, the effects of PBZ on the vegetative growth, yield and quality of crops. This review will be useful for the professionals and researchers working on plant growth regulators to improve crop production through the use of PBZ.

Reprints and Permissions

Paclobutrazol (PBZ) is a fungicide that can regulate inhibition of plant growth. PBZ has been mainly used in agriculture and horticulture to improve plant growth in the open field and in greenhouse soils. PBZ is a synthetic compound with two chiral centers; i.e., it bears two asymmetric carbons. Therefore, two pairs of enantiomers may exist, (2R, 3R)- and (2S, 3S)-PC, and (2S, 3R)- and (2R, 3S)-PC. However, steric hindrance allows the production of only the first pair of enantiomers. (2S, 3S)-PC is a stronger regulator of plant growth inhibition than (2R, 3R)-PC in the case of apple seedlings. In contrast, (2R, 3R)-PC inhibits cell growth more potently and accounts for the composition of sterols in suspension culture of celery. Wu et al. (2015) carried out the in vitro enantioselective metabolism of PC by employing rat liver microsomes. The results suggested that the degradation of both enantiomers followed first-order kinetics, and that (2S, 3S)-PC degraded faster than (2R, 3R)-PC ($t_{1/2}$ values of 10.93 and 18.60, respectively), evidencing the significant enantioselectivity of the degradation process. Furthermore, the results showed significant differences in the kinetic parameters of (2R,3R)-PC and (2S,3S)-PC: K_M values of 2.96 and 7.33 μmol L^{-1} and V_{MAX} values of 150.22 and 397.95 nmol min^{-1} mg^{-1} of protein, respectively. However, the CL_{int} values of (2R, 3R)-PC and (2S, 3S)-PC were almost the same, 50.79 and 54.31 mL min^{-1} mg^{-1}, respectively. The clearance results obtained as the ratio between the clearances of (2R, 3R)-PC and (2S, 3S)-PC provided a value of 0.94, which suggested that the hepatic clearance of this pesticide had a small degree of stereo selectivity.

References

1. Abod SA, Jeng LT (1993) Effects of Paclobutrazol and its method of application on the growth and transpiration of Acacia mangium Seedlings. Pertanika J Trop Agric Sci 16(2): 143-50.
2. Abraham SS, Jaleel CA, Chang-Xing Z, Somasundaram R, Azooz MM, Manivannan P, Panneerselvam R (2008) Regulation of Growth and Metabolism by Paclobutrazol and ABA in Sesamum indicum L. under drought condition. Glob J of Mol Sci 3(2): 57-66.
3. Achard P, Genschik P (2009) Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. J Exp Bot 60:1085-1092.
4. Adil OS, Rahim A, Elamin OM, Bangerth FK (2011) Effects of paclobutrazol (PBZ) on floral induction and associated hormonal and metabolic changes of beinnially bearing mango (Mangifera indica L.) cultivars during off year. ARPN J Agric Biol Sci 6: 55-67.
5. Aguirre R, Blanco A (1992) Pattern of histological differentiation induced by paclobutrazol and GA_3 in peach shoots. Acta Hort 315: 7-12.
6. Ahmad Nazarudin MR, Mohd Fauzi R, Tsan FY (2007) Effects of paclobutrazol on the growth and anatomy of stems and leaves of Syzygium campanulatum. J Trop Forest Sci (2): 86-91.
7. Ahmad Nazarudin MR, Tsan FY, Mohd FR. (2012) Morphological and physiological response of Syzygium myrtifolium (Roxb) Walp, to paclobutrazol. Sains Malays 41(10): 1187-1192.
8. Alkhassawneh NM, Karam NS, Shibli RA (2006) Growth and flowering of black iris (Iris nigricans Dinsm.) following treatment with plant growth regulators. Sci Hort 107: 187-193.
9. Almekinders CJM, Struik PC (1967) Shoot development and flowering in potato (Solanum tuberosum L.). Potato Res 39: 581-607.