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Paclobutrazol Applications in Agriculture, Plant Tissue Cultures and Its Potential as Stress Ameliorant: A mini Review

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PACLOBUTRAZOL (PBZ) is a plant growth regulator and it is a type of triazole, which could stop the biosynthesis of growth and development hormone (gibberellin). PBZ is also used to decrease the canopy volume and increase the flowering strength of many crops. PBZ can shorten the plant height, increase the stem diameter and number of leaves as well. PBZ could be also used to control flowers blooming and fruits set. PBZ efficiency in plant tissue cultures have been reported as one of the most important inhibitory substances, which slow growth and decrease the numbers of subcultures. Moreover, PBZ has been widely applied *in vitro* to reduce growth and induce flowering. On the other hand, a lot of studies confirmed the promoting effect of PBZ for plants tolerance/resistance to different abiotic/biotic stresses. However, more studies concerning the ecotoxicology of PBZ in different environmental compartments are still needed to be investigated. In addition, the impact of PBZ on cultivated plants under combined stresses like heat-drought or drought-salinity under biotic stress is still needed to examine.

Keywords: Triazole, PBZ retardant, Gibberellin, *In vitro*, Ameliorant, Drought, Salinity stress.

Introduction

Paclobutrazol, triazole-type plant growth regulator or retardant, is well known as anti-gibberellins. PBZ can block the conversion of *ent*-kaurene to *ent*-kaurenoic acid during the gibberellin biosynthesis pathway by inhibition of kaurene oxidase (Detpitthayanan et al. 2019). Foliar application of PBZ usually reduces plant height and root length through increasing the stiffness of cell wall along with decreasing cell wall expansion (Yang et al. 1996). Many studies reported about the reduction of vegetative growth *via* applied PBZ on many cultivated crops such as potato (Tekalign and Hammes 2005), mango (Upreti et al. 2013), *Leonotis leonurus* L. (Teto et al. 2016) and olive (Ajmi et al. 2020), as well as its potential under stress like drought (Mohammadi et al. 2017; Fan et al. 2020; Iqbal et al. 2020), and salinity (Detpitthayanan et al. 2019; Forghani et al. 2020).

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Several studies confirmed that applied PBZ increased various kinds of compatible solutes and osmo-protectants such as proline, which increase plant tolerance to water deficit (Tesfahun 2018; Detpitthayanan et al. 2019). Many benefits of PBZ application have been intensively reported including improving crop productivity, plant stress tolerance, fruit/grain quality, plant water relation, membrane stability index (Soumya et al. 2017; Mohammadi et al. 2017; Zhao et al. 2017; Detpitthayanan et al. 2019; Fan et al. 2020; Iqbal et al. 2020), preventing sucker re-growth of banana (Luo et al. 2018), promoting fruit set in many crops like olive (Ajmi et al. 2020), inhibiting the biosynthesis of gibberellin, early fruit set, and reduced stem growth (Lucho et al. 2021). Concerning toxicity of PBZ for living organisms, it showed low toxicity *via* the dermal route in animals, whereas it caused moderate toxicity *via* the oral and inhalation routes for human. Based on the available researches, PBZ is considered unlikely to be genotoxic or carcinogenic to humans (Wang et al. 2019; Kumar et al. 2021).

Therefore, this review focused on different applications of paclobutrazol, in the agriculture, the function of PBZ in the field of plant tissue culture and its potential as plant stress ameliorant. Also, the potency of applied PBZ against some pathogens *in vitro* has been handled in this review.

Paclobutrazol and agricultural applications

Paclobutrazol could be widely used in agriculture based on its special properties (**Table 1**) in several sides as follow: (1) arrests vegetative growth as plant retardant (Tesfahun 2018; Mog et al. 2019), (2) improving plant tolerance against different stresses by increasing proline content and enzymatic antioxidants (Fan et al. 2020; Iqbal et al. 2020), (3) increases grain/fruit yield due to relatively stouter canopy of PBZ-treated plants, improving rooting system, which may increase the uptake of water and nutrients (Mehmood et al. 2021), (4) regulating photosynthetic capacity and delaying leaf senescence under semi-arid conditions (Kamran et al. 2020), (5) inducing lodging resistance of some cereals like wheat and maize by stem physical strength, mediation of plant height, and lignin biosynthesis (Kamran et al. 2018a, b), (6) reducing seed shattering under rainfed conditions (Mehmood et al. 2021), (7) improving the resistance against many plant pathogens (Roseli and Ahmad 2019), (8) inducing inhibition of shoot apical meristem activity at dormancy (MacDonald 2017), (9) acting as systemic fungicide against several economically fungal diseases (Desta and Amare 2021), and (10) regulating the flower abscission (Tian et al. 2021). PBZ could be also applied in the floricultural industry to control their size and quality at rates of 1–90 mg L^{-1} to perennials and other pot crops (Desta and Amare 2021), although some crops have a response to higher doses (300 mg L^{-1}) for sesame (Mehmood et al. 2021), and maize (Kamran et al., 2018b) or 500 mg L^{-1} for *Ligustrum lucidum* (Yang et al. 2019) or mulberry (Mohan et al. 2020). When PBZ is sprayed as a foliar, it is absorbed by stems and petioles and then is translocated through the xylem to the growing tip (Desta and Amare 2021). The applications of PBZ in plant tissue cultures for micropropagation of several crops will be presented in the next section.

There are a lot of literatures cited about PBZ applications in several crops grown under different conditions like potato (Jiang et al. 2019; Ellis et al. 2020), Chinese bayberry (Hu et al. 2017), okra (Iqbal et al. 2020), mulberry (Mohan et al. 2020), rice (Dewi and Darussalam 2018; Khunpon et al.2018; Detpitthayanan et al. 2019; Hui et al. 2019), sweet sorghum (Forghani et al. 2018, 2020), mung bean (Kuo et al. 2019), wheat (Kamran et al. 2018a; Dwivedi et al. 2017, 2019), maize (Kamran et al. 2018b, 2020), tomato (Zhao et al. 2017; Zhu et al. 2019; Chen et al. 2020; Li et al. 2020), sesame (Mehmood et al. 2021), olive (Ajmi et al. 2020), banana (Luo et al. 2018; Indrayanti et al. 2019), as well as many ornamental transplants (Collado and Hernández 2021) and medicinal plants (Zhang et al. 2021), as plant growth retardants and other mentioned previous purposes.

Paclobutrazol in plant tissue cultures

During the last three decades, several investigations have been carried out concerning the applications of PBZ in the field of plant biotechnology especially plant tissue cultures (**Table 2**). Plant *in vitro* culture is a promising technique, which allows to produce a huge amount of plants, but significant losses may occur during the *ex-vitro* stage or the acclimatization period (Gimenes et al. 2018). The main purpose of PBZ used *in vitro* may include inducing of flowering and promoting the reduced growth through the blockade of *ent*-kaurene oxidation to GA12-aldehyde by P450 monooxygenase during the synthesis of gibberellin, which acts on cell elongation and inhibits plant growth (Bisht et al.

2018; Mendes et al. 2021). Several doses of PBZ could be used, which depend on the physiological characteristics of each species, but there are crops are needed to establish applied doses of PBZ during *in vitro* culture (Deswiniyanti and Lestari 2018; Mog et al. 2019). *In vitro* conservation of many crops like citrus would be more efficient with the applied PBZ, which acts as a growth reducer, thereby reducing contamination and increasing its maintenance period as well as reduce the costs associated with labor and materials due to less manipulation (Mendes et al. 2021).

TABLE 1. Most common characterizations of paclobutrazol (PBZ) and its applications.

Item or property of PBZ	Value or details	Reference
Chemical formula	$C_{15}H_{20}ClN_3O$	Kumar et al. (2021)
IUPAC name	[(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol]	
Molar mass or weight	293.8 $g \cdot mol^{-1}$	
Density	1.19 g cm^{-3}	
Appearance	off-white to beige solid	
Half-life in soil for degradation	43–618 days with average 182 days	Jiang et al. (2019)
Melting point	165-166 □	
Boiling point	460.9 °C	
Solubility in water	26 mg L^{-1} at 20 °C	
Residual level of PBZ in soil	1.1–150 mg kg^{-1}	Kuo et al. (2019)
PBZ concentration in seawater	119.6 ng l^{-1} in Jiulong, West Sea, China	Li et al. (2012)
Soil microbial community	PBZ decreases the microbial community and soil dehydrogenase activity	Xiang et al. (2017)
Main applications of PBZ	Inhibits the biosynthesis of gibberellin, early fruit set, and reduced stem growth	Lucho et al. (2021)
	Promoting fruit set in many crops like olive	Ajmi et al. (2020)
	Plant growth retardant and triazole fungicide	Ashraf and Ashraf (2020)
	Improves crop yield by increasing drought tolerance, and resistance to pathogen attack	Fan et al. (2020); Iqbal et al. (2020)
	Improves plant water relation, membrane stability index; leaf relative water content	Hu et al. (2017); Soumya et al. (2017)
	Enhances plant photosynthetic pigments, protects ultra-structure of chloroplast	Soumya et al. (2017)
	Encourages flowering, fruiting; increases chlorophyll content; prevents sucker re-growth of banana	Bhattacherjee and Singh (2015); Luo et al. (2018)
Applied both of gibberellin (GA_3) *and PBZ to some crops*		
In vitro applied on *Stevia*	Applied of GA_3 (2 mg L^{-1}) reverse negative role of PBZ	Lucho et al. (2021)
Applied PBZ to tomato roots	Applied 0.1 mM PBZ or GA_3 altered fruit shape and ripening or tomato fruits	Chen et al. (2020)
Sprayed 150 mg L^{-1} GA_3 and PBZ, 20 mg L^{-1} on celery leaves	Spraying exogenous GA_3 or PBZ changed the gibberellin content in plants; interaction between these hormones is still unknown	Duan et al. (2019)
PBZ-treated sweet leaf	PBZ treatment decreased plant growth, but both GA and PBZ treatments effectively increased metabolites and antioxidants	Hajihashemi (2018)
PBZ-treated *Stevia*	Gibberellin and PBZ (10 mg L^{-1}) was studies shared steps between GA and steviol glycosides biosynthesis in stevia	Hajihashemi and Geuns (2017)

Abbreviations: **IUPAC**, the International Union of Pure and Applied Chemistry

TABLE 2. Some published studies on paclobutrazol in field of plant tissue culture.

Applied dose of PBZ	plant (Scientific name)	Main findings of the study	Reference
PBZ at 100, 200 and 400 mg L^{-1}	Greenberry (*Rubus brasiliensis* Mart)	PBZ (200 mg L^{-1}) recorded 20% rooting, but when PBZ (400 mg l^{1-}) added with IBA (1 g L^{-1}) rooting of cuttings was 36%	Bueno et al. (2021)
Supplemented with 0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1} PBZ	Citrus rootstocks (*Citrus* spp.)	PBZ used *in vitro* germplasm conservation purpose by establishment of a protocol of citrus rootstocks	Mendes et al. (2021)
PBZ at 0.1, 0.5, 1, 2 mg L^{-1}	*Lilium monodelphum* M.	To induce micro-bulbs formation and generation, highest formation of micro-bulb was observed in medium of 2.0 mg L^{-1} PBZ, 0.2 mg L^{-1} NAA and 0.1 mg L^{-1} GA_3	Topaloglu and Öztürk (2021)
Rooting medium contained 2 µM IAA or 2 µM PBZ	*Pinus massoniana* Lamb.	Optimum rhizogenesis *in vitro* shoot culture by combined exogenous NAA, PBZ increased rooting rate and its number	Wang and Yao (2021)
PBZ at 2, 4, 8 µM	*Pinus massoniana* Lamb.	Plantlet regeneration promoted by PBZ *in vitro* cultures, improved performance after long-term (3 to 4 y) subculture	Wang and Yao (2020)
PBZ at 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.40 g L^{-1}	Pathogenic soil fungi like *Phellinus noxius*	PBZ inhibited up to 90% when treated with at least 0.05 g L^{-1} of fungi *Rigidoporus microporus*	Roseli and Ahmad (2019)
Applied PBZ at 2.5 and 5.0 ppm as a growth retardant	Banana (*Musa* spp.)	PBZ improved number of shoots and leaf shape, plant height of banana after 6 months *in vitro* storage at acclimatization	Indrayanti et al. (2019)
50 or 500 µM PBZ was added to the MS medium	Oriental lily 'Sorbonne' (*Lilium* sp.)	Low PBZ stimulated biomass of bulblet, but higher suppressed leaves and roots the growth with increase soluble carbohydrate and starch contents	Wu et al. (2019)
PBZ at rate of 0.5; 1.0; 1.5 mg L^{-1} (a.i)	*Zygopetalum crinitum* Orchid	PBZ promoted root thickening and reduced length of aerial parts and roots	Gimenes et al. (2018)
PBZ added at 2 mg L^{-1} to the media	*Stevia rebaudiana*	PBZ and GA increased ROS scavenging and osmolytes in stevia calli under drought stress compared to control	Hajihashemi et al. (2018)
PBZ at 1, 2, 4 mg L^{-1}	*Stevia rebaudiana* Bertoni,	PBZ treatments increased glycine betaine, proline, α-tocopherol, and protein contents, reduced drought stress damage	Hajihashemi and Ehsanpour (2013)
0.4, 0.8, 1.2 mg L^{-1} PBZ	Orchid (*Dendrobium nobile* L.)	PBZ improved the transfer efficiency of plantlets from *in vitro* culture to pots because of its impacts on root growth	Wen et al. (2013)

Notes: Active ingredient (a.i), naphthalene acetic acid (NAA), reactive oxygen species (ROS)

It is well known that, plant tissue culture has been applied in several genetic and physiological studies for many crops (Teixeira da Silva et al. 2019). These studies included several *in vitro* fields or themes using many phytohormones (e.g., ethylene, gibberellin, and cytokinins) or plant growth retardants like PBZ or abscisic acid (ABA), which focused on following studies:

1- Controlling adventitious root formation (Wang and Yao 2021)
2- Improving the responses of plantlets to abiotic/biotic stress like drought (Hajihashemi and Ehsanpour 2013, 2014; Dwivedi et al. 2017) or salinity (Hu et al. 2017; Forghani et al. 2020)
3- Producing plantlets originating from somatic embryos (Yao and Wang 2020)
4- Optimization of the "*rhizogenesis*" for *in vitro* shoot culture (Wang and Yao 2021)
5- Conservation of citrus rootstocks *in vitro* using PBZ with high genetic stability (Mendes et al. 2021)
6- PBZ can control dwarfing in apples by elevating auxin and abscisic acid, reducing gibberellins and zeatin and modulating their transporter genes in apple rootstocks (Opio et al. 2020)
7-Enhancing the formation of micro-bulb plantlets of a native bulbous flower by micropropagation (Topaloglu and Öztürk 2021)
8- Promoting regeneration and conservation of some endangered plant species like orchid of *Laelia anceps* Lindl (Ramírez-Mosqueda et al. 2019)
9- The strong relationship between PBZ and GA_3 was confirmed by several *in vitro* studies like the capability of GA_3 (Falcioni et al. 2017), Hajihashemi (2018); Hajihashemi et al. (2018), Lucho et al. (2021)
10- Optimizing the direct/indirect regeneration of commercial bulbs or flowers, their number and size using PBZ (Youssef et al. 2019).

Paclobutrazol as plant stress ameliorant

Plant hormones or phytohormones are universal regulators that playing a key role in the growth of cultivated plants, development, and its adaptation to adverse environments. These phytohormones could be divided into two main categories including stimulators (e.g., auxin or AUX, gibberellin or GA, cytokinin or CK, melatonin) and the inhibitors (e.g., abscisic acid (ABA), ethylene (ET); salicylic acid (SA), jasmonic acid (JA), paclobutrazol (PBZ) (Li et al. 2021). The production of global foods often suffers from several global threats including the climate changes and environmental stresses (mainly biotic and abiotic stress). Drought, salinity, flooding, waterlogging, heat and cold stress are considered as abiotic stresses, whereas plant pathogens cause biotic stresses. Paclobutrazol has been used to support plant protection against several abiotic stresses such as drought or water deficit stress (Dwivedi et al. 2017, 2019; Zhao et al. 2017; Fan et al. 2020; Mohan et al. 2020), flooding or submerging (Elanchezhian et al. 2015; Hui et al. 2019), cold stress (Yang et al. 2019; Peng et al. 2019, 2020), salinity (Keramati et al. 2016; Hu et al. 2017; Khunpon et al. 2018; Detpitthayanan et al. 2019; Forghani et al. 2018, 2020), and light stress (Collado and Hernández 2021). Few studies focused on the effect of paclobutrazol on different pathogens like (Roseli and Ahmad 2019; Sun et al. 2021). More impacts of PBZ on plant pathogens could be presented in the **Figs. (1), (2), (3), and (4)** as well as many other stresses in **Table 3**.

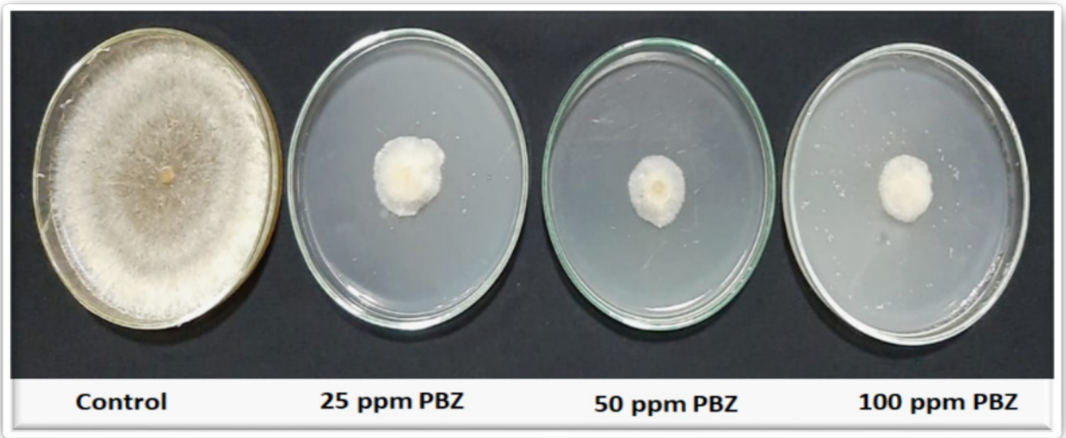

Fig.1. Impact of PBZ on inhibition the growth of *Rhizoctonia solani*.

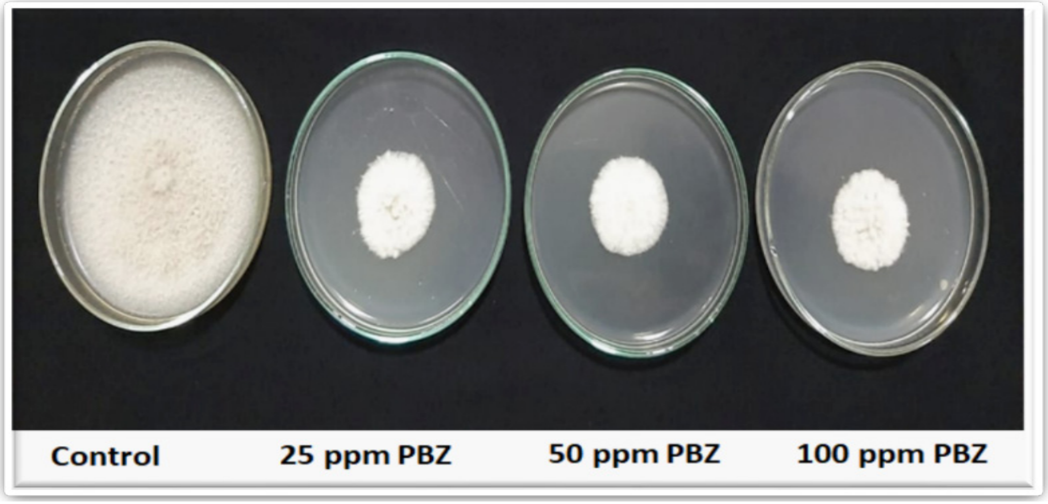

Fig. 2. Impact of PBZ on inhibition the growth of *Fusarium oxysporum* of eggplant.

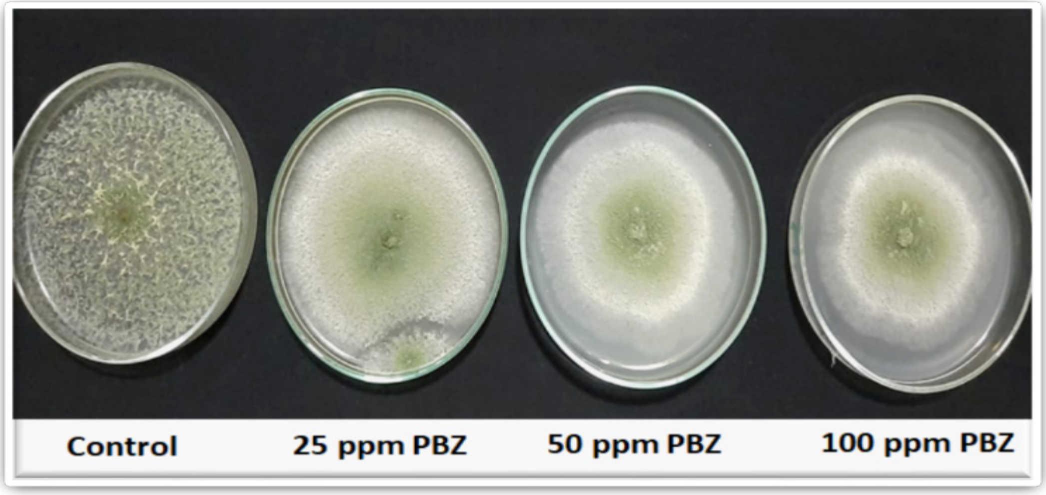

Fig. 3. There is no effect of PBZ on inhibition the growth of *Trichoderma harzianum* (a very important microorganism used in the biocontrol).

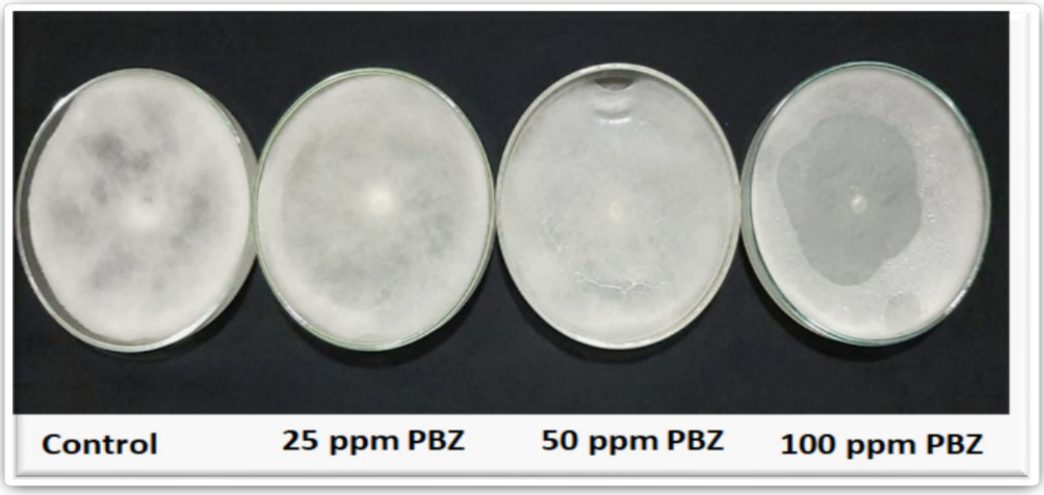

Fig. 4. There is no effect of PBZ on inhibition the growth of *Pythium* sp.

TABLE 3. Some published studies on paclobutrazol (PBZ) as plant stress ameliorant.

Applied dose of PBZ	Cultivated plant (Scientific name)	Main findings of the study	Reference
Drought stress			
PBZ drenched into soil at 10, 50, 100, 150, and 200 mg L^{-1}	Seedlings of *Amorpha fruticosa* L.	Optimum dose (150 mg L^{-1}) of PBZ improved peroxidase activity, growth rate, contents of soluble sugars, relative water content and chlorophyll under stress	Fan et al. (2020)
Foliar applied 20, 40, 80 ppm PBZ	Okra (*Abelmoschus esculentus* L.)	PBZ enhanced growth and yield of okra by mediating physio-biochemical traits	Iqbal et al. (2020)
PBZ applied as a soil drench at 5, 10, 25, 50, 100, and 500 mg L^{-1}	Mulberry (*Morus alba* L.)	PBZ mitigated drought stress and rainfed conditions by improving leaf biomass as a result of increasing net photosynthetic rates	Mohan et al. (2020)
PBZ foliar sprayed at 30 mg L^{-1} after 62 d from sowing	Wheat (*Triticum aestivum* L.)	PBZ-induced alleviation of drought damage during wheat reproductive stage due to high proline & photosynthetic items	Dwivedi et al. (2017)
Spray PBZ at 0.42 kg ha^{-1} on pots	Ryegrass (*Lolium perenne* L.)	PBZ enhanced drought tolerance through restoring visual quality of stressful plants	Mohammadi et al. (2017)
Salinity stress			
In hydroponic, applied 17 µM PBZ	Sweet sorghum (*Sorghum bicolor* L.)	Under 100, 200 mM NaCl, PBZ role was detoxification of toxic compounds like H_2O_2 by increasing proline content and total antioxidant capacity (GR, CAT)	Forghani et al. (2020)
Sprayed PBZ at 50, 100, 150 ppm on leaf blade in pots	Rice (*Oryza sativa* L.)	Foliar spray PBZ (100 ppm) under 0.4% NaCl mimic salt stress; increased proline, improving grain quality of rice	Detpitthayanan et al. (2019)
Foliar spray at 15 mg L^{-1} PBZ	Rice (*Oryza sativa* L.)	PBZ mitigated salt stress (150 mM NaCl) in rice seedlings through promoting metabolism of glutathione and glyoxalase	Khunpon et al. (2018)
Pots contained 17 µM of PBZ or GA_3	Sweet sorghum (*Sorghum bicolor* L.)	PBZ mitigated salt stress (up to 250 mM NaCl) by accumulation photosynthetic pigments; regulated by polyamines, ABA	Forghani et al. (2018)
Applied PBZ at 2.0 µmol L^{-1})	Chinese bayberry [*Myrica rubra* Zucc.]	PBZ ameliorated negative effects of salt stress (up to 0.4% NaCl) by increasing proline content, a relative water content, and antioxidant enzyme activities	Hu et al. (2017)
Light stress			
Applied PBZ doses were from 2.5 to 10 mg L^{-1}	Ornamental plants (petunia, geranium, pansy, dianthus)	PBZ reduced leaf area and, consequently, the benefit of light intensity on plant growth (reduced light capture)	Collado and Hernández (2021)
Cold stress			
Foliar sprayed PBZ at 10 µM	*Tetrastigma hemsleyanum* Diels & Gilg	PBZ promoted cold tolerance (0 °C) by regulating the potential candidate genes related to cold tolerance	Peng et al. (2020)
Seedlings subjected to PBZ at 100, 300, and 500 mg L^{-1}	Privet tree (*Ligustrum lucidum* L.)	Highest dose of PBZ at 500 mg L^{-1} was stronger freezing tolerance during natural cold acclimation (–13 °C) by accumulated proline, soluble proteins, soluble sugars	Yang et al. (2019)
Foliar sprayed PBZ at 50, 100 mg L^{-1}	Teak (*Tectona grandis* L.f.)	Both AMF and PBZ alleviated cold stress (0 °C) by increasing activity of SOD and POX, photosynthetic pigments, and by decreasing membrane lipid peroxidation	Zhou et al. (2012)
Flooding stress			
Sprayed PBZ at 200 mg L^{-1}	Rice (*Oryza sativa* L.)	PBZ application inhibited leaf senescence, a greener leaf color and GA concentration	Hui et al. (2019)
Sprayed PBZ at 100 mg L^{-1}	Rice (*Oryza sativa* L.)	PBZ alleviated the stress by enhancing gas exchange, yield parameters, chlorophyll fluorescence	Elanchezhian et al. (2015)

Abbreviations: glutathione reductase (GR), catalase (CAT); abscisic acid (ABA); Arbuscular Mycorrhiza (AMF); peroxidase (POX)

Based on the kind of stress, the cultivated plants have particular response including molecular, biochemical and physiological responses, which could enhance stress tolerance. The physiological response to salinity stress for example may include the adjustment of growth and development, cell water potential and turgidity, stomatal conductance and ion homeostasis. The biochemical responses involve the modulation of transport proteins leading to ion extrusion and sequestration, phytohormones, ROS detoxification and compatible osmolyte production, whereas the molecular ones include the upregulating of gene expression for protective functions, signal transduction, ion transport, osmolyte biosynthesis, cell wall and membrane remodeling, and photosynthesis and energy metabolism (Mansour et al. 2021). In general, PBZ acts as stress protectant by maintaining relative water content, membrane stability index, photosynthetic activity, photosynthetic pigments and protects the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield.

Conclusions

PBZ is a plant growth retardant, which is a compound used to reduce plant growth without changing developmental patterns or being phytotoxic. The group of PBZ is the largest group of plant growth retardants, which include chemicals antagonistic to gibberellins (the hormone of plant growth). PBZ also has some fungicidal activity because of its action as a triazole to inhibit sterol biosynthesis. PBZ may also induce many morphological changes in plant leaves including thicker leaves, smaller stomatal pores, increased the number of surface appendages and their size, and increased root density, which may support the plant tolerance against environmental stress and disease resistance as well. Under different stress, PBZ may alleviate the stress by modifying the electrolyte leakage, phytohormones; reducing glutathione and lipid peroxidation in many cultivated crops grown in field or in *in-vitro*. The best application rate of PBZ may depend on many factors including plant species, cultivation method, plant growth stage, extent of vegetative growth and method of PBZ application. Generally, the PBZ amount, which required to promote flowering and fruiting in fruit crops is very low, whereas this rate may reach up to 150 mg L^{-1} to improve the economic yield and its quality for some crops and may be up to 500 mg L^{-1} for others. The field of plant tissue culture like others has several applications of PBZ, but this is still in an urgent need for certain studies particularly the combined stresses or multiple cases.

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