

Does Application of Brassinosteroids mitigate the Temperature Stress in Plants?

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Abstract

The temperature across the globe is constantly changing for the worse of the biotic beings viz., the flora and fauna. Rapid temperature changes are the result of continuous interference of human beings as a bane post the rapid industrialization and urbanization. The ever increasing and decreasing ranges of temperature in the environment are leading to the malfunctioning of various physiological and biochemical processes in plants thereby resulting in severe temperature stresses in terms of high temperature/heat stress as well as low temperature stress (chilling and freezing stress). Brassinosteroids (BRs) are a novel group of plant growth regulators (PGR's) with significant growth promoting activity. BRs were initially extensively studied for their profound growth promoting physiological responses viz., growth and yield, seed germination, photosynthesis, senescence, photomorphogenesis, flowering etc. BRs have been further explored for stress-protective properties in plants against a number of abiotic stresses like heat, chilling, freezing, drought, flooding, oxidative, salt, allelochemicals, radiation, light, wind, heavy metals stresses etc., and can be aptly stated that BRs induce plant tolerance to a wide spectrum of stresses. The present review is a study on the role of BRs in mitigating the effect of temperature stress in plants viz., high temperature/heat stress as well as low temperature stress (chilling and freezing stress).

Keywords: Brassinosteroids; Chilling stress; Freezing stress; High temperature stress; Low Temperature stress.

Introduction

Brassinosteroids (BRs) are a novel type of polyhydroxy steroidal phytohormones that are capable of emphatically exhibiting pronounced growth-promoting influence [1,2]. The discovery of this new group of PGRs (plant growth regulators) way back in the early 70's [3-5] followed by the research work in the late 70's by Grove et al. [6] led to the recognition of BRs as a potential 6th group of PGRs. BRs are usually classified as C₂₇, C₂₈ or C₂₉ BRs according to the number of carbons in their structure and brassinolide (BL), 28-homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-EpiBL) are the three potential BRs of the present world of research and development [7] are represented in figure 1.

BRs was first studied as the regular PGRs capable of modulating a wide range of physiological functions like source/sink relationships, seed germination, photosynthesis, senescence, photomorphogenesis, flowering and responses to different abiotic and biotic stresses [8]. The research on BRs exhibited their ability in overcoming various abiotic stresses like high temperature [9], low temperature in terms of chilling [10,11] as well as freezing [12], salt [13,14], light [15], water in terms of drought [16,17] as well as flooding [18], heavy metals [19-21], osmotic [22], herbicide [23], pesticide [24], inorganic pollutants [25,26] as well as organic pollutants [27,28] stresses. Further, BRs were also

capable of overcoming different biotic stresses caused by viruses [29,30], nematodes [31,32], fungi [33], insects [34], bacteria [35] etc. The recent studies on BRs also revealed their ability in overcoming certain unique stresses like newly reclaimed sandy soil stress [36], shade stress [37], preservative stress [38], petroleum polluted soil stress [39] etc. The present review focuses on the ability of BRs in mitigating temperature stress in different plants viz., high temperature/heat stress as well as low temperature stress (chilling and freezing stress).

role of BRs as effective PGRs in mitigating various temperature stresses in different plants and their potentiality in combating the negative effect of temperature stresses in plants.

BRs in mitigating different Temperature Stresses in Plants

BRs are known to mitigate various stresses including temperature stress which includes high temperature/heat stress and low temperature stress (chilling and freezing). Application of 24-EpiBL at 10^{-11} , 10^{-9} and 10^{-7} M to three *Brassica* species (*B. carinata*, *B. juncea* and *B. napus*) under high as well as low temperature (4, 14, 34 and 44°C) for 5 hours mitigated high and low temperature stresses in all the three *Brassica* species by decreasing the lipid peroxidation in terms of MDA (malondialdehyde) content and accumulation of the osmolyte, proline [40]. Chen et al. [11] reported that cold-induced oxidative stress in grapevine seedlings was mitigated by foliar treatment of 24-epiBL by regulating the ascorbate-glutathione cycle. Further, Zhao et al. [41] also reported that application of 24-epiBL mitigated a combination of drought and heat stress in *Triticum aestivum* L. seedlings by increasing the rate of photosynthesis and Rubisco activase gene expression.

BRs in mitigating Heat/High Temperature Stress in Plants

Cukor et al. [42] reported that application of BRs positively regulated the seed germination of Scots pine cultivated under standard and heat stress conditions. BRs were reported to play a positive role in mitigating the high temperature or heat stress in plants [43,44]. Wilen et al. [45] in the early 90's studied that supplementation of 24-EpiBL markedly enhanced the tolerance to high temperature stress in brome grass cell suspension cultures by enhanced accumulation of ABA-inducible heat stable proteins. Hayat et al. [46] observed that treatment of 28-homoBL to *Vigna radiata* c.v. T-44 plants mitigated the stress generated by high temperature by improved membrane stability index (MSI), leaf water potential (ψ), increased activities of antioxidative enzymes as well as proline levels. Cao and Zhao [47] studied that foliar application of 0.005 mg/L of BR to two varieties of *Indica* rice (*Oryza sativa* L.) seedlings viz., *Xieqingzao* B (heat-sensitive) and 082 (heat-tolerant) mitigated high temperature stress by enhanced activities of peroxidase (POD), super oxide dismutase (SOD) isozymes expression levels and reduced MDA levels and leakage of leaf electrolytes. BRs were found to enhance the rate of photosynthesis by increasing the CO₂ fixation and the antioxidative system activities in tomato plants by mitigating high temperature stress [48]. Mazorra et al. [49] studied that the pre-incubation with 24-epiBL or MH₅ (polyhydroxylated spirostane analogue of BR) for around 24 hours mitigated heat stress in tomato leaf discs by enhanced the activities of catalase (CAT), peroxidase (POD) and super oxide dismutase (SOD). Further, a study on mitochondrial

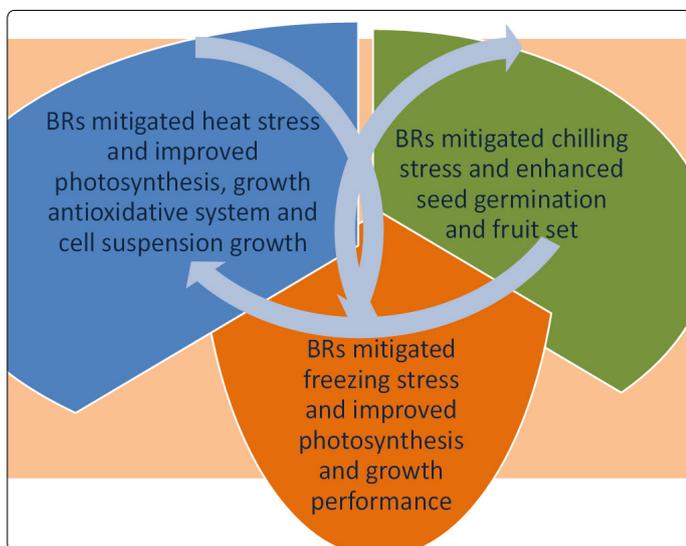
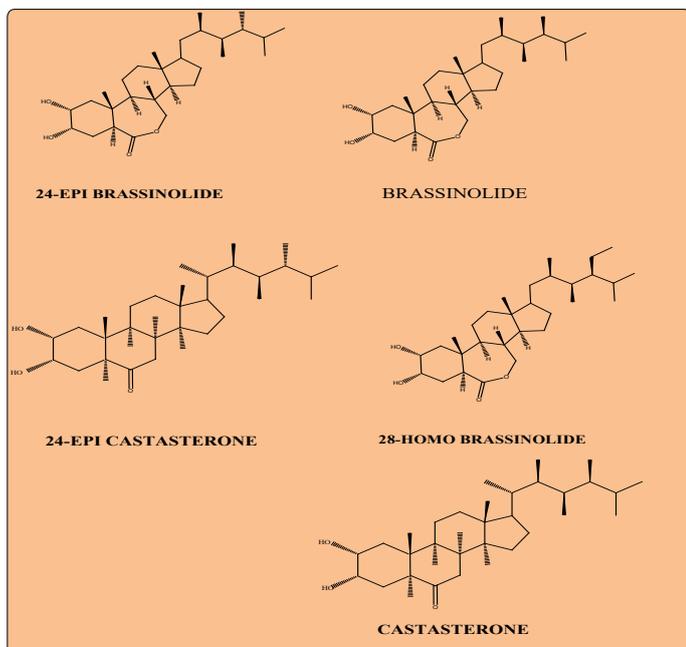


Figure 1. Potential BRs in Research.

Temperature causes a lot of physiological changes in plants especially in the disruption of the enzymes which are basically proteins capable of solarization as well as crystallization due to high or low temperature. This in turn affects the major metabolic processes like photosynthesis and respiration. Application of PGRs especially BRs mitigated the different stresses in plants caused by different temperature regimes. The recent changes in the environment across the globe due to various reasons especially global warming is posing a severe threat to the plants due to fluctuations of temperatures. Hence the present review article is to show the

small heat shock proteins (MT-sHSPs) of tomato showed the leaves did not preferentially accumulate in 24-epiBL treated plants at 25°C but accumulated at 38°C [50]. Further, Mazorra et al. [51] also reported that application of EpiBL induced tolerance to heat shock (HS) in tomato seedlings [BR-deficient mutant (*extreme dwarf d(x)*), a partially BR-insensitive mutant *curl₃*(-abs) allele (*curl₃* altered brassinolide sensitivity) and a line over expressing the dwarf, BR-biosynthesis gene (35SD)] by reduced ion leakage, lipid peroxidation and increased antioxidative systems.

Homo BL was found to mitigate the negativity of heat stress in growth of apical meristems of banana shoots cultured *in vitro* conditions [52]. Janeczko et al. [53] studied that application of 24-epiBL mitigated heat stress and improved the physiological functions of barley. Dhaubhadel et al. [54] observed that application of 24-epiBL resulted in enhanced basic thermo tolerance of tomato seedlings which might have been due to the protection of the translational machinery as well as heat-shock protein synthesis by BR-application [55]. BRs mitigated heat-induced inhibition of photosynthetic capability by enhanced carboxylation efficiency as well as antioxidative enzyme system in *Lycopersicon esculentum* [48]. Foliar treatment of 24-epiBL mitigated the ill effects of high-temperature-induced inhibition of photosynthesis in two cultivars of melon (*Cucumis melo* L.) seedlings [56]. A preliminary laboratory research established that the tomato leaf ultra structure was less affected in Bio Bras 6-treated leaves subjected to high temperature stress [57]. Further, Sam et al. [58] observed that a BR-analogue (Bio Brass-6) mitigated the negative effect of high temperature stress (40°C for 1.5 h) on leaf ultra structure of tomato plants and improved the internal membrane system of chloroplasts and mitochondria. Even, Niu et al. [59] also observed that foliar treatment of BRs to (Trin.) Tzvelev grown under high temperatures mitigated the stress and improved the morphological and physiological traits of *Leymus chinensis*.

Krishna et al. [60] observed that supplementation of 24-epiBL resulted in enhanced basic thermotolerance of tomato seedlings. BRs mitigated the high-temperature injury in *Ficus concinna* seedlings by enhanced antioxidative defense mechanism and improved glyoxalase systems [61]. BRs were also found to improve the rate of photosynthesis, lipid peroxidation, and rice seed set under high temperature stress [62,63]. 24-EpiBL supplemented tomato pollen showed higher *in vitro* pollen germination and increased tube growth subjected to high temperature stress [64]. Further, exogenously treated BRs enhanced the development of heat-stressed rice pollens [65]. Recently, Liu et al. [66] reported that BRs improved the lipid productivity and enhanced the stress tolerance of *Chlorella* cells subjected to high temperature.

BRs in mitigating Low Temperature in Plants

Plants are prone to low temperature stress during winter or autumn. Low temperature stress includes chilling stress as well as freezing stress. The research study conducted by

Janeczko et al. [67] showed that BR infiltration prior to cold treatment reduced the ion leakage in rape plants and stated that BRs are potential mitigators of low temperature stress.

BRs in mitigating Chilling/Cold stress in Plants

BL mitigated the ill effect of chilling stress and increased the growth of cucumber [68] and maize [69] seedlings. The supplementation of BL markedly enhanced the seed germination and seedling growth of rice subjected to low-temperature stress [70]. The treatment of BL exhibited enhanced lamina joint-cell elongation under low-temperature stress in rice [71]. He et al. [72] stated that BL enhanced the growth of maize subjected to chilling stress. Xi et al. [73] observed that supplementation of 24-epiBL resulted in enhanced antioxidative defense mechanisms as well as modulated osmoregulatory systems in young grapevines (*V. vinifera* L.) grown under chilling stress. Liu et al. [74] studied that treatment of 24-EpiBL to *Chorispora bungeana* cell suspension cultures exposed to 4 and 0° C for 5 days of chilling stress showed mitigation of oxidative damage due to over production of ROS (reactive oxygen species) by increased antioxidative defense mechanism viz., enhancement in the activities of antioxidative components like APX (ascorbate peroxidase), CAT (catalase), POD, SOD, ASA (ascorbic acid) and decreased contents of GSH (reduced glutathione). Kumar et al. [75] reported that foliar application of 24-epiBL to *Brassica juncea* L. seedlings grown under 4°C of chilling stress exhibited reduced H₂O₂ concentration by enhanced antioxidant defense system (enhanced activities of various antioxidative enzymes viz., CAT, APX and SOD). Hu et al. [76] studied that foliar supplementation of 24-epiBL mitigated the 12/8°C chilling-induced inhibition of photosynthetic capacity of cucumber (*Cucumis sativus* L.) plants by not only decreasing the production of ROS accumulation, but also enhancing the activities of SOD, APX; decreasing H₂O₂ and MDA. Further, Hu et al. [77] also studied that cucumber plants pretreated with 24-epiBL as well as 0.3 and 1.0 mmol·L⁻¹ chlorpyrifos mitigated the phytotoxicity as well as chilling stress by enhancing the oxidative stress and regulating antioxidative enzymes (APX, GR [glutathione reductase], CAT and GPX).

Earlier research indicated by Ohshiro et al. [78] showed that 24-epiBL capably regenerated the bulbets of *Lilium japonicum* by breaking the dormancy. BL effectively mitigated the ill effects of chilling stress in tomato [54] and increased the growth of cucumber seedlings [79]. Fariduddin et al. [80] observed that 10⁻⁸, or 10⁻⁶ M 28-homoBL mitigated chilling stress (10/8°C, 5/3°C) by enhanced growth, photosynthesis, activities of antioxidant enzymes like CAT, POD, SOD and the osmolyte, proline in cucumber (*Cucumis sativus* L.) subjected to stress. Jiang et al. [81] studied that BRs were capable of protecting the photosynthetic apparatus from cold-induced damage in *Cucumis sativus* plants by enhancing the activities of Calvin cycle enzymes and enhancing the antioxidative system which in turn resulted in mitigation of the photo oxidative stress during the process of recovery from chilling

injury. Wang et al. [82] reported that BRs (5, 10 and 15 μM) efficiently decreased the chilling injury of pepper fruit during 18-day storage at 3°C by decreasing the electrolyte leakage, MDA content and enhancing antioxidative enzyme activities (CAT, POD, APX and GR). Aghdam et al. [83] studied that application of 0, 3 and 6 μM BRs to tomato fruits stored at 1°C for 21 days decreased the chilling injury, electrolyte leakage, MDA content while increased proline levels, total phenols, phenylalanine ammonia-lyase (PAL) activity and maintained the membrane integrity.

Anwar et al. [84] observed that 24-EpiBL mitigated the endogenous hormone levels to enhance low-temperature stress tolerance in cucumber seedlings. BR-supplemented tomato (*Lycopersicon esculentum*) plants grew better than control plants under low temperature conditions [85]. BL supplementation resulted in increased energy status and proline metabolism in bamboo shoots during postharvest stage under chilling stress [86]. Watanabe et al. [87] studied that foliar application of Ts303, a BL analogue before one week of flowering enhanced fruit set in 15 year old trees of Japanese persimmon and 12 year old grape vines. Exogenous application of BL mitigated chilling stress in *Leymus chinensis* (Trin.) Tzvel by modulating morphological, physiological and biochemical traits [88]. Dong et al. [89] studied that treatment of a BR-analogue (BR-TS303) increased the resistance of *Arachis hypogaea* plant grown under chilling stress. Foliar treatment of 24-epiBL resulted in enhanced photosynthesis, anti-oxidant defenses and protected eggplant (*Solanum melongena* L.) seedlings from chilling stress [90].

Seed supplementation with TNZ303 which is mixture of jasmonic acid and BR-derivatives mitigated the formation of deformed leaves in cucumber plants treated subjected to cold stress [91]. Treatment of 0.01% BL solution increased the yield as well as the resistance to autumn low-temperature damage in rice crop [10]. Even a proteomics study also revealed the mitigative ability of BRs subjected to chilling stress in mung bean epicotyls [92]. Aghdam & Mohammadkhani [93] observed that postharvest supplementation of BL resulted in enhanced chilling stress tolerance in tomato fruit where as Wu [94] observed that supplementation of BRs enhanced the chilling resistance in *Dendrobium huoshanense*.

Supplementation with BRs increased the winter survival of winter rye (*Secale cereale* L.) by increased photosynthetic capacity [95]. Further, Pociecha et al. [96] also studied that pre-treatment with 24-EpiBL modified the cold-induced photosynthetic acclimation mechanisms and PGR responses of perennial ryegrass in cultivar-dependent manner. 24-EpiBL enhanced plant tolerance to low temperature stress in *Lycopersicon esculentum* Mill [97] and also mitigated the chilling-induced oxidative stress in pepper by enhancing antioxidative systems as well as maintenance of photo system II [98]. Hirai et al. [99] reported that BL improved the ripening of rice plants subjected to low temperature condition. Recently, Tavallali [100] observed that vacuum infiltration of 24-epiBL significantly delayed the chlorophyll degradation

and maintained the quality of lime fruit during cold storage, thus increasing its shelf life. Even, Xia et al. [101] observed that BR-mediated apoplastic H_2O_2 -glutaredoxin 12/14 cascade that regulated the antioxidant capacity in response to chilling stress in tomato plants.

BRs in mitigating Freezing/Frost Stress in Plants

Eremina et al. [102] reported that BRs participated in controlling the basic and acquired freezing tolerance of plants. Ma et al. [103] observed that foliar treatment of BRs ($1 \times 10^{-6} \text{ mol L}^{-1}$) increased the growth and photosynthesis in terms of Stomatal conductance (Gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr) and photosynthetic saturated light intensity (LSP) in rapeseed (*Brassica napus* L.) subjected to freezing stress. Gallo et al. [12] observed that BRs mitigated the late frost stress in *Fagus sylvatica* L. plantation by improved growth performance and resistance.

Conclusion

The ability of BRs in mitigating different temperature stresses like heat, chilling as well as freezing is an established fact and the research of BRs as potential mitigators of various abiotic stresses especially temperature stress is gaining much importance in the current scenario of environmental stress research. Sadura and Janeczko [104] aptly stated that BRs are capable of inducing tolerance to high and low temperature in plants by modulating various physiological and molecular mechanisms. Further, Filek et al. [105] studied that BRs mitigated low temperature stress in winter wheat seedlings by regulating its membrane structure. Kaur et al. [9] observed that application of 28-homoBL regulated the antioxidant enzyme activities and gene expression in response to temperature-induced oxidative stress in *Brassica juncea*. Vardhini and Anjum [106] stated that BRs have the ability in overcoming various abiotic stresses in plants by positively modulating the antioxidative system of the plants. It is a well known fact that there is always a threat for the plants to face extreme heat or extreme cold temperatures due to the constant changes in the environmental conditions across the globe [107]. Hence, the present review article focuses on the role of BRs as potential PGRs that are capable of mitigating temperature stress (high temperature, low temperature and freezing) which is one of the main abiotic stresses that the plants are facing in the current scenario of ever changing temperature regimes.

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