Chapter 16

Abiotic Stress Alleviation with Brassinosteroids in Plant Roots

Sevgi Marakli and Nermin Gozukirmizi

Additional information is available at the end of the chapter

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Abstract

This chapter covers the advances in establishment and optimization of brassinosteroids (BRs) in the alleviation of abiotic stresses such as water, salinity, temperature, and heavy metals in plant system, especially roots. Plant roots regulate their developmental and physiological processes in response to various internal and external stimuli. Studies are in progress to improve plant root adaptations to stress factors. BRs are a group of steroidal hormones that play important roles in a wide range of developmental phenomena, and recently they became an alleviation agent for stress tolerance in plants. This review is expected to provide a resource for researchers interested in abiotic stress alleviation with BRs.

Keywords: Water stress, salt stress, temperature stress, heavy metal stress

1. Introduction

Abiotic stress responses in plants occur at various organ levels among which the root-specific processes are of particular importance. Under normal growth condition, root absorbs water and nutrients from the soil and supplies them throughout the plant body, thereby playing pivotal roles in maintaining cellular homeostasis. However, this balanced system is altered during the stress period when roots are forced to adopt several structural and functional modifications. Examples of these modifications include molecular, cellular, and phenotypic changes such as alteration of metabolism and membrane characteristics, hardening of cell wall, and reduction of root length [1, 2]. The root system has the crucial role of extracting nutrients and water through a complex interplay with soil biogeochemical properties and of maintaining these functions under a wide range of stress scenarios to ensure plant survival and reproduction [3].

Water stress is characterized by a reduction of water content and leaf water potential, closure of stomata, and decreased growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism, and finally the death of plant [4]. This water loss causes a loss of turgor pressure that may be accompanied by a decrease in cell volume depending on the hardness of the cell wall [5]. The cells of the root must activate processes to limit water loss and mitigate its harmful effects.

Salinity also affects plant growth, activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis, causing oxidative stress and programmed cell death, reducing nutrient uptake, genetic and epigenetic effects, metabolic toxicity, inhibition of photosynthesis, decreasing CO_2 assimilation, and reducing root respiration [6, 7, 8, 9]. Salt stress affects the root in all developmental zones. Cell division decreases in the meristematic zone and cell expansion attenuates in the elongation zone, resulting in reduced overall growth [10]. Cells also expand radially in the elongation zone [11], and root hair outgrowth suppresses in the differentiation zone [12]. Salt stress additionally results in agravitropic growth [13] as well as reduced lateral root number under high-salt conditions and enhanced lateral root number under moderate-salt conditions [14, 15]. Salt stress developes from excessive concentrations of salt, especially sodium chloride (NaCl) in soil. Root is the primary organ of exposure and hence responds rapidly [16]. Salt stress is known to increase Na^+/K^+ ratio in the root that leads to cell dehydration and ion imbalance [17, 18, 19].

High temperature increases the permeability of plasma membrane [20], and also reduces water availability [21]. Moreover, low temperature (chilling and frost stress) is also a major limiting factor for productivity of plant indigenous to tropical and subtropical climates [22]. Chilling stress has a direct impact on the photosynthetic apparatus, essentially by disrupting the thylakoid electron transport, carbon reduction cycle, and stomatal control of CO_2 supply, together with an increased accumulation of sugars, peroxidation of lipids, and disturbance of water balance [23].

Heavy metal contamination in soil could result in inhibition of plant growth and yield reduction and even poses a great threat to human health via food chain [24]. Among heavy metals, Cadmium (Cd) in particular causes increasingly international concern [25]. Cd-contaminated soil results in considerable accumulation of Cd in edible parts of crops, and then it enters the food chain through the translocation and accumulation by plants [26, 27]. Another metal, chromium (Cr III or VI), is not required by plants for their normal plant metabolic activities. On the contrary, excess of Cr (III or VI) in agricultural soils causes oxidative stress for many crops. Reactive oxygen species (ROS), like hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and superoxide radical (O_2^-) generated under Cr-stress, are highly reactive and cause oxidative damages to DNA, RNA, proteins, and pigments [28, 29]. Nickel (Ni) is one of the most abundant heavy metal contaminants of the environment due to its release from mining and smelting practices. It is classified as an essential element for plant growth [30]. However, at higher concentrations, nickel is an important environmental pollutant. Ni^{2+} ions bind to proteins and lipids such as specific subsequences of histones [31] and induce oxidative damage. Copper (Cu) is also an essential micronutrient for most biological organisms. It is a cofactor for a large array of proteins involved in diverse physiological processes, such as photosynthesis, electron transport chain, respiration, cell wall metabolism, and hormone signaling [32, 33]. Cu has emerged as a major environmental pollutant in the past few decades because of its excessive use in manufacturing and agricultural industries [34]. Zinc (Zn) is one of the other essential microelement, the second most abundant transition metal, and plays roles in many metabolic reactions in plants [35, 36]. However, high concentrations of Zn are toxic, induce structural disorders, and cause functional impairment in plants. At organism level, Zn stress reduces rooting capacity, stunted growth, chlorosis, and at cellular level alters mitotic activity [37, 38].

The key to find out abiotic stress tolerance resides in understanding the plant's capacity to accelerate/maintain or repress growth. Most plant hormones play a role in development and have been implicated in abiotic stress responses. One of these hormones, BRs, are a group of steroidal hormones that play significant roles in a wide range of developmental phenomena including cell division and cell elongation in stems and roots, photo-morphogenesis, reproductive development, leaf senescence, and also in stress responses [39]. Mitchell et al. [40] discovered BRs which were later extracted from the pollen of *Brassica napus* by Grove et al. [41]. To date, more than 70 BR-related phytosteroids have been identified in plants [42].

BRs increase adaptation to various abiotic stresses such as light [43, 44], low or high temperature [45], drought [46, 47, 48], salt stress [9], and heavy metal stress [49, 50]. BRs may be applied/supplied to plants at different stages of their life cycle such as meiosis stage [51], anthesis stage [52], and root application [9, 53].

In this chapter, the potential role of BRs in alleviating the adverse effects of water, salt, low/high temperature stresses, and heavy metals on plants, especially roots, were discussed.

2. Water stress

Water shortage is predicted as one of the most important environmental problem for the 21st century that limits crop production [54]. Although drought stress inhibites the plant–water relations, exogenous application of BRs maintaines tissue–water status [55] by stimulating the proton pumping [56], activating nucleic acid and protein synthesis [57] and regulation of genes expressions [58]. It has been shown that 24-epibrassinolide (24-epiBL)-treated *Arabidopsis* and *B. napus* seedlings had a higher survival rate when subjected to drought [59], and in another study BR-treated sorghum (*Sorghum vulgare*) showed increased germination and seedling growth under osmotic stress [60].

Root nodulation is a fundamental developmental event in leguminous crops, and is sensitive to water shortage [61, 62, 63]. As endogenous hormones play an important role in the organogenesis and initial growth of nodules in roots, attempts have been made to increase root nodulation by growth regulator treatments [64, 65]. The potential of BRs in the improvement of root nodulation and yield have been reported in groundnut [66]. Upreti and Murti [67] also studied the effects of two BRs, epibrassinolide (EBL) and homobrassinolide (HBL), on root nodulation and yield in *Phaseolus vulgaris* L. cv. Arka Suvidha under water stress. They concluded that water stress negatively influenced nodulated root, but BRs increased tolerance to water stress and EBL was relatively more effective than HBL.

Several researchers have found that increased proline levels can protect plants from water stress. BR treatment increased the contents of proline and protein under water stress [68]. Zhang et al. [69] also indicated that BR treatment promoted the accumulation of osmoprotectants, such as soluble sugars and proline. It may be due to the fact that BRs activated the enzymes of proline biosynthesis, which caused an additive effect on the proline content [70].

Drought stress causes increment in H_2O_2 due to decrease in antioxidative enzyme activities [71]. Plants have improved various defense mechanisms to respond and adapt to water stress [72]. Vardhini et al. [73] studied with sorghum seedlings grown under PEG-imposed water stress and investigated the effects of HBL and 24-epiBL on the activities of four oxidizing enzymes: superoxide dismutase (SOD), glutathione reductase (GR), IAA oxidase, and polyphenol oxidase (PPO). They found that supplementation of both the BRs resulted in enhanced SOD and GR but lowered IAA oxidase and PPO. Li and Feng [68] also reported that treatment of brassinolide significantly increased peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities of seedlings under normal water and mild water stress. Therefore, increment in enzyme activities provided tolerance of *Xanthoceras sorbifolia* seedlings to drought stress. It has been found that BRs can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD, POD, CAT, and APX [74, 75].

3. Salt stress

Salinity stress is one of the most serious abiotic stress factors. It causes morphological, biochemical, cytogenetic, and molecular changes in plants [9, 76, 77, 78]. Root lengths, shoot lengths, and root numbers decrease in plants exposed to salt stress [7]. Moreover, salinity also induces oxidative stress in plants due to production ROS [79, 80]. These ROS are produced in the cell and interacted with a number of vital cellular molecules and metabolites, thereby leading to a number of destructive processes causing cellular damage [81].

BRs reduce impacts of salt stress on ROS, gene expression, mitotic index, nutrient uptake, and growth [9, 82, 83–88]. There are lots of studies to analyse alleviation of salt stress by using BRs. In these studies, different parameters have been investigated to understand the mechanism of BRs on salt stress (Table 1).

References	[9]	[84]	[85]	[86]	[87]	[88]
Effects on gene expression	---	---	---	---	Increased *Cu/Zn-SOD*, *APX*, *CAT*, *GR* and *OsBRI* expressions	---

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					but reduced *Fe-SOD* and *Mn-SOD*, *OsDWF4* and *SalT*	
Effects on protein content	Increased	---	Increased	---	Increased	---
Effects on enzyme activities	Increased SOD and CAT activities	Increased CAT, GR, POX and SOD activities	Increased POX and SOD activities	Increased SOD and POD activities	Showed varying results depending on 24-epiBL concentration for SOD, APX, CAT, GR	Increased CAT, POX and SOD activities
Effects on growth and/or cell division	Increased	Increased	Increased	Increased	Increased	Increased
Methods of salt and BRs applications	Seeds were grown under both 150–250 mM salt concentrations and 0.5 and 1 μM HBR at 48 h and 72 h.	Plants received 100 mM NaCl as well as 0.01 μM of HBL during 18 days after sowing	25, 50, 100, and 150 mM NaCl were applied and then sprayed twice with 0.05 ppm brassinolide during 25 days. At 45 days from sowing, the plants were collected	Seedlings were exposed to 90 mM NaCl with 0, 0.025, 0.05, 0.10, and 0.20 mg dm^{-3} 24-epiBL for 10 days	Seeds were soaked for 8 h in different concentration s of 24-epiBL (10^{-11}, 10^{-9} and 10^{-7} M). After 24-epiBL application, The seeds were sown in autoclaved sand moistened with different concentration s of NaCl (0, 75, 100, 125 mM) during 12 days	The 15-day-old plants were exposed to 100 mM NaCl and they were subsequently treated by exogenous 24-epiBL (10^{-8} M). The plants were harvested after 30 days of growth
Plant Species	*Hordeum vulgare* L.	*Vigna radiata* L.	*Vigna sinensis* L.	*Solanum melongena* L.	*Oryza sativa* L. var. Pusa Basmati-1 cv. indica	*Cucumis sativus L.*

Table 1. Effects of BRs on plants subjected to salt stress Dashes indicate that there are no results in study.

4. Temperature stress

4.1. High temperature

In general, a transient elevation in temperature (usually 10–15°C above environment) causes heat shock or heat stress [89]. High-temperature effects can be seen at the biochemical and molecular level in plant organs (especially leaves). Heat stress induces decrease in duration of developmental phases, leading to fewer organs, smaller organs, reduce light perception over the shortened life cycle, and finally play an important role in losing the product [90, 91, 92].

High-temperature stress often induces the overproduction of ROS [93] which can cause membrane lipid peroxidation, protein denaturation, and nucleic acid damage [94, 95]. Many studies have demonstrated that ROS scavenging mechanisms play an important role in protecting plants from high-temperature stress [96, 97]. BRs applications decrease ROS levels and increase antioxidant enzyme activities to provide thermotolerance to elevated temperatures [98].

4.2. Low temperature

Chilling and frost stresses affect growth, development, survival, and crop productivity in plants [99, 100, 101]. However, BRs treatments enhance seedling tolerance to chilling stress [101] and increase the height, root length, root biomass, and total biomass of rice under low-temperature conditions [102, 103]. In another study, Krishna [104] reported the same results in maize. They postulated that treatments with BRs promoted growth recovery of maize seedlings following chilling treatment (0–3°C).

Chilling stress increases the proline, betaine, soluble protein, soluble sugar contents of plants [79, 105]. Studies showed that BRs treatment enhanced proline content and therefore increased plant chilling resistance and cell membrane stability [99, 100, 106, 107].

Chilling stress could trigger the production of antioxidant enzymes in plants to prevent the chilling injury [108]. In the previous investigations, it was reported that treatment with BRs further increased the activities of antioxidant enzymes under chilling stress as well [99, 100, 107, 109]. The enhanced activities of the antioxidative enzymes as a result of BRs applications may occur with increasing de novo synthesis or activation of the enzymes, which is mediated through transcription and/or translation of specific genes to gain tolerance [57].

5. Heavy metal stresses

5.1. Cd stress

Cd toxicity has emerged as one of the major agricultural problems in many soils around the world [110]. It has been shown to interfere with the uptake, transport, and utilization of essential nutrients and water, change enzyme activities, cause symptoms (chlorosis, necrosis), decrease in fresh and dry mass of root and shoot and also their lengths [110, 111, 112].

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There are lots of studies to investigate the effects of BRs on Cd stress in plant species [110, 113, 114]. In these studies, results showed that BRs change different parameters such as germination, plant dry biomass, protein content, and antioxidant enzyme activities (Table 2). It is proposed that the changes induced by BRs are mediated through the repression and/or derepression of specific genes [58]. Microarray experiments evaluating gene expression changes in *Arabidopsis* roots and shoots under Cd stress were performed [115]. Moreover, studies showed that gene expression in response to Cd mimics a BR increase, and Cd exposure most probably induces an activation of the BR signaling pathway in *Arabidopsis* [116].

5.2. Cr stress

Cr (III or VI) is not required by plants for their normal plant metabolic activities [117]. The entry of Cr into a plant system occurs through roots via using the specialized uptake systems of essential metal ions required for normal plant metabolism [118]. On the contrary, excess of Cr (III or VI) in agricultural soils causes oxidative stress to many crops. Reduced seed germination, disturbed nutrient balance, wilting, and plasmolysis in root cells and thus effects on root growth of plants have been documented in plants under Cr stress [118, 119].

Choudhary et al. [120] reported that EBL treatment improved seedlings growth under Cr (VI) stress. Ability of EBL to increase seedling growth under this metal stress could be attributed to the capacity of BRs to regulate cell elongation and divisional activities, by enhancing the activity of cell wall loosening enzymes (xyloglucan transferase/hydrolase, XTH) [121]. Studies also indicated that increment in antioxidant activities as a result of BRs application (Table 2) provide plant tolerance to grow under Cr stress.

5.3. Ni stress

The heavy metals that affect (either positively or negatively) plants include Fe, Cu, Zn, Mn, Co, Ni, Pb, Cd, and Cr, but out of them, nickel has recently been defined as an essential micronutrient, because of its involvement in urease activity in legumes [122]. Excess Ni causes different problems. These symptoms include the inhibition in root elongation, photosynthesis and respiration, and interveinal chlorosis [123]. Moreover, the toxic concentration of Ni also inhibits enzyme activities and protein metabolism [124]. This metal also accelerates the activities of antioxidative enzymes [125, 126].

BRs effect on Ni stress in plants has been studied to understand the relationship between BRs and this stress (Table 2). One of these studies was carried out by Yusuf et al. [49]. They showed that seed germination and seedling growth were significantly reduced by Ni treatment, but HBL treatment enhanced germination percentage as well as shoot and root lengths in Ni-stressed seedlings. BRs confer tolerance against heavy metals either by reducing their uptake or by stimulating the antioxidative enzymes in *B. juncea* [127, 128]. The exogenous application of BRs in nickel-stressed *R. sativus* L., and *Triticum aestivum* L. plants enhanced the pool of antioxidant enzyme activity, thus alleviating the toxic effects of this stress [129].

5.4. Cu stress

Among the pollutants of agricultural soils, Cu has become increasingly hazardous due to its involvement in fungicides, fertilizers, and pesticides [130]. In addition, Cu present in excess has been known to decrease root biomass and alter plant metabolism [131, 132]. Sharma and Bhardwaj [127] demonstrated decrease in growth parameters of *Brassica juncea* grown under Cu stress. The reduction in growth parameters due to the Cu stress occurred as a result of decreasing mitotic activity and cell elongation [133, 134]. Moreover, Chen et al. [130] suggested a different opinion. They concluded that Cu-induced inhibition in root growth of rice seedlings was due to the stiffening of the cell wall. Moreover, excess of Cu ion leads to the generation of harmful ROS via the formation of free radicals [135].

Effects of exogenous application of BRs were studied on *Raphanus sativus* seedlings under Cu stress. It was found that 24-epiBL promoted the shoot and root growth by overcoming the Cu toxicity [136]. The growth-promoting effects of BRs on seedlings under Cu stress may be linked to the general ability of BRs to promote cell elongation and cell cycle progression [137, 138] as well as the stimulation of genes encoding xyloglucanses and expansins [139]. BRs applications also increase antioxidant enzyme activities [140, 141]. Increasing all parameters as a result of BRs application improves plant tolerance against Cu stress, and finally plant development (Table 2).

5.5. Zn stress

Zn is an essential microelement, the second most abundant transition metal after iron (Fe), and has a role in many metabolic reactions in plants [35, 36]. However, high concentrations of Zn are toxic, induce structural disorders, and cause functional problems in plants. At organism level, Zn stress causes reduced rooting capacity, growth, and at cellular level alters mitotic activity [37, 38]. It induces oxidative stress by promoting ROS production as a result of indirect consequence of Zn toxicity [142].

Application of BRs on plants alleviates Zn stress via increasing protein content and antioxidant enzyme activities (Table 2). Çağ et al. [143] reported that EBL application effectively enhanced the protein content in *Brassica oleraceae* cotyledons. Sharma et al. [144] also reported that pre-sowing treatments of HBL lowered the uptake of metal and enhanced the activities of antioxidative enzymes and protein concentration of *B. juncea* seedlings under Zn stress. Moreover, Ramakrishna and Rao [145] also reported that the application of 24-epiBL significantly alleviated the Zn-induced oxidative stress.

References	[114]	[146]	[30]	[147]	[141]	[145]
Effects on gene expression	---	---	---	---	---	---
Effects on protein content	---	Increased	Decreased protein content	---	Showed varying results	---

Effects on enzyme activities	Increased CAT, POX and SOD activities	Decreased CAT level	Decreased SOD but increased POD activities	Increased SOD, CAT and POX activities	Showed varying results	Increased
Effects on growth and/or cell division	Alleviated	Improved	Improved	Improved	Increased	Increased
Methods of metal and BRs applications	Soil amended with 50 μM Cd and foliage sprayed with 10^{-8} M HBL at 20 days after sowing. The plants were sampled at 30 days after sowing	Seeds were treated with eEBL (10^{-9} M) and 1.2 mM Cr(VI) (K_2CrO_4) solution at 7 days	Seeds were soaked for 8 h in different concentrations of 24-epiBL (0, 10^{-7}, 10^{-9} and 10^{-11} M). Then, seeds were grown under different (0, 0.5, 1.0, 1.5, and 2.0 mM) Ni concentrations	Seeds were grown in different levels of Cu^{2+} (50 or 100 mg kg^{-1} of soil. At 15 and 20 days stage, 10^{-5} mM HBL was applied. At 45 days, plants were collected	Seeds were grown under both 30 μM – 40 μM Cu and 2 μM HBR at 48 h and 72 h	EBR (0.5, 1, and 2 μM) and 5 mM of Zn were applied to seeds. Seven day old seedlings were collected
Plant Species	*Triticum aestivum* L.	*Raphanus sativus* L.	*B. juncea* L.	*Vigna radiata* L.	*Helianthus annuus* L.	*Raphanus sativus* L.
	Cd Stress	**Cr Stress**	**Ni Stress**	**Cu Stress**		**Zn Stress**

Table 2. Effects of BRs on plants subjected to Cd, Cr, Ni, Cu and Zn stresses. Dashes indicate that there are no results in study

6. Conclusion

Roots are very important plant organs whose architecture is determined by endogenous and environmental conditions to adjust water and nutrient uptake from soil [148, 149]. BRs, one of the plant hormones, have both positive and negative effects on root growth related to hormone concentrations [150]. Experimental condition is one of the most important factors for analysing BRs effects on root development. The procedures using BRs to alleviate abiotic systems generally are easy, time saving, and one of the most reliable systems [53]. Therefore, BRs open up new approaches for plant tolerance against hazardous environmental conditions [151]. Morphological, biochemical, and molecular analyses have been performed to analyse the effects of BRs. However, detailed analyses should be performed to investigate the relationship between abiotic stresses and BRs, especially gene expression studies will provide knowledge about interaction at molecular level in plants [152]. We tried to cite as many papers as possible. Yet we apologize to authors whose works are gone unmentioned in this chapter.

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Author details

Sevgi Marakli* and Nermin Gozukirmizi

*Address all correspondence to: sevgi.marakli@istanbul.edu.tr

Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, Vezneciler, Istanbul, Turkey

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