

## Advances on exogenous applications of brassinosteroids and their analogs to enhance plant tolerance to salinity: A review

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### Abstract

Soil salinity is one of the abiotic stresses, which reduces plant growth and limits crop productivity. Brassinosteroids are considered as the sixth class of plant hormones and they have pleiotropic effects on plants, among them, they can protect plants under abiotic stresses and especially under salt stress. This paper reviews the information published during the last sixteen years related to the effects of brassinosteroids and their spirotanic analogs in plants submitted to saline stress when they are used by means of seed treatments, through the application of foliar sprays or by rooting media. Brassinosteroids stimulated salt stress tolerance in plants is associated to a decrease of oxidative damage generated by this stress and the interaction between brassinosteroids and other plant hormones, for example: abscisic acid, ethylene and salicylic acid.

**Keywords:** Plant growth regulators, brassinosteroids, spirotanic analogs, abiotic stress, salt stress.

**Abbreviations:** ABA\_abs cisic acid, BR\_brassinosteroid, BL\_brassinolide, EBL\_24-epibrassinolide, HBL\_28-homobrassinolide DAA-6 – 2α, 3α-dihydroxy-5α-spirostan-6-one, DI-31\_3α,5α-dihydroxy-5α-spirostan-6-one

### Introduction

Salt stress is frequently caused by NaCl, but the saline soils may present different combinations of salts such as sodium or magnesium or calcium chloride and sulfate (Lamz and González, 2013). Salinity causes an osmotic stress, ionic toxicity and also disturbs the uptake and translocation of mineral nutrients in plants (Turkan and Demiral, 2009). Plant adaptation to salinity mainly concerns tolerance to salt-induced osmotic stress, tissue tolerance to accumulated Na<sup>+</sup> or Cl<sup>-</sup> ions and exclusion of these ions in the cells and at the whole plant level (Munns and Tester, 2008).

On the other hand, the salinity affects plant growth and it has been suggested that this behavior is at least in part, associated to a depression of photosynthesis activity (Munns, 2002; Hajlaoui et al., 2006; Stepien and Klobus, 2006), one of the processes affected severely during the saline stress. It is due to stomatal limitations resulting in reduction of stomatal conductance, transpiration rate, intercellular CO<sub>2</sub> concentration and net photosynthesis (Wilson et al., 2006; Lu et al., 2009) or to non-stomatal limitations including the depression in photosystem II activity, electron transport and photophosphorylation activity (Xia et al., 2004; das Neves et al., 2008). All these and other altered processes give rise to a plant growth and a crop productivity decrease (Rady, 2011). Salinity tolerance involves a complex of responses at molecular, cellular, metabolic, physiological, and whole-plant levels. Among

various salinity responses, mechanisms or strategies controlling ion uptake, transport and balance, osmotic regulation, hormone metabolism, antioxidant metabolism, and stress signaling play critical roles in plant adaptation to salinity stress. Genetic engineering has been proved to be an efficient tool to the development of salinity-tolerant plants, and this approach will become more powerful as more candidate genes associated with salinity tolerance are identified and widely utilized (Gupta and Huang, 2014). Other strategies have also been used as the application of different natural compounds or plant growth regulators which are capable of mitigating the salt stress-induced adverse effects in plants. Among them, brassinosteroids (BRs) are natural compounds of steroidal structure which have demonstrated to protect plants under saline stress. Particularly, Hamada (1986) demonstrated that brassinolide (BL) improved *Oryza sativa* salt-tolerance grown in nutritive solutions under greenhouse conditions. Other authors reported that this same compound protected nucleus and chloroplast ultrastructure of *Hordeum vulgare* leaf segments exposed to NaCl (Kulaeva et al., 1991) and *Eucalyptus camaldulensis* seed germination was stimulated in the presence of 24-epibrassinolide (EBL) and NaCl (Sasse et al., 1995). Afterwards, Vardhini and Rao (1997) reported that application of BL, EBL and 28-homobrassinolide (HBL)

removed the salt-induced inhibitory effects in peanut seedlings.

Various authors have reviewed the role of brassinosteroids in alleviating abiotic stress in plants (Krishna, 2003; Bajguz and Hayat, 2009; Javid *et al.*, 2011; Núñez, 2012; Fariduddin *et al.*, 2014). However, this paper will review the progress reached, during the last sixteen years, in the field of the exogenous application of brassinosteroids and their analogs in plants submitted to saline stress and the action mode of natural brassinosteroids under these conditions.

### **Seed treatments with brassinosteroids**

The seed treatment with BRs to protect plants to salt stress has been used by different researchers who have tested different compounds, concentrations and ways of treatment application.

Thus, Anuradha and Rao (2001) soaked rice seeds for 24 hours in solutions of NaCl supplemented with EBL or HBL. BRs enhanced seed germination and completely recovered the inhibitory effects of salt stress on seedling growth. This effect was associated to increased levels of nucleic acids and soluble proteins.

Özdemir *et al.* (2004) and Arora *et al.* (2008) demonstrated that seed treatment with EBL or HBL considerably alleviated oxidative damage in rice and maize seedlings, respectively, under NaCl-stressed conditions.

Previously, Anuradha and Rao (2003) had reported that rice seed treatments with BL or EBL reduced the impact of salt stress on growth and considerably restored pigment levels and increased nitrate reductase activity. In the same crop, Phap (2006) found that EBL improved both the earliness and the total germination of salinity-tolerant cultivar MTL119 but had no effect on the salinity-sensitive cultivar IR28. Lucerne seed priming with BL improved germination and seedling growth under high-saline soils and it was associated to a significant reduction of the malondialdehyde (MDA) accumulation (Zhang *et al.*, 2007).

Other EBL-induced effect by the seed treatment was the reduction of the chromosomal abnormalities present in root tips of barley plants grown in a medium containing NaCl (Tabur and Demir, 2009). However, the applications of HBL and NaCl to barley roots stimulated root lengths and showed more mitotic abnormalities when compared to salt-treated samples alone (Marakli *et al.*, 2014).

Other application method was used by Ali *et al.* (2007) who treated chickpea seeds for four hours with solutions of NaCl and then transferred to HBL for additional four hours or vice versa. They showed that the adverse effects induced by salt stress were overcome by HBL, although, this treatment was given before or after NaCl treatment. Similar methodology was also used by Shahid *et al.* (2011) in pea seeds and they concluded that EBL treatments, especially with a 10  $\mu\text{M}$  concentration, given prior to NaCl treatment significantly overcame the NaCl-induced deleterious effects.

### **Foliar application with brassinosteroids**

Various authors have used foliar sprayings with BRs in order to enhance plant tolerance to saline stress. For example, Ali *et al.* (2006) reported that the foliar application of two wheat cultivars with EBL significantly improved the seedling growth and  $\text{Ca}^{2+}/\text{Na}^+$  and  $\text{K}^+/\text{Na}^+$  ratio by enhancing uptake

of  $\text{Ca}^{2+}$  and  $\text{K}^+$  and reducing that of  $\text{Na}^+$ , which contributed to the salt tolerance of both wheat cultivars.

On the other hand, Shahbaz and Ashraf (2007) worked with the same cultivars and they found that exogenous application of EBL ameliorated the growth of salt-tolerant cultivar (S24), however this application did not have a prominent role in accumulation of different nutrients which contribute to salt tolerance in both wheat cultivars.

Similar results were reported by Shahbaz *et al.* (2008) who associated the growth response of the salt-tolerant wheat cultivar to the improvement of EBL-induced photosynthetic capacity and the increase of peroxidase and catalase enzyme activities. Talaat and Shawky (2012) demonstrated that EBL detoxified the oxidative stress generated by salinity and significantly improved some physiological and biochemical indicators of other salt-tolerant wheat genotype, which confirms the above presented results. Also, EBL prevented diamine oxidase and polyamine oxidase enzymes inhibition, indicating a positive correlation between salt tolerance and polyamines accumulation. These authors also reported that EBL foliar spray stimulated organic solutes, antioxidant system and  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  levels in two wheat cultivars under saline conditions and this increases proved to be more pronounced in the salt-tolerant cultivar (Talaat and Shawky, 2013). According to Bajguz and Hayat (2009), BRs exert a protective action on wheat seedlings via a considerable decrease in the salt-induced ABA and wheat germin agglutinin accumulation in roots.

Foliar EBL application to two strawberry cultivars grown under saline conditions, significantly increased shoot and root dry matters, stomatal conductance, leaf relative water content and leaf chlorophyll values and leaf and root macro-micro element content of plants, except for  $\text{Na}^+$ ; also demonstrating that foliar application can overcome the effects of salinity stress on plant growth (Karlidag *et al.*, 2011).

In rice seedlings under salt stress conditions, EBL foliar application restored growth in the seedlings due to a decrease in oxidative stress, evidenced by a reduction of lipid peroxidation and proteins oxidation, and maintenance of chlorophylls concentration (Reyes *et al.*, 2015).

Similar protective effects with EBL foliar application were also reported by Houimli *et al.* (2010) on pepper; Ekinçi *et al.* (2012) on lettuce; Cheng *et al.* (2015) on watermelon and Çoban and Bayda (2016) on peppermint plants.

On the other hand, HBL foliar spray in mustard plants attenuated the NaCl-induced adverse effects on nitrate reductase and carbonic anhydrase activities, chlorophyll content, rate of photosynthesis and seed yield (Alyemeni *et al.*, 2013).

In legumes, López *et al.* (2016) demonstrated that BRs are involved in the regulation of the nodulation in the *Medicago truncatula*-*Sinorhizobium meliloti* symbiosis. The co-treatment with NaCl and EBL augmented the foliar spermine (Spm) concentration. This increment of the Spm levels was followed by a reduction of the membrane oxidative damage and a diminution of the proline accumulation.

Furthermore, BRs have been sprayed along with other compounds such as polyamines and salicylic acid. Thus, Slathia *et al.* (2013) found that EBL and putrescine (Put) were not only able of restoring the adverse effects on photosynthetic pigments induced by salinity but they also increased contents of ascorbic acid, total phenols,

glutathione, proline and glycine betaine, indicating positive and complementary impact of EBL and Put interactions on antioxidant system of tomato plants.

Previously, Hayat *et al.* (2012) demonstrated that foliar sprays of HBL and salicylic acid to mustard plants completely counteracted the toxic effects generated by salinity stress.

BRs can also diminish the injurious effects induced by salt and heavy metal stresses. Thus, Ali *et al.* (2008a) reported that EBL foliar spray of mustard plants detoxified the stress generated by NaCl and NiCl<sub>2</sub> and significantly improved the growth, the level of pigments and photosynthetic parameters of plants. Similar results were obtained on bean plants grown under salinity and cadmium stress conditions (Rady, 2011). Furthermore, BRs and spermidine can completely overcome the toxic effects induced by salt and Zn metal stress on *Vigna radiata* plants (Mir *et al.*, 2015).

#### **Brassinosteroid applications through rooting media**

Brassinosteroids have been also applied to the rooting media to evaluate their effects in plants submitted to saline stress. For example, in *Brassica napus*, Kagale *et al.* (2007) showed that the presence of EBL in the medium significantly enhanced germination and initial plant growth under saline conditions.

On the other hand, Ali *et al.* (2008b) demonstrated that EBL application to roots of two wheat cultivars improved growth and yield of both cultivars and it was suggested that the increase in the total grain yield was due to the increase in grain size by EBL induced increase in translocation of photoassimilates towards grains.

Durigan *et al.* (2011) found that the presence of EBL in the nutritive solution increased plant fresh weight, shoot dry weight, leaf area, leaf and root water content, photosynthetic pigments, sugar concentration, photosynthetic rate and water use efficiency of *Cajanus cajan* plants grown under NaCl stress. An alleviated growth reduction, a decrease of oxidative damage and a significantly increased chlorophyll content and photosynthetic attributes were obtained on eggplant plants by Ding *et al.* (2012) and Wu *et al.* (2012), respectively.

Previously, Hayat *et al.* (2010) demonstrated that HBL treatment detoxified the stress generated by temperature and/or NaCl and significantly improved the growth and photosynthetic activity of *Vigna radiata* young plants.

The addition of BRs to the soil in field conditions may not be efficient due to the fact that they may be partially or completely degraded by soil microorganisms (Ashraf *et al.*, 2008) and also, the application to the soil is not economically feasible by the high cost of these products. For this reason, further research is needed to fine-tune the efficiency of the BRs use as a supplement through soil to reduce the adverse effects of any abiotic stress, including salinity (Ashraf *et al.*, 2010).

#### **Modes of brassinosteroids action**

EBL and HBL application enhanced the protein level, antioxidant metabolites and enzyme activities, the compatible solutes accumulation in plants subjected to salt stress, which provided plant tolerance against this kind of stress (Rattan *et al.*, 2014).

It is essential for plant tolerance to salinity that reactive oxygen species (ROS) production and scavenging are controlled in the chloroplast (Miller *et al.*, 2010), and the results here discussed demonstrate that BRs are able to protect plants against oxidative stress generated by salinity, so BR-induced plant growth under saline conditions may be associated to the increases in the activities of antioxidant enzymes and scavenging metabolites in this organelle. Moreover, BRs induce oxidative stress tolerance by triggering the accumulation of apoplast H<sub>2</sub>O<sub>2</sub> which subsequently up regulates the antioxidant system (Fariduddin *et al.*, 2014). On the other hand, exogenous application of EBL modified the activity of enzymes of proline metabolism (Yusuf *et al.*, 2017) and it also increased the expression of various oxidative stress marker genes, although to different levels (Sharma *et al.*, 2013). Thus, Vardhini and Anjum (2015) reviewed the reports on the role of BRs in the modulation of enzymatic and non-enzymatic components of antioxidant defense system in abiotic stressed plants.

BRs induce the alternative respiratory pathway in *Nicotiana benthamiana* (Deng *et al.*, 2015) and *Brassica napus* (Derevyanchuk *et al.*, 2017) under saline stress conditions. In the first case, it was also evidenced that BR-induced H<sub>2</sub>O<sub>2</sub> production is necessary for this induction and the activation of phospholipid signaling by measuring levels of diacylglycerol and phosphatidic acid was observed in the second case.

Furthermore, Divi *et al.* (2010) reported that the redox-sensitive protein NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1), a master regulator of salicylic acid-mediated defense genes is likely a critical component of EBL-mediated increase in salt tolerance, but this protein is not required for EBL-mediated induction of PR-1 (PATHOGENESIS RELATED 1) gene expression.

It is well known the interaction between BRs and other plant hormones. Thus, Zhang *et al.* (2011) suggested that BR-induced NO production and NO-activated ABA biosynthesis are important mechanisms for BR-enhanced oxidative stress tolerance in maize. Previously, Zhang *et al.* (2009) had reported that ABA signaling regulates the primary signaling outputs of BRs, and, recently, Nawaz *et al.* (2017) confirmed that genes upregulated by ABA were down-regulated by BRs under salt stress conditions.

Wang *et al.* (2011) demonstrated that the mitigating effect of BR on salt stress-induced inhibition of seed germination may occur through its interaction with ethylene synthesis and Choudhary *et al.* (2012) indicated that BR signaling components mainly interact with the signaling elements of other hormones at the transcriptional level and, so, it could provide a unique tool for the genetic improvement of crop productivity in a sustainable manner.

Nitric oxide plays an important role in hydrogen peroxide dependent induction of plant stress tolerance by BRs in cucumber young plants (Cui *et al.*, 2011). In parallel, Yang *et al.* (2011) updated the mechanisms of BR action and they underscored the importance of antioxidant enzymes and H<sub>2</sub>O<sub>2</sub> in the action of BRs, and its involvement in conveying environmental cues and growth responses.

Later, Cui *et al.* (2012) reported that the stress-induced ubiquitin conjugating enzyme, UBC32, plays a role in the BR-mediated salt stress response.

All these results demonstrate that BR-induced salt tolerance is associated to a decrease of oxidative stress by means the increase of antioxidant metabolites and enzyme activities and of the expression of oxidative stress marker genes; thus, as to the interaction of brassinosteroids with other plant hormones at transcriptional level. Recently, Sharma *et al.* (2017) pointed out that addition of knowledge on transcriptional and post-transcriptional BR signaling as well as translational and post-translational events regulated by BRs will be critical in modulating the regulatory role of BRs in abiotic stress tolerance in plants.

### **Treatments with analogs of brassinosteroids**

Spirostanic analogs of brassinosteroids, synthesized in Cuba, have been tested as plant protectors against drought and high temperature (Mazorra and Núñez, 2003) or salt stress (Reyes *et al.* 2010) in tomato plants.

Particularly, Núñez *et al.* (2007) demonstrated that seed treatment of two rice cultivars with Biobras-6 (DAA-6, 2 $\alpha$ ,3 $\alpha$ -dihydroxy-5 $\alpha$ -spirostan-6-one as active ingredient) or Biobras-16 (DI-31, 3 $\alpha$ ,5 $\alpha$ -dihydroxy-5 $\alpha$ -spirostan-6-one as active ingredient) formulations reduced the salt-induced inhibition on seedling shoot and root lengths and fresh and dry weights of both cultivars.

Another experiment was performed in order to compare the effects of the application of EBL or Biobras-16 in two rice cultivars grown under salt conditions for 13 days. EBL concentrations stimulated the shoot and root lengths of both cultivars and seedling dry weight in the salt-tolerant cultivar, while BB-16 (0.1  $\mu$ M) only stimulated the shoot length and dry weight. However, BB-16 (0.01 and 1.0  $\mu$ M) completely recovered the salt-induced inhibition on the shoot length and dry weight in the salt-sensitive cultivar; while EBL (0.1  $\mu$ M concentration) increased the shoot dry weight. These results revealed the potentialities of both products at low concentrations to revert the salt-induced adverse effects on rice young plant growth (Núñez *et al.*, 2013).

The effectiveness of another formulation, Biobras-25 (a second formulation containing the spirostanic analog, DI-31, and a cholestanic brassinosteroid analog as active ingredients), was also studied and the authors demonstrated that it was better than Biobras-16, since it not only significantly enhanced seedling growth under NaCl stress but also significantly increased pigment concentration and decreased the proline content in leaves (Núñez *et al.*, 2016). Recently, Reyes *et al.* (2017) demonstrated that the foliar application of BB-16 (0.1  $\mu$ mol L<sup>-1</sup>) to rice young plants increased the shoot dry weight under saline conditions. This response appears to be associated with increased antioxidant defenses as well as an increase of the concentration of chlorophyll a in the leaves.

These results were confirmed in another experiment included in this paper, in which foliar application of BB-16 not only stimulated rice young plant growth seven days after NaCl treatment but also 14 days after recovering.

Significant results have also been reported with the application of DI-31 (active ingredient of BB-16) in lettuce plants, demonstrating that this brassinosteroid analog partially reverted the negative effects of salt stress in plants, concluding that this compound has a protective effect against salinity (Serna *et al.*, 2015).

The spirostanic analog of brassinosteroids, Biobras-16, has demonstrated to stimulate crop growth and yield, (Núñez *et al.*, 2014), so it has been introduced in Cuban agriculture as a biostimulant. In this review, protection of young plants against salt stress by seed treatment or foliar spray of Biobras-16, at low concentrations, is also showed. So, it is necessary to confirm these results under field conditions and thus, this brassinosteroid analog, whose synthesis is more economic than that natural brassinosteroids might be become an ecological and practical alternative to mitigate the adverse effects of saline stress in plants. However, further research is needed for elucidating its mechanism of action and compare to the natural brassinosteroids.

### **Conclusion**

Exogenous application of brassinosteroids by means of seed treatments, foliar sprays or through rooting medium counteracts the salinity-induced growth and yield inhibition in plants. From a practical point of view, the seed treatments and foliar sprays are the alternatives less expensive in field conditions. In general, the plant response to the BRs application was associated to the decrease of oxidative stress generated by salinity caused by increased expression of oxidative stress marker genes, the increase of activities of antioxidant enzymes and metabolites and their interaction with other plant growth regulators as salicylic acid, ethylene and ABA. Spirostanic analogs of brassinosteroids, also, enhanced plant growth under salt stress conditions and decreased oxidative damage generated by this stress. However, it is necessary to confirm these results under field conditions and establish practical strategies for the application of these compounds in agriculture to mitigate the increase of soil salinity associated to global climate changes.

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