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### Impact of brassinosteroids on salt stress tolerance in horticultural crops

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#### Abstract

Phytohormones are the most important chemicals these are produced in parts of the plant and transferred to the other plant parts, where they can regulate an indispensable role in responses to saltiness conditions. Brassinosteroids are a new plant hormone for promoting or enhancing the growth activities in plants. Salinity is the extensive abiotic stresses that disapprovingly disturb the cultivation of the crop. Reducing the consequence of saltiness enhances the implementation of important cultivar regulators which regulate the wide range of physiological and metabolic activity of plants. Plant steroid hormones Brassinosteroids are pertinent for controlling the cellular divisions, cell distinction, and cell elongation of the number of cell types in its life cycle. 24-epibrassinolide and 28-homobrassinolide are applied to plants and it will increase the growth and protect the plant from adverse environmental stresses. That article includes the major aspects of the hormone stresses. That article includes the major aspects of the hormone involved in horticultural crops for maintaining the biotic factors and gave concentrates on various hormones at molecular and biochemical levels for getting the desirable stress response.

Keywords: Brassinolides, horticultural crops, 24-epibrassinolide, 28-homobrassinolide, salt stress

#### 1. Introduction

Brassinosteroids are new plant growth hormones with several plant growth-promoting activities. Brassinosteroids were founded on the rapeseed pollen, Brassica napus L. It can regulate the plant processes for plant development and plant growth, plant cell proliferation, elongation, reproduction phase, photomorphogenesis, fruit ripening, cell division, flowering development, cell senescence, root initiation and resistance to the eco-physiological and biota stresses are cold, pesticides, heat, and drought. Brassinosteroid occurrence has been identified in almost all parts of the plant, such as leaves, stems, flower buds, fruits, roots, shoots, seeds, vascular cambium, and pollen. In order to increase the development, productivity, and fruit quality of agri-horticulture plants, brassinosteroids are a natural, safe, non-genotoxic, phytohormone that may be employed (Ghosh et al., 2022)<sup>[24]</sup>. The loss in global crop production is mostly caused by soil salinity and drought, in particular (Boscaiu & Fita, 2020) <sup>[7]</sup>. Various physiologic and metabolic functions, involving growth, gaseous exchange, relative moisture content, cell permeability, and the presence of pigments involved in photosynthesis activities, are adversely impacted by high salt values in plants (Azarmi et al., 2010)<sup>[2]</sup>. Nacl stress can lower overall chlorophyll concentrations by degrading chlorophyll contents, and such degradation has been connected to Na+ toxicity (Shahzad et al., 2019)<sup>[53]</sup>. As a result of salt, plants respond by restricting their growth, limiting the formation of lateral shoots, decreasing the size of their leaves and fruits, and decreasing the content of both their fresh and dry components (Sinha et al., 2022)<sup>[44]</sup>. By interfering with the evapotranspiration mechanism, salinity in water lowers the rate at which plants transpire, hence lowering agricultural output (Habib *et al.*, 2016) <sup>[27]</sup>. Salt stress considerably decreased the N, P, and K concentrations in the leaves when it was present in irrigation water (Alam et al., 2020) [5]. Brassinosteroids are steroid phytohormones found in plants that assist in a number of physiological processes, including gas exchange; stalk elongation, and cell multiplication and expansion, which enhances the ability of plants to respond to salt stress (Gupta et al., 2017)<sup>[22]</sup>. Plant efficiency can be enhanced by rhizosphere microorganisms, especially helpful bacteria and fungus (Mondal & Kaur, 2017)<sup>[40]</sup>. The Brassinosteroids associated with the phytohormones including the polyamines for regulating the different developmental and plant physiological processes (Saini et al., 2015) [57]. The fruit flavour is significantly influenced by acidity, which may be significantly reduced by exogenous administration of 24-epibrassinolide, while retaining fruit quality (Wang et al., 2020)<sup>[67]</sup>.

#### The Pharma Innovation Journal

24- Epibrassinolides promotes the genetic and physiological changes in pepper varieties under salt stress condition. BRs affect root and flower development, cell division, photomorphogenesis, tissue vascular matrix, proton pumps, membrane polarization, and stress modulation (Clouse, 2011) <sup>[10]</sup>. Water and salt shock adversely impact plants by impeding osmotic processes, nutrient uptake, and the reduction of photosynthetic activity (Stepien & Klobus, 2006) <sup>[52]</sup>. Brassinosteroids promote the exertion of catalyzing and decrease the action of peroxides and ascorbic acid oxide undergoing salinity conditions (Sadeghi & Shekafandeh, 2014)<sup>[56]</sup> and also regulates the secondary metabolites (Rao et al., 2002) <sup>[47]</sup>. By enhancing proline synthesis in harsh environmental circumstances, Homobrassinolide confers stress tolerance. It also induces flowering and boosts fruit set and development (Wang et al., 2019) [66]. Additionally, BRs

control the activity of defense-related enzymes, allowing for the development of robust defenses against various pathogens (Pavani *et al.*, 2020) <sup>[46]</sup>. In cultivars susceptible to salinity, phytohormones preserved the physiological viability of the seeds and the development traits of the seedlings, reducing the negative impacts of salt on horticultural crops (Junior *et al.*, 2021) <sup>[30]</sup>.

#### 2. Structure

Brassinosteroids are identified as C28, C27 & C29 based on different substitutions of the alkyl sequence of the side bonds. The trans-ring system merged with some two –OH (hydroxyl radical) at ring-A and 7-oxa-6-ketone system at B-ring for BR. The total number BRs is 49, out of the 25 are related to 6-oxo, 5 to 7-oxalactone, 17 relate to 6-dexoand 2 relate to given (Table-1).

Table 1: Brassinosteroids structure

Carbon	B-ring	A-ring (substituent)	Characteristics (s)	References
C28	7-Oxalactone	С(2а,3а)-ОН	24-epiBL, BL, DL	(Schmidt et al., 1996) <sup>[50]</sup>
		С(2α,3β)-ОН	3-epiBL	(Konstantinova et al., 2001) [36]
		С(2β,3α)-ОН	2-epi-23-dehydroBL	(Watanabe et al., 2000) <sup>[64]</sup>
		С(2β,3β)-ОН	2,3-diepi-23-dehydroBL	
		С3а-ОН	7-oxTY	(Katsumata <i>et al.</i> , 2008) <sup>[32]</sup>
		С3β-ОН	7-oxTE	
	6-Oxo	С(2а,3а)-ОН	24-epiCS, CS	(Schmidt et al., 1995) <sup>[49]</sup>
		С(2α,3β)-ОН	3,24-diepiCS,3-epiCS	(Kim et al., 1987) <sup>[33]</sup>
		С(2β,3α)-ОН	2-epiCS	(Kim et al., 1987) <sup>[33]</sup>
		С(2β,3β)-ОН	2,3-diepiCS	
	6-Deoxo	С3β-ОН	6-deoxoCT	
		С3а-ОН	3-epi-6-deoxoCT	(Yokota <i>et al.</i> , 1990) <sup>[68]</sup>
C27	6-Oxo	С3а-ОН	28-norTY	(Katsumata <i>et al.</i> , 2008) <sup>[32]</sup>
		С(2а,3а)-ОН	28-norCS	(Gamoh et al., 1990) <sup>[21]</sup>
	6-Deoxo	С3β-ОН	6-deoxo-28-norCT	(Bancos et al., 2002) <sup>[6]</sup>
		СЗа-ОН	3-epi-6-deoxo-28-norCT	
C29	6-Oxo	С(3а,2а)-ОН	28-homoDS	(Yokota <i>et al.</i> , 1983) <sup>[69]</sup>
		С3β-ОН	3-epi-2-deoxy-25-MeDS	(Park et al., 2000)
	6-Deoxo	С(3а,2а)-ОН	6-deoxo-25-MeDS	
	7-Oxalactone	С(3α,2α)-ОН	28-homoDL,28-homoBL	(Yokota <i>et al.</i> , 1984) <sup>[70]</sup>

#### 3. Effect of brassinosteroids on cell physiology

The plant expansion is inhibited along with salt in rhizoid water for two purposes. First, it impairs the plant's proficiency to imbibe water, succeeding in unperceptive expansion. The water and osmotic-deficit effect actualization is saline. Secondly, this might infiltrate the denitrification stream and harm cells in an emerging leaflet, slowing even supplementary, that is the ion-excess or alkali-specific impact of salinity. Because of osmotic fragility generate by elevated salt aggregation in plants and soil, the hydrophilic magnitude of root systems decreases, and dehydration from leaflets speeds up in the preliminary of salt stress, which is also dubbed hyper osmotic stress (Munns, 2005) [38]. The presence of salinity solution inhibits leaves and to a mild extent, rootage (Munns, 1993) <sup>[39]</sup>. The salt taken by the plant concentration in old leaves, the long-term transfer into transpiring leaves leads to the leaves dying because of extremely high Na+ and Cl- concentrations. The injury is most likely caused by a salt load that exceeds the cells' ability to digest salinity in the vacuoles. Salinity then swiftly accumulates in protoplasm, inhibiting the synthesized action. Otherwise, it could accumulate in the plasma membrane and cause damage and that cell is dehydrated. BRs cause

conversion in enzyme campaign, RNA, membrane potential, DNA and protein production, photosynthesis, and the stability of other androgenic plant hormones, among other physiological reactions (Mandava, 1988). Under salt stress, BRs also restored chlorophyll levels and boosted the nitrate dehydrogenate activity. The leaflets of salinity plants have nitrate reductase activity. Because nitrate transfer to the roots is impeded by salt, this is a consequence of nitrate intake hindrance loading of the xylem (Anuradha & Rao, 2003) <sup>[4]</sup>. The application of 28-homoBL to the leaves or the delivery of 28-homoBL through the roots of plants grown from NaCl-soaked seeds improved growth, seed yield, ethylene content, and nucleic acid content (Hayat *et al.*, 2006, Hayat *et al.*, 2007) <sup>[24, 25]</sup>.

Table 2: Effect of brassinosteroids on cell physiology

S. No.	Cell physiology characteristics	Reference
1.	Increasing flowers	Yokota et al. (1984) <sup>[70]</sup>
2.	Increasing fruit set	Kamuro and Takatsuto (1999) <sup>[34]</sup>
3.	Reducing fruit drop	Suzuki et al. (1990) <sup>[48]</sup>
4.	Reducing fruit cracking	Peng et al. (2004) [45]
5.	Influence fruit ripening	Chai et al. (2013) <sup>[11]</sup>

#### 4. Induction of thermal tolerance in plants

One of the active substances synthesized during BL biosynthesis is 24-epibrassinolide (EBL), which can enhance a variety of metabolic functions in plants, including the uptake of carbon dioxide, nucleic acid synthesis, and ROS metabolism, and light absorption (Tanveer et al., 2018)<sup>[61]</sup>. Since numerous preceding research suggested that EBL may be useful in reducing various abiotic stressors, hightemperature sensitivity can be achieved by exogenous administration of EBL (Shahzad et al., 2018) [53]. EBL strengthens a variety of morphological and physiological characteristics to stimulate plant growth and production under High-temperature stress. Eggplant growth and yield were considerably reduced while exposed to HT stress, however, with the application of EBL, the plant increased in stature by 26%, stem girth by 42%, and root and shoot volume by 43 and 55%, respectively. It is feasible that EBL can stimulate the carbon incorporation process, which will accelerate plant expansion under HT stress. HT stress primarily inhibits plant blossoming and fruit set, which subsequently diminishes agricultural production. A larger number of tomatoes per plant and increased pollen germination, pollen tube development with less pollen bursting, and EBL-induced tomato output enhancement were all linked to HT stress (Singh et al., 2005) [51]. By improving antioxidant activity, diminishing lipid peroxidation in rice seedlings, and accumulating heat shock protein, EBL promotes thermal tolerance (Cao and Hua, 2008) [9]. Under both stress and nonstress situations, BLs are also involved in influencing the structure, plasticity, and transparency of cell membranes (Siddiqui et al., 2018)<sup>[54]</sup>. EBL treatment enhances the carbon absorption process and shields it against HT-induced oxidative damage, which in turn boosts plant growth and productivity.

#### 5. Effectiveness of foliar nutrition additives on Horticultural Crops

Recent hostile climatic and edaphic situations have increased the incidence of reproductive abnormalities in horticultural crops, comprising intense air volatility, dehydration shock and desiccation, excessive illumination stress, subsurface salinity and alkalinity, and nutrient deficiency (Kallsen, 2017)<sup>[37]</sup>. The accessibility of micronutrients for plant incorporation is diminished by soil acidification in addition to surface salinity owing to the generation of recalcitrant aggregates or adversarial effects. Foliar micronutrients should be delivered in this situation when fertilizer demand is at its peak, in theory, because soil availability and root absorption may not be ample to meet expectations even with a suitable amount of soil-applied fertilizer. Supplemental aerial nourishment with zinc, potassium, nitrogen, calcium, and boron might be efficacious in alleviating the consequences of environmental stressors, perhaps immediately direct synthesis, phloem loading and conveyance of photo-assimilates, and allocation appropriate to sink need, or vicariously by safeguarding plants from ROS damage (Wimmer et al., 2013)<sup>[65]</sup>. Plant growth regulators (PGRs) may strengthen crop quality aspects such as nutrient effectiveness and susceptibility to abiotic stress (Colla and Rouphael, 2015) <sup>[12]</sup>. Brassinosteroids (BRS) participate in a diversity of physiological activities, such as respiration control, antioxidant systems, and other associated areas, which promote the overall effectiveness of plants (Fariduddin *et al.*, 2013) <sup>[15]</sup>. Under challenging atmospheric conditions, brassinosteroids (BRS) influence a comprehensive spectrum of biological processes like blossoming and fruit development (Nolan *et al.* 2020) <sup>[43]</sup>. PGRs have been shown to improve the efficiency of nutrient exporters in cell membranes and augment nutrition activity, each of which has been associated with enhanced plant nutrient efficiency (De Pascale *et al.*, 2017) <sup>[14]</sup>.

#### 6. Brassinosteroids' effect on salt tolerance

BRs have a wide range of protective and stimulatory effects on plant yield and quality (Khripach et al., 2000) [35]. Different developing portions of originating seeds, including the epicotyls, peduncles, and epicotyls in coleoptiles, mesocosms, and dicot seeds in monocotyledon seeds, were all elongated when brassinosteroids were applied at very low concentration (Clouse et al., 1996)<sup>[8]</sup>. Crop yield augmentation, differentiation, cell division and cell elongation, senescence, the biology of reproduction (flowering), and ethylene induction are all physiological processes that are impacted by BRs (Houimli et al., 2008)<sup>[26]</sup>. The destructive effects of insufficient salinity on crop maturation may be linked to hydrodynamic stress, apoptosis brought on by an excess of sodium (Na+) and chloride (Cl), nutritional imbalances, diminished turgor, and modifications in the anatomical structure of the leaves (Farouk & Al-Huqail., 2022)<sup>[20]</sup>. Reactive oxygen species (ROS) which are hazardous and may destabilize cellular processes and drastically impact enzymatic processes were produced in excess as a result of excess Na+. ROS accumulation often compromises with antioxidant homeostasis, causing photosynthetic productivity to deteriorate (Farhangi & Torabian, 2017) <sup>[18]</sup> reducing nutritional absorption, influencing the incorporation of nitrogen and osmolytes, influencing the profile of phytohormones, and regulating the expression of genes. In addition to transforming the plasma membrane and stimulating metabolic activities in a demanding environment, BRs also promoted ion absorption, promoted the transport of chlorophyll to the sink, and modified the plasma membrane (Ali et al., 2008) <sup>[1]</sup>. Salt shock also facilitates the delineation of the hydrodynamic and ionic components, albeit with significant discrepancies. The plants should contend with a substantial discrepancy in osmotic pressure when they are confronted with a salt shock that contributes to plasmolysis and the evacuation of the nourishing solution from the apoplast. Roots cells efficiently activate genes connected to osmotic responses. Salt is promptly carried through the ground to the branches during this phase because the plants are unwilling to restrict the passage of the solutes (Franzoni et al., 2019)<sup>[19]</sup>.

S. No.	Name of fruit	Type of brassinosteroids	Tolerance attribute	Reference
1.	Orange	BL	Decreased fruit drop	Kuraishi et al. (1991) [31]
2.	Grapes	EBL	Promoted ripening	Symons <i>et al.</i> (2006)
3.	Watermelon	EBL	Increased yield (20%)	Wang et al. (1994) <sup>[63]</sup>
4.	Litchi	BL	Reduced fruit cracking	Peng et al. (2004) [45]
5.	Jujube (Ber)	BS	Reduced postharvest losses	Zhu et al. (2010) <sup>[71]</sup>
6.	Cucumber	BS	Early fruit Development	Fu et al. (2008) [17]
7.	Papaya	BS	Quick ripening	Fabi et al. (2007) [16]
8.	Apple	BL	Increased protein level	Sharma <i>et al.</i> (2021) <sup>[60]</sup>

#### Table 3: Effect of brassinosteroids on salt tolerance

## 7. Effects of Brassinosteroids on numerous horticultural crops under salt stress conditions

In orange trees, the application of brassinolides at the time of flowering will increase the fruit set. When the brassinolides are applied at the fruit growth stage the physiological drop will be decreased and the aggregate yield of fruits plant<sup>-1</sup> will be increased. (Kuraishi *et al.*, 1991) <sup>[31]</sup> was found that in citrus (*Citrus unshiu*) the acidity/Brix ratio and juice production will also be increased.

In grapes (*Vitis vinifera* L.) trees the application of BSs. Application is done with the number of seedless vines grape cultivars, it can utilize the numbers of BRs solutions concentration (0.1 mg L<sup>-1</sup>, 0.5 mg L<sup>-1</sup>, and 1 mg L<sup>-1</sup>) on twelve years old grapevine crops. As a result, the length, cluster weight, and width will be increased considerably by the application of 0.5 and 1 mg L<sup>-1</sup> BL. Fruit firmness, TSS content, and Total Phenolics content will be increased when we applied 0.5 mgL<sup>-1</sup> (Champa *et al.*, 2015) <sup>[13]</sup>.

In watermelon, the foliar application of 24-epibrassinolides increased the yield by 20% by 0.01 mg  $L^{-1}$  (Ikekawa and Nagai, 1987) <sup>[28]</sup>. The application of EBL will increase the fruit set, delay in senescence, and the number of flowers.

BS improves the fruit ripening, its quality as well as fruit yield. However, when spraying the solution of brassinolides on litchi before the blossom on the leaves increased the pectin methyl esterase activities and water-soluble content of pectin, calcium, and protopectin in the pericarp of the fruits, and the rate of fruit cracking will be reduced, providing an essential role through the increment in litchi's commercial worth fruit (Peng et al., 2004)<sup>[45]</sup>. As shown, (Zhu et al., 2010)<sup>[71]</sup> also found that brassinosteroids use will reduce the postharvest decay at a concentration of 5M found by Penicillium expansum on ber and also delayed the maturation by prohibiting the rate of ethylene production and the respiration rate, by that lifespan of the fruits will also be increased. Jujube fruit trees have more potential to resist salinity conditions as compared to other fruits. The two wild varieties of Indian jujube (Ziziphus nummularia and Ziziphus rotundifolia) were found to be more resistant under saline conditions due to higher CO2 assimilation, stomata conductance high, higher hormonal regulation, and better translocation of nutrients by the accumulation of the higher amount of Na+ in roots by the restricted translocation and higher ratio of K+/Na+ to keep the ions balance in leaves (Mohammadkhani, 2018)<sup>[41]</sup>.

24-epiBL enhanced the genetic as well as a physiological alternate in the diversity of pepper underneath the salinity. However, they keep reducing the deleterious effect of salt stress in pea (*Psidium Sativa*) and increasing its growth rate. Treated the bean (*Phaseolus vulgaris*) with 5uM epiBL the stress has to be detoxified and brought out by improving the rate of growth and sodium chloride, the intensity of pods

protein, and pod quantity in beans. Further found that the utilization of brassinolides (24-EpiBL) will reduce these biochemical and physiological effects of salt stress conditions in cucumber. 28-homoBL can enhance the cucumber growth, leaf chlorophyll, and antioxidant enzymes level by applying 1umol L<sup>-1</sup> (Ahmad *et al.*, 2019) <sup>[3]</sup> reported that the NaCl will reduce the root and shoot growth, and its dry and wet weight is also reduced by the salt stress. The application of Homobrassinolide with AMF (arbuscular mycorrhizal fungi) will result in increasingly dry and wet weight and the root and shoot length.

In papaya, the application of BRs on a specific leaf area as well as on the whole canopy could promote the degradation of chlorophyll and plant development, as well as the abscission and leaf senescence. The height increment was determined for every plant by calculating the difference between the present height and the height recorded after the first day of measurements.

In the stress conditions, the plants are stressed in different manners: oxidative damage, and ionic and osmotic stress (Su *et al.*, 2020) <sup>[59]</sup>. Apple trees are highly affected by the serious salt stress, arising from the loss in quality and production of apples. Attenuate the effect of salt stress conditions. Brassinolides (BLs) were applied which the anti-oxidative activities, ion homeostasis, and osmotic balance will influence. This will find that the enhancement in the application and inspection of brassinolides physiologic effect under the salt stress conditions. Treat the seeds with different components of nutrients solution to examine the desired results. Seed were drenched along 0.05 mg L<sup>-1</sup>, 0.1 mg L<sup>-1</sup> and 0.2 mg L<sup>-1</sup> of BLs.

Homobrassinolide implementation can stimulate the campaign of peroxides, catalase, and membrane catabolism decreased in groundnut (Verma *et al.*, 2012)<sup>[62]</sup>. And by this foundation, the lipid peroxidation is decreased, which seems to reduce reactive oxygen species (ROS) and it can remove stressful situations by the use of antioxidant that is activated by the BR hormone. Germination of spinach was enhanced up to 54% to 72% by soaking the seeds in brassinolides aqueous solutions (Kim *et al.*, 1987)<sup>[33]</sup>.

#### 8. Conclusion

Stresses can significantly decrease the yield potential and the cultivation area will shift for important commercially grown fruit crops by the change in the climate. They have also increased the quantity and the quality of horticultural plants such as apple, papaya, pea, bean, cucumber, orange, litchi, watermelon, grapes, etc. BS also protected the plants from different biotic stresses such as pathogens attack. 28-homobrassinolide overcame the salt stress effect on the cucumber plants and enhanced the growth attributes, antioxidants enzymes, and leaf chlorophyll.

The Pharma Innovation Journal

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