Role of brassinosteroids in horticultural crops (Reviews)

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DOI: https://doi.org/10.22271/chemi.2020.v8.i3a.9458

Abstract
With the progress of chemical synthesis technology, structurally modified brassinosteroids (BRs) with greater stability, under field conditions have been synthesized on a commercial scale and registered as plant growth regulators for specific horticultural crops. In both fundamental and application-oriented research, BRs and their analogues play prominent roles in various physiological processes including, seed development and germination, flower sex expression, fruit development, improvement of quantity and quality of crops, and resistance to various biotic and abiotic stresses. It is worthy to note here that the involvement of BRs in plant protection from adverse environmental stress and pesticides seems to have good prospects, since BRs appear nontoxic and environmentally friendly. It is well known that horticultural crops have a great variety of produce organs as well as high yield and output values. Moreover, their production is susceptible to sub-optimum environmental conditions, especially in facilities cultivation. Thus, practical application of BRs to horticultural crops for enhancing crops production and protection may have a promising prospect in the near future.

Keywords: brassinosteroids, horticultural crops and chemical synthesis technology

Introduction
Brassinosteroids are a class of polyhydroxysteroids that have been recognised as a sixth class of plant hormones. These were first explored nearly 40 years ago, when Mitchell et al. reported promotion in stem elongation and cell division by treatment of organic extracts of rapeseed (Brassica napus) pollen. Brassinolide was the first isolated brassinosteroid in 1979, when pollen from Brassica napus was shown to promote stem elongation and cell divisions, and the biologically active molecule was isolated. The yield of brassinosteroids from 230 kg of Brassica napus pollen was only 10 mg. Since their discovery, over 70 BR compounds have been isolated from plants.

Plant Metabolism
Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds in Brassica having a wide range of biological activities and the ability to confer tolerance to Brassica plants against a wide spectrum of biotic and abiotic stresses. These stress factors include, low and high temperatures, drought, high saline concentrations, pathogen attack and exposure to heavy metals. Sterols have been recently recognized not only as precursors of brassinosteroids and membrane constituents, but also as modulators of plant development. Also, there is evidence of cross-talk between BRs and abscisic acid, GA and ethylene. Treatment with 24-epibrassinolide, a brassinosteroid, increases tolerance to several environmental stresses such as basic thermo-tolerance in B. napus, or to drought and cold stress in the case of seedlings of both Arabidopsis and B. napus, aside from helping them to overcome salt-stress-induced inhibition of seed germination. The expression of the B. napus steroid sulfotransferase genes was found to be induced by SA, suggesting that in addition to increased accumulation of an antimicrobial protein, plants respond to pathogen infection by modulating steroid-dependent growth and developmental processes. Brassinosteroids also proved to be able to protect the membrane integrity of the radish seedling from Cd-induced oxidative stress, minimizing the impact of reactive oxygen species by increasing antioxidant
enzyme activity, a possible secondary defence mechanism against oxidative stress. Since the isolation and identification of brassinolide from the pollen of rape as the first bioactive steroid with high plant growth-promoting activities, brassinosteroids (BRs) have received increasing attention as a new class of phytohormone. Studies have shown that BRs are essential for plant growth and development, and are actively involved in many physiological processes. BRs have pleotropic effects and can induce a broad spectrum of cellular responses including stem elongation, pollen tube growth, leaf bending and Epinasty, root inhibition, induction of ethylene biosynthesis, proton-pump activation, xylem differentiation and regulation of gene expression. Use of BRs has also been investigated in agricultural production. Several studies have demonstrated that BRs influence plant growth, seed germination, nitrogen fixation, senescence, and leaf abscission, and enhanced tolerance against cold stress, salt stress, and diseases. As a consequence, extensive research has been undertaken to develop BRs as plant growth regulators for agricultural production. Although improved plant growth has been shown in field trials, the physiological basis of these effects is poorly understood. Previous studies found that a BR-deficient Arabidopsis mutant had an impaired carbohydrate metabolism and reduced biomass, and application of BRs can increase sugar content in plants such as wheat and groundnut. However, these results do not necessarily mean that BRs have a direct role in the regulation of photosynthesis since gas exchange was usually measured in plants after several days or weeks of treatment. To date the mechanistic basis of BR-induced stimulation of CO2 assimilation in leaves is not known. EBR pretreatment significantly alleviated chilling injury and photo-inhibition in cucumber seedlings. During this experiment, a sharp increase in net photosynthetic rate was observed after EBR treatment.

Translocation
The BR is biosynthesized from campesterol. The biosynthetic pathway was elucidated by Japanese researchers and later shown to be correct through the analysis of BR biosynthesis mutants in Arabidopsis thaliana, tomatoes, and peas. The sites for BR synthesis in plants have not been experimentally demonstrated. One well-supported hypothesis is that all tissues produce BRs, since BR biosynthetic and signal transduction genes are expressed in a wide range of plant organs, and short distance activity of the hormones also supports this. Experiments have shown that long distance transport is possible and that flow is in an acropetal direction, but it is not known if this movement is biologically relevant. Brassinosteroids are recognized at the cell membrane, although they are membrane-soluble.

Parthenocarpic Capacity
Fu et al. (2008) [3] studied the role of brassinosteroid in early fruit development in cucumber. Brassinosteroids (BRs) are essential for many biological processes in plants, however, little is known about their roles in early fruit development. To address this, BR levels were manipulated through the application of exogenous BRs (24-epibrassinolide, EBR) or a BR biosynthesis inhibitor (brassinazole, Brz) and their effects on early fruit development, cell division, and expression of cyclin and cyclin-dependent kinases (CDKs) genes were examined in two cucumber cultivars that differ in parthenocarpic capacity. The application of EBR induced parthenocarpic growth accompanied by active cell division in Jinchun No. 4, a cultivar without parthenocarpic capacity, whereas Brz treatment inhibited fruit set and, subsequently, fruit growth in Jinchun No. 2, a cultivar with natural parthenocarpic capacity, and this inhibitory effect could be rescued by the application of EBR. RT-PCR analysis showed both pollination and EBR induced expression of cell cycle-related genes (CycA, CycB, CycD3:1, CycD3:2, and CDKB) after anthesis cDNA sequences for CsCycD3:1 and CsCycD3:2 were isolated through PCR amplification.

Metabolism of pesticide
Xia et al. (2009) [11] studied the effect of Brassinosteroids Promote Metabolism of Pesticides in Cucumber. Brassinosteroids (BRs) are known to protect crops from the toxicity of herbicides, fungicides and insecticides. It is shown here that application of 24-epibrassinolide (EBR) accelerated metabolism of various pesticides and consequently reduced their residual levels in cucumber (Cucumis sativus L.). Chlorpyrifos, a widely used insecticide, caused significant reductions of net photosynthetic rate (Pn) and quantum yield of PSII (ΦPSII) in cucumber leaves. EBR pretreatment alleviated the declines of Pn and ΦPSII caused by chlorpyrifos application, and this effect of EBR was associated with reductions of chlorpyrifos residues. To understand how EBR promotes chlorpyrifos metabolism, the effects of EBR on activity and expression of enzymes involved in pesticide metabolism were analyzed. EBR had a positive effect on the activation of glutathione S-transferase (GST), peroxidase (POD), and glutathione reductase (GR) after treatment with chlorpyrifos, although the effect on GR was attenuated at later time points when plants were treated with 1 mM chlorpyrifos. In addition, EBR enhanced the expression of P450 and MRP, which encode P450 monooxygenase and ABC-type transporter, respectively. However, the expression of GST was consistently lower than that of plants treated with only chlorpyrifos. Importantly, the stimulatory effect of EBR on pesticide metabolism was also observed for cypermethrin, chlorothalonil, and carbendazim, which was attributed to the enhanced activity and genes involved in pesticide metabolism. The results suggest that BRs may be promising, environmentally friendly, natural substances suitable for wide application to reduce the risks of human and environment exposure to pesticides.

Germination and Root Formation
Swamy and Rao (2010) [9] studied the effect of brassinosteroids on rooting and early vegetative growth of calceus [Plectranthus for skohlii (wild) Briq.] stem cuttings. Brassinosteroids are new class of phytohormones and now considered as sixth group of hormones in plant. They influenced varied growth and developmental processes such as growth, germination of seed, rhizogenesis, flowering, Senescences and abscission and they are considered as plant hormones with pleiotropic effects. The present paper emphasizes importance of 28-homobrassinolides and 24-epibrassinolides in the root formation and root growth in case of calceus [Plectranthus for skohlii (wild) Briq.]. The study revealed the ability of Brassinosteroids in increasing the root formation and root growth of treated cutting over control.

Stress Resistance
Yuan et al. (2010) [12] studied the effect of Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. The effect of brassinosteroid (BR) on relative water content (RWC), stomatal conductance (gs), net photosynthetic rate (PN),
intercellular CO2 concentration (Ci), lipid peroxidation level, activities of antioxidant enzymes and ascorbic acid concentration (ABA) in tomato (Lycopersicon esculentum) seedlings under water stress was investigated. Two tomato genotypes, Mill cv Ailsa Craig (AC) and its ABA-deficient mutant notabilis (not), were used. Water stress was achieved by withholding water and both the AC and not plants were treated with 1M 24-epibrassinolide (EBR) or distilled water as a control. The RWC, gs, Ci and PN were significantly decreased under water stress. However, EBR treatment significantly alleviated water stress and increased the RWC and PN. EBR application also markedly increased the activities of antioxidant enzymes (catalase, ascorbate peroxidase and superoxide dismutase) while it decreased gs, Ci and the contents of H2O2 and malondialdehyde (MDA). Interestingly, ABA concentration in AC and not plants was markedly elevated after EBR treatment although the increasing rate and amplitude of ABA in not plants treated by EBR was significantly lower than those in AC plants. Our study suggested that amelioration of the drought stress of tomato seedlings may be caused by EBR-induced elevation of endogenous ABA concentration and/or the activities of antioxidant enzymes.

Shahid et al. (2011) [5] observed the effect of Brassinosteroid (24-epibrassinolide) enhances growth and alleviates the deleterious effects induced by salt stress in pea (Pisum sativum L). The response of pea (Pisum sativum L) cv. Climax seeds imbibed with 24-epibrassinolide (EBL) and sodium chloride (NaCl) prior to sowing was evaluated. Soaking of seeds in two different concentrations of EBL (5 and 10 µM) for 4 hours, caused an increase in germination, embryo axis length and most of the aspects of shoot and root growth at seedling stage, maturity stage (90 DAS) along with seed yield at the time of harvest. Both the EBL treatments (5 and 10 µM) improved the above mentioned attributes but maximum improvement was observed in response to EBL concentration of 10 µM with respect to the control. At seedling stage, EBL (10 µM) significantly enhanced the fresh and dry biomass, seedling height (shoot+root), photosynthesis rate (Pn), stomatal conductance (gs), total chlorophyll contents (Chl), Proline contents, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), nitrate reductase activity (NRA) and nitrite reductase activity (NiRA) as compared to control (water soaked alone). Similarly, at maturity stage the plants grown from seeds pre-imbibed in EBL (10 µM) also exhibited the augmentation in dry biomass, physiological aspects (Pn, gs and Chl), enzymatic activities (NRA, NiRA, SOD, POD, CAT), leaf Proline contents, nodule number and nodule dry biomass in comparison to water imbibed control. Seed attributes like seed yield, seed number and seed protein contents also showed the improvement in response to EBL (10 µM) at the time of harvest. Although, plants subjected to saline stress exhibited a reduction in all the morphophysiological and enzymatic attributes (NRA and NiRA) but proline contents and enzymatic activities of antioxidants were enhanced in response to NaCl stress. However, deleterious effects induced by salinity were reduced if seeds were treated with EBL before or after NaCl imbibition.

Hayat et al. (2012) [4]. Studied the effect of foliar spray of brassinosteroid enhances yield and quality of Solanum lycopersicum under cadmium stress. The presence of cadmium in the soil above a particular level is proposed to check not only plant growth but also productivity and fruit quality. Therefore, in the present study investigations are directed to evaluate the effect of four levels of cadmium (3, 6, 9, 12mg kg⁻¹) in interaction with two analogs of brassinosteroids on the growth, fruit yield and quality of tomato. Under greenhouse conditions plants were analyzed for antioxidant system activity and photosynthetic assimilation efficiency. Cd stressed plants exhibited poor growth and biological yield. The metal also had a negative impact on the antioxidant system of the resulting fruits. However the follow up application of BRs (108M) neutralized the damaging effects of the metal on the plants.

Serna et al. (2015) [6] studied the effect of a brassinosteroid analogue prevented the effect of salt stress on ethylene synthesis and polyamines in lettuce plants. The present study was carried out to analyze the effect of treatment with DI-31 a brassinosteroid analogue (0.1 or 1 M) on alleviating salt stress in lettuce plants. After application of DI-31, 100 mM NaCl treatments were administered. Samples that were separated into shoots and roots were taken 5 days after this application. DI-31 alleviated the reduction of weight due to NaCl with a greater effect at 1 than at 0.1 M. The emission of ethylene increased with the application of NaCl and the DI-31 treatment decreased this effect in shoots and roots. Similar results were obtained in ACC since its concentration was increased by salinity and DI-31 was effective in reducing it. The Putrescine content decreased with salinity and DI-31 reversed the effect in the shoots, while it remained constant in the roots. The spermidine and spermine contents in roots increased with salinity. The DI-31 reversed the effect of NaCl in both shoots and roots in the two polyamines, except in shoots where the spermine content was maintained. Since the DI-31 brassinosteroid has partially reversed the negative effect of NaCl on lettuce plants, we conclude that the brassinosteroid has a protective effect against salinity.

Sex expression
According to Manzano et al. (2011) [3] the role of ethylene and brassinosteroids in the control of sex expression and flower development in Cucurbita pepo. The sensitivity of different squash genotypes to ethylene and brassinosteroids by studying the effects of different ethylene and brassinosteroid treatments on the sexual expression and flower development of different C pepo genotypes: Bolognese (Bog) and Vegetable Spaghetti (Veg) two contrasting lines for ethylene production and sensitivity, as well as Cora, a standard commercial hybrid. Results have demonstrated that ethylene has a much greater effect on sexual expression and flower development in C. pepo than brassinosteroids. Ethephon increases the number of female flowers per plant and reduces the first male phase of development, while treatments with the ethylene inhibitors AVG and STS reduce the number of female flowers per plant and expand the first male phase of development. The differential response observed between genotypes appears to be related to ethylene production and sensitivity. Bog, which produces more ethylene and is more sensitive to this hormone, responded much better to AVG and STS, reducing the number of female flowers per plant, while Veg, which is characterised by lower production of and sensitivity to ethylene, responded better to ethephon by reducing the first male phase of development and increasing the number of female flowers per plant. The differential abortion of female and male flowers in ethephon, AVG and STS treatments, as well as the occurrence of bisexual flowers in the AVG and STS treated plants of the more ethylene sensitive genotypes, demonstrate that ethylene is also involved in the development of female flowers. Female flower buds require a minimal level of ethylene not only to complete their development and maturation without a

 divider
premature abortion, but also to arrest the development of stamens in the third whorl and to promote the appropriate growth of the carpels. On the contrary, the role of brassinosteroids in the sexual expression of C. pepo was not so evident. The application of brassinazole, an inhibitor of brassinosteroid biosynthesis slightly changes the production of ethylene in the three analysed genotypes, but those changes have little effect on their sexual phenotypes, and they do not alter the development of the unisexual flowers.

### Root Growth
Vardhini et al. (2011) [10]. Studied the effect of brassinosteroid on the qualitative changes in the storage roots of radish. Brassinosteroids stimulates the growth of radish roots which was associated with increased levels of carbohydrates in terms of reducing sugar and starch. The soluble proteins were also elevated. Minerals like phosphorus, calcium and iron increased whereas the level of potassium and sodium decreased. Brassinosteroids also considerably increased the contents of vitamins i.e. ascorbic acid and niacin preresent in the roots indicating their ability to improve the quality of storage roots of radish as roots are the consumable part of the plant.

### Leave and Pod Growth
El-Bassiony et al. (2012) [12]. Studied the ameliorative Effects of Brassinosteroids on growth and productivity of snap beans grown under high temperature. The effect of brassinosteroids (Brs) was tested, in order to assess the possibility of alleviation of the adverse effects of high temperature stress on snap bean plants during delayed summer cultivation, therefore, two field experiments were carried out in successive seasons 2010 and 2011, with two bean cultivars ‘Paulesta’ and ‘Oxzira’, spraying with Brs of the following concentrations 0 (control), 25, 50 and 100 ppm. Plant growth, yield and pods quality of beans were studied. Spraying bean plants with Brs at a concentration of 25 and 50 ppm increased vegetative growth, total yield and quality of pods with no significant difference between both treatments. Using Brs at 25 ppm increased the total free amino acids (FAA) in leaves and total phenolic acids in the pod in comparison to control-treatment. ‘Oxzira’ cultivar resulted in the highest number of leaves, number of branches, dry weight of whole plant, total yield, total FAA in leaves and total phenolic acids in pod. Whereas, ‘Paulesta’ cultivar had the highest of plant length and total FAA in pods.

### Increase in Yield
Serna et al. (2012) [7]. Brassinosteroid analogues effect on yield and quality parameters of field-grown lettuce (Lactuca sativa L). Lettuce is a common crop in Spain. It is the most widely used fresh-cut vegetable product for salads in Europe. So increasing its yield is of great interest. The effects from two brassinosteroid (BR) analogues, DI-31 (BB16) and DI-100, were evaluated at concentrations of 4, 8 and 12 ppm together with a seaweed extract and amino acids called Tomex Amin (2.5 l/ha) to enhance their activity. These were sprayed fourfold to foliage of Lactuca sativa L var Beliva grown under field conditions. All treatments with BR analogues increased production. Treatments with DI-31 and DI-100 at a dose of 12 ppm (30 mg/ha) resulted in the highest production increases which were 25.93% and 31.08% respectively relative to the control with Tomex Amin (T02). We could not correlate the slight increase in net photosynthesis with increasing yield produced by both BR analogues except for treatment at the highest concentration of DI-100, which produced the maximum yield increase and also the biggest net photosynthesis with significant differences with respect to the control. This increased yield was caused by an increase in weight lettuce (which causes diameter and length increases) caused by increase of water absorption. These treatments produced no significant changes in the organoleptic quality of lettuce. The chemical variables related to lettuce quality, such as humidity, carbon and nitrogen content, sugar and organic acid content, total antioxidant activity (TAA) and phenolic content were similar in the control and treated lettuces. The results showed that sprayed brassinosteroid analogues may play an important role in increasing the yield of field grown lettuces due to an increase in lettuce size without any undesirable effects on their nutritive and organoleptic properties.

### Reducing Pollutant Residue
Ahammed et al. (2012) [11] studied role of brassinosteroids in alleviation of phenanthrene-cadmium co-contamination-induced photosynthetic inhibition and oxidative stress in tomato. Heavy metal pollution often occurs together with organic contaminants. Brassinosteroids (BRs) induce plant tolerance to several abiotic stresses, including phenanthrene (PHE) and cadmium (Cd) stress. However, the role of BRs in PHE+Cd co-contamination-induced stress amelioration is unknown. Here the interactive effects of PHE, Cd, and 24-epibrassinolide (EBR a biologically active BR) were investigated in tomato plants. The application of Cd (100 µM) alone was more phytotoxic than PHE applied alone (100 µM); however, their combined application resulted in slightly improved photosynthetic activity and pigment content compared with Cd alone after a 40 d exposure. Accumulation of reactive oxygen species and membrane lipid peroxidation were induced by PHE and/or Cd, however the differences in effect were insignificant between Cd and PHE+Cd. The foliar application of EBR (0.1 µM) to PHE- and/or Cd-stressed plants alleviated photosynthetic inhibition and oxidative stress by causing enhancement of the activity of the enzymes and related transcript levels of the antioxidant system, secondary metabolism and the xenobiotic detoxification system. Additionally, PHE and/or Cd residues were significantly decreased in both the leaves and roots after application of EBR, more specifically in PHE+Cd-stressed plants when treated with EBR, indicating a possible improvement in detoxification of these pollutants. The findings thus suggest a potential interaction of EBR and PHE for Cd stress alleviation. These results advocate a positive role for EBR in reducing pollutant residues for food safety and also strengthening phytoremediation.

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