Brassinosteroids: A Multifunctional Phytohormone of Plant Development and Stress Responses

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors JP and LB wrote the first draft of the manuscript. Authors SD and GRR edited the manuscript. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/CJAST/2020/v39i2530896
Editor(s):
(1) Dr. Hanuman Singh Jatav, S.K.N. Agriculture University, Jobner, India.
Reviewers:
(1) Fabiana Aparecida Cavalcante Silva, Federal University of Pernambuco, Brazil.
(2) Amina Yaqoob, University of the Punjab, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59978

Received 14 June 2020
Accepted 19 August 2020
Published 29 August 2020

ABSTRACT

Brassinosteroids (BRs) act as immune-modulators for generating plant growth and development. It regulated either activation or suppression of various key enzymatic reactions, activation of synthesis of protein, and generation of various defense modulating compounds for the plant kingdom. BRs play a vital role in regulating cellular differentiation, pollen development, fruit ripening, and quality seed formation. BRs regulates the various physiological process including root growth during nutrient deficiency such as nitrogen, phosphorus, boron and tends to signal the nutrient distribution in the rhizosphere level for better growth and high yield in crop plants. This review highlighted the role of BRs in plant growth and development and stress response, understanding the BR pathway, the molecular mechanism of BR signaling in various tissues, crosstalk between BRs and other phytohormones, gene involves in the brassinosteroids signaling pathway, biosynthesis and role of BRs in biomass production and crop yield.

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1. INTRODUCTION

Growth of the plant and its metabolic activities are fully regulated by different plant growth hormones and biostimulants. It is mostly regulated in a coordinated manner. Plant growth-promoting substances are derived naturally which influences several physiological processes at a low concentration [1]. Plant growth and metabolism are solely responsible for the power and building blocks of a plant cell. Plant hormones regulate the growth of the tissues and metabolic action. At the beginning of the nineteenth century, it has been demonstrated that the development of plant growth is regulated by the hormone that moves from one part to another part of the plant. Plant growth is basically synchronized by plant hormones and synergistically affects various physiological, metabolic, and cellular processes. There are different phytohormones which include auxins, cytokinins, gibberellins (GAs), brassinosteroids (BRs), abscisic acid (ABA), and ethylene [2]. Brassinosteroids (BRs) are a plant-derived polyhydroxysteroids and recognized as a plant hormone [3]. BRs is an important group of plant steroid hormones, which regulate many processes in plant growth and development, cell elongation and proliferation, division of the cell, cell senescence, vascular differentiation, phases of reproduction, photo-morphogenesis, seed germination, initiation of the root, development of flowering, fruit ripening, tolerance response to various biotic and abiotic stresses [4–9]. In past decades, high thought research has been made in the application of brassinosteroids in plant growth and metabolism, interaction with other phytohormones and networking signaling pathways [10-13]. brassinolide, a plant growth promoting steroid was first isolated by Grove et al. [14] from the pollen of Brassica napus and showed growth-promoting activity named as ‘brassinosteroids’ [15]. Hayat and Ahmad [16] reported that these steroidal compounds are considered as another group of phytohormones and essential for plant growth and developmental processes. Coll et al. [17] reported that the BRs are natural, non-genotoxic, safe, and eco-friendly phytohormone and used for plant growth, fruiting quality, and yield performance in agricultural and horticultural crops. Due to their various significant properties, BRs are considered as plant hormones having pleiotropic properties [18]. These BRs have an active role in a large number of physiological processes in plants as reported by Khrpach et al [19]. Saini et al. [20] reported that BRs associated with phytohormones including polyamines for regulating different physiological and developmental activities in plants. There are more than 70 BRs have been isolated from different plant species including 37 angiosperms, 5 gymnosperms, a pteridophyte (Equisetum arvense), and an alga (Hydrilla reticulatum). Among all, only three i.e., brassinolide (BL), 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) are the most important derived from different plant parts (Fig. 1) and actively used in the physiological process. The BR-concentrations were more in pollen and immature seeds as reported by Bajguz and Tretyn [21]. BRs have a common 5α-cholestan skeleton and classified as C-27, C-28, or C-29 compounds on the basis of alkyl-substitutions in the side chain, Oxygen at C-6 and hydroxyl group on the side chain at C-22 and C-23 positions are essential for the activity of BRs. However, BL is the most active, low abundance plant steroids and an oxygen moiety at C-3 and additional ones at the C-2, C-6, C-22, and C-23 carbon atoms [22,23,16]. The present review highlighted the present scenario on role of brassinosteroids on plant growth and metabolism, understanding the BR pathway, molecular mechanism of BR signaling, crosstalk between BRs and other phytohormones and its regulation.

2. REGULATORY MECHANISMS OF BRs IN PLANTS

In last two decades, the BR signal transduction pathway has been extensively studied and reported as a complex pathway. The transduction pathway has a critical role in plant growth and development. The signal transduction pathway shows that BR is perceived by BRASSINOSTERIOD INSENSITIVE 1 (BRI1) receptor kinase at the cell surface and activates BRASSINAZOLE RESISTANT 1 (BZR1) and BRI1-EMS SUPPRESSOR 1 (BES1) transcription factors to induce stress tolerance. Exogenous application of BR binds to transcription factor (BRI1) inducing an association with BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and disassociation of BRI1 KINASE INHIBITOR 1 (BKI1). Sequential transphosphorylation between

Keywords: Brassinosteroids; biostimulants; gene regulation; phytohormone; plant stress; signal transduction.

Praveena et al.; CJAST, 39(25): 174-196, 2020; Article no.CJAST.59978
BRI1 and BAK1 is necessary to activate BRI1 and furthermore to phosphorylate BR-SIGNALING KINASE 1 (BSK1) and enhance BRI1 SUPPRESSOR 1 (BSU1) activity. The activated BSU1 inhibits BRASSINOSTEROID INSENSITIVE 2 (BIN2) through dephosphorylation of the phospho-tyrosine residue of BIN2, which allows accumulation of unphosphorylated BZR1 and BZR2/BES1 transcription factors. The dephosphorylated BZR1 and BES1 enter to the nucleus and subsequently regulating BR-targeted genes for enhancing the activity of antioxidant enzymes, regulating the accumulation of endogenous hormones and upregulating many genes for plant stress tolerance [24–28].

It has been reported that both abiotic and biotic stresses have been recorded as the main potential threats to the normal plant growth and agricultural productivity. The stresses such as UV radiation, alkalinity, salinity, water lodging,
temperature, and heavy metals alter plant growth and metabolism. Plant growth hormones play a vital role in the regulation of plant growth and are actively involved in counteracting the stresses through signaling cascades for better response. There are many published literature that BRs actively participate in chlorophyll synthesis, antioxidant activity, accumulation of osmolyte, nitrogen metabolism, nutrient management and plant–water relationship between normal plant growth as well as stressful conditions [29–35]. BRs have also regulated the growth and imparting the regulation of genes under stress and other defense mechanism for adaptability and a significant role in modulating oxidative damage of reactive oxygen species (ROS), amelioration of components of the antioxidant defense system, osmoprotectant regulation, detrimental effects on pigments and photosynthesis, production of secondary compound and biosynthesis of other plant growth regulators as well as the expression of genes involved in defense responses [36-39] (Fig. 2). It has also been noted that BRs impart growth stimulation and stress mitigation in a concentration-dependent manner. Exogenously application of BRs either through feeding along with nutrients, spraying or priming helps the stress tolerance mechanisms [40–44].

2.1 Role of BRs in Drought Stress

Many researchers reported that the crop growth reduction due to a decrease in photosynthetic rate, changes in nitrogen and antioxidant metabolism, accumulation of secondary product, and alteration of mineral nutrients [6,45,46]. However, the drought effect in plants has been reduced on the application of growth regulators including BRs. Uperti and Murti [47] reported that the application of either EBL or HBL to water-stressed plants enhances the plant growth including root nodulation and nitrogenase activity. Further, Fariduddin et al. [40] noted that the application of 0.01 μM HBL to drought-stressed plants of Brassica juncea at two different developmental stages enhanced the stomatal conductance, photosynthetic rate, and increasing the accumulation of proline. It has been widely reported that exogenous BR application up-regulates the activity of antioxidant enzymes and the levels of non-enzymatic antioxidants for mediating efficient removal of reactive oxygen species (ROS) and protect the membrane lipids for maintaining membrane integrity [30,48–50]. Behnamnia [51] noted that the application of EBL reduced oxidative damage in tomato plants during drought stress by down-regulating lipoxygenase activity and upregulating the antioxidant defense system by enhancing the expression of antioxidant isozymes. Therefore, BRs plays a vital role in expressing genes involved in the mechanism of drought tolerance in various crops. Sahni et al., [52] reported that the over-expressing the BR biosynthetic gene, DWF4 in transgenic Brassica napus for enhancing the drought stress tolerance. It has also been reported that application of EBL develops the drought tolerance by up-regulating the transcription factors regulating the expression of the drought-responsive element, DRE, in Arabidopsis thaliana and Brassica napus [53].

2.2 Role of BRs against Salinity Stress

Salinity stress imparting both the osmotic and ionic imbalances in plants which reduces the growth and productivity [12,31,46,54]. Salinity disturbed the different activities of the plant growth and metabolism which includes ionic toxicity, elevated production of ROS leading to oxidative damage, osmotic stress, disturb the photosynthetic organelles, reduced nitrogen metabolism, and reduced the uptake and translocation of mineral nutrients. The application of BRs in plants regulating salinity tolerance mechanisms has been reported in several crops including rice [55], Brassica species [10,11,12,29], Vigna sinensis [56], and Mentha piperita [57]. Shahbaz and Ashraf [58] reported that the foliar application of EBL has the adverse effects of salinity in wheat by increasing the oxidative activity of peroxidase and catalase with regard to salinity tolerance. Song et al. [59] noted that the inclusion of EBL in salt-stressed peanut, caused the enhancement in growth by up-regulating the oxidative enzyme activity concomitant with reduced electrolyte leakage and malondialdehyde content. BRs are also known to regulate a number of genes involved in key metabolic processes in plants exposed to saline stress. Divi et al. [60,61] suggested that BRs share transcriptional targets with other plant hormones. In Arabidopsis thaliana, the application of EBL enhanced the expression of phytohormone marker genes and it rescued the ethylene-insensitive ein2 mutant and the ABA-deficient aba-1 mutants from salt stress.

2.3 Role of BRs against Temperature Stress

According to the Intergovernmental Panel on Climate Change [62] report, the mean land
surface air temperature has increased by 1.53°C while global mean surface (land and ocean) temperature (GMT) increased by 0.87°C. Both high and low temperatures can adversely affect plant growth. Both high and low temperatures are potential environmental factors affecting physiological processes, biochemical and molecular changes [10,11,63–66]. The application of BRs showed positive impact on plants particularly different agricultural crops with enhancing the efficiency of key metabolic pathways. There are several reports on exogenous application of BRs in different crops which mitigate the adverse effects of either high temperature [42,52,67–70] or low temperature [66,71–73] regimes regulating the metabolic action. It has been reported that the BRs have an ameliorative effect in low-temperature stress in different crop plants for up taking and translocation of both water and nutrients. Xi et al. [74] noted that the application of BR in grapevines reduces the cold-induced ion leakage by stabilizing membrane integrity through improvement in antioxidant and osmoregulatory components. The foliar spray of HBL mediated growth enhancement of Cucumis sativus under chilling stress by improving the activities of antioxidant enzymes and providing protection to the photosynthetic system from the ROS-induced oxidative damage [32]. Jiang et al. [75] reported that the exogenous application of BR activated photosynthetic and antioxidant enzymes leading to improved photosynthesis through the alleviation of chilling induced pho-oxidative damage. Heat shock proteins have been extensively studied in plants and their potential role in high-temperature tolerance. It is an evident fact that BRs can also promote the expression of heat shock proteins (hsp100, hsp90, hsp 70) in Brassica species and tomato under thermal stress [76]. Lee et al [77] reported that the application of BRs affects physiological responses and improves heat stress tolerance in Kimchi cabbage. They observed that after 3 days of heat stress, catalase and peroxidase enzyme activities was increased by 1.76- to 2.08-fold as compared to the control. It indicates that the foliar application of biostimulants reduced physiological damage and enhanced the antioxidant enzymes, thereby improving heat stress tolerance.

2.4 Role of BRs against in Nutrient Stress

The plant’s vegetative and reproductive process is severely affected by the deficiency of mineral nutrients. Phytohormones are not able to replace the nutrients for regulating the physiological and biochemical process of plant metabolism. But, they can compensate for the need for nutrients to some extent. However, phytohormones including BRs are able to compensate some extent the uptake of mineral nutrients. Janeczko et al. [78] reported that the foliar application of EBL caused a significant enhancement in nutrient uptake in Triticum aestivum under salt stress. The nutrients like potassium and calcium are able to maximize uptake and stabilizing the K/Na ratio. They also noted that the exogenous application of EBL (0.01 M) either foliar or soaking increased uptake of potassium, magnesium, and calcium, and reduced-sodium and iron in wheat. Yuan et al. [79,80] reported that EBL-treated plants maintained a higher K+/Na+ ratio and improved the activity of Ca2+-ATPase and prevent toxicity of excess Ca2+ from the cells. Song et al. [59] achieved to reduce the production of ROS including superoxide and H2O2 due to the application of EBL to Arachis hypogea. They also noted that EBL application mitigates the Fe-deficiency-induced oxidative stress by up-regulating the activity of nitrate reductase, antioxidant activity, and the accumulation of osmoles. Furio et al. [81] reported that BRs is able to induce calcium signaling pathway and overexpressed calmodulin-like (CML) proteins and CMLs genes e.g. FaCML1a, FaCML36, FaCML42 and FaCML45 and other defense-related genes (PR1, ERF1, and GLS5). Zhao et al. [37] demonstrated that the treatment of BR reduced ammonium toxicity by down-regulating the expression of ammonium transporter-1 (AMT1) gene expression in roots of Arabidopsis. The expression of AMT1 transporters (AMT1:1, AMT1:2, AMT1:3) is directly regulated by BR signaling transcription factor, BES1, and NH4+-mediated repression of AMT1 transporters were observed to suppress in a gain-of-function in ammonium-sensitive BES1 mutant (bes1-D). They concluded that BR-induced regulation of nitrogen uptake and assimilation occurs via the BR signaling pathway. Yan et al. [82] reported that phytohormone brassinosteroids (BRs) have a significant role in pollen viability, pollen germination, and seed development in tomato plants. They observed that overexpressing the BR biosynthetic gene DWARF (DWF) or BR signaling regulator DWARF RESISTANT 1 (BZR1) and exhibited the opposite effects. Loss or gain of function in the DWF or BZR1 genes altered the timing of reactive oxygen species (ROS) production and programmed cell death (PCD) in tapetal cells and
resulting in delayed tapetal degeneration. BZR1 could directly bind to the promoter of RESPIRATORY BURST OXIDASE HOMOLOG 1 (RBOH1), and that RBOH1-mediated ROS promote pollen and seed development by triggering PCD, tapetal cell degradation in *Solanum lycopersicum*.

### 2.5 Role of BRs against Heavy Metals

Brassinosteroids have the ability to regulate the uptake of ions into plant cells and also used to reduce the accumulation of heavy metals in plants. Sharma et al. [83] reported that the application of 28-HBL in the plant which regulate the ameliorative properties to detoxify the zinc toxicity in *Brassica juncea*. Bajguz and Hayat [38] noted that BRs has the ability to minimize the toxic effects by an excess of heavy metals. Sharma et al. [83] reported that BR's application helps to regulate the antioxidant enzymes and mitigating the toxic effect of zinc in *Brassica juncea*. Hayat et al. [41] reported that the application of HBL signifies the positive effect on Cd stress in *Brassica juncea*. They observed that the plants under Cd stress exhibited a decline in growth, chlorophyll content, the activity of nitrate reductase, the activity of carbonic anhydrase, nitrate, and sugar content. But, it has been overcome the toxic effect by the application of HBL. It enhanced proline accumulation and oxidative enzyme activities and reduced the peroxidase and ascorbic acid oxidase activities. Anuradha and Rao [39] further reported that the application of BRs helps the reduction of lipid peroxidation induced by Cd. The aluminum toxicity is the major growth-limiting factor for crop cultivation on acidic soils. Ali et al. [29] studied the application of either EBL or HBL through spraying resulted in the reduction of aluminum stress in *Vigna radiata*. The activities of superoxide dismutase, peroxidase, catalase, and proline content increased in response to the Al stress and maximum in the HBL or EBL treated plants. The increase in the aluminum resistance conferred by BRs was reflected in the improvement of plant growth, photosynthesis efficiency, etc in the presence of aluminum [29]. Nickel is an essential element, its high concentration is toxic to plant system and inhibits photosynthesis, respiration, enzyme activities, and protein content. Kaya et al. [84] reported that 0.5 μM 24-epibrassinolide (EBR) sprayed every other day for 10 days to pepper plants enhanced the defense mechanism against Cd stress. EBR reduced leaf Cd²⁺ content and oxidative stress, enhanced plant growth, regulated water relations, and led to further increases in proline content, AsA-GSH cycle-related enzymes’ activities, antioxidant defense system-related enzymes as well as NR activity and endogenous nitric oxide content. They also noted that nitrate reductase (NR) participated in brassinosteroid (BR)-induced cadmium (Cd) stress tolerance primarily by accelerating the ascorbate-glutathione (Asa-GSH) cycle. The EBR and the inhibitor of NR reversed the positive effects of EBR by reducing NO content. It is evident that nitrate reductase could be a potential contributor of EBR-induced generation of NO which plays an effective role in tolerance to Cd in pepper plants by accelerating the Asa-GSH cycle and antioxidant enzymes.

### 3. BRs AND BIOTIC STRESS

In the natural environment, plants are facing different kinds of biotic stresses including bacteria, viruses, and harmful insects and pests. The agricultural production is hampering due to the biotic stresses. Plants have their own immune system, which provides resistance to external stressors. Plants make use of pre-existing physical and chemical barriers, as well as inducible defense mechanisms, which become activated upon attack by microbes and pathogens. The plant defense mechanism reduces the harmful effects of biotic stresses. During stress atmosphere, the plant induced defense system is regulated by complex interconnected signal transduction pathways in which plant hormones such as abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and BRs play a vital role [26,85]. Bajguz and Hayat [23] reported that the application of BR at low concentrations in horticultural crops helps to improve the growth and yield and increases resistance to pathogens. However, the levels of protection and effectiveness depend upon the method of application of BR. Lu et al. [38] reported that secondary compounds like flavonoid, anthocyanin, and catechin, induced MYB genes which were increased in rust infected tissues of apple. The *MYB30* genes directly regulated *BES1* in *Arabidopsis*. *BES1* is a key gene of the BR signal transduction pathway, and both *MYB30* mutants and *BES1* interact with each other and promote BR targeted genes as reported by Kim and Wang [86]. Lu et al. [38] reported that the application of BR along with other plants hormones like ABA, JA, and SA help in reducing rust disease in apple plants. They noted that BR functions via synergistic crosstalk with SA, JA, and ethanol (ETH) signaling...
pathways to respond to chilling stress and play an essential role in biotic stress tolerance by activating enzymes, resistance genes, antioxidants, hormones, transcriptional factors, and signaling pathways to reduce biotic stress damage. Brassinosteroids perform various functions due to its interplay with other phytohormones like auxins, cytokinins, ethylene, ABA, GA, SA & JA and to regulate myriad aspects of plant growth and developmental processes [87,88].

4. INTERACTION OF BRASSINOSTEROIDS (BRS) WITH OTHER PHYTOHORMONES

Cross-talk between BR and auxin regulates the plant growth and developmental process [89-92]. Interaction of BRs and auxin are involved in hypocotyl or root development and also regulating stress responses [93]. Mouchel et al. [94] reported that both the CPD gene and DWF4 gene required for BR biosynthesis and in auxin signaling. There is a network connection between BR and auxin for plant growth and improvement via BIN2 and auxin reaction factors (ARF2). Vert et al. [95] reported that the BR signaling part BIN2 can specifically collaborate with an auxin signaling segment ARF2, an individual from the auxin response factor group of transcriptional controllers [96].

BR is also found to interact with gibberellic acid (GA) to coordinate different physiological processes [48,97]. Several reports indicate that BR-GA antagonistic interaction help in defense processes against Pythium graminicola. They demonstrated that in several GA-deficient and/or insensitive mutants, the disease occurrence were more severe. It implies a positive role of GA in providing resistance against P. graminicola. Further, it has been noted that susceptibility similar to those observed in BR treated plants was detected when endogenous GA level was disrupted using uniconazole. GA biosynthesis inhibitor [98]. The abundance of BR repressors, DELLA and SLR1 is positively regulated by BR. They reported that this phenomenon leads to BR mediated suppression of the GA biosynthetic genes such as GA20ox and GA3ox3 induce GA2ox expression which is involved in suppression of GA signaling and its deactivation. Tong et al. [99] reported that the crosstalk between BR and GA has been established in regulating plant cell elongation in rice. They suggested that BR promotes GA accumulation by inducing the expression of D18/GA3ox-2, one of the GA biosynthetic genes. However, exogenous application of high concentration of BR leads to the activation of GA2ox-3, a GA inactivation gene, resulting in inhibition of cell elongation. Moreover, GA inhibits BR signaling as well as its biosynthesis in a feedback inhibiting loop but facilitate cell elongation through activating primary BR signaling pathway upon applying exogenous high GA concentration, indicating brassinosteroid (BR) - gibberellic (GA) crosstalk in regulating cell elongation [99]. Hu et al. [100] reported the interaction between BR, IAA, and GA on cotton fiber development has been studied in Gossypium hirsutum. A class of DELLA proteins GhGAI is down-regulated by BR and auxin treatment during cotton fiber initiation and elongation, suggesting its importance in cotton fiber improvement through genetic modulation of phytohormone strategy. Hui et al. [101] reported that the levels of gibberellic acids have positively correlated with BRs at three developmental stages with the expression levels of CsCPD (Cytochrome P450 90A1) and CsDWF4 (Dwarf4), which are involved in BRs biosynthesis pathway. The expression of CsGA20ox1 (Gibberellin 20 oxidase 1), a gene involved in the GAs biosynthesis pathway. They suggested that CsGA20ox1, CsCPD, and CsDWF4 might play regulative roles in the crosstalk between GAs and BRs at the developmental stages of tea leaves. Further, Li et al. [102] reported the crosstalk between gibberellin (GA) and brassinosteroid (BR) in co-regulating rice seed germination. On the basis of the isobaric tags for relative and absolute quantitation (iTRAQ) proteomic approach, they identified 42 differentially abundant proteins in both BR-deficient and BR-insensitive rice plants, and most were altered consistently in the two groups. Gene Ontology analysis revealed the enrichment in proteins with binding and catalytic activity. A potential protein-protein interaction network was constructed using STRING analysis, and five Late Embryogenesis Abundant (LEA) family members were markedly down-regulated at both mRNA transcript and protein levels. These LEA genes were specifically expressed in rice seeds, especially during the latter stages of seed development. Mutation of LEA33 affected rice grain size and seed germination, possibly by reducing BR accumulation and enhancing GA biosynthesis [102].

Cytokinin-brassinosteroid indirectly crosstalks through modulation of auxin transport in regulating lateral root development. BR
enhances the expression of auxin efflux carriers i.e. PIN genes which probably aids to maintain local auxin maxima required for root primordium development [103]. Cytokinin inhibits the lateral root development and disturbs auxin accumulation by down-regulating the expression of PIN genes, indicating an indirect interaction between BR and cytokinin (CK) [104]. Cytokinin stimulates the accumulation of endogenous BR suggesting the synergistic interaction between brassinosteroid and cytokinin in Chlorella vulgaris [105]. Upon exogenous treatment of 10 µM trans-zeatin (IZ) to the C. vulgaris culture, there was considerable increase in the level of all endogenous BR by 27–46%. The co-application of both BL and trans-Zeatin (IZ) lead to highest stimulation in the number of C. vulgaris cells and endogenous accumulation of proteins, chlorophylls and monosaccharides, whereas, the lowest was observed upon treatments with 28-homocastasterone (28-homoCS) and 1,3-diphenylurea (DPU) indicating brassinosteroids and cytokinin crosstalk [105].

Brassinosteroid and ethylene crosstalk regulate the plant growth and developmental processes. BR has been identified as a negative regulator of shoot gravitropism, whereas ethylene has been shown to promote gravitropic reorientation in light-grown seedlings [106]. They suggested that BR and ethylene interact indirectly in regulating shoot gravitropic responses through involving auxin signaling genes [107]. BR activates AUX/IAA (a negative regulator of auxin signaling) and inactivates ARF7 and ARF19 (positive regulator of auxin signaling), thus inhibiting shoot gravitropic responses. Ethylene is a key regulator of hyponastic growth, which is employed by plants to cope with biotic and abiotic stresses. ROT3/CYP90C1 encodes an enzyme that mediates C-23 hydroxylation of BR. A mutation in ROT3 reduces hyponastic growth leading to impairment of local cell expansion and inhibition of BR biosynthesis, indicating that hyponastic growth induced by ethylene is mainly regulated by BR [108]. Vandenbussche et al. [106] reported that ethylene down-regulate AUX/IAA and enhances ARF7 and ARF19 genes to positively regulate shoot gravitropic responses. So, ethylene and BR have been found to have opposite effects on the upward growth of etiolated shoots. Ethylene-BR antagonism has also been observed in the case of roots. Ethylene reduces root gravitropic responses, while BR enhances root gravitropic bending probably by modulating auxin transport [106,109-111]. In BR-insensitive mutants, bri1-301 and bak1, delayed root growth and reduced response to the gravitropic stimulus [110]. However, in ethylene insensitive mutants, ein2-5 and etr1-3 reduced inhibition toward root gravitropic responses was reported, indicating antagonistic interaction between BR and ethylene in regulating gravitropic responses in plants [109]. The exogenous application of BR enhanced ethylene biosynthesis in Arabidopsis seedlings [112]. Muday et al., [113] reported that BR help to up-regulates the expression of 1-aminocyclopropane-1-carboxylate synthase (ACS), the key gene required for ethylene production. Further, BR acts post-transcriptionally and also increases the stability of ACS proteins such as ACS5, ACS6 and ASC9 by preventing its ubiquitination mediated by 26S proteosome. Thus, in response to various endogenous and exogenous signals, ACS is regulated by BR to continuously adjust ethylene biosynthesis in various tissues [112]. The synergistic interaction between ethylene and BR in regulating hyponastic growth has also been demonstrated [108].

BR promotes seed germination indicating the antagonistic interaction with abscisic acid [114,115,116]. Genetic, physiological and biochemical studies have revealed that BR and ABA can co-regulate the expression of genes [117,118]. They reported that in BR biosynthetic and signaling mutants such as det2-1 and bri1, does not rely upon BR perception, but depends on BIN2, a negative regulator of BR signaling [118]. Abscisic acid (ABA) on BR signaling largely depends upon ABI2 and slightly on ABI1, a PP2C family serine/threonine phosphatase. It is also noted that ABA and BR crosstalks through BR signaling components (BIN2) and, ABA signaling components (ABI1 and ABI2). Furthermore, BR and ABA have been suggested to play antagonistic roles in regulating seed germination and post-germinative growth processes [119]. ABA inhibits while BR enhances seed germination and post-germinative growth processes. Another factor undertakes that BIN2, positively regulates ABA responses by physically interacting with ABI5. So, BIN2 stabilizes ABI5, by phosphorylating it, thus mediating ABA responses during seed germination. However, BR application inhibits the regulation of ABI5 by BIN2 to antagonize ABA mediated inhibition reported by Hu and Yu [119]. Zhou et al., [120] observed that there is a synergistic correlation between BR and ABA in inducing various responses such as H₂O₂ production, respiratory burst oxidase homolog1
Brassinosteroid (BR) – Polyamine (PA) crosstalk is involved in enhancing the stress tolerance mechanism in plants. Takahashi and Kakehi [121] noted that the application of BR maintains the optimum amount of spermidine concentration required for normal plant growth and specifically increases the production of putrescine necessary for stress tolerance but decreases the concentration of cadaverine which generates oxidative burst to counteract heavy metal stress. The co-application of Cu and BR also decreases cadaverine content enhancing superoxide dismutase (SOD) activity necessary for stress tolerance [122]. It has key role of BR-PA interaction in providing abiotic stress tolerance [123].

The crosstalk between brassinosteroid (BR) and salicylic Acid (SA) is mediated via non-expressor of pathogenesis-related genes 1 (NPR1) and WRKY70, encoding a transcription factor working downstream of NPR1 [60,61]. It has been reported that SA mediated gene NPR1, is an essential module of 24-epibrassinolidedemediated increase in temperature and salinity tolerance in Arabidopsis thaliana. Nakashita et al. [124] reported that an application of brassinosteroid tends to increase the resistance to the tobacco mosaic virus, the bacterial pathogen Pseudomonas syringae pv. tabaci, and the fungal pathogen Oidium sp. But, in case of rice, the BR enhances resistance to the fungal pathogen Magnaporthe grisea and the bacterial pathogen Xanthomonas oryzae. They have further studied that in case of tobacco, the enhancement in the BR mediated resistance does not necessary SA. It indicates that BR and SA act independently in providing resistance against pathogens [124].

There is a synergistic connection of brassinosteroids (BRs) and jasmonic acid (JA) and has key roles in the plant growth. Kitanaga et al. [125] reported that BR improves JA level in rice under stress condition, which increases the thionin qualities encoding antimicrobial peptides having a potential crosstalk between BRs and JA. Peng et al. [126] noted that brassinazole application help in accumulation of anthocyanins in Arabidopsis which mediated JA pathway. The transcript levels of JA biosynthesis and JA-initiated signaling gene were down-controlled during the application of low concentration of BR. At higher concentration, the transcript levels of JA biosynthesis and signaling gene were up-regulated. Nahar et al. [127] further reported that two BR biosynthesis and signaling gene, OsDWF4 and OsBRI1 showing counter communication among BR and JA in the rice roots. Additionally, BR biosynthesis controlled by improved JA-antecedent, 12-oxo-phytodienoic destructive, and subsequently joining BR and JA pathway initiation.

5. ROLE OF BRASSINOSTEROIDS IN PLANT PHYSIOLOGY AND BIOCHEMISTRY

Brassinosteroids (BRs) at very low concentration (mM or µM) play important roles in regulating plant growth [128]. It affects the coordination of morphogenic events throughout plant ontogeny, seed germination and seedling elongation to maturity and seed development. Cell elongation, division and differentiation, enhancement of crop yield, reproductive biology (flowering), senescence, induction of ethylene biosynthesis, root growth and development, pollen tube growth, activation of proton pump, activation of photosynthesis and antioxidant system have been affected by brassinosteroids [129,130,131]. Wang et al. [132] demonstrated that BRs have able to stimulatehypocotyl elongation by increasing cell wall relaxation without a concomitant change in wall mechanical properties in Brassica chinensis. In addition, physiological measurements revealed that BRs could stimulate cell wall loosening in epicotyls of soybean and hypocotyls of Brassica chinensis and Cucurbita maxima [128,133,134]. Low concentration of BRs induces the 10-fold increase in cell numbers, cell division and xylem differentiation [135,136]. Some researchers reported that BRs also promote cell elongation by regulating the transport of water via aquaporins as well as regulating the activity of a vacuolar H+-ATPase subunit [137,138]. BRs play a key role in Arabidopsis cell division in mutant det2 (de-etiolated2) suspension cultures and also caused an increase in transcript levels of the gene encoding cyclin-D3, a regulatory protein of the cell cycle [139]. Bajguz and Czerpak [140] reported that BRs-induced cell expansion and hyperpolarization of cell membrane and also stimulates the growth of cell cycle. BRs are involved in the process of cell enlargement through their effects on gene expression and
enzyme activity [141]. BRs stimulate the cell division by increasing the transcript levels of genes encoding cyclin D3, a regulatory protein of the cell cycle [139,142]. The role of cyclins and CDK genes has also been investigated in early fruit development of tomato [143]. Yamamoto et al. [144] suggested that BRs are synthesized immediately prior to secondary cell wall development and cell death. In maize (Zea mays L.) roots, Li et al. [145] observed that BRs actively take part in the control of the gravitropic response of Arabidopsis roots. They also noted that externally application of BRs increased the activity of ROP2, a GTPase, and an improved gravitropic response. Kim et al. [111] suggested that BRs interacted with auxin differently in the root elongation as in gravitropic responses. They demonstrated that BRs promoted an increased gravitropic response in Arabidopsis roots at low concentration of IAA and had reduced activity at high levels of IAA. Li et al. [146] highlighted the role of brassinosteroid (BR) and redox signal hydrogen peroxide in breakdown of starch which is the major storage carbohydrate in plants. They reported that the brassinosteroid and redox signal hydrogen peroxide (H$_2$O$_2$) induce the breakdown of starch in guard cells, which promotes stomatal opening. BRASSINAZOLE-RESISTANT1 (BZR1) interacts with the basic leucine zipper transcription factor G-BOX BINDING FACTOR2 (GBF2) to promote the expression of β-AMYLASE1 (BAM1), which is responsible for starch degradation in guard cells. H$_2$O$_2$ induces BZR1 oxidation, enhancing the interaction between BZR1 and GBF2 to increase BAM1 transcription. Mutations in BAM1 lead to starch accumulation and reduce the effects of BR and H$_2$O$_2$ on stomatal opening.

Liu et al. [147] reported that brassinosteroids help to increases the essential inorganic ions, decreased toxic ions, and promoted ion homeostasis especially in leaves, root, and epicotyls of canola under abiotic stress. At low temperature and low light stress, 24-epibrassinolide enhanced the metabolic activity of nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthases and glutamate dehydrogenase enzymes [59,149]. Furthermore, exogenous brassinosteroids application increased H+$^+$ ATPase and Ca$^{2+}$ ATPase activities in root and leaf, which are responsible for establishing an electrochemical potential gradient to maintain ion balance in plants to alleviate stress effect [115]. Brassinosteroids have the potential to maintain ion homeostasis either directly or indirectly in plants [149-151]. It is observed that Brassinosteroids have positive effect on the activity of high affinity K$^+$ transporters and are associated with the reduction in Na$^+$ and enhancement in K$^+$ concentration to improve the K$^+$/Na$^+$ ratio. Brassinosteroids have also been found to improve the Ca$^{2+}$/Na$^+$ and K$^+$/Na$^+$ ratios of the wheat cultivars by enhancing Ca$^{2+}$ and K$^+$ uptake, and thus enhance salt tolerance [152].

Brassinosteroid (BR) increases ethylene biosynthesis at the step between s-adenosyl methionine (AdoMet) and 1-aminoacyclopropane-1-carboxylic acid (ACC) by stimulating ACC synthase activity [153,154]. BR-induced ethylene can be inhibited by aminooxyacetic acid (AOA), fusicoccin (a fungal toxin) and the transport inhibitors 2,3,4-tri-iodobenzoic acid and 2- (p-chlorophenoxy)-2- methylpropionic acid. Schlagnhauer and Arteca [155] reported that the promotion of ethylene production in plant parts as well as in a whole plant system due to role of brassinosteroid. Exogenous application of BR significantly increased chlorophyll content and enhanced photosynthetic characteristics of plants under stresses. Chlorophyll is an important parameter used as an indicator of chloroplast development and photosynthetic activity [148]. BR is also regulating the combination of chlorophyll molecule (by regulating chlorophyllase activity) with membrane protein and maintains stability of the thylakoid membranes. Brassinosteroids alleviate the adverse effect of different stress conditions and regulate the defense system by regulating transcription levels of defense genes as reported in cucumber [156]. BR is also regulating the Rubisco carboxylase activase (RCA) gene, which plays a key role in photosynthesis under drought and temperature stress in wheat and significantly increases the activities of antioxidant enzymes and the process of photosynthesis. Many researchers reported that the total chlorophyll contents increased in the leaves of various crops by application of by application of 24-epibrassinolide and 28-homobrassinolide [40,157-159]. Li et al. [102] reported that the brassinosteroid and redox signal hydrogen peroxide (H$_2$O$_2$) induce the breakdown of starch in guard cells, which promotes stomatal opening. BRASSINAZOLE-RESISTANT1 (BZR1) interacts with the basic leucine zipper transcription factor G-BOX BINDING FACTOR2 (GBF2) to promote the expression of β-AMYLASE1 (BAM1), which is responsible for starch degradation in guard cells. H$_2$O$_2$ induces BZR1 oxidation, enhancing the interaction between BZR1 and GBF2 to
increase BAM1 transcription. Mutations in BAM1 lead to starch accumulation and reduce the effects of BR and H₂O₂ on stomatal opening. brassinosteroids application has significant role in the enhancement of nitrate reductase in various crop plants [6,33,159,160]. Babalik et al [161] reported that the application of 24-epibrassinolide (24-eBL) in grape plants increased yield, quality, and antioxidant compounds. The concentration 0.2 mg L⁻¹ of 24-eBL applied to vines at three times (7 days after berry set, day of change of colour of the berries, 30 days after veraison) was the most suitable application providing the highest yield and some quality properties such as cluster weight, berry weight, and specific gravity.

6. BRASSINOSTEROIDS ON CROP YIELD

Fruit development and crop yield have been intensively studied over the past few decade and a key challenge for scientists is to improve yield per unit area. Hayat et al., [157,158] reported that brassinosteroids helps significantly to increase yield and yield components in plant. Either foliar application or seed priming of brassinosteroids significantly enhanced growth of fruits as well as number of fruits [162,163]. Brassinosteroids played appositive role in fruit ripening and fruit growth and in the quality [156,162]. Brassinosteroids also reduce the harmful effect of stress by activating a plant defense system (antioxidants) against stress conditions and leading to significantly increased growth, yield, and yield components [164,165]. Foliar application BL also improved the yield of wheat and mustard, rice, corn and tobacco [166,167]. Brassinosteroids were also found to increase the growth and yield of sugar beet [168], legumes [169] and rape seed [157,158,170]. Treatment of 28-homobrassinolide and 24-epibrassinolide significantly increased the yield of potato, mustard, rice and cotton [171] and Vigna radiata [172] respectively.

7. BRASSINOSTEROIDS SIGNAL TRANSDUCTION PATHWAY AND MODE OF ACTION

Major brassinosteroid (BR) effects such as the promotion of growth require mRNA and protein synthesis. The identification of BR-responsive genes proved to be highly useful for the exploration of signal perception and downstream signaling [173-175]. The gene products ultimately mediate BR responses in the nucleus and also extra cellular part of the transmembrane LRR receptor kinase BR1 binds BR [176,177]. BR1 interacts with the LRR receptor kinase BAK1. BR binding to BR1 inactivates the BIN2 kinase [174,178,179]. BIN2 phosphorylates BES1, thus targeting the protein for ubiquitination and subsequent proteasome-dependent degradation. In the presence of BR the BIN2 kinase becomes inactivated and the nuclear phosphatase BSU1 promotes BES1 dephosphorylation. The accumulation of hypophosphorylated BES1 correlates with changes in transcription of BR-responsive genes (Fig. 3) [174]. The basic helix loop-helix protein BIM1 interacts with BES1. Both proteins synergistically bind and activate BR-induced promoters [180]. The BZR1 protein is closely related to BES1 and represses BR biosynthetic genes. Abundance of the BRZ1 protein is also affected by the BIN2 kinase. Further BES1 homologues (BEH1-4, BES1/BZR1 homologue 1-4) function redundantly in BR signaling.

8. GENES INVOLVED IN THE BIOSYNTHESIS OF BRASSINOSTEROIDS

The brassinosteroid (BR) biosynthetic pathways consist of two major parts, sterol biosynthesis and a BR-specific pathway. Analysis of BR metabolic gene expressions indicate that BR homeostasis is maintained through feedback expressions of multiple genes, each of which is involved not only in BR-specific biosynthesis and inactivation, but also in sterol biosynthesis [181]. They reported that there are two groups of BR dwarf loci reported in Arabidopsis. The first group includes dwf1 to dwf8, and dwf12. The dwf1, dwf5, and dwf7 mutants are defective in sterol biosynthesis. The second group including dwf3,dwf4, dwf6, and dwf8 belong to the BR specific pathway. Only dwf2 and dwf12 mutants are insensitive to bioactive BRs. Except for dwf2 and dwf12 alleles, all of the dwf mutants are highly responsive to exogenously application of BRs. Choe et al. [182-184] first identified the dwf1 as BR-related gene. The three alleles dwf1 (dwf1), dimandcbb1 were unable to synthesis of campesterol from 24-methylenecholesterol [185,186,187]. BR intermediates showed that 24-methylenecholesterol in dwf1 accumulated 12 times the level more than the wild type [184]. In the lkb mutant type, the levels of BL, castasterone (CS), and 6-deoxyCS, campestanol, and campesterol were severely reduced in young shoots, however, levels of 24-
methylencholesterol were elevated, compared to those of wild-type plants. Some mutant alleles i.e. dwf3 have only been rescued by 23-alpha-hydroxylated BRs. The dwf4 mutant was also shown to be defective in the BR biosynthetic pathway, more specifically in a steroid hydroxylation presenting 513 amino acids and 43% identity and 66% similarity with the cpd gene, which catalyzed a key regulatory step in BRs biosynthesis [188]. Choe et al [184] observed that transgenic Arabidopsis plants over expressing dwf4 (aad4) and enhances the hypocotyl length as compared to wild type. Bishop and Yokota [22] reported that dwarf5 (dwf5) allele has been shown to be defective in the reduction of 5-dehydroepisterol to 24-methylenecholesterol. Choe et al. [183] reported that dwf7 allele resided before the production of 24-methylenecholesterol in the sterol biosynthetic pathway, more specifically the dehydrogenation of episterol to 5-dehydroepisterol, indicating that dwf7 was an allele of the previously cloned Arabidopsis sterol1 (ste1) gene [189]. An another allele dwarf11, a rice (Oryza sativa) dwarf mutant, was defective for a novel cytochrome P450 (CYP724B1), which showed homology to enzymes involved in BR biosynthesis. The dwarf11 gene is feedback-regulated by BL. They suggested that the dwarf11/cyp724b1gene plays a role in BR synthesis and may be involved in the supply of 6-deoxotyphasterol and typhasterol in the BR biosynthesis network in rice.

**Fig. 3. Model of BR signal transduction pathway**

The plasma membrane localized receptor kinase BRI1 is the major BR receptor. Brassinosteroids bind directly to the 70-amino acid island in the extracellular domain of BRI1. BAK1 is a likely co-receptor. Downstream from BRI1/BAK1 in the BR pathway is the GSK3 kinase BIN2 which functions as a negative regulator. The transcription factors BES1 and BZR1 are substrates of BIN2. Phosphorylation of BES1 and BZR1 triggers their proteasome-mediated degradation. In the presence of BR, both proteins accumulate in the hypophosphorylated form and bind to specific promoter elements of BR-responsive genes. The nuclear phosphatase BSU1 dephosphorylates BES1 and thus counters the effects of BIN2. The subcellular localization of BIN2 and other components is not yet clear.

(Vert et al. [174] & Mussig et al. [200])
8.1 Genes Involved in the Signal Transduction Pathway of BRs

Zurek and Clouse [190] identified brl1 in soybean, a gene specifically regulated by BRs during the early stages of elongation. BR application resulted in increased plastic extensibility of the elongating soybean epicotyl walls within 2h, with a concomitant increase in BRU1 mRNA levels. A direct relationship between the level of BRU1 transcripts and both, magnitude of BR-induced stem elongation and plastic extensibility of the cell wall was also demonstrated. BRU1 encodes a protein that shows significant homology to various xyloglucan endotransglycosylases (XETs), enzymes that specifically cleave xyloglucan chains and transfer a fragment of the cleaved chain to an acceptor xyloglucan, being the mechanism of BR regulation of BRU1 post transcriptional [191].

Vert et al. [174] reported that brassinolide binds directly to both, native and recombinant BRI1 proteins. Friedrichsen et al. [192] reported that a BRI1::GFP (GFP, green fluorescent protein) fusion protein was located at the plasma membrane, which, along with the protein acting Ser/Thr phosphorylation suggested that BRs were perceived at the cell surface. There are more than 20 BR-insensitive mutants reported, cbb2, 18 bin and 3 alleles of dwf2, were all allelic to brl1.BRI1 was the only unique and specific component of the BRs signal transduction pathway [3,177,193]. Li et al [48] identified mArabidopsis a dominant genetic suppressor of bri1,bak1-1d (bri1-associated receptor kinase 1-1Dominant). This gene encodes an LRR-RLKSerine/threonine protein kinase, which interact with bri1. bri1and bak1 can phosphorylate each other, being the autophosphorylation activity ofbak1 enhanced by bri1. The BRI1-BAK1 receptor complex is now thought to initiates BR signaling [194]. The gene bak1 is believed to act as a co-receptor and/or downstream target of bri1 [174]. Wang et al. [195] reported that BZR1 is a transcriptional repressor that binds directly to the promoters of regulated BR biosynthetic genes. The BZR1 protein accumulates in the nucleus of elongating cells of dark-grown hypocotyls and has been shown to be a positive regulator of the BR signaling pathway. Thus, BZR1 coordinates BR homeostasis and signaling by playing dual roles in regulating BR biosynthesis and downstream BR responses. BZR1-BES1 family of proteins directly binds to and regulates BR-responsive genes, which establish a link between hormonal signal transmission in the cytoplasm and transcriptional status change in the nucleas [196]. Choe et al. [184] identified two new BR-insensitive mutants (dwarf12-1d and dwarf12-2d). The semi dominant dwarf12 mutants displayed the typical morphology of previously reported BR dwarfs but they also exhibited several unique phenotypes such as severe downward curling of the leaves. Friedrichsen et al. [197] identified three genes (bree1, bree2, and bree3)in Arabidopsis, which shared high sequence identity, encoding putative basic helix-loop-helix (bHLH) proteins called BR Enhanced Expression (BEE1, BEE2, and BEE3). These functionally redundant, transcription factors, are induced within 30 min of treatment with BL being this induction dependent of functional BRI1 and independent of de novo protein synthesis, making these genes the first early response genes characterized in the BR signal transduction pathway. BEE1, BEE2, and BEE3 were active throughout the plant, and mutants lacking all three proteins were less responsive to BRs.

Sasuga et al. [198] identified a novel cDNA from BL-treated rice seedlings, tentatively named BR-up regulated gene2 (bru2). The bru2 could encode an actin effector protein that control polymerization of actin molecules, which provided evidence for the involvement of BRs onthe orientation of microtubules in plant cells. Coll-Gracia et al. [199] identified an exordium (exo) protein which acts as a regulator of BR-responsive genes in A. thaliana. The exo gene was characterized as a BR-up-regulated gene. Over expression of exo resulted in increased transcript levels of the BR-up-regulated kcs1, exp5, delta -tip, and agp4 genes, thought to be involved in the BR-promoted growth. In addition, to that exo over expressing lines showed enhanced vegetative growth, resembling the features of BR treated plants.

9. CONCLUSION

Brassinosteroids (BRs) are efficiently used in plants as immune-modulators. BRs are implicated in plant responses to abiotic and biotic stresses with activation or suppression of key enzymatic reactions, production chemical defense compounds, induction of protein synthesis and induction of ethylene biosynthesis, root growth and development, pollen tube growth, activation of proton pump, activation of photosynthesis and antioxidant system. It has been utilized for plant resistance against different
stress environmental conditions and involved in the process of osmotic regulation, photosynthesis, nitrogen metabolism, antioxidant activity and water relationship in plants. BRs have been used in different horticultural crops particularly application of exogenously which influences better growth, vascular differentiation, stress tolerance, pathogen resistance, pollen viability, fruit setting, yield and quality etc. Based on published literatures, it has been concluded that BRs and their analogues are the sixth group of phytohormones, which promote the growth and metabolism besides the classical growth regulators. It has interaction with other phytohormones and polyamine for enhancing the growth and metabolism. Molecular studies underlying those BRs provide new approaches for creating best root systems for efficient water and nutrient uptake abilities that can sustain crop biomass and yield.

ACKNOWLEDGEMENT

The first author (JP) wishes to acknowledge to University Grant Commission, Government of India for providing research fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Praveena et al.; CJAST, 39(25): 174-196, 2020; Article no.CJAST.59978


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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59978