

Brassinosteroids Application Responses in Fruit Crops – A Review

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ABSTRACT

Brassinosteroids (BRs) are a class of novel plant hormones gaining importance as potential allies of agricultural development worldwide. Research findings on influences of exogenous BRs application on various aspects of growth and development like vegetative growth, flowering, fruit set, fruit growth, ripening, storage, and tolerance to various biotic and abiotic stresses in fruit plants' have been reviewed and discussed. The molecular or biochemical aspects of BRs responses to modulate growth and productivity by activating or withholding enzymatic reaction of different biochemical pathways of fruit plants have been elaborated. BRs are involved in ripening and fruit quality development of climacteric and non-climacteric fruits; as per reports of many researchers, they improve fruit color, phenolics, anti-oxidant activities and post-harvest life of many fruits. Fruit cracking in litchi has been found to be significantly reduced due to BRs application. This group of plant hormones possess the capacity to negate genotoxicity and pesticidal residues in many horticultural crops; it reflects the extension of their uses in producing consumer-friendly fruits through BRs application. One of the biggest advantages of BRs application is controlling favorable plant responses under abiotic and biotic stresses; plants have been reported to manage these stresses through modifications in various gene expressions and physiological processes under the influence of the BRs. The need for in-depth studies has been speculated for optimizing the concentration of BRs application together with the standardization of critical stages for harnessing the benefit of quality orchard productivity under varied growing conditions.

Highlights

- ① Brassinosteroids are a class of essential plant hormones regulating broad aspects of plant vegetative and reproductive development.
- ① Having an inextricable connection with germination, root, and shoot proliferation; they have been recognized to have potential in the promotion of plant propagation and nursery growth.
- ① Plant physiological functions and biomass production are influenced greatly by the BRs application hence modulation of several horticultural traits like flowering, fruit set, fruit growth and yield etc. of various fruit crops is possible.
- ① BRs can be exploited as potential orchard stress busters; they help the plants to maintain electrochemical gradient, ionic balance, and other stress-fighting attributes during stress.
- ① Woody fruit tree species need more attention for detailed research on harnessing benefits of BRs application for cost-effective orchard management.

Keywords: Brassinolide, Homobrassinolide, Plant Propagation, Flowering and fruiting, Ripening and storage, Biotic and abiotic stress

Agricultural development throughout the world has been revolutionized by the usages of plant growth regulators (PGRs); these have become great allies for modulation of plant

biochemical functions and modification of plant

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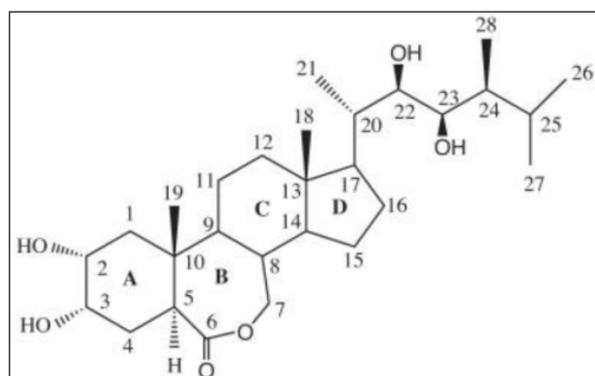


constituents for enhancement of yield and quality of agricultural produce. Commercial formulations of Indol-3-Acetic Acid (IAA), Indol-3-Butyric Acid (IBA), 2,4-Dichlorophenoxyacetic acid (2,4-D), 1-Naphthalene Acetic Acid (NAA), 6-Benzyl Amino Purine (BAP), Benzyl Adenine (BA), Gibberellic Acid (GA_3), ethylene or ethephon (CEPA), Abscisic Acid (ABA) etc. have been in use since 1930 and contributing significantly by regulating vegetative growth, susceptibility towards biotic and abiotic stress, bud and seed dormancy, flowering and fruit set, fruit quality, acceleration or delay of fruit ripening for optimal harvesting, yield, optimization of fruit storage and ripening, etc. under varied growing conditions (Alcázar *et al.* 2010; Bergstrand 2017; Fahad *et al.* 2016; Choudhury *et al.* 2013; Rademacher 2015). New classes of plant hormones are being discovered which possess a very high potential for modulation of plant growth functions at very minute concentrations. As agriculture and horticulture is getting more and more mechanized and science is increasing the possibilities for pushing the limits of classical crop production systems, the role of knowledge in extrinsic modulation of the growth processes is vital for effective tapping of these versatile resources to enhance productivity and quality of agricultural produce under the dwindling agroclimatic conditions.

Plant growth and development is an outcome of the integration of a variety of environmental and intrinsic genetic programs for utilization and translation of a variety of growth substances (nutrients, proteins, carbohydrates, fats, etc.) into biomass. It involves the regulation of cellular processes by a variety of chemical messengers called plant hormones which act as signal molecules for regulation of these functions in the targeted cells. Hormone possesses a specific affinity for protein receptor, embedded either in the plasma membrane or in the cytoplasm (depending on the receptor system), which causes the protein's conformation to a change that stimulates the production of 'relay molecules' in the cytoplasm. These relay molecules trigger various responses in plants at a cellular level in relation to the original signal. The responses produced are unique depending upon the tissue and stage of growth and development of the plant. Also, the hormones produce the responses in a given tissue in a concentration-dependent manner. When

these plant growth and development stimulating chemical messengers are synthetic in nature and applied exogenously, these are called as Plant Growth Regulators (PGRs). Besides the classical hormones/ PGRs, a new class of biomolecular stimulants like brassinosteroids, jasmonic acid, salicylic acid etc. have been identified in the recent past which governs the ability of plants to adapt to changing environments by mediating growth, development, nutrient allocation and source/sink relationship. Among these biostimulants, Brassinosteroids (BRs) are the ones that have been most extensively researched for their uses in plant growth and development. Being organic in nature and having the ability to act at cellular or whole plant level, they regulate almost all aspects of plant growth and are thus considered equivalent to plant hormones (Santner *et al.* 2009). This is a group of polyhydroxysteroidal plant hormone which is present ubiquitously in the plant system and regulate various plant developmental processes like induction of stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, ethylene biosynthesis, proton pump activation, gene expression and photosynthesis, gravitropism and adaptive responses to environmental stress (Clouse and Sasse 1998; Dhaubhadel *et al.* 1999; Khripach *et al.* 2000; Steber and McCourt 2001; Yu *et al.* 2004; Vandebussche 2011). About seventy BRs have been identified from different plants, and most of these are classified as C_{27} , C_{28} or C_{29} depending upon the alkyl substitution on C-24 (Bajguz 2011). Among the identified BRs, brassinolide (BL), 24-epibrassinolide (EBL), and 28-homobrassinolide (HBL) have been reported to be the most effective and extensively used in physiological studies. Brassinolide (BL) is the first BR that was extracted from rapeseed (*Brassica napus*) pollen, and its structure was defined by Grove *et al.* (1979). Though, the growth-promoting activities of rapeseed pollen were known to Mitchell *et al.* (1970). Brassinosteroids got recognition as a novel phytohormone during the 1990s, when several genes involved in BR biosynthesis and perception were identified. Numerous deformities like short hypocotyl, dwarfism of seedlings and mature plants, short petioles, dark-green leaves, delayed flowering, and reduced male fertility was recognized under the situations of loss-of-function mutation of the BRs (Li *et al.* 1996; Szekeres *et al.* 1996; Li and Chory, 1997). Their biological activity has been

attributed to their peculiar structural features, which render them very potent in hormonal cross-talks, activation of protein and nucleic acid synthesis, enzyme activity, and resistance to biotic and abiotic stresses. They play a regulatory role in the control of cell-cycle progression and differentiation in many plants. Exogenous application of these steroidal compounds at seed level or as a foliar spray is now known to enhance antioxidant defense mechanism and accumulation of osmoprotectants such as proline and glycine betaine under stress conditions (Sirhindi 2013).



Source: Clouse (2011)

Fig. 1: Chemical structure of Brassinolide - one of the naturally occurring brassinosteroids

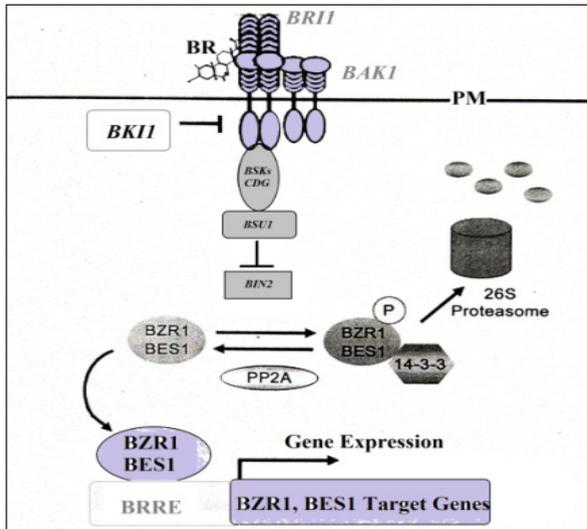
Biosynthesis and Mechanism of Action

Brassinosteroids biosynthesis pathway was illustrated by Fujioka and Sakurai (1997), describing the synthesis of BRs from plant sterol, campesterol to brassinolide through early or late C6-oxidation pathways. Though the sites for BR synthesis in plants have not yet been experimentally demonstrated, it is believed that all plant tissues produce BRs. This hypothesis was supported by the fact that BR biosynthesis and signal transduction genes are expressed in a wide range of plant organs, and their activities are limited to a short distance only (Clouse and Sasse 1998 and Li and Chory 1997). However, later the research proved that long-distance transport of BRs is possible, and the flow is from the base to the tips (acropetal); though, the biological relevance of this movement is still not clear (Clouse and Sasse, 1998). A recent enzymatic analysis of cytochrome P_{450} enzymes and re-evaluation of the endogenous amount of BRs in BR-deficient mutants also suggested a campestanol independent pathway of the BR biosynthesis (Ohnishi, 2018).

As far as signal transduction is concerned, it is believed that a BR synthesized in the endoplasmic reticulum is transported through the apoplast where it binds with plasma membrane-localized receptor, and the signal is then transduced to the nucleus where thousands of genes are modulated to confer various biological responses (Clouse 2011; Guo *et al.* 2013; Dejonghe *et al.* 2014; Nolan *et al.* 2017). The receptors binding to BR molecules are plasma membrane bound kinase receptors which transduce the signal from the cell surface to the nucleus by the initiation of an intracellular cascade of protein-protein interactions involving kinases, phosphatases, 14-3-3 proteins, and nuclear transcription factors. The receptors BAK1 interacts with BRI1 and gets phosphorylated. Phosphorylated BRI1 interacts with 14-3-3 nuclear proteins and releases BZR1 and BZR2 (also named BES1 for BRI1-EMS-suppressor 1) (Wang *et al.* 2011). Activated BRI1 phosphorylates BR-signaling kinases (BSKs), and constitutive differential growth 1 (CDG1) gets phosphorylated by the activated BRI1, which then activates the BRI1-suppressor 1 (BSU1) phosphatase. BSU1 inactivates Brassinosteroid-Insensitive 2 (BIN2) through dephosphorylation. BIN2 is a negative regulator in BR signaling pathway. BZR1 and BES1 are the positive regulators of BR signaling pathway just down to BIN2. The accumulation of BZR1 and BES1 is increased by BR treatment; BZR1 and BES1 are mostly in phosphorylated form, and BR treatment induces dephosphorylation and accumulation of protein. The regulation of BZR1 and BES1 degradation by BIN2 phosphorylation regulates the accumulation of un-phosphorylated BZR1 and BES1 that in-turn regulates BR target genes in the nucleus (Li and Chory 1997; Wang *et al.* 2002; Yin *et al.* 2002; He *et al.* 2002; Li and Nam 2002; Mora-Garcia *et al.* 2004; Caño-Delgado *et al.* 2004; Tang *et al.* 2008; Kim *et al.* 2009, 2011).

The increased endosomal localization of the BR receptor and enhancement in signaling indicates that the BR signaling is also regulated by the endophytic machinery of the plants, and thus like other hormones BRs also alter the gene expression before the onset of cellular or physiological changes that impact the activity of complex metabolic processes contributing to the regulation of cell division and differentiation. They modulate the processes which are more specific to growth

and development viz. phoshomorphogenesis, skotomorphogenesis and cellular expansion (Clouse and Feldmann 1999). Cell expansion is critical for cellular differentiation and organogenesis, which is controlled by coordinated alterations in cell wall mechanical properties, biochemical processes, and gene expression (Cosgrove 1997).



Source: Li et al. 2013

Fig. 2: Pictorial representation of BR signal transduction and mode of action

For turgor-driven expansion to proceed, the cell wall must yield through breakage of hemicellulose teathers and further to be followed by incorporation of more wall polymers to prevent thinning and weakening of cell wall. The hormones involved in elongation then target the regulation of synthesis and activity of wall-modifying enzymes such as xyloglucan, sucrose synthase, and cellulose synthase. Similar activity of BRs resulting in cell wall loosening has also been reported by Clouse (1997) and Zurek et al. (1994) in *Arabidopsis*, soybean, and tomato. Later, Clouse and Sasse (1998) demonstrated that the BRs increase the rate of cell division under auxin and cytokinin limiting situations. They stimulate cell division by way of promoting the kinetics of the cell cycle and regulate the expression of genes associated with S phase, including H2B and high mobility group_1 protein (Nakajima et al. 1996; Oh and Clouse 1998). In the dark, the BRs promote the expression of cyclin-dependent kinase, whereas it is reported to remain unaffected under light (Yoshizumi et al. 1999). Magnitude of BR-mediated gene expression changes is, however, small but appears to affect

cell expansion processes largely. Nemhauser et al. (2004) reported that BRs work in association with auxins for cellular expansion, and a similar association with gibberellins was reported by Bai (2012). As far as cellular differentiation is concerned, Fukuda (1997) and Caño-Delgado et al. (2004) reported that BRs promote vascular differentiation by increased differentiation of tracheary elements. It was supported by the findings that BRs reverted back the adverse action of unicanozoles on vascular differentiation.

BRs Uses in Fruit Crop Production

Brassinosteroids have a multifarious role in crop production. A wide range of critical plant growth and developmental processes like seed germination, allometric growth, reproductive growth, fruit development, ripening, fruit quality, post-harvest life, and plant responses to several biotic and abiotic stresses are now known to be influenced by very minute concentrations of BRs application. Some commercial applications of BRs in fruit crop production and associated plant functions are discussed hereunder:

Plant Propagation and Nursery Growth

Success of an orchard enterprise is largely governed by the quality of the nursery being produced and planted. The production of quality planting material, however, faces several challenges right from germination of rootstock seed to successful establishment of the grafts in the orchard. The use of growth hormones in achieving faster multiplication of elite plant material has been demonstrated widely across the globe. Recently, BRs have also been reported to contribute to the nursery business by way of influencing various nursery production activities right from seed germination to mass multiplication through tissue culture and other vegetative plant production techniques. There are reports suggesting beneficial effects of pre-sowing seed treatment with BRs on enhanced germination and plant performance under varied growing conditions. Takematsu and Izumi (1985) reported that the application of brassinolide and related compounds as a pre-sowing seed treatment for 4 hours in 1 ppm solution enhances seed germination and the yield of tomato plants under greenhouse conditions. Promotion of germination in non-

photodormant tobacco seeds has also been reported, but it was attributed to partial influence on the signal transduction pathways and promotion of xyloglucan endo-transglycosylase (XET) enzyme activity in the embryo and endosperm of germinating seeds due to BRs application (Leubner-Metzger 2003).

The role of BRs in the promotion of germination is different from that of gibberellins and light, which are supposed to act in a common pathway to release photodormancy. BR does not release photodormancy; it promotes seed germination by enhancing the growth potential of the emerging embryo in a GA-independent manner. Brassinolide, 24-epibrassinolide, and 28-homobrassinolide were tested for germination of peanut seed, all these three brassinosteroids promoted and accelerated the seed germination. Early seedling growth was also accelerated by the brassinosteroidal application (Vidyavardhini and Rao 1996). A similar effect of 24 epi-brassinolide was observed on germination of *Eucalyptus camaldulensis* seeds (Silva and Gracia-Martinez 2016). Soaking the spinach seeds in 10–12 ppm aqueous solution of EBL for 8 hours has been found to enhance germination from 54% to 72% (Ikekawa and Akutsu 1987). Contrarily, there are some studies that have reported that brassinolide and other similar compounds have an inhibitory effect on potato germination during storage (Kazakova *et al.* 1991); it is otherwise an advantage that enhances the storage life of potatoes.

Apart from germination, BRs have been reported to influence seedling growth and help in maintaining plant height and branch number in nursery plants. Megbo (2010) reported that brassinolide together with GA₃ increased petiole growth when applied at the rate of 100mL of 150mg/L solution three weeks after germination of sweet pepper seeds. Kumari and Thakur (2018) have also observed enhanced growth of apple seedlings due to BRs application.

Due to the lengthy generation cycle of woody perennial species, clonal propagation has a great practical significance in the propagation of horticultural plants (Legue *et al.* 2014; Wei and Li, 2016). The capacity of the plants to form abundant roots is highly desirable in almost all vegetatively propagated species. The role of adventitious root formation is thus of utmost importance in vegetative and clonal propagation of horticultural crops, and the phytohormones are essentially involved

in root growth and development (Pacifci *et al.* 2015). BRs have also been reported to enhance root meristem size and adventitious root development in a concentration-dependent manner. The lower innate concentrations of BRs have been reported to promote root growth whereas it is found to be restricted at higher concentrations (Roddick *et al.* 1993; Clouse *et al.* 1996; Mussig *et al.* 2003; González-García *et al.* 2011; Hacham *et al.* 2011; Chaiwanon and Wang 2015; Gupta *et al.* 2015 and Lee *et al.* 2015). Swamy and Rao (2006) reported that the exogenous application of 50 to 100 µM brassinosteroids for 5 minutes significantly affected rhizogenesis and enhanced both root formation and root growth in *Geranium* stem cuttings. 28-homobrassinolide and 24-epibrassinolide were tested for promotion of rooting in grapes cuttings. It was found that the effect of 28-homobrassinolide was marginally more prominent than 24-epibrassinolide. Lower concentrations of homo-brassinolide (BL) enhanced root number in cuttings of grape rootstock (Kaplan and Gokbayrak 2012). Stimulation of adventitious root formation in soybean by epibrassinolide was also observed by Sathiyamoorthy and Nakamura (1990). The promotion of root formation by BRs application is primarily through their effect on the meristem size, root hair formation, and lateral root initiation. Promotional effects of auxins on root growth in propagules are also reported to have linkage with BRs, which are supposed to help in the initiation of root growth in plants (Kim *et al.* 2000; Bao *et al.* 2004). Contrarily, Guan and Roddick (1988) reported inhibition of adventitious root formation in tomatoes by brassinosteroids application.

Micropropagation

Micropropagation of many woody perennial fruit species has been a challenge due to poor shoot elongation and proliferation in-vitro. BRs application has shown efficient use in tissue culture of woody perennial species (Pereira-Netto *et al.* 2003 and 2006). The brassinosteroids like 28-homocasterone have been found to stimulate branch elongation in *in-vitro* cultured shoots of Marubakaido apple rootstock. Sasaki (2002) discovered that brassinosteroids also have light-dependent mode of action; he found that adventitious bud formation from hypocotyl segments was stimulated by BRs application in *in-vitro* cultured of cauliflower under lighted condition.



In the dark, this regeneration was much lower due to a possible increase in ethylene synthesis. Azpeitia *et al.* 2003 found that coconut explants responded well to the BRs application; better growth of callus, production of embryogenic callus, and somatic embryos were obtained due to brassinosteroidal application. The first ever protocol for mass multiplication of epiphytic orchid (*Liparis elliptica*) through *in-vitro* culture was developed by Malabadi *et al.* (2009), who used 4.0 μM concentration of 24-epiBL in basal medium and attained the highest percentage (93%) of protocorm-like bodies (PLBs) – transverse thin layer (TCLs) explants in a period of 12 weeks. Further, during *in-vitro* establishment of ground nut cultivars, it was found that shoot multiplication potential was highest when the application of brassinosteroids is made at a concentration of 1 mL L^{-1} with benzyladenine at 3 mg L^{-1} , the rhizogenesis was noticed best when BRs have applied alone. Overall growth in terms of multiple shoots, chlorophyll content, hill reaction activity, activities of catalase, peroxidase, polyphenol oxidase and ascorbate peroxidase were found best in BR containing cultures (Verma *et al.* 2011).

Physiological Functions and Biomass Production

As mentioned earlier, the brassinosteroids have a crucial role in regulating the growth and development processes of plants in a coordinated manner for providing energy and the building blocks that generate the form that we recognize as a plant. The ubiquitous distribution of BRs throughout the plant system influences various aspects of plant life. Evidence are increasing day by day that BRs are generating a significant impact on photosynthesis, transpiration, ion uptake and transport, besides specific changes in leaf anatomy and chloroplast structure. As the plant growth is largely an outcome of photosynthetic apparatus and the building blocks synthesized through the uptake of essential nutrients, the efficiency of light energy transformation, CO_2 productivity; the potential of light and dark reactions and the photosynthetic rate depends on the balance of nutrient ion influx (Talaat 2013 and Song *et al.* 2016). In a recent study conducted on *Canola* species, it was observed that BRs increase essential inorganic ions, decrease toxic ions, thereby promotes ion homeostasis,

especially in leaves, root, and epicotyl (Liu *et al.* 2014). 24-epibrassinolide has been found to enhance nitrogen metabolism under low temperature and week light stress. Steber and McCourt (2001) found that BRs promoted the activity of nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase enzymes and promoted photosynthesis in tomato seedlings.

A number of reports are available which show an increase in plant biomass after brassinosteroids application (Sairam 1994; Gomes *et al.* 2006). Kagale *et al.* (2007) reported that treatment with EBR produces visible morphological changes in plants. Foliar application of spirosanic analog of brassinosteroid has been found to cause physiological changes related to chlorophyll metabolism depending upon the leaf ontogeny (Gomes *et al.* 2013). More than a hundred times increase in the number of leaves, petiole length, total leaf area, and a number of crowns in strawberry has been reported by Pipattanawong (1996). In cactus pear (*Opuntia ficus-indica*) vegetative bud initiation was observed one week earlier due to BB-6 and BB-16 (types of BRs) application; the growth of cladodes was also increased (Cortes *et al.* 2003). In a recent study in Valencia sweet oranges, bi-weekly application of homo-brassinolide at the rate of 0.1 micromolar concentration (18.6ml/100 gallons) has been found to improve canopy area photosynthesis, fruit yield, sugar/acid ratio (Sutton *et al.* 2020).

Flowering, Fruit Set, Fruit Drop, and Yield

It is now recognized that brassinosteroids have a definite role in the flowering of many plants, including fruit crops (Pipattanawong *et al.* 1996; Clouse and Sasse 1998; Domagalska *et al.* 2007; Clouse 2008; Li *et al.* 2010). However, Yoshiok *et al.* (1990) reported that the BRs application was effective in increasing the number of flowers in autumn but reduced flowering in late winter crops of grapes. The application of BRs modulates the metabolic pathways for defining the pattern of branching and flower formation by modifying the nutrient allocation and signaling pathways. Papadopoulou and Grumet (2005) also reported that exogenous application of epi-brassinosteroids (EBRs) increased precocious bearing and female flower production in cucumber.

Evidence of involvement of BRs in early fruit

development are not many, but it has been reported that exogenous BRs application increases fruit set (Kamuro and Takatsuto 1999). In Navel orange (*Citrus sinensis* L.) cv. Morita, the brassinolide spray at a concentration of 0.01 ppm increased fruit set (Sugiyama and Kuraishi 1989). Watanabe *et al.* (1997) also observed an increase in fruit set of Fuyu (Persimmon) by BRs application at 0.01 ppm. The fruit set was also improved by 76.2% and 70.60%, respectively, when applied seven days before blooming and at the full bloom stage. Initial fruit development also gets influenced by exogenous BR application, in strawberry BR have been noticed to be involved in downregulating the expression of BRs receptors and promote initial fruit development by way of stimulating cell division (Chai *et al.* 2013).

Apart from fruit set BRs application has also been found effective in the reduction of fruit drop. Suzuki *et al.* (1988) found that application of BR decreased fruit drop significantly in persimmons when sprayed at anthesis. It was found to advance the maturity and reduced pre-harvest drop in sweet oranges (Alferez 2019). In grapes, Isci and Gökbayrak (2015) observed that 22S-, 23S-homobrassinolide applied at high concentration resulted in stronger attachment between the pedicel and the stalk and thus reduce fruit drop and improved fruit retention.

Rajan *et al.* (2017) found that post-shooting spray of banana bunches with brassinosteroid at the rate of 2.0 mg L⁻¹ resulted in a yield of 114.46 t ha⁻¹ in cultivar Grand Naine as against 84.24 t ha⁻¹ in control; the improvement in yield was attributed to the effect of brassinosteroids on cell elongation by increasing the cell permeability to water and osmotic solutes of the cells. The application of BR analog (BR-3) during a period of reproductive development has been reported to increase yield by 65% in passion fruit over control. It stimulated better accumulation of photosynthates resulting into increased fruit number (Gomes *et al.* 2006). Similarly, the increased yield was also observed in Navel orange and sweet cherry due to BRs application (Sugiyama and Kuraishi 1989; Roghabadi and Pakkish 2014). In a study conducted on carrots, Que *et al.* (2017) observed that exogenous application of 24-EBL resulted in better root weight than control. Commercial application of BRs is there in practice in countries like Belarus, Japan, Russia, and China for improvement in the production of potato, cucumber,

pepper, tomato, etc. (Moiseev 1998). BRs influence growth processes in consonance with other plant hormones. In a study on 10-year-old sugar apple trees, Mostafa and Kotb (2018) found that BRs when sprayed at weekly intervals after anthesis (up to five sprays) at the rate of 1ppm, produced effects similar to those of 1500 ppm GA₃ or 0.5 ppm BRs + 1000 ppm GA₃. These applications resulted in the highest fruit set percentage, fruit retention, number of fruits/tree, and yield. Also, BRs helped in getting seedless sugar apple fruits with high fruit quality. In grapes, Bhat *et al.* (2011) concluded that exogenous application of CPPU and 0.4 mg/liter BR enhanced berry size, berry length and diameter, and berry number considerably in grapes.

Fruit Cracking

Foliar spray of brassinolide solution on litchi leaves before blossom has been found to increase the enzyme activity. Peng *et al.* (2004) reported that calcium content and water-soluble pectin, protopectin improve following BRs spray in the fruit pericarp and reduced fruit cracking rate in litchi fruits; suggesting an important role of BRs in increasing the commercial value of litchi fruits.

Ripening, Pre and Post Harvest Life of Fruits

Human health and nutrition have direct linkage to fruit ripening. The consumer needed physicochemical attributes of the fruits develop during the process of fruit ripening which is regulated by different hormone-mediated pathways leading to a series of pre- and post-harvest changes associated with the development of fruit quality and post-harvest life. Brassinosteroids have been reported to trigger the ripening process by stimulating ethylene biosynthesis between S- adenosylmethionine and 1-amino-cyclopropane 1- carboxylic acid (ACC) pathway (Schlagnhauser *et al.* 1984). In Kensington Pride mango role of hormones in modulating the ripening process was investigated by Zaharah *et al.* (2012). They monitored the endogenous level of BRs, ABA, IAA, ethylene, and the respiration rate of the fruits at 2 days interval in a period of 8 days of ripening at ambient temperature 21±1°C. BRs were found to be present in the fruit only in trace amounts, but their presence was found to be continuous. Effect of exogenously applied Epi-brassinolide (E-BL) at the rate of 45 and 60



ngg⁻¹ fresh weight basis was found to advance the climacteric peak of ethylene production and respiration rate by two and one days, respectively. These application rates resulted in the production of 4.81 and 5.74 nmol C₂H₄ kg⁻¹ h⁻¹ ethylene and 4.87 and 5.06 mmol CO₂ kg⁻¹ h⁻¹ rate of respiration at climacteric peak, respectively.

Furthermore, full fruit peel color development was obtained by these treatments between the second day and the seventh day of fruit ripening. Improved peel color of EBL treated mango fruits was attributed to the enhanced activity of chlorophyll degrading enzymes and also due to probable accumulation of carotenoids. Further, the pulp rheological attributes like cohesiveness, firmness, and mellowness of ripe fruit were also significantly influenced by the different concentrations of Epi-BL. However, the effect of exogenous BRs application did not significantly affect total sugars, soluble solids' concentration, acidity, and sugars acid ratio of ripe fruit. Though, the post-harvest application of epibrassinolide advances and accelerates the climacteric ethylene evolution and respiration rate, which eventually reduces fruit firmness and promotes peel color development. The fruit ripening was found to be hastened without affecting the quality of ripe fruit. The BRs application has been found to accelerate the ripening of other climacteric fruits, also. The effect of 28-homobrassinolide and 24-epibrassinolide was studied on the ripening of tomato pericarp discs by Vardhini and Rao (2002). They treated mature green equatorial pericarp discs of tomato maintained at ambient temperature i.e. at 20±1 °C with brassinosteroids at concentrations viz. 0.5, 1.0 and 3.0 µM. The increased application of these steroidal hormones resulted in elevated levels of lycopene, carbohydrates, and ethylene and lowered chlorophyll and ascorbic acid levels. The fruit-senescence was found to be accelerated with brassinosteroids application. Contrarily, Zhu *et al.* (2010) reported that BRs helps in slowing down the fruit senescence by lowering ethylene production and respiration rate in Chinese ber and other fruits. Mostafa and Kotb (2018) found that application of BRs (0.5 or 1.0 mg/L) recorded the highest total sugars. Regarding other physicochemical properties of sugar apple, BR application improved the fruit length, diameter, weight, pulp weight, and peel

weight, etc., but the reduction in fruit acidity was recorded.

The ripening of non-climacteric fruits such as grape (*Vitis vinifera*), the hormonal control of ripening is not well understood as in the case of climacteric fruits. Earlier, the BRs were not supposed to be involved in the ripening of non-climacteric fruits, but Symons *et al.* 2006 confirmed that it is the endogenous level of BRs, not the level of indole-3-acetic acid (IAA) or gibberellins, which is associated with ripening in grapes. They isolated putative grape homologs of genes encoding BR biosynthesis enzymes (*brassinosteroid-6-oxidase* and *dwarf1*) and the BR receptor (*brassinosteroid insensitive 1*) and confirmed the function of the grape *brassinosteroid-6-oxidase* gene by transgenic complementation of the tomato (*Lycopersicon esculentum*) *extreme dwarf* mutant. Further, it was found that exogenous application of BRs to grape berries significantly promoted ripening, while brassinazole, an inhibitor of BR biosynthesis, delayed fruit ripening.

These results provided sufficient evidence to prove that changes in endogenous BR levels influence the fruit ripening process in non-climacteric fruits also. Association of BRs in non-climacteric fruit ripening was also observed by Chai *et al.* (2013) in strawberries. They observed that FaBR-1, a BR receptor, gene expression instantly increased during the shift from white to initial red stages of strawberry fruit development. These findings were, however, helpful in managing market-oriented ripening and market logistics of grapes and non-climacteric fruits. For improving the commercial appeal of berries, Xu *et al.* (2015) recommended pre-harvest application of 24-EBL for having better anthocyanin and color development in Cabernet Sauvignon grapes. Xi *et al.* (2013) observed that 24-EBL application also enhances the activity of anti-oxidants and concentration of phenols in grapes. The other fruit quality attributes and yield were also reported to be improved by exogenous BR application in grapes, 24-epibrassinolide when sprayed at a concentration of 0.01 and 0.1 ppm have been found to improve the number of grape berries per bunch and the total yield by 66.7 and 29.9% over untreated control (Pozo *et al.* 1994). Increased CO₂ assimilation and increase in fruit biomass due to BB-16 (a polyhydroxylated spirostanoic BR analogue)

were reported by Gomes *et al.* (2003). Champa *et al.* (2015) also reported benefits of BRs application, grape cluster weight and diameter, berry weight, length, and diameter were considerably enhanced when BRs were sprayed on developing berries. Application of GA₃ at 50 ppm together with 1 ppm BRs at fruit set found to increase the fruit sugar content due to the increased capacity of fruits to draw more carbohydrates through BRs induced increase in auxin content (Singh *et al.* 1993). BRs were reported to be involved in increasing ABA content which activates the sugar metabolic pathway (Symons *et al.* 2006).

Therefore, the application of BRs has been found to increase the sugars in fruits. HBR application has been found to induce early maturation of sweet cherry cv. 'Tulare' and 'Bing' together with increased fruit firmness, skin color development, and force required to remove the fruits from stem at harvest (Mandava and Wang 2016). BRs application has also been found very effective in advancing the maturity of oranges. An increase in sugar content and fruit weight of sweet oranges due to BRs application was also reported by Wang *et al.* (2004). Treatment of grapes and litchies with BRs results in an enhanced accumulation of anthocyanin, organic acids, and phenol contents have also been reported by Luan *et al.* (2016), Roghabadi and Pakkish (2014).

Even under cold storage, the BRs helped in maintaining fruit peel color, and reducing the rate of total soluble solids, fruit acid degradation. Synergistic interaction between GA₃ and BRs was reported by Padashetti *et al.* (2010) while observing their effects on Arka Neelamani and Thompson seedless grapes. The activity of pectin degrading enzymes and calcium content which are major determinants for fruit firmness, have also been found to be influenced by BRs application. Better berry firmness, lower weight loss, and berry decay have been reported by Liu *et al.* (2016) in grape berries during cold storage. BRs application also reduces pulp browning under storage due to reduced activity of phenylalanine ammonia-lyase, polyphenol oxidase enzymes (Liu *et al.* 2014). In contrast, there are reports which mention that BRs like 24-epibrassinolide hasten senescence in plant systems (Ding *et al.* 1995 and He *et al.* 1996).

Stress Tolerance

Under the current scenario of climate change and global warming, the horticultural plantations face a number of abiotic and biotic stresses such as drought, cold, heat and high salinity, herbivory, disease, and allelopathy etc. Being non-mobile, plants can't avoid the unfavorable situations and are required to go through these growth and developmental challenges. The response of the plants largely depends upon their genetic tolerance mechanisms and complex signaling pathways that start with the perception of stress stimulus. The stimulus led to the synthesis of chemical messengers – the phytohormones in some parts of the plant and transported to the other parts, where they take part in decisive roles in controlling the response of the plant to stress at exceptionally low concentrations (Javid *et al.* 2011). Besides the five classical phytohormones (auxins, GAs, ABA, cytokinins, ethylene), BRs induce an extensive variety of adaptive responses in plants to several biotic and abiotic stresses (Kagale *et al.* 2007).

There are reports which illustrate that BRs have a shielding effect against the stresses and thereby enhance tolerance or resistance of the plants against several extreme conditions, such as temperature, water stress/drought, salt stress, toxicity of heavy metals like Cd, Cu, Al and Ni and disease-causing microorganisms (Aghdam and Mohammadkhani 2014; Upreti and Murti 2004; Fariduddin *et al.* 2014; Krishna 2003; Ali *et al.* 2008; Gomes *et al.* 2013; Nakashita *et al.* 2003). They control stress response either by activating or sustaining the enzymatic system of various biochemical pathways or by protein biosynthesis induction for the production of a wide range of defense imparting bio chemicals (Bajguz and Hayat 2009). Thus, it is the ability of the plant to switch between growth activation and repression that governs its stress tolerance capacity (Bechtold and Field 2018; Feng *et al.* 2016). Though the key pathway that controls plant's responses to environmental stresses under the abscisic acid (ABA) signaling pathway are not known (Yoshida *et al.* 2014; Zhu *et al.* 2017) however, there are strong evidence that indicate that BRs have a prominent role in controlling the balance between normal growth and resistance against various stresses. Their



mode of action is either independent or through hormonal cross-talks for activation of ABA pathway. Brassinosteroids are supposed to either fine-tune stress-responsive transcript machinery (Ye *et al.* 2017) or activate antioxidant mechanisms (Xia *et al.* 2009; Kim *et al.* 2012; Lima and Lobato 2017; Tunc-Ozdemir and Jones 2017; Zou *et al.* 2018). The BRs also act via promoting the production of osmoprotectants (Fàbregas *et al.* 2018) and strengthening membrane stability for sustaining the performance of the plants under stress conditions (Wang and Zeng 1993; Sharma and Bhardwaj 2007 and Sharma *et al.* 2008).

Among the major plant responses related to growing under stressful environment is the escalation in the generation of various reactive oxygen species such as superoxide radicals, hydroxyl radicals, alkoxy radicals, perhydroxyl radicals, hydrogen peroxide, singlet oxygen, etc. (Anjum *et al.* 2010, 2012, 2014, Gill and Tuteja 2010). Therefore, another mechanism in plants for stress tolerance is associated with activation of complex metabolic activities. For the continuation of plant growth under stress conditions, the BRs maintain antioxidative pathways of the plants for ROS-scavenging within the cells (El-Mashad and Mohamed 2012). Recent research has established that BRs and associated compounds have a great role in the modulation of both enzymatic and non-enzymatic components of antioxidant defense system in stressed plants. BRs have been found to regulate the transcription of such genes that encodes protective proteins having a vital role for the activation of antioxidant defense system. The impact of such genes is usually less prominent under normal conditions, but their beneficial effects are quite tangible under stressful conditions. In papaya, Gomes *et al.* (2006) reported that BRs application improved functions of papaya genotypes under water stress by way of inducing physiological changes related to chlorophyll metabolism without affecting leaf biomass and leaf area. In an experiment with papaya grown under irrigated and non-irrigated condition, Gomes *et al.* (2013) found that BRs treatment do not alter chlorophyll content, not even in older leaves of plants under of irrigated conditions. They concluded that action of BRs is prominent only when these are applied under water deficit conditions where the stress acted as a triggering event for induction of

senescence. Kumari and Thakur (2018) reported that application of BRs @ 0.05 to 0.1 ppm 15 to 30 days prior to induced-water stress-regulated the broad spectrum of plant growth and physio-biochemical processes in apple seedlings.

Influence of BRs on vital plant physiological parameters like net photosynthetic rate, relative water content, intercellular CO₂ concentration, stomatal conductance, and ABA concentration was investigated under water stress in tomato seedlings by Yuang *et al.* (2010). Two tomato genotypes, Mill. cv. Ailsa Craig (AC) and its ABA-deficient mutant *notabilis* (*not*), were used in these studies. Water stressed plants of both the genotypes were treated with 1 μM 24-epibrassinolide (EBR) or distilled water as a control. The net photosynthetic rate, relative water content, intercellular CO₂ concentration, stomatal conductance, which were hampered under stress, were found to be normalized, and water stress was alleviated in the plants receiving EBR treatment. The activity of antioxidant enzymes (catalase, ascorbate peroxidase and superoxide dismutase) was markedly increased by EBR application, whereas; it decreased intercellular CO₂ concentration and stomatal conductance. Interestingly, EBR treatment increased the ABA concentration both in AC and *not* plants, but the amplitude of ABA in *not* plants was significantly lower than AC plants. The studies concluded that in tomato seedlings, the drought stress might be mitigated with EBR-induced elevation in endogenous ABA concentration and the activities of antioxidant enzymes. Apart from activation of stress tolerance machinery of the plants, the BRs have also been found to delay fruit quality deterioration under stress conditions. Behnamnia *et al.* (2009) verified that carotenoid content of BR-treated tomato plants was significantly higher than the control under drought stress conditions. Gomes (2011) also illustrated that brassinosteroids promote the antioxidative activity of antioxidant enzymes and antioxidative compounds (ascorbate, carotenoids, and proline) under water stress conditions.

Disease Tolerance

Though, specific research work on the involvement of brassinosteroids in disease tolerance of fruits and fruit crops is very less, but there are certain reports



which elaborate the role of BRs in fighting diseases of fruits and fruit crops. BRs have been reported to have a crucial role in stimulating disease tolerance/resistance of some horticultural crops against pathogenic organisms by way of their association of interacting signal transduction pathways and strengthen the defense mechanism for establishing systemic acquired resistance. BRs application has been found to improve the expression of genes such as chitinase and beta 1, 3-glucanase, which enhance the ability of the plants to fight against pathogens (Sticher *et al.* 1997).

In Chinese ber, it has been found that at very minute concentration, the BRs application improved activities of, such as phenyl alanine ammonia-lyase, polyphenol oxidase, superoxide dismutase, and catalases- the enzymes related to plant defense and inhibited the growth of *Penicillium expansum* (Zhu *et al.* 2010). In another study on Satsuma mandarin, Zhu *et al.* (2016) found that EBR treatment was very effective in reducing the postharvest diseases by way of enhancement in the accumulation of H₂O₂, stress-related metabolites and through the induction of stress-related genes. Further, Champa *et al.* (2015) added that the effects of BRs on reducing fruit decay is associated with induction of disease resistance in fruits and delay of senescence rather than direct toxicity of the BRs to the fungal pathogens. Other reports are also available, which reveal that diseases like citrus greening caused due to *Candidatus liberibacter asiaticus* can be managed successfully with exogenous application of EBL (Canales *et al.* 2016). Alferez *et al.* (2019) reported that application homo-brassinolide (HBr) at a concentration of 0.01 to 1 µM alleviated the symptoms of greening and better tree health after HBr treatment in HLB-affected citrus trees. In grape berries, the incidence of grey mold rot was reduced by EBR application resulting in an increase in the activities of superoxide dismutase, peroxidase, catalases, and phenylalanine ammonia-lyase enzymes (Liu *et al.* 2016). Even the plants containing a high amount of brassinosteroids are reported to influence the disease resistance of the plants in the surrounding. Roth *et al.* (2000) reported that *Lychnis viscaria* plants contain a high amount of Brassinosteroids; the disease tolerance of other plants growing in the vicinity of this plant was observed to be higher than that of plants not growing in closer association with *Lychnis viscaria*.

Reversal of genotoxicity/ pesticidal toxicity

Brassinosteroids also possess a good potential for negating the negative impact of growth retarding/inhibiting substances and genotoxicity in some plants. 24-epi-brassinolide (EBL) isolated from *Aegle marmelos* Correa (Rutaceae) was tested for anti-genotoxicity of maleic hydrazide (MH) induced in *Allium cepa* chromosomal aberration assay by Sondhi *et al.* (2008). They found that the percentage of chromosomal aberrations caused due to 0.01% maleic hydrazide application was decreased significantly by the EBL application. Pesticidal toxicity or residual toxicity is a big problem in horticulture; removal of the residual impact of pesticides can be of great benefit for improving the food quality by way of reducing the level of pesticide residues in the horticultural products raised under non-organic growing conditions. Xia *et al.* (2009 a) found that BRs increased the metabolism and reduced the residue levels considerably in cucumber.

Temperature Stress

Apart from salt, drought or other abiotic stresses, chilling or frost-induced freezing stress also hampers the normal metabolism of plants (Fariduddin *et al.* 2014). It is a fact that the geographical distribution of plants and their productivity limits are strongly influenced by cold stress. Chilling or cold injury is one of the major physiological problems of several tropical and subtropical fruits. These fruits are very sensitive to low-temperature storage and easily get spoiled, thereby affecting their quality (Han *et al.* 2006). The plants possess a typical signaling chain of reactions that enables them to survive low-temperature stress. A molecular model showing the participation of BRs in the regulation of freezing tolerance of plants was presented by Eremina *et al.* (2016). In juvenile grapevines, 24-EBL treatments were observed to augment osmoregulation material and amount of antioxidant enzymes viz., phenylalanine ammonia-lyase, polyphenol oxidase, catalase, and superoxide dismutase, and ascorbate peroxidase. The damage caused by reactive oxygen species and lipid peroxidation was found to be decreased due to these treatments (Xia *et al.* 2009). As far as cold tolerance of fruits under storage is concerned, some reports have indicated that cold



stress tolerance in fruits can be enhanced by treating them with BRs. For instance, Li *et al.* (2010 and 2012) observed that 10 μ M brassinolide (BL) was able to regulate plasma membrane proteins such as remorin, abscisic stress ripening-like protein, type II SK2 dehydrin and temperature-induced lipocalin, and genes encoding these proteins get up-regulated under low-temperature stress condition (5 °C) in mango and finally suggested that BL has the important capability in enhancing tolerance of fruits to cold temperature stress. Further, lower phase transition and higher un-saturation was observed in the plasma membrane lipids in the BRs treated fruits. The BRs treatment maintained higher membrane fluidity under low-temperature stress conditions.

In another study, it was found that BRs are quite effective in inducing chilling tolerance in harvested banana fingers. Minimum chilling index, reduced electrolyte leakage, and melondialdehyde (MDA) content was recorded at 8°C during 12 days of storage of 24-epibrassinolide (EBL) treated fruits. The chlorophyll fluorescence, total soluble solids, and titratable acid content were also improved in the treated fruits. Gel based proteomic studies suggested that the proteins related to energy biosynthesis, stress response, and cell wall modification were also upgraded due to EBR treatment. In contrast, proteins related to protein degradation and energy consumption were suppressed, consequently contributing to cold tolerance of EBR treated bananas (Li *et al.* 2018). Roghabadi and Pakkish (2014) suggested that antioxidant activity in sweet cherry fruits can be induced by BRs application. They found that cold tolerance of sweet cherry was considerably improved when stored at 1 °C and BRs have been reported as the key factor in lowering oxidative damage caused by cold stress.

Brassinosteroids also play a crucial role during heat stress. Dhaubhadel *et al.* (2002) reported that there occurs increased accumulation of heat shock proteins (HSP) in seedlings due to their higher synthesis in EBR treated plants. They further added that this synthesis in the treated seedlings was higher even when the mRNA levels were lower than in untreated seedlings. Several translation initiations and elongation factors were found to be present at significantly higher levels in EBR-treated seedlings. It was concluded that EBR treatment limits the loss

of some components of the translational apparatus during prolonged heat stress and increase the level of expression of some components of the translational machinery during recovery; this is correlated with a more rapid resumption of cellular protein synthesis following heat stress and a higher survival rate of EBR treated seedlings.

Future perspectives

During the past two decades, considerable research has been made elaborating the mechanisms involved in brassinosteroids signaling and transduction in a plant system. The majority of the findings are based on the studies conducted on the model plant species like *Arabidopsis*. But, these findings may have deviated relevance as far as the higher woody species are concerned. Though BRs have been found to influence several developmental and physiological processes in higher plants, but very few studies are there illustrating the molecular mechanisms involved in regulating the biological processes of woody perennials. The advancement in research on these lines will improve our understanding of genetic determinants of BR biosynthesis at the molecular level, and it will further explore the possibilities of BR uses for targeted horticultural applications. More emphases are also required for elucidation on the role of BRs in tolerance to different types of stresses being faced by the fruit orchards. In-depth studies on hormonal cross-talks with other hormones like abscisic acid, jasmonic acid, auxins, GAs, etc., will facilitate the uses of these hormones for enhanced horticultural output and extending the shelf or storage life of climacteric and non-climacteric fruits. Also, fruit crop specific scientific studies are needed for the optimization of concentration and standardization of stage and mode of BRs application for cost-effective orchard management.

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