



PHYSIOLOGICAL ROLE OF BRASSINOSTEROIDS : AN UPDATE

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SUMMARY

The entire range of the developmental processes in plants is largely regulated by the shift in the hormonal balance. Out of the recognized hormones, attention has largely been focused on auxins, gibberellins, cytokinin, abscisic acid and ethylene. Brassinosteroids (BRs) were initially assigned a position of a lesser importance than the above recognized plant growth regulators. At present BRs have evolved as essential regulators of growth and development. Much progress has been achieved in their isolation, characterization and possible mechanism of action. However, their practical applicability has lacked far behind. BRs are recognized as regulators of transcription and translation thereby changing the pattern of total proteins, enzymes, and the rate of photosynthesis and finally the seed yield, at harvest. In this review we have discussed the importance of BRs in regulating the enzyme level, enhancing photosynthetic rate and other related aspects, determining biological yield, under natural conditions.

Key words: Brassinosteroids, enzymes, growth, photosynthesis, yield

INTRODUCTION

Growth is an organized, well-coordinated complex process where metabolism provides the energy and the building blocks. However, it is the relative hormone level that regulates the pace of growth of each individual plant part, to produce a form that is recognized as a plant. Earlier, only five groups of hormones (auxins, gibberellins, cytokinins, abscisic acid and ethylene) were designated as regulators of plant growth. However, in the recent past, compelling evidences have been put forward to classify an additional group of steroidal substances (brassinosteroids), first isolated from rape (*Brassica napus* L.) pollen, as a new class of phytohormones.

Mitchell *et al.* (1970) screened the pollens of nearly sixty species, out of which the extract from about thirty

species generated growth in bean seedlings. This growth promoting substance was then called as "Brassin". The search for its active factor(s) was collectively approached in 1974 by the USDA scientists working at Northern Regional Research Centre (NRRC), Peoria; Eastern Regional Research Centre (ERRC), Philadelphia and Beltsville Agricultural Research Centre (BARC), Maryland. Bee-collected pollens (500 lb) were processed through a pilot plant-size solvents (2-propanol) extraction procedure at ERRC and succeeded in partial purification at BARC. However, it was crystallized at NRRC and was subjected to X-ray analysis to establish its structure. This biologically active plant growth promoter was found to be steroidal lactone (C₂₈H₄₈O₆) and was named as "brassinolide" which was renamed as "brassinosteroid" (Rao *et al.* 2002). All natural BRs have a common 5 α -cholestane skeleton and its structural variants come from

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the type and the orientation of functionalities on the skeleton. Their low level in plants is not uniform throughout its body but young growing tissues have comparatively a larger share than the mature tissues (Yokota and Takahashi 1986). Their richest sources are pollen and immature seeds where its concentration ranges between 1-100 ng per g fresh mass, whereas shoots and leaves have about 0.01-0.1 ng per g fresh mass (Takatsuto 1994). Till now 70 BRs, structurally and functionally different from each other, have been characterized (Rao *et al.* 2002, Bajguz and Tretyn 2003). Out of which, three (brassinolide, 24-epibrassinolide and 28-homobrassinolide) are being largely applied to have an economical impact on plant metabolism, growth and productivity (Khripach *et al.* 2000).

OCCURRENCE OF BRASSINOSTEROIDS

Since the discovery of brassinolide (BL), 70 BRs (65 unconjugated and 5 conjugated) have been isolated from 60 plant species including 51 angiosperms (12 monocotyledons and 39 dicotyledons), 6 gymnosperms, 1 pteridophyte (*Equisetum arvense*), 1 bryophyte (*Marchantia polymorpha*) and 1 chlorophyte, the alga (*Hydrodictyon reticulatum*) (Bajguz and Tretyn 2003). Thus, the BRs are widely distributed in the plant kingdom, including higher and lower plants. BRs have been detected in all the plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, and grain (Bajguz and Tretyn 2003). The galls of *Castanea crenata* and *Distylium racemosum* have higher levels of BRs (several mg/kg) than the normal, healthy tissues (Bajguz and Tretyn 2003). Another tissue having BRs is the crown gall cells of *Catharanthus roseus* which have higher contents of BL and castasterone (CS) than the normal cells (30-40 mg/kg). Also, young growing tissues contain higher levels of BRs than mature tissues. Pollen and immature seeds are the richest sources with a range of 1-100 ng g⁻¹ fresh weight, while shoots and leaves usually have lower level of 0.01-0.1 ng g⁻¹ fresh weight. In the pollen of *Cupressus arizonica* the concentration of 6-deoxo typhasterol (TY) may be about 6400-fold greater than BL. BRs occur endogenously at quite low levels. Compared to the pollen and immature seeds, the other plant parts contain BRs in the nanogram or subnanogram levels of BRs per gram fresh weight. The highest concentration of BR, 6.4 mg per 1 kg pollen, was

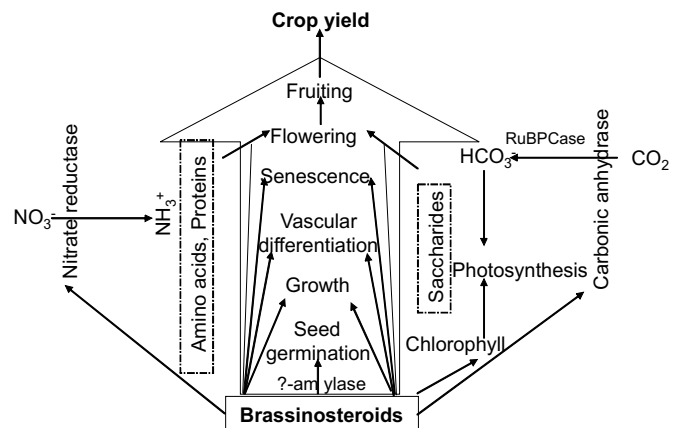


Fig. 1. Effect of brassinosteroids on plant under natural condition

detected in *Cupressus arizonica* (Griffiths *et al.* 1995, Clouse and Sasse 1998, Fujioka 1999).

Among the BRs, CS is the most widely distributed (50 plant species), followed by BL (34), TY (25), 6-deoxo CS (19), teasterone [TE] (19), and 28-nor CS (12) (Bajguz and Tretyn 2003). To the present day 34 other BRs and 5 BR conjugates have been found in only one plant species. Among all naturally occurring BRs, CS and BL are the most important BRs because of their wide distribution as well as their potent biological activity (Kim 1991, Fujioka 1999). Among the plant sources investigated, immature seeds of *Phaseolus vulgaris* contain a wide array of BRs (25 free and 2 conjugates). The wide occurrence of BRs was also reported in dwarf mutant of *Catharanthus roseus* (19 compounds), *Arabidopsis thaliana* (18 compounds), *Cryptomeria japonica* and *Cupressus arizonica* (9 compounds), *Dolichos lablab*, *Oryza sativa*, *Thea sinensis* and *Secale cereale* (8 compounds), *Lilium longiflorum* (7 compounds), *Distylium racemosum* (6 compounds) (Bajguz and Tretyn 2003).

DISTRIBUTION IN MONOCOTYLEDONS

The occurrence of BRs in monocotyledons has been demonstrated from four families including twelve plant species. BRs are represented by 18 compounds: 7-oxalactone (1, BL), 6-oxo (16, including two conjugates) and 6-deoxo (1 – 6-deoxoCS) types. Seven BRs, such as secasterone (SE), 2,3-diepi secasterone (2,3-diepi SE), TY, 3-dehydroteasterone (3-DT), teasterone-3-laurate

(TE-3-La), teasterone-3-myristate (TE-3-My) and secasterol were also isolated in plants (Bajguz and Tretyn 2003).

DISTRIBUTION IN DICOTYLEDONS

The presence of BRs in dicotyledons has been reported from three subclasses. The first, the Apetalae is represented by 6 families including 8 plant species. Total quantity of BRs amount to 7 compounds. The second, the Chloripetalae is represented by 8 families including 21 plant species. There are 49 BRs, among them 25 compounds belong to 6-oxo type, 17 belong to 6-deoxo type, 5 belong to 7-oxalactone type and 2 belong to 5-en type. Furthermore, from immature seeds of *Phaseolus vulgaris* a large quantity of 23 unconjugated and 2 conjugated BRs have been isolated so far. Among plants of this subclass, 44 BRs were detected for the first time. The third, the Sympetalae is represented by 7 families including 10 plant species. Among the BRs, compounds that belong to 6-deoxo type are widely distributed (13), 6 belong to 6-oxo type, 2 belong to 5-en type, and one compound belongs to 7-oxalactone (BL) and 6-hydroxy (6a-OH-CS) types (Bajguz and Tretyn 2003).

DISTRIBUTION IN GYMNOSPERMS

The occurrence of BRs in gymnosperms has been reported from six conifers. The presence of new 6 BRs was shown in *Cupressus arizonica* and *Cryptomeria japonica*. Among plant species so far reported, the level of BR in the mature pollen of *Cupressus arizonica* is highest (6.4 mg/kg 6-deoxoTY) (Bajguz and Tretyn 2003).

DISTRIBUTION IN LOWER PLANTS

BRs have been identified in lower plants such as green alga (*Hydrodictyon reticulatum*), a pteridophyte (*Equisetum arvense*) and a bryophyte (*Marchantia polymorpha*). Total quantity of BRs amount to 9 compounds, among them 6-oxo type of BRs dominates (8 compounds) (Bajguz and Tretyn 2003).

PHYSIOLOGICAL EFFECTS

Effect of brassinosteroids on seed germination and growth

Endogenous BRs have been identified in the seeds of several species, including pea (Yokota *et al.* 1996), *A. thaliana* (Schmidt *et al.* 1997) and *Lychnis viscaria* (Friebe *et al.* 1999). It is well documented that BRs promote seed germination, like gibberellic acid (Leubner-Metzger 2003). The treatment of the seeds of *Lepidium sativus* (Jones-Held *et al.* 1996) and *Eucalyptus camaldulensis* (Sasse *et al.* 1995) with brassinolide improved per cent germination. Similarly BRs promoted seed germination in case of rice (Dong *et al.* 1989), wheat (Sairam *et al.* 1996, Hayat *et al.* 2003a), tomato (Vardhini and Rao 2000), chickpea (Ali *et al.* 2005) and tobacco (Leubner-Metzger 2001). Moreover, brassinolide, 24-epibrassinolide and 28-homobrassinolide promoted seed germination in groundnut (Vardhini and Rao 1997). BR application has been reported to enhance the germination of certain parasitic angiosperms (Takeuchi *et al.* 1991, 1995), cereals (Gregory 1981, Yamaguchi *et al.* 1987), *Arabidopsis* (Steber and McCourt 2001). Pretreatment with brassinolide stimulated the germination and seedling emergence of aged rice grains (Yamaguchi *et al.* 1987) and seed treatment of barley accelerated subsequent seedling growth (Gregory 1981). It is, however, not certain, whether the promoting effect of BR in cereal grains is actually manifested only at the level of seedling growth and / or also at the level of germination *per se*.

In *A. thaliana*, BR promotes the germination of pre-chilled (i.e. non-dormant) seeds of BR-deficient biosynthesis mutant *det2-1* and the BR-insensitive response mutant *bri1-1* imbibed in the light (Steber and McCourt 2001). Seed germination of *det2-1* and *bri1-1* is more strongly inhibited by ABA than the wild type and BR is, able to partially overcome the inhibition of germination by ABA (Zhang *et al.* 2009). BR treatment rescues the germination phenotype of the severe GA-deficient biosynthesis mutant *gal-3*, which normally requires GA treatment for dormancy release and

germination. It also partially rescues the germination phenotype of the severe GA-insensitive response mutant *sly1* (*sleepy1*), which cannot be rescued by treatment with GA. Interestingly, a new allele for *sly1* was identified in a screen for BR-dependent germination and also proposed an interaction between BR and GA signalling in seeds (Steber *et al.* 1998, Steber and McCourt 2001). This is further supported by the germination phenotype of the *gpa1* mutant of *Arabidopsis* (Ullah *et al.* 2002). BR promotes seedling elongation and germination of non-photodormant tobacco seeds, but do not appreciably affect testa rupture and subsequent induction of β Glu I in the micropylar endosperm (Leubner-Metzger 2001, 2003). Treatment with BR accelerates endosperm rupture of tobacco seeds imbibed in the light. Promotion of endosperm rupture by BR is dose-dependent and 0.01 μ M brassinolide is most effective. The growths of the seedlings are also reported to increase by the BRs in *Zea mays* (Bhardwaj *et al.* 2007, Arora *et al.* 2008), *Brassica juncea* (Sharma *et al.* 2007, Sharma and Bhardwaj, 2007, Sirhindi *et al.* 2009).

Effect of brassinosteroids on flowering

There has been very limited use of steroids in regulating flowering. The number of flowers in strawberry increased by the application of BRs at the foliage (Pipattanawong *et al.* 1996). However, in case of grapes, the application of BRs in autumn improved the number of flowers but inhibited if the time of application is delayed to late winter (Rao *et al.* 2002).

Effect of brassinosteroids on senescence

Senescence is the process, which refers to, endogenously regulated deteriorative, changes that become the natural cause of death of cells, tissues, organs or that of the whole organism (Arteca 1997). Like other hormones (Rao *et al.* 2002), BRs also play a crucial role in regulating the processes leading to senescence. The brassinolide promotes senescence in *Xanthium* and *Rumex* explants (Mandava *et al.* 1981) and in wheat leaves (Saglam-Cag 2007). In addition to it, BRs also accelerate senescence in leaves of mung bean seedlings (He *et al.* 1996). However,

brassinosteroid deficient *Arabidopsis* mutants exhibited delayed senescence of chloroplast (Li *et al.* 1996). Similarly, the senescence of the leaves of mungbean and mustard was delayed, if supplied with 28-homobrassinolide at early stage of growth due to chlorophyll biosynthesis (Fariduddin 2002, Fariduddin *et al.* 2005). During a search of senescence associated genes, He *et al.* (2001) developed a preliminary model for leaf senescence regulating network in *Arabidopsis*, where signals such as abscisic acid, jasmonic acid, ethylene, darkness, dehydration and aging activated 147 senescence associated enhancer trap line. 24-epibrassinolide could activate some of these but associated genes have not yet been cloned (He *et al.* 2001).

Effect of brassinosteroids on photosynthesis

The aqueous solution of 28-homobrassinolide, applied to the foliage of wheat and mustard (Sairam 1994, Hayat *et al.* 2000, 2001a, Fariduddin *et al.* 2009) or applied as seed soaking treatment to mungbean (Fariduddin *et al.* 2003, 2004a), *Lycopersicon esculentum* (Hayat *et al.* 2010), *Raphanus sativus* (Anuradha and Rao 2009) and dialkylaminoethylalkanoate or 24-epibrassinolide, in association with GA₃, to spinach enhanced the photosynthetic rate (Liang *et al.* 1998). Application of 24-epibrassinolide and 28-homobrassinolide through root was also reported to increase the net photosynthetic rate in *Cucumis sativus* (Kang *et al.* 2009) and in *Lycopersicon esculentum* (Ali *et al.* 2006). Foliar spray of aqueous solution of BR to wheat and mustard (Braun and Wild 1984), 24-epibrassinolide to seedlings of cucumber (Ding *et al.* 1995) and brassinolide to rice (Fujii *et al.* 1991) increased the rate of CO₂ assimilation. Likewise, the foliar application of 24-epibrassinolide enhanced the light saturated net CO₂ assimilation rate and carboxylation rate of rubisco, thereby increasing the capacity of CO₂ assimilation in the Calvin cycle (Yu *et al.* 2004). However, the epicotyl of cucumber, did not respond to epibrassinolide but the transport of the labeled (¹⁴C) glucose towards the epicotyl was favoured (Nakajima and Toyama 1995). Similarly, Hill activity in the foliage of *Vigna radiata* was favourably affected, on being supplemented with aqueous solution of 28-homobrassinolide (Bhatia and Kaur 1997).

Effect of brassinosteroids on chlorophyll

The total chlorophyll contents or its fractions increased in the leaves of *Vigna radiata* (Bhatia and Kaur 1997) and *Brassica juncea* (Hayat *et al.* 2001a) by 28-homobrassinolide and in *Cucumis sativus* (Yu *et al.* 2004) by 24-epibrassinolide, applied directly to their foliage. Similarly, the values for the above parameters increased in the leaves of rice (Wang 1997), *Brassica juncea* (Hayat and Ahmad 2003b, Fariduddin *et al.* 2009), *Vigna radiata* (Fariduddin *et al.* 2003), *Raphanus sativus* (Anuradha and Rao 2009) and *Lycopersicon esculentum* (Hayat *et al.* 2010) raised from the seeds given presowing treatment with 28-homobrassinolide. Moreover, the water stressed wheat plants treated with 28-homobrassinolide possessed high chlorophyll level (Sairam 1994).

Effect of brassinosteroids on carbonic anhydrase activity

Carbonic anhydrase (CA, E.C. 4.2.1.1) is the second most abundant soluble protein, other than RuBPCase, in C_3 -chloroplast (Reed and Graham, 1981, Okabe *et al.* 1984). It is a zinc containing protein with a molecular weight of 180 KDa (Lawlor 1987) and is ubiquitous enzyme, among living organisms. It catalyzes the reversible inter conversion of bicarbonates (HCO_3^-) and CO_2 (Sultemeyer *et al.* 1993). The rate of conversion of HCO_3^- to CO_2 is normally slow in alkaline conditions. However, CA activates the use of HCO_3^- in the production of CO_2 (Lawlor 1987). In C_3 plants, CA has a close association with RuBPCase where it elevates the level of CO_2 at its active site (Badger and Price 1994). An increase in the activity of CA, in the leaves, was attained by the application of 28-homobrassinolide to the shoot of the *Brassica juncea* (Hayat *et al.* 2000, 2001a) and through root dipping in tomato (Ali *et al.* 2006). Moreover, the seedlings of wheat, mungbean, radish and tomato raised from the grains treated with 28-homobrassinolide or 24-epibrassinolide possessed high CA activity in their leaves (Hayat *et al.* 2001b, Fariduddin *et al.* 2003, 2006, Anuradha and Rao 2009, Hayat *et al.* 2010).

Effect of brassinosteroids on nitrate reductase activity

The process of reduction of nitrate is catalyzed by the enzyme, nitrate reductase (E.C. 1.6.6.1), the level of which increased in the plants of rice (Mai *et al.* 1989), maize (Shen *et al.* 1990), wheat (Sairam 1994, Hayat *et al.* 2001b), *Lens culinaris* (Hayat and Ahmad 2003a, b), *Vigna radiata* (Fariduddin *et al.* 2004b, 2006), *Raphanus sativus* (Anuradha and Rao 2009), *Lycopersicon esculentum* (Hayat *et al.* 2010) and in the seeds of wheat (Hayat and Ahmad 2003c) by the application of BRs.

Effect of brassinosteroids on the vascular tissue

The first report of a role for BRs in the differentiation of vascular tissues came in 1991 (Clouse and Zurek 1991). Jerusalem artichoke (*Helianthus tuberosus*) cells transferred to the xylem differentiation medium, in the presence of auxin and cytokinins will differentiate into xylem elements in 72 - 96 hours. Very few vascular elements develop in the first 24 hours following transfer into this medium. However, nanomolar concentrations of BL included in the medium, resulted in a 10-fold increase in xylem differentiation, this was observed in the first 24 hours (Castle *et al.* 2003). Also significant increases in cell numbers were observed, indicating a role for BRs in cell division and differentiation (Clouse and Zurek 1991). Homobrassinolide are also reported to increase the cell division in barley (Kartal *et al.* 2009).

Zinnia elegans has been used extensively to study the formation of xylem/tracheary elements, a process that has three distinct stages (Fukuda 1997). BRs have been implicated in the transition between Stage II, and Stage III where secondary wall formation and cell death occurs (Fukuda 1997). It had previously been shown that the effects of uniconazole (a putative BR biosynthesis inhibitor) prevent differentiation of *Zinnia* mesophyll cells into tracheary elements and this inhibition was overcome by exogenous BR application (Iwasaki and Shibaoka 1991). Uniconazole appears to suppress the transcription

of genes involved in the final stages of differentiation but could be recovered by the application of BL (Yamamoto *et al.* 1997). It suggests that BRs are synthesized immediately prior to secondary cell wall development and cell death and possibly induces entry into this stage (Yamamoto *et al.* 1997).

Effect of brassinosteroids on the yield of crops

Once the presence of BRs in plants was established, the next phase was to explore the possibilities of using these new substances in improving the yield of economically useful plants. Brassinolide has been used to improve the yield of lettuce, radish, bush bean and pepper (Meudt *et al.* 1983, 1984). Foliar application of dilute aqueous solution of BL also improved the yield of wheat and mustard (Braun and Wild 1984), rice, corn and tobacco (Yokota and Takahashi 1986). BRs were also found to increase the growth and yield of sugar-beet (Schilling *et al.* 1991), legumes (Kamuro and Takatsuto 1991) and rape seed (Hayat *et al.* 2000, 2001b, 2003b). Application of 28-homobrassinolide and 24-epibrassinolide significantly increased the yield of potato, mustard, rice and cotton (Ramraj *et al.* 1997), *Lens culinaris* (Hayat and Ahmad 2003a, b), *Vigna radiata* (Fariduddin *et al.* 2003) and that of corn, tobacco, watermelon, cucumber and grape (Ikekawa and Zhao 1991) respectively. Foliar application of brassinolide, 24-epibrassinolide (Vardhini and Rao 1997) and 28-homobrassinolide (Vardhini and Rao 1998) was highly effective in enhancing the yield of groundnut and tomato. Role of BRs in increasing the crop productivity of tomato is also reported recently (Hasan 2009). Moreover, in China, 28-homobrassinolide has been registered as a plant growth regulator in case of tobacco, sugarcane, rapeseed and tea (Hayat and Ahmad 2003).

CONCLUSION

BRs are implicated in mediating the expression of classical phytohormones as auxins, cytokinins and GA etc. They are recognized as mediators of transcription and translation thereby changing the pattern of total proteins, enzymes, and the rate of photosynthesis and finally the seed yield, at harvest. The key growth regulating enzymes includes Rubisco, RuBPCase, CA, NR, α -amylase and components viz. PS assemblage,

flowering, senescence, chlorophyll and key regulators of cell differentiation (Fig.1). When hormones are applied exogenously under normal conditions they curtail their production slowing the flux of biochemical pathways, and redirecting the central intermediates of Krebs cycle towards assimilation and enhanced growth. This is achieved by higher enzymatic activity (for harvesting of carbon, nitrogen to synthesize mono-saccharides, fulfilling raised energy demand, polysaccharides and amino acids to build up plant body under accelerating growth conditions, and those for other regulatory molecules), pathway flux, cell enlargement and division, tissue differentiation (in newly formed tissues and acceleration in slow differentiating tissues) viz. seed germination, vigorous vegetative growth, vascular differentiation, chlorophyll formation, enhanced transport, flowering, pollen differentiation, fruiting, yield and senescence etc. It is matched with nutrient availability, their transport and rate of assimilation. Limit in availability switches towards next phase of regulation where spatial expression of hormones best regulate the growth and development under changed conditions of supplement. The specific tissues, cells, or internal metabolic profile could be sacrificed even, sensing the essentiality of the conditions. The intermediary growth regulator, like BRs, thus work better integrating the expression of super and subregulators well within due time so that the plant have not to let fall back coping up between repair and growth maintenance.

FUTURE PROSPECTS OF BRASSINOSTEROIDS

Thirty five years of research, on BRs has brought into light several vital functions of this class of phytohormones in the regulation of plant growth, development and productivity. Further progress in the investigation of mechanism of BRs action in plants, on the one hand, and elaboration of economically feasible schemes of synthesis of natural BRs and their analogs, on the other hand, will surely make a basis for the inclusion of this new class of plant hormones in the regular package of chemicals used for optimizing agricultural production. Hopefully, as the research will progress, much more knowledge will be added to the present literature. It has been stated earlier that the application of these steroids to plants generates varied

physio-morphological changes by involving the genome and also do not initiate co-evolution of pests, enriching our arsenal of plant protection strategies, in the twenty first century. Moreover, the knowledge of the physical and chemical properties of these steroids is tempting us to consider them highly promising, environment friendly protectors and promoter of agricultural productivity. One of the major constraints, to employ BRs at larger scale, in the fields is their high cost. However, recent progress, in chemical synthesis of BRs and their analogues has led us to economically feasible approaches that has brought practical application very near to the reach of the farmers. Pesticidal companies in China and Japan have started synthesizing BRs, on a commercial scale. In India, also Godrej Agrovet Ltd., Mumbai, introduced 28-homobrassinolide in the market. We predict a better future for BRs in realizing crop yields, during the 21st century.

BRs can act efficiently in plants as immunomodulators when applied at the appropriate concentration and at the correct stage of plant development. BRs are implicated in plant responses to abiotic environmental stresses and to undergo profound changes in plants interacting with bacterial, fungal and viral pathogens. BRs open up new approaches for plant resistance against unfriendly environmental conditions.

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