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Brassinosteroid-Mediated Stress Responses

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ABSTRACT

Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds with wide-ranging biological activity that offer the unique possibility of increasing crop yields through both changing plant metabolism and protecting plants from environmental stresses. In recent years, genetic and biochemical studies have established an essential role for BRs in plant development, and on this basis BRs have been given the stature of a phytohormone. A remarkable feature of BRs is their potential to increase resistance in plants to a wide spectrum of stresses, such as low and high temperatures, drought, high salt, and pathogen attack. Despite this, only a few studies aimed at understanding the mechanism by which BRs promote stress resistance have been undertaken. Studies of the BR signaling pathway and BR gene-regulating properties indicate that there is cross-talk between BRs and other hormones, including those with established roles in plant defense responses such as abscisic acid, jasmonic acid, and ethylene. Recent studies aimed at understanding how BRs modulate stress responses suggest that complex molecular changes underlie BR-induced stress tolerance in plants. Analyses of these changes should generate exciting results in the future and clarify whether the ability of BRs to increase plant resistance to a range of stresses lies in the complex interactions of BRs with other hormones. Future studies should also elucidate if BRI1, an essential component of the BR receptor, directly participates in stress response signaling through interactions with ligands and proteins involved in plant defense responses.

Key words: Brassinosteroid; Hormone; Environmental stress; Stress responses; Thermotolerance; Disease resistance

INTRODUCTION

Structurally related to animal and insect steroid hormones, brassinosteroids (BRs) are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. Although it was well documented in the 1970s and 1980s that BRs can induce a broad spectrum of cellular responses, such as stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, and root inhibition (reviewed by Clouse and Sasse 1998; Mandava 1988), interest in BRs by the plant scientific community at large remained low due to the lack of definitive proof of an essential role of BRs in plant growth and development. The molecular cloning and characterization of the *Arabidopsis DET2* and *CPD* genes in 1996 revealed that these genes encode enzymes in the BR biosynthesis pathway (Li and others 1996; Szekeres and others 1996) and sparked much interest in the role of BRs in plant

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**Corresponding author; e-mail:* pkrishna@uwo.ca development. The observation that deficiency in BR results in a dwarf phenotype instigated the study of several dwarf mutants in *Arabidopsis* and other plant species, resulting in the confirmation of an essential role of BRs in plant development and in the recognition of BRs as a phytohormone (reviewed in Clouse and Sasse 1998; Li and Chory 1999; Mussig and Altmann 1999). Much remains to be learned about the signaling pathways and gene-regulating properties of BRs, although several critical components of the signaling pathway have been identified (reviewed by Clouse 2002; Peng and Li 2003).

Prior to 1996, it was noted in several preliminary studies that treatment with BRs enhances plant resistance to a variety of environmental stresses (Ikekawa and Zhao 1991; Kamuro and Takatsuto 1991). Such observations generated much interest in developing BRs for application in agriculture, but the findings typically carried attendant problems, such as poor reproducibility of BR efficacy. To date, BRs remain as compounds with much promise that has not yet come to fruition. As with the role of BRs in plant development, definitive genetic and biochemical proof of BRs' ability to modulate plant stress responses is required before BRs can be generally used in mainstream commercial agriculture. Some recent findings based on molecular and biochemical approaches have provided convincing evidence in this direction. Although the study of this aspect of BR is still in its infancy, this review brings together evidence for macro effects of BRs on plant stress tolerance and the recent progress made towards understanding the molecular basis of BR-induced effects.

PRELIMINARY INVESTIGATIONS OF BR ANTISTRESS EFFECTS

The ability of BRs to increase plant resistance against environmental stresses has been explored under laboratory, greenhouse, and field conditions. In most such studies, aspects of plant growth, such as vegetative and reproductive growth, germination, rooting, fruit setting, greening of leaves, and others, were followed in BR-treated and untreated plants under stress conditions. The results of these investigations have been summarized in several reviews and books (Clouse and Sasse 1998; Kamuro and Takatsuto 1999; Khripach and others 1999, 2000; Mandava 1988; Sasse 1999). Only a few of the studies, primarily those for which original references are available, have been cited here.

Temperature Stress

Because temperature changes are likely to occur more rapidly than other stress-causing factors in nature, temperature stress has been the research focus of many studies. Maize seedlings are highly sensitive to chilling stress during germination and the early stages of growth. Treatment with BR promoted growth recovery of maize seedlings following chilling treatment (exposure to 0–3°C for increasing number of days). BR also promoted greening of etiolated maize leaves, especially at lower temperature in light (He and others 1991). Similarly, cucumber seedlings germinated from seeds soaked in BR solution during imbibition had greater growth as compared to controls (without BR treatment) under cold conditions (5°C for 3 days). The chlorophyll content was maintained in BR-treated seedlings during the cold treatment, increasing even further during recovery from cold (Katsumi 1991). Fruit setting was higher in tomato plants sprayed with BR under winterlike conditions. This difference between treated and untreated plants was less apparent under optimal growing conditions (Kamuro and Takatsuto 1991). The growth-promoting effects of BR in rice also were obvious under low-temperature conditions but not under optimal growing conditions (Kamuro and Takatsuto 1991).

The effects of high-temperature stress in BR-treated and untreated wheat leaves were examined at the level of total protein synthesis and leaf cell ultrastructure. Protein synthesis was maintained in BR-treated leaves at 43°C at levels similar to those at 23°C, whereas in untreated leaves it decreased 2.5-fold at 43°C as compared to samples at the control temperature (Kulaeva and others 1991). During heat stress in plants, small heat shock proteins aggregate to produce highly ordered cytoplasmic complexes referred to as heat shock granules (HSGs). It is believed that HSGs represent storage and protection sites for housekeeping mRNPs, which are released following removal of stress (Nover and others 1989). Kulaeva and others (1991) observed that HSGs in wheat leaf cells aggregated in small clusters. The average number of HSGs in clusters and the average cluster size were both higher in BR-treated leaves versus untreated leaves.

Salt Stress

The effect of BR on barley leaf cell ultrastructure was examined under salt stress. Leaf segments were preincubated in either BR solution or water and then incubated in 0.5 M NaCl solution in the presence or absence of BR. BR had no effect on the leaf cell ultrastructure under normal conditions, but damage induced by salt stress on nuclei and chloroplasts was significantly reduced by BR treatment (Kulaeva and others 1991). Interestingly, germination of *Eucalyptus camaldulensis* seeds in the presence of 150 mM salt was enhanced by BR, but when seedlings were grown hydroponically in salt, uptake of BR through roots caused more damage (Sasse 1999). In another study, rice seeds soaked in water or 150 mM NaCl in the presence or absence of BR were tested for germination and seed growth. When the salt solution was supplemented with BR, the inhibitory effect of salt on germination was reduced considerably. The promotion of growth by BR under salt stress conditions was associated with enhanced levels of nucleic acids and soluble proteins (Anuradha and Rao 2001).

Drought Stress

Exposure of sugar beet plants to drought stress leads to a reduction in taproot mass in proportion to stress severity. Treatment with BR fully compensated for the reduction in biomass caused by mild drought stress. The increase in root growth in BR-treated plants versus untreated plants was seen only under water stress conditions. Increases in biomass correlated with increases in acid invertase activity in young leaves, which likely provided more assimilates to the plant due to their larger sizes (Schilling and others 1991). Applied either as seed treatment or foliar spray to drought-tolerant and drought-susceptible wheat varieties, BR had a stimulatory growth effect under stress conditions. Although subtle differences in BR effects on the two wheat varieties were noted, overall the drought-tolerant variety showed a higher response to BR application under water stress conditions. Increased water uptake and membrane stability and higher carbon dioxide and nitrogen assimilation rates in BR-treated plants under stress were correlated with BR-induced drought tolerance (Sairam 1994). In a study with cucumber plants it was also demonstrated that BR treatment improves resistance to desiccation and high-temperature stress. The content of free amino acids and amides in the leaves of treated plants was higher as compared to untreated plants (Pustovoitova and others 2001).

Aquaporins are membrane water channels that play critical roles in controlling the water content of cells (Chrispeels and Agre 1994). Measurements of the osmotic permeability of protoplasts isolated from hypocotyls of wild-type *Arabidopsis*, and of BR-deficient (*cpd*) and BR-insensitive (*bri1*) mutants indicated that BR treatment caused an increase in the osmotic permeability of protoplasts prepared from *cpd* plants. The authors extrapolate from their results that aquaporins may have a role in the BR-mediated increase in water permeability and that, because there was no ''fast'' effect of BR on the osmotic permeability of protoplasts, BR does not directly affect the plasma membrane in the process (Morillon and others 2001). Although in this study changes in osmotic permeability were correlated with hypocotyl growth, in the future BR effects on cellular water flux under stress conditions could be examined.

Pathogen Attack

The potential of BRs to enhance plant resistance against fungal pathogen infection has been investigated in several studies (reviewed in Khripach and others 1999, 2000). It is noted that in some investigations BRs proved to be more effective in their protective effects than standard fungicides. Potato plants sprayed with BR solution had less incidence of infection by *Phytophthora infestans*. The increase in resistance in BR-treated potato tubers was associated with enhancement of abscisic acid (ABA) and ethylene levels and the presence of phenolic and terpenoid substances. BR-induced disease resistance was also noted in barley and cucumber plants. In cucumber plants increased activities of peroxidase and polyphenoloxidase enzymes, which are involved in the metabolism of polyphenols, was suggested as a factor contributing to BR-induced disease resistance. The general conclusion, based on these observations, is that BRs have potential as fungicides. However, certain concentrations of BR, and application of BR at certain developmental stages of the plant, can stimulate fungal growth and disease progression. Thus, the BR concentration and the timing and method of BR application are important considerations if BRs are to be developed and used as fungicides.

A preliminary study was carried out to determine if exogenous application of 24-epibrassinolide (EBR), a BR, affected the expression of disease symptoms in tomato plants inoculated with *Verticillium dahliae*. Tomato seedlings grown for 14 days in the presence of 1 μM EBR were root inoculated with *V. dahliae* and assessed for disease symptoms 28 days after inoculation. EBR-treated plants either showed no disease symptoms or had the lowest disease scores, whereas the majority of untreated plants showed moderate to severe symptoms (Figure 1). Short-term exposure (24 hours) of seedlings to EBR prior to inoculation with *V. dahliae* did not reduce disease symptoms (Underwood, Krishna, and Dobinson, unpublished results). The resistance mechanism mediated by EBR in tomato remains to be investigated.

Recently, it has been demonstrated that treatment with brassinolide (BL), the biologically most active BR, induces resistance in tobacco plants against tobacco mosaic virus, the bacterial pathogen *Pseudomonas syringae*, and the fungal pathogen *Oidium* sp., and in rice against *Magnaporthe grisea* and *Xanthomonas oryzae* which cause rice blast and bacterial blight, respectively (Nakashita and others 2003). BL was applied to tobacco and rice plants by foliar spraying and by the soil-drench method, respectively. Plants were challenged with the pathogen 5 days after treatment with BL. BL-induced resistance in tobacco did not correlate with increase in salicylic acid levels or induction in pathogenesis-related (*PR*) gene expression, suggesting that the mechanism by which BL induces resistance is distinct from systemic acquired resistance (SAR) and wound-inducible resistance. Simultaneous induction of SAR and BL-induced resistance by treatment of plants with SAR inducers and BL, respectively, produced additive protective effects against pathogens, indicating that parallel activation of defense responses leads to this enhancement. Further studies of the molecular changes accompanying BL-induced resistance in these plant–pathogen systems should reveal the mechanism by which BL confers resistance to a broad range of diseases.

BRs have also been noted for their antiviral properties. Potato cuttings cultured in a medium containing BR were more resistant to viral infection through all stages of development (Khripach and others 2000). Furthermore, BRs and their synthetic derivatives are reported to be good inhibitors of herpes simplex virus type 1 (HSV-1) and arena virus replication in cell culture. The arena virus was susceptible to the compounds throughout its replicative cycle, and the HSV-1 was likely affected at a late step in multiplication (Wachsman and others 2000). A subset of BR derivatives also showed good antiviral activity against the measles virus in cell culture (Wachsman and others 2002).

Other Stresses

BRs are reported to be effective in reducing damage caused by pesticides and herbicides and in controlling insect development (Cutler 1991). Only a few studies appear to have been carried out in these directions; further exploration of BR effects are needed before BRs can be said to have potential as plant protectants or insecticides.

It is clear from the studies described above that the promotive effects of BRs on plant growth and yield are more obvious under stress conditions as compared to optimal growing conditions. However, these preliminary investigations have just scratched the surface of an area that needs intense exploration. The role of BRs in plant development was confirmed using genetic, molecular, and biochemical approaches. A systematic and in-depth investigation using a battery of approaches is also needed to confirm and further our knowledge of BR effects and BR mode of action under stress conditions. Few such studies have been undertaken, and the data forthcoming are beginning to provide a glimpse of the molecular changes underlying BR-mediated stress tolerance. Before discussing those studies, I review the circumstantial evidence, derived through analyses of BR-deficient and -insensitive mutants as well as of BR signaling pathway, that links BRs with plant stress responses.

CIRCUMSTANTIAL EVIDENCE LINKING BR TO PLANT DEFENSE RESPONSES

The *CPD* gene of *Arabidopsis* encodes a cytochrome P450 protein (CYP90) that shares sequence similarity with mammalian steroid hydroxylases (Szekeres and others 1996). Feeding experiments with different BRs indicate that CYP90 acts at the C-23 hydroxylation step in the BR biosynthetic pathway. Overexpression of CPD in the homozygous *cpd* mutant resulted in significant induction of the *PR* genes *PR1*, *PR2*, and *PR5*. Correspondingly, the expression of these genes in the *cpd* mutant was remarkably lower than in wild-type plants (Szekeres and others 1996). PR proteins accumulate in plants in response to infection by pathogens and are believed to contribute to the innate immunity of plants. Some PR proteins have antifungal activities; the molecular activities of others are unknown (Kitajima and Sato 1999). Although the results of Szekeres and others (1996) suggest a link between BRs and plant defense responses, it is unclear if it is the overproduction of BRs that influences *PR* gene expression or the overexpression of CYP90 itself. Pleiotropic effects of the *cpd* mutation and the activation of *PR* genes in *CPD* mRNA overexpressing lines have led to the suggestion that CYP90 itself may be involved in a subset of signaling pathways (Szekeres and others 1996). That CYP90 may have a signaling function is supported by the observation that in a yeast two-hybrid screen CYP90 interacted with a sterol-binding protein and signaling proteins with RING-finger motifs of protease inhibitors (Salchert and others 1998).

The finding that BRI1, an essential component of the BR receptor complex (Wang and others 2001), encodes a leucine-rich repeat receptor-like kinase (LRR–RLK) and is structurally similar to LRR–RLKs involved in pathogen defense (Gomez–Gomez and Boller 2000; Song and others 1995), provides yet another link between BRs and plant defense responses. BRI1 is a plasma-membrane-associated LRR–RLK consisting of an N-terminal signal peptide, a putative leucine-zipper motif, extracellular LRRs, a transmembrane domain, and a cytoplasmic protein kinase domain (Li and Chory 1997). It is proposed that BRI1 may dimerize with other LRR–RLKs through its LRRs. Such heterodimerization could provide several combinations of BR-mediated control of developmental and pathogenic signaling pathways (Koncz 1998).

Disease resistance specificity in plants is governed by specific interactions between the pathogen *avr* (avirulence) genes and the corresponding plant disease resistance (*R*) genes. The R products recognize *avr*-dependent signals and trigger signaling events that result in the induction of defense responses, leading to disease resistance (Dangl and Jones 2001). At present there is no evidence that BRI1 forms heterodimers with a *R* gene-encoded LRR–RLK that mediates recognition of fungal or bacterial pathogens; however, an LRR–RLK BAK1 has been demonstrated to interact with BRI1 and modulate BR signaling related to plant growth (Li and others 2002; Nam and Li 2002). BRI1 and BAK1 physically associate with each other in plant cells and their interaction in yeast activates their kinase activities through transphosphorylation. Based on these results it is proposed in a model that BRI1 and BAK1 exist as inactive monomers; BR binding promotes or stabilizes heterodimerization, which leads to the activation of the BRI1/BAK1 complex via transphosphorylation and the subsequent recruitment of downstream signaling components (Li and others 2002; Nam and Li 2002). The interaction of BRI1 with LRR–RLKs other than BAK1 is easily accommodated in this model. It is likely then that a specific signaling pathway is activated based on ligand binding and kinase specificity of the receptor complex that is influenced by the BRI1-interacting kinase. Experimental evidence in support of this model remains to be obtained.

Systemic wound signaling in plants, leading to the expression of defense response genes, is caused by herbivore and insect attack and also occurs in response to some pathogens. Systemin is a Solanaceae family-specific peptide of 18 amino acids that is released from the wound site, triggering a cascade of signaling events that result in the expression of defense response genes. Signaling by systemin occurs via increased synthesis of jasmonic acid (JA) (Ryan 2000). An intriguing recent finding is that the tomato systemin receptor SR160, also an LRR–RLK, is identical to the putative tomato BR receptor tBRIl (Montoya and others 2002; Scheer and Ryan 2002), implying that BR and systemin use the same receptor for signaling in tomato (Szekeres 2003). It remains to be determined how the two ligands interact with the same receptor and how signaling, leading to the activation of different cellular responses, is regulated. From what we currently know about the BR and the systemin signaling pathways, it is difficult to hypothesize how the two pathways may connect. Further research should unravel the novel and elegant mechanisms by which plants defend themselves against environmental stresses.

EBR AND THERMOTOLERANCE: A SYSTEMATIC STUDY

In a systematic study aimed at obtaining a comprehensive understanding of the mechanism by which EBR confers stress tolerance in plants, the effects of EBR were first examined on a bromegrass cell suspension culture known to develop cold and thermotolerance in response to ABA. EBR increased the freezing tolerance of bromegrass cells by only 3–5°C, but markedly enhanced cell viability following exposure to high-temperature stress (Wilen and others 1995). The study of the effects of EBR was next extended to include plant seedlings; *Brassica napus* and tomato seedlings when grown in the presence of 1 μM EBR were significantly and consistently resistant to a heat treatment that is lethal to untreated seedlings. Because a mild heat treatment prior to the usual lethal heat stress was not required to observe this effect, it is concluded that EBR treatment increases the basic thermotolerance of seedlings (Dhaubhadel and others 1999). An examination of the expression of heat shock proteins (hsps) before, during, and after heat stress revealed that hsps belonging to four major classes—hsp100, hsp90, hsp70, and low-molecular-weight hsps—accumulate to higher levels in EBR-treated *B. napus* seedlings following heat stress but not at control temperatures. A link between hsps and thermotolerance is well established (Parsell and Lindquist 1993). Thus, the higher accumulation of hsps in EBR-treated seedlings contributes, at least in part, to enhanced thermotolerance in these seedlings.

Figure 1. *Lycopersicon esculentum* cv. Craigella R-GCR218 (resistant) and S-GCR26 (susceptible) isolines grown in the absence or presence of EBR were inoculated by root dip with a spore suspension of *V. dahliae* and transplanted into pots. Symptoms of verticillium wilt, such as wilting, necrosis, and stunting, are most obvious in the susceptible (Sus) cultivar grown in the absence of EBR (EBR−) as compared to the susceptible cultivar grown in the presence of EBR (EBR+) and the resistant (Res) cultivar.

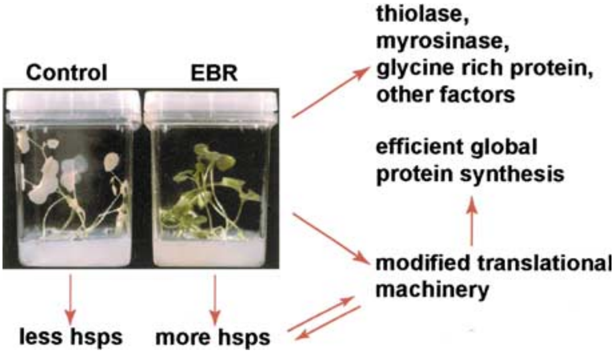

Figure 2. Summary of the molecular changes in EBR-treated *B. napus* seedlings that may correlate with increased tolerance to heat and other stresses (reproduced from Dhaubhadel and others 2002 with kind permission from Blackwell Publishing Ltd). Photograph of the seedlings taken at 7 days of recovery at 20°C following exposure to 45°C for 4 h (reproduced from Dhaubhadel and others 1999 with kind permission of Kluwer Academic Publishers).

Surprisingly, the higher level of hsps in EBR-treated seedlings did not correlate with hsp mRNA levels during the recovery period (Dhaubhadel and others 1999). Further investigation into the mechanism leading to higher accumulation of hsps in EBR-treated seedlings revealed that this is a result of higher hsp synthesis in these seedlings, even when the mRNA levels are lower than in untreated seedlings (Dhaubhadel and others 2002). Consistent with this finding, several translation initiation and elongation factors were detected at significantly higher levels in treated seedlings as compared to untreated seedlings, particularly during the recovery period. These results suggest that EBR treatment limits the loss of some of the components of the translational apparatus during a prolonged heat stress and increases the level of expression of some of the components of the translational machinery during recovery, which correlates with higher hsp synthesis during heat stress, a more rapid resumption of cellular protein synthesis following heat stress, and a higher survival rate.

Although higher levels of hsps must contribute to increased thermotolerance in EBR-treated seedlings, factors other than hsps that may directly or indirectly contribute to EBR-mediated increase in stress tolerance were searched for using differential display. Four cDNAs characterized thus far that were upregulated in treated seedlings encode 3-ketoacyl CoA thiolase, myrosinase, glycine-rich protein 22 (GRP22), and a hypothetical protein (Dhaubhadel and Krishna, unpublished). The thiolase transcript levels were higher in treated seedlings as compared to untreated seedlings during heat stress, but transcripts of the other three cDNAs were present at higher levels in treated seedlings prior to any stress. Although the glucosinolate–myrosinase system is implicated in defense against pathogens and pests (Rask and others 2000), and the GRPs are postulated to have a role in cell wall strengthening (Sanchetto–Martins and others 2000), experimental verification is required to substantiate a link between these genes and EBR-mediated stress resistance. In conclusion, it is hypothesized that a modified translational machinery coupled with increased expression of genes involved in a variety of physiological responses and other as yet unidentified factors in treated seedlings may contribute to increased overall stress tolerance in these seedlings (Figure 2). The next logical step is to examine global gene expression in *B. napus* seedlings in response to EBR treatment under both nonstress and stress conditions using DNA microarrays.

BR INTERACTIONS WITH OTHER HORMONES

Earlier analyses of the growth-promoting effects of BRs revealed that BRs interact with other plant hormones (reviewed by Khripach and others 1999; Mandava 1988). For example, the interaction of BRs with auxin produces synergistic effects, and with gibberellin (GA) the effects are additive. ABA is an antagonist of BR action, and some differences in gene regulation by these two hormones have been noted (Abraham and others 2003; Wilen and others 1995). Recent molecular studies strongly support the notion that there is cross-talk between BRs and other plant hormones, in addition to parallel hormone signaling pathways regulating the expression of common gene targets. Cross-talk between BRs and auxins, GA, ABA, ethylene, and JA includes alteration in the expression of hormone biosynthetic genes and/or signaling intermediates (Bouquin and others 2001; Friedrichsen and others 2002; Goda and others 2002; Lin and others 2003; Mussig and others 2000, 2002; Yi and others 1999). Thus, when determining molecular changes associated with BR-induced stress tolerance, it should be borne in mind that not all changes in gene expression will represent BR primary responses; it is likely that some changes will result from BR cross-talk with other hormones. Given that the roles of ABA in cold, salt, and drought stresses (Zhu 2002) and those of JA and ethylene in plant defense responses (Liechti and Farmer 2002; Wang and others 2002) are well documented, the involvement of these hormones in BR-mediated stress tolerance remains a strong possibility.

FUTURE DIRECTIONS

Results of both past and recent studies indicate that BRs have the ability to confer tolerance in plants against a wide spectrum of biotic and abiotic stresses, but the mechanisms by which BRs induce stress tolerance remain largely unexplored. Recent observations of the molecular changes accompanying BR-induced heat stress resistance suggest that complex transcriptional and translational reprogramming occurs in response to BR and stress. I hypothesize that the ability of BRs to confer tolerance against a wide range of stresses results primarily from cross-talk with other hormones. The ability of BRI1 to heterodimerize with LRR–RLKs involved in plant defense responses, resulting in several combinations of BR-mediated control of stress response signaling, also remains a strong possibility. Comparisons of genome-wide expression profiles of BR-treated plants exposed to different environmental stimuli should allow the identification of what may be termed as ‘‘general stress responses'' (genes upregulated in response to different stresses) and ‘‘specific stress responses'' (genes upregulated in response to a specific stress or related stresses). In such screening it is possible that novel stress genes as well as novel regulators of stress responses are identified. Elucidating the functions of these genes will not only shed light on how BR mediates increase in stress resistance, but it will also enhance our current understanding of the molecular mechanisms of stress tolerance. An enormous task for the future lies in the identification of signaling pathways leading to BR-mediated stress responses and in comprehending cross-talk between signaling by BRs and other hormones. The vast repertoire of genetic mutants, both hormone deficient and hormone insensitive, as well as transgenic plants overexpressing hormone biosynthetic genes, could be utilized in answering the above questions. Once the mechanism of BR action is better understood, new opportunities for agricultural biotechnology may become evident. Alongside unraveling the BR mode of action, other aspects such as uptake, transport, and stability of BRs, as well as the development of BR analogues with high activity, should continue to be explored. It is only with this combined knowledge that unique mechanisms of stress resistance can lead to implementations, with predictable effects of BR application in the field, allowing for the full potential of BRs to be harnessed in the future.

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