Mohd Tanveer Alam Khan
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Brassinosteroids Signalling

Intervention with Phytohormones and Their Relationship in Plant Adaptation to Abiotic Stresses

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Dedicated to my Parents
(MOHD AYYUB KHAN AND ANJUM AFROJ)

Preface

The book attempts to update on the state of the art of the knowledge on brassinosteroids signaling and crosstalk with phytohormones and their relationship in plant adaptation to abiotic stresses involving physiological, biochemical, and molecular processes. Due to progressively adverse environmental conditions and scarce natural resources, high-efficient crops become more important than ever. More importantly, sustainable agriculture and food security are a major concern, especially for the areas prone to abiotic stress conditions. Abiotic stress such as cold, drought, salt, and heavy metals largely influences plant development and crop productivity. It is becoming a major threat to food security due to the constant change of climate and the deterioration of the environment caused by human activity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses. Better understanding of plant responsiveness to abiotic stress will aid in both traditional and modern breeding applications towards improving stress tolerance. For successful development of stress-tolerant plants, it is important to understand precise signaling mechanisms that plants use to tolerate stresses and how much these mechanisms are induced by phytohormones. Moreover, it is debatable at which point plants could have acquired brassinosteroids (BRs) signaling from an evolutionary perspective. BRs are involved in modulating a large array of important functions throughout a plant's life cycle. BRs are considered as one of the most important plant steroidal hormones that show varied role in observing a wide range of developmental practices in plants. At cellular levels, BRs regulate cell elongation, division, and differentiation. At whole plant levels, BRs regulate male fertility, flowering time, root meristem size, and development of stomata and are involved in diverse abiotic and biotic stress responses. Exogenously applied BRs have the ability to substantially enhance plants yield and improve stress tolerance by inducing cellular changes like stimulation of nucleic acid and protein synthesis, activation of ATPase pump, antioxidant enzymes and accumulation of osmoprotectants, induce other hormone responses, regulate expression of stress-responsive genes, and improve

photosynthetic efficiency. Our grip of brassinosteroids signaling has rapidly expanded over the past two decades, due in part to the isolation of the components involved in the signal transduction pathway. The book offers a helpful guide for plant scientists and graduate students in related areas.

Chapter 1 of this book (which represents a total of 16 chapters) talks about molecular links between BR and several other signaling pathways under abiotic stress. In this chapter, we provide a summary of the highly incorporated BR signaling network and elucidate how this steroid hormone functions as a master regulator of plant growth, development, and metabolism. Chapter 2 discusses the specific role of BRs at different stages of seed germination, focuses on the signaling factors, and categorizes the signaling mechanisms. However, all the details have been provided with a special focus on proteins associated with BR. The chapter has also enlisted the BR-sensitive proteins along with their specific roles in cell physiology and metabolism. It describes the details of BR-sensitive proteins at three stages of seed germination and differentiates BR signaling into two distinct pathways. A total number of 88 protein species have been found to be BR-sensitive, for which the international identifiers and cellular activities have been described. Nitric oxide and brassinosteroids positively influence plant responses to abiotic stresses, such as temperature stress, heavy metal stress, water stress, oxidative stress, salt stress, and UV radiation, which is discussed in Chap. 3. The intent of the chapter is to explain how BRs and NO interact with each other and regulate various metabolic processes in plants and improve growth, photosynthesis, antioxidative defense system, and ROS homeostasis under normal and abiotic stress conditions. Chapter 4 provides an overview of current understanding on the signaling of BRs and H_2O_2 and their interplay in modulating plant growth and development, in particular seed germination, root growth, stomatal movement, leaf senescence, and fruit ripening, in addition to providing an overview of their interaction under diverse abiotic stress factors. More importantly, gene expression by mitogen-activated protein kinases, BZR1, BES1, SlNAC2, and other transcription factors which modulate abiotic stresses in plants have also been sectioned. In Chap. 5, we provide some insights on brassinosteroids and strigolactones signaling pathways and emphasize on recent findings on the mechanisms and networks for BR and SL-regulated gene expression and various transcriptional networks involved in the signaling pathways. Chapter 6 describes brassinosteroids (BRs) and gibberellins (GAs), which play their role to promote plant growth-related developmental processes. Recent advancements in molecular tools have now provided a better understanding of phytohormones biosynthesis, signaling, and degradation pathways. For the elaboration of signaling crosstalk between BRs and GAs, different studies have been performed with the conclusion that, to control cell elongation in *Arabidopsis*, signaling crosstalk between BRs and GAs is mediated by the interaction between BZR1/BES1 and DELLA proteins which are the transcriptional regulators from BR and GA signaling pathways. Chapter 7 examines the interrelation of ethylene and BRs during different developmental stages. It also highlights the two hormones' role

during fruit ripening, stomatal closure, reproduction, abiotic stresses, and biotic stresses. The BRs and ethylene possess an antagonistic influence on the expansin gene AtEXPA5 expression. That antagonistic interrelation is responsible for the hook formation during the gravitropic growth of hypocotyls. The ethylene and BRs crosstalk comprises a complex network of signaling pathways, e.g., the ACC synthase pathway. Chapter 8 is devoted to different groups of plant hormones (Auxin and BRs), which regulate many processes from seed germination to fruit development independently. But in recent years, several studies have revealed a common link between these two hormones in regulation of plant developmental processes. A recent advancement in molecular tools has made it possible to better understand the mechanism of signal transduction of the interaction of BRs and auxin. So, in this book chapter we discuss the physiological responses of plants induced through the interplay of BRs and auxin and its detailed mechanism of signal transduction pathway. In Chap. 9 we provide an overview of the role of BR in plant growth and development and then discuss how BRs react under different environmental stress conditions. We will also highlight how BRs function with ABA to regulate plant growth and development. At the end, we review our understanding of BRs crosstalk with ABA and elaborate its genetic basis to overcome the gap in our knowledge related to BR crosstalk with ABA. Chapter 10 inspects the interrelation of cytokinins and BRs throughout diverse developmental points. It also highlights the physiological response of plants convinced through interaction of BRs and cytokinins and its detailed mechanism of signal transduction pathway. Chapter 11 gives us an opportunity to improve the growth efficiency of plants and their adaptation under heavy metal stress through modulation in BR signaling pathway, hormone interactions, and crosstalk at organ, tissue, and cell levels to better understand how plants respond to heavy metal stress. In Chap. 12 an attempt has been made to give a comprehensive idea over the uptake, transportation, effect, and detoxification mechanism of pesticides in plants. However, BRs strengthen the plant's defense potential by stimulating the enzymatic and nonenzymatic antioxidative mechanisms which scavenge the generated ROS and activate the pesticidal detoxifying transcripts. Therefore, understanding the BRs-mediated pesticide degradation process in plants is vital for global food security. Chapter 13 specially debates the role of glyphosate and brassinosteroids applications in plants. So, this chapter offers to reveal the function of BRs in the management of glyphosate, and current research illuminates the detoxification of BR-regulated glyphosate in plants. Chapter 14 focuses on the basic information regarding distribution of important SM and in vitro strategies involved for optimal metabolite production with special reference to the use of BR as abiotic elicitor in improving metabolite yields in hairy root cultures. Chapter 15 discusses how heat stress could function in protein folding during BR action is poorly understood. This chapter focuses on the current status of our understanding about the role of BRs in protein folding under high temperature stress. In Chap. 16, we focus on representing the molecular mechanism, genes, and cascades in plants (both *Arabidopsis* and crop plants) for controlling growth-related factors. These techniques upon allocation in crops can set out

perceptible biological and cellular BR mechanism and its future application in controlling traits that can serve as a potential tool for enhancing yield and quality.

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About the Editors

Mohd Tanveer Alam Khan is a Leibniz-DAAD postdoctoral fellow at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. His main focus of research is in understanding the integrative analysis of low temperature stress defense responses in *Arabidopsis thaliana* with respect to brassinosteroids signaling and metabolite patterns. He completed his BSc, MSc, and PhD from the Department of Botany at Aligarh Muslim University, Aligarh, India. Before joining the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, he has worked as a postdoctoral fellow at National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, P.R. China. His area of research is to dissect the abiotic stress tolerance mechanism in plants through engineered signaling, proteomics, metabolomics, and biochemical traits in the presence and absence of phytohormones. During the span of eight and half years as researcher, he has published more than 21 research articles in the journal of international repute, with a total impact factor of more than 50 and 500 citations along with an h-index of 16 and also contributed two book chapter to book edition published by Springer. During his PhD and post-PhD tenure, he was awarded several research fellowships, including CST-UP-RA, SERBNPDF, and international PDF at Huazhong Agricultural University in Wuhan, China, and at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

Mohammad Yusuf is Lecturer in the Department of Biology at United Arab Emirates University, Al Ain, UAE. Mohammad received his PhD in Plant Physiology and MSc in Botany (Plant Physiology) from Aligarh Muslim University, India. He has worked as the SERB-Young Scientist and also an awardee of the prestigious Dr. D.S. Kothari Postdoctoral Fellowship from the Government of India. He has also been invited to present his work at Huazhong Agricultural University (HAU), Wuhan, China. Mohammad's research contribution includes more than 50 research

articles in the international journal of repute and also more than 2500 google scholar citations with 26 h-index. He is exploring phytohormones-mediated abiotic stress tolerance mechanism in crop plants through proline metabolism.

Fariduddin Qazi is Professor of Botany at Aligarh Muslim University, Aligarh, India, where he has been serving as faculty since 2006. He has been extensively working in the field of agricultural biotechnology to explore the abiotic stress tolerance mechanism in plants through physiological and molecular approaches. The findings of his work have revealed that brassinosteroids (BRs) and salicylic acid improved the yield and quality of plants under low temperatures, salt, water, and heavy metal stress and could be exploited as a farmer-friendly tool to overcome the menace of crop losses due to various abiotic stresses. Moreover, his findings have also revealed the potential role of hydrogen peroxide and polyamines in conferring tolerance to abiotic stresses in crop plants. His lab is extensively using proteomic approaches to reveal the novel pathway protein expressed under various abiotic stresses in plants. He had visited Göttingen University, Göttingen, Germany, for six months under BOYSCAST Fellowship and conducted experiments related to the topic "Molecular studies of salt tolerance in *Arabidopsis thaliana*." He has visited Michigan State University, Michigan, USA, on an International Research Project with a specific objective to generate information on "Host target modification as a strategy to counter pathogen hijacking of the jasmonate hormone receptor" (Published in PNAS, 2015). He has published more than 80 research papers in the international journal of high impact factor such as *Proceedings of the National Academy of Sciences*, USA, *Food Chemistry*, *Plant Physiology and Biochemistry*, *Chemosphere*, *Journal of Integrative Plant Biology*, *Environmental and Experimental Botany*, *Ecotoxicology and Environmental Safety*, and many more with a total citation of 4925 and an h-index of 32. He had presented his findings in various conferences held in the USA, Germany, China, Malaysia, etc. He has successfully completed various funded research projects from reputed funding agencies. He has also supervised six doctoral students and three MPhil students and a number of master's students, and presently five students are enrolled under his supervision for PhD degrees.

Aqeel Ahmad is a young and energetic researcher, working in the field of stress physiology. He earned his doctoral degree (PhD) from the University of the Punjab, Lahore, Pakistan, and performed his research work at the University of Florida, USA. Then, he joined Huazhong Agricultural University as a Postdoctoral Fellow, and now he is serving Guangdong Academy of Agricultural Sciences as Postdoctoral Fellow. He extensively studied plant defense responses (i.e., defense genes, hormones, PR proteins, and biochemicals) against the plant pathogens. His core skills are proteomics (profiling, characterization, and supramolecular kinetics) and metabolomics, by using which he elucidated cell signaling to understand plant defenses, based on oxidative enzymes of the phenylpropanoid pathway. Transcriptomics further strengthened his scientific findings concerning cell responses towards pathogenic invasions and abiotic stresses. He has devised

bioactive metabolites (e.g., benzimidazole and benzenedicarboxylic acid) to control plant pathogens and to augment the nutritional quality of our plant-based foods. He has developed techniques to make edible plants tolerant against environmental stressors by reharmonizing their osmoregulatory systems, oxidative machinery, and physiological responses. He has published 59 research manuscripts in world-renowned journals and won three research grants and one research honor award at such a young age of 32 years. A wide spectrum of publication platforms is evident from his scientific articles including the leading journals of *Food Chemistry*, *Chemosphere*, etc. His editorial activities in multiple Impact Factor journals have made him a distinctive and progressive figure in the researchers' pool.

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Chapter 1
Signal Transduction of Brassinosteroids Under Abiotic Stresses

Mohd Tanveer Alam Khan, Mohammad Yusuf, Waheed Akram, and Fariduddin Qazi

Abstract Plants live in regularly fluctuating surroundings that are critical for progression and enlargement. Divergent environmental circumstances comprise biotic and abiotic stress. The opposing things of abiotic indications are impaired by environmental variation, which has been forecast to outcome in an improved rate of dangerous climate. However, brassinosteroids (BRs), a unique polyhydroxy steroidal hormones in plants and capable for endogenous signals for the directive of plant growth and enlargement. It plays an imperative function in plant like seed sprouting, flowering and elongation of hypocotyl, etc. Moreover, BRs have capability to ameliorate the numerous abiotic difficulties like metal stress, temperature stress, water stress, oxidative damage, and salt injury. Furthermore, BR signaling is transduced by a receptor kinase-mediated signal transduction pathway, which is distinct from animal steroid signaling systems. Newest studies entirely associated with the signal pathway of BR have recognized numerous BR marker genes, associating with BR signaling to several cellular practices. This chapter summarizes the BR signaling system in wide detail and discusses how steroid hormone plays a key role in controlling plant growth, size, and metabolism.

Keywords Abiotic stress · Brassinosteroid · Signaling · Target genes

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Introduction

Plants live in regularly fluctuating surroundings that are critical for progression and enlargement. These opposing environmental circumstances comprise biotic and abiotic stress. The opposing things of abiotic practices are impaired by environmental alteration, which has been forecast to outcome in an improved rate of risky climate (Fedoroff et al., 2010). Plants acclimate to opposing environments through stress signals acting as biological queries. Plant stress encounter is dangerous for farming and environmental sustainability due to the excessive consumption of water and manure resources to load the environment. However, plant growth regulators recover over all plant development and productivity (He & Zhu, 2008; Khan et al., 2019). Wang et al. (2005) revealed that environmental stresses influence the endogenous concentration of many phytohormones, as a result alter numerous signaling pathways. These modifications cause severe metabolic complaints most important to embarrassment of overall plant growth performance in stress environments (Lerner & Amzallag, 1994). A decent strategy to overcome abiotic stresses is the exogenous use (either through the seed or soil management) of PGRs (Ashraf et al., 2008). Brassinosteroids (BRs) show dynamic roles in improving growth and enlargement of plants and can upgrade the opposing things of numerous abiotic stresses in a varied range of plant species (Fariduddin et al., 2011; Jiang et al., 2013; Khan et al., 2015, 2019; Nazir et al., 2021). In this chapter, we deliver the summary of latest improvements in revealing the signaling trails for BRs under abiotic stresses. Furthermore, this chapter emphasizes on the possible mechanisms to decipher the molecular and biochemical levels of BR signaling linked to upstream sensing and to downstream alterations in gene expression, metabolic rate, physiology, growth, and expansion.

Physiological Roles of Brassinosteroids

Brassinosteroids are the steroidal growth controllers related to plant easiness. These entities show essential roles in many biological practices like cell division, cell elongation, xylem disparity, initiation of stem elongation, proton pump activation, leaf epinasty, tissue disparity, morphogenesis, pollen tube progression, and photosynthesis (Clouse & Sasse, 1998; Xia et al., 2009; Clouse, 2011). BRs have been used to upgrade the adversarial response of plants contrary to various stresses such as metal stress (Yusuf et al., 2011), cold stress (Fariduddin et al., 2011), salinity stress (Deng et al., 2012), and oxidative impairment (Cao et al., 2005). The foliar practice of BRs can upregulate the manifestation of stress connected genes, resultant stimulation of antioxidant enzymes, proline, repairs of photosynthesis activity, and some other favorable retorts (Divi & Krishna, 2009; Fariduddin et al., 2015; Khan et al., 2015, 2019; Nazir et al., 2020).

Effect of Brassinosteroids on Seed Germination

Numerous studies have provided that BRs promote seed sprouting. It has been renowned that BRs encourage seed propagation in tobacco (Leubner-Metzger, 2001), wheat (Hayat & Ahmad, 2003), tomato (Ahammed et al., 2012), *Brassica juncea* (Sirhindi et al., 2009), and *Arachis hypogaea* (Vardhini & Rao, 1997). BRs stimulated the sprouting of pre-chilled seeds of BRs-lacking biosynthesis *det2-1* mutant and the BRs-unresponsive reply mutant *bri1-1* exposed to light in *Arabidopsis thaliana* (Zhang et al., 2009). Seed germination of *det2-1* mutant and *bri1-1* is further powerfully repressed by ABA associated with their wild type. Further, pre-treatment with BL encouraged growth and sprout appearance of old rice grains. Hayat and Ahmad (2003) reported that seeds soaked in BRs had increased activity of α-amylase in *Lens culinaris*. In *Arabidopsis*, BR-signal reversed the ABA-convinced dormancy, therefore encouraging the sprouting (Steber & McCourt, 2001). BRs promoted the break of endosperm in tobacco in dose dependent method (Leubner-Metzger, 2001).

Effect of Brassinosteroids on Growth

BRs have imperative character in plant developmental courses comprising cell division, cell elongation, pollen tube progression, xylem disparity, proton pump activation, initiation of stem elongation, leaf epinasty, tissue disparity, morphogenesis, and photosynthesis (Xia et al., 2009; Clouse, 2011; Gudesblat & Russinova, 2011). Mussig et al. (2003) have reported that BRs deficient mutants of *Arabidopsis* showed increased root elongation after exogenous applications of BRs and auxins. Sun et al. (2010) revealed that improved plant growth could be recognized to the BRs skill to control cell growth and central events over the upregulation of xyloglucan endo-transglycosylase. It has also been stated that BRs improved the growth of *Raphanus sativus* seedlings (Choudhary et al., 2012).

Brassinosteroids and Plant Abiotic Stress Tolerance

Various researches over the years have indicated the active involvement of BRs in plants when showing to different abiotic practices such as low temperature (Khan et al., 2015, 2019), high temperature, and chilling stresses (Janeczko et al., 2009, 2011). Some previous studies highlight the status of BRs and associated composites in diverse plants under drought (Mahesh et al., 2013), light (Li et al., 2012a), salinity (Abbas et al., 2013), heavy metal (Yusuf et al, 2011), submerging (Liang & liang, 2009), herbicide (Sharma et al., 2013a). Therefore, recent reports regarding the role of BRs in the modulation of abiotic stresses in plants are appraised in Table 1.1.

Table 1.1 Effect of brassinosteroids and abiotic stress tolerance in plants

BR analogues	Abiotic stress	Plant species	Responses	References
BRs (EBL or HBL)	Cd	*Raphanus sativus*	Activated antioxidant enzymes like catalase, superoxide dismutase, peroxidase, and glutathione in the plantlets treated by cd and BRs	Anuradha and Rao (2007)
BRs (EBL/HBL)	Low temperature	*Lycopersicon esculentum*	BRs facilitated enhancement in photosynthetic machinery and proline content	Khan et al. (2015)
BRs (EBL/HBL)	Cd	*Lycopersicon esculentum*	BRs mediated upgradation in stomatal conductance, transpiration rate, proline accumulation, and antioxidant system	Hasan et al. (2011)
BR	Drought	*Glycine max*	Raised the activities of POX and SOD, augmented the concentration of soluble sugars and proline that eventually caused reduced MDA concentration and electrical conductivity	Zhang et al. (2008)
EBL/HBL	Water stress	*Raphanus sativus*	Mediated a decline in the deleterious outcome of water stress on seed development and sprout progression by enhancing the antioxidant and free proline	Mahesh et al. (2013)
EBL	Mn	*Brassica juncea*	Enriched growth, water relations, and photosynthesis and improved several antioxidant enzymes like CAT, POX, and SOD and proline	Fariduddin et al. (2015)
EBL	Salinity	*Cucumis sativus*	Better seedlings growth as outcome upgraded activities of several antioxidant enzymes	Lu and Yang (2013)
EBL	Drought	*Chorispora bungeana*	Deliberated tolerance to drought-stress by reducing the lipid peroxidation, membrane permeability as consequence of augmented antioxidant enzymes and non-enzymatic antioxidants like ascorbate and GSH	Li et al. (2012b)
EBL	Cd	*Brassica napus*	EBL reduced the lethal result of cadmium on photochemical practices by falling injury of photochemical reaction centers also O_2 developing centers as well as retaining effective photosynthetic electron transport	Janeczko et al. (2005)
EBL	Cd	*Raphanus sativus*	EBL minimized the harmful role of cd on plant growth,	Anuradha and Rao (2007)

(continued)

Table 1.1 (continued)

BR analogues	Abiotic stress	Plant species	Responses	References
			photosynthesis related attributes, and enzymes activity	
HBL	Cu	*Vigna radiata*	Improved photosynthetic associated traits and carbonic anhydrase activity	Fariduddin et al. (2014)
EBL	Ni	*Raphanus sativus*	Elevated activities of antioxidant that ultimately caused in dropping lipid peroxidation. Greater proline and protein contents, and upgraded the overall plant growth	Sharma et al. (2011)
EBL	Co	*Brassica juncea*	EBL improved the stress created by co and suggestively improved the activities of antioxidant enzymes	Arora et al. (2012)
EBL	Zn	*Brassica juncea*	Augmented activities of superoxide dismutase, catalase, ascorbate peroxidase, MDHAR, DHAR, and the GSH contents	Arora et al. (2010)
EBL	Pb	*Raphanus sativus*	Decreased Pb harmfulness and improved overall plant growth and activities of antioxidant enzymes and reducing peroxidase	Anuradha and Rao (2007)
HBL	B	*Vigna radiata*	Upgraded the growth, water relationships, net photosynthesis, stomatal conductance, and transpiration rate by improving antioxidant enzymes and level of proline	Yusuf et al. (2011)
HBL	Zn	*Raphanus sativus*	Conferred tolerance to Zn harmfulness by improving antioxidant enzymes, establishment of GSH metabolic rate and redox grade, and enlightening the contents of non-enzymatic antioxidants	Ramakrishna and Rao (2013)
BR	High temperature	*Oryza sativa*	Displayed significant improvement in the expression of POX and SOD; decreased level of MDA and electrolytes leakage	Cao and Zhao (2007)
EBL	High temperature	*Lycopersicon esculentum*	Significantly improved high temperature convinced reduction of photosynthesis via improving the antioxidant enzymes and decreasing H_2O_2 and MDA contents	Ogweno et al. (2008)
HBL	Chilling	*Cucumis sativus*	Improved growth and photosynthesis by improving proline content	Fariduddin et al. (2011)

(continued)

Table 1.1 (continued)

BR analogues	Abiotic stress	Plant species	Responses	References
BR	Cold	*Cucumis sativus*	Protected photosynthetic related cold convinced harm by triggering the enzymes of Calvin cycle and improving the antioxidant capacity, alleviated the influence of photo-oxidative stress and impairment	Jiang et al. (2013)
EBL	Low temperature	*Brassica juncea*	Improved the lethal consequence of H_2O_2 through improving the activities of several enzymes involved in antioxidant defense systems such as CAT, APX, and SOD	Kumar et al. (2010)
EBL	Low temperature	*Vitis vinifera*	Improved antioxidant defense and osmoregulation	Xi et al. (2013)
EBL	Cd	*Phaseolus vulgaris*	Mediated improved activity of antioxidant enzymes, proline content, and later enhancement in the membrane stability index and relative water content	Rady (2011)
EBL	Ni	*Brassica juncea*	Ameliorated Ni-stress by improving the movement of antioxidant enzymes	Kanwar et al. (2013)
EBL	Cu and NaCl	*Cucumis sativus*	Greater the actions of several antioxidant enzymes such as CAT, POX, SOD that ultimately enhanced growth, nitrate reductase activity, and photosynthetic efficacy	Fariduddin et al. (2013)
EBL	Salinity	*Oryza sativa*	Displayed enhancement in growth, levels of protein, proline contents, and activities of antioxidant enzymes over the expression of several BRs and salt responsive genes	Sharma et al. (2013b)

Brassinosteroids and Low Temperature Stress

BRs have been successfully used to make plants resistant contrary to cold stress. BRs could be exogenously functional either by seed soaked, root dipping, and foliar application. However, foliar spray and seed soaking methods have been generally adopted. Janeczko et al. (2009) stated that application of EBL earlier to cold stress minimized the ion leakage in freezing showing rape plants, while it improved the antioxidant system and proline in freezing worried young grapevines (Xi et al., 2013). The characters of BRs in cold stress are concise in Table 1.2.

Table 1.2 Effect of brassinosteroids and abiotic stress tolerance in plants

BR analogues	Abiotic stress	Plant species	Responses	References
HBL	Chilling	*Cucumis sativus*	Improved the growth photosynthesis and water relation by improving antioxidant enzymes such as CAT, POX, and SOD	Fariduddin et al. (2011)
BR	Cold	*Cucumis sativus*	Protected the photosynthetic tool from cold convinced impairment by triggering the enzymes of Calvin cycle and improving the antioxidant ability	Jiang et al. (2013)
BL	Chilling	Maize	Improved the growth and rescue of seedlings after freezing treatment	He et al. (1991)
EBL	Low temperature	*Brassica juncea*	Improved the lethal outcome of H_2O_2 over improving the accomplishments of several enzymes intricate in antioxidant defense arrangement such as CAT, APX, and SOD	Kumar et al. (2010)
EBL	Low temperature	*Vitis vinifera*	Augmented antioxidant system and osmoregulation	Xi et al. (2013)
BL	Chilling	*Solanum lycopersicum*	Inhibited the events of phospholipase D and lipoxygenase in fruits, subjected to chilling stress	Aghdam and Mohammadkhani (2014)
BL	Chilling	*Campsicum annum*	Effectively reduced freezing damage of *Campsicum annum* fruit put in storing on 3 °C for longer duration via decreasing the ion leakage, MDA content; aggregate the activities of antioxidant enzymes like CAT, POX, APX, and GR	Wang et al. (2012b)
EBL	Chilling	*Cucumis sativus*	Improved the chilling-convinced embarrassment of photosynthesis in *Cucumis sativus* by minimizing ROS generation and accumulation over increased activities of antioxidants	Hu et al. (2010)
EBL	Chilling	*Chorispora bungeana*	Alleviated chilling-prompted oxidative injury over the antioxidant defense mechanism and decreased the intensities of ROS as well as lipid peroxidation, thereby improved the freezing tolerance	Liu et al. (2009)

(continued)

Table 1.2 (continued)

BR analogues	Abiotic stress	Plant species	Responses	References
BRs	Cold	*Brassica napus*	BR perception earlier to cold action reduced the leakage of ion in chilling showing rape leaves in plants	Janeczko et al. (2005)
EBL	Chilling	*Solanum melongena*	Chilling injury, triggered an upsurge MDA, total phenolic contents and ion leakage that were declined by EBL	Gao et al. (2016)
BRs	Chilling	*Solanum lycopersicum*	Reduced chilling injury in fruits kept at 1 °C for 21 days by dropping the electrolyte leakage, MDA content, improved proline, total phenol contents, PAL activity, and retained membrane reliability	Aghdam et al. (2012)
BRs	Chilling injury	*Citrus sinensis*	Induced cold tolerance through encouragement of antioxidant enzymes and similarly providing defense contrary to the oxidative injury of membrane; diminished lipid peroxidation and H_2O_2 content in fruits	Ghorbani and Pakkish (2014)
EBL/ HBL	Low temperature	*Lycopersicon esculentum*	Improved growth, photosystem II, leaf water potential, stomatal conductance, transpiration rate, proline content and yield	Khan et al. (2015, 2019)

Brassinosteroids and Crop Yield

BRs have also played a key role to improve yields by modulating plant metabolic rate and protection against environmental stresses. Application of BRs significantly improved yield of *Lens culinaris* (Hayat & Ahmad, 2003), *Brassica juncea*, *Oryza sativa*, cotton and potato (Ramraj et al., 1997), watermelon, cucumber, corn, grape, and tobacco (Ikekawa & Zhao, 1991), and mung (Fariduddin et al., 2006). Additionally, Hayat et al. (2012) informed that BRs have been used for the improvement of yield of several other plant species. Foliar application of BL upgraded the crop yield in mustard and wheat (Braun & Wild, 1984). However, Schilling et al. (1991) conveyed that BRs initiate to upsurge the growth development and yield of sugar beet and *Brassica* seed. BR referents which are biologically active, constant when integrated in plant fleshy tissue, could improve the amount and quality of varied varieties of crop plants. Moreover, modifying endogenous BR activity by direct manupulation of genes involved in either BR biosynthesis or signaling could have way for much improved crop yield.

Signaling Pathway of Brassinosteroids

BR signaling has been widely considered at both molecular and biochemical intensities in plants. It has been exposed in *Arabidopsis thaliana* that BR signaling initiated from ligand perception going on the cell membrane to gene appearance in the nucleus. However, Li and Chory (1997) revealed that BR impasse to plasma membrane attached BRASSINOSTEROID INSENSITIVE1 (BRI1), a leucine rich repeat (LRR) receptor-like kinase (RLK) receptor, to provoke signaling cascade modulating the appearance of genes over cytosolic and nuclear transcription kinases and phosphatases (Kim & Wang, 2010; Wang et al., 2012a). Moreover, Nam and Li (2002) and Wang and Chory (2006) specified that perception of BRs, BRI1, quickly discharges BRI1 KINASE INHIBITOR1 (BKI1), a harmful controller at the C-terminal domain of BRI1 and triggers its kinase action by several autophosphorylations and consecutive transphosphorylation of BRI1 with BAK1. This insulated BKI1 augments BRs signaling by mortifying 14-3-3 proteins, accountable for the cytoplasmic custody of two master transcription factors (TFs), such as BRASSINAZOLE RESISTANT 1 (BZR1) and BRI1-EMS SUPPRESSOR 1 (BES1) of BRs signaling (Yin, 2002; Ryu et al., 2010; Jaillais et al., 2011; Choudhary et al., 2012). Moreover, phosphorylation of BSKs (BRs signaling kinases) activated BRI1, subsequently activated the BRI1 SUPPRESSOR 1 (BSU1) phosphatase. The triggered BSU1 in try to neutralizes BRASSINOSTEROID INSENSITIVE 2 (BIN2) through dephosphorylation (Tang et al., 2008; Clouse, 2011; Kim & Wang, 2010). The inactivation of BIN2 releases its suppression of the master TFs; BZR1 and BES1. The triggered BZR1 and BES1 transport into the nucleus to control BR-associated gene expression willingly or via collaboration with additional TFs (Yan et al., 2009; Luo et al., 2010).

In the absence of BRs, BRI1 rests in inactive state by the company of BKI1 (Gudesblat and Choudhary et al., 2012). BIN2, a GSK3 kinase repressor protein which is existent in nucleus, cytoplasm, and plasma membrane, phosphorylates two nuclear transcription factors, BZR1 (brassinazole-resistant 1) and BZR2/BES1 (bri1-EMS suppressor 1), in that way stops their activities. Consequently, Vert and Chory (2006) revealed that BZR1 and BZR2/BES1 link with other proteins or TF is repressed creating them non-functional TF (Vert & Chory, 2006). The scheme of BR signaling is shown in Fig. 1.1.

Target Genes of BR Signaling

Genome-wide protein–DNA interaction analyses combined with expression profiling have identified several thousand in vivo binding targets of BZR1, including more than thousand BR-controlled BZR1 target genes (Sun et al., 2010). However, Gudesblat and Russinova (2011) testified that a smaller set of targets, which operlaps significantly with the BZR1 target, has been identified for BZR2. These

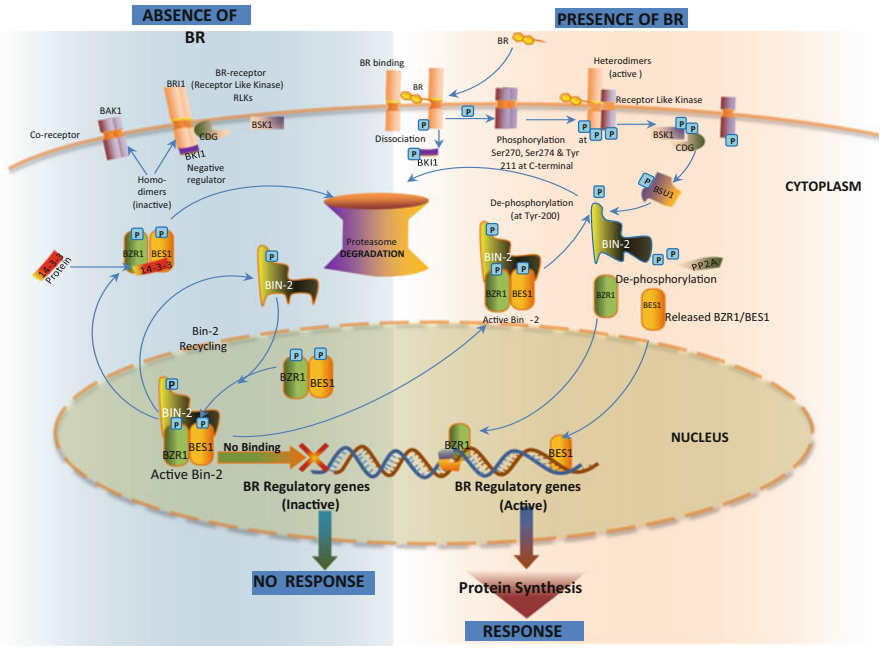

Fig. 1.1 Effect of brassinosteroids and abiotic stress tolerance in plants. (Yan et al., 2009; Luo et al., 2010; Ryu et al., 2010; Jaillais et al., 2011; Choudhary et al., 2012)

BZR1/BZR2 mark genes showed many molecular associates related to cellular, developmental, and metabolic practices. In specific, cell partition amendment and cellular transportation remain the main cellular utilities directed by BR, constant with its belongings on cell elongation and development (Sun et al., 2010). Extraordinarily, Wolf et al. (2012) indicated that negotiated cell partition reliability triggers BR signaling, signifying a response mechanism for the BR-facilitated stability among cell extension and reliability of the cell partition. The TF and mechanisms of several other signaling trails, like light, gibberellin (GA), and auxin pathways are similarly extremely symbolized in the BZR1 targets (Sun et al., 2010).

Particularly, He et al. (2005) and Yu et al. (2011) informed that BZR1 and BZR2 can affect the appearance of genes encrypting BR biosynthetic enzymes and upstream BR signaling modules. Furthermore, Wu et al. (2011) reported that BR encourages the expression of the SUPPRESSOR OF BRI1 (SBI1) leucine carboxylmethyltransferase, which methylates PP2A and stimulates PP2A localization to membranes, where it dephosphorylates and deactivates the suppressed BRI1, providing alternative mechanism of feedback regulation.

BZR1 and BZR2 mediate the expression levels of BR-responsive gene expression along with the additional interactions with TF. In adding to BIM1 (Yin et al., 2005), BZR2 interrelates with the TF MYB30 (Li et al., 2009), INTERACTS WITH SPT6 1 (IWS1) (Li et al., 2010), EARLY FLOWERING6 (ELF6), and the histone H3 lysine 27 demethylase RELATIVE OF ELF6 (REF6) (Yu et al., 2008; Lu et al.,

2011). However, genetic and transgenic trials showed that BZR2-interrelating proteins have negligible role in BR-controlled growth retorts like elongation of hypocotyl, whether they intermingle with BZR1 remains unidentified. Together BZR1 and BZR2 interrelate with the phytochrome-interacting factor and the GA signaling DELLA proteins to coregulate the appearance of enormous quantity of genes, cell elongation, and photomorphogenesis (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Oh et al., 2012).

Conclusion

This chapter discusses the signal transduction of BRs under abiotic stress. Numerous studies indicate that abiotic practices can ground molecular responses in plant soft tissue. However, BRs have been used to improve crop production by modulating plant metabolic rate and defending plants from environmental cues, and plentiful proof nowadays backings the awareness that altering the BR retort trail can be a great approach for scheming enhanced-reformed crops. Though our accepting of the key purposes of BR signaling through stress is simply nonspecific, and the exploration of exact spatiotemporal and context-specific controlling appliances has only unbiased originated. Further research are needed to get extra systematic appreciative of the universal and confined schedules of the BR pathway. This understanding will help to increase both the growth amounts of plants and their reworking to the environment by only shifting the hydrotropism that is independent of the BRI1 pathway, signifying that the retort to diverse stresses might be focused by BR receptors in particular cell types, like stem cells and vascular tissues. The credentials of these BR receptor-driven variance signals will further demonstrate how altered tissues manage their tissue growth and can be useful for engineering new plants. Lastly, we courage that this chapter has not only delivered novel awareness into plants stress retort mechanisms, which are essentially designed for persistent improvement of genetically engineered stress-tolerant crop plants, nevertheless also has emphasized the consequence of learning modifications in BRs signaling in reply to abiotic stress.

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Chapter 2
Plant Proteomics and Metabolomics Investigations in Regulation of Brassinosteroid

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Abstract Brassinosteroids (BRs) are plant-specific, intrinsically steroidal, and key hormones synthesized by the plant cells. The hormone mediates plant growth and development events, right from the decisive event of seed germination. Proteins are also the functional factors of a cell, which respond and regulate almost all physiological processes. The chapter discusses the specific role of BRs at different stages of seed germination, concentrates the signaling factors, and categorizes the signaling mechanisms. However, all the details have been provided with a special focus on proteins associated with BR. The chapter has also enlisted the BR-sensitive proteins along with their specific roles in cell physiology and metabolism. It describes the details of BR-sensitive proteins at three stages of seed germination and differentiates BR signaling into two distinct pathways. A total of 88 protein species have been found BR-sensitive, for which the international identifiers and cellular activities have been described. Although there are many gaps in understanding the BR responses and the mechanisms behind them, the current article would be helpful to understand the behavior of the hormone and the dimensions of its cellular responses.

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Introduction

Brassinosteroids (BRs) belong to a group of polyhydroxylated steroidal hormones specifically related to plants. They play a pivotal role in the development and growth of the plant species, e.g., elongation of plant cells, germination of seeds, and photomorphogenesis.(Wu et al., 2008) Any abnormality in BR contents leads to deformation in the plants. However, several plant species have been reported for producing BR deficient or insensitive mutants. Major examples of such plants are rice, maize, *Arabidopsis*, pea, tomato, and barley (Wang & Mao, 2014; Hasan et al., 2015). BR deficient plants of these species show several development and growth defects, among which dark green leaves, dwarfism, delayed flowering, photomorphogenesis in the dark, and male sterility are the most commonly found issues. Numerous investigations have been conducted on the proteomic, genetic, and molecular basis by taking Arabidopsis as a model plant. Now, the BR signaling pathway is one of the best-understood signal transduction pathways in plants (Wang et al., 2012).

Types of Brassinosteroid Signaling

Based on the mechanism involved BR signaling is divided into two different types:

1. Genomic signaling.
2. Non-genomic signaling

Genomic Signaling

Cognate nuclear steroid receptors are involved in the perception of genomic signaling of steroid hormones by complementary binding process. The steroid receptors are located in the cytoplasm of the cell. Upon successful binding, the receptor complex is translocated into the cell nucleus to activate the respective genomic cascade. It results in the activation or repression of a specific set of genes sensitive toward the hormone (Beato et al., 1995). This complete process is termed as genomic signaling of brassinosteroid due to the involvement of genes in generating physiological outcomes.

Non-genomic Signaling

Sometimes, the plant cells are densely occupied by the proteins or transcriptional inhibitors, which prevent the genes from being overexpressed. In that case, a non-genomic signaling mechanism is imperative for steroidal hormones, including BR. BRs have been reported for effective elicitation of cellular responses during the heavy biosynthesis of proteins. The mechanism adopted by BR is termed as non-genomic signaling of the hormone due to the absence of genetic instructions. It includes direct involvement of the nuclear receptors for BR and many other biochemical signaling mediators.

Branching of Non-genomic Signaling

Non-genomic signaling is not a strict but somewhat complicated pathway of biochemically interacting factors in a cell, adopted by all types of plant cells. A cell can change the downstream physiological responses based on the physical stimuli, type of stress, and environmental factors. However, the type of the cell, nature of the stimuli, and environmental factors determine the alterations adopted by the cells in the signaling pathway. These alterations in the pathway can be observed in second messenger levels, which mean the differences in ion fluxes and protein kinase activities. These alterations are mainly involved by the proteins which are not characterized yet, steroid carrier proteins and receptors located in plasma membrane (Falkenstein et al., 2000; Shafique et al., 2014a). Another factor responsible for the altered downstream outcomes is the absence of specialized receptors for steroid molecules (or close homologs of the receptors) in the plant cells. It has been proved after annotating the complete genome sequence of *Arabidopsis* (Analysis of the genome sequence of the flowering plant Arabidopsis thaliana, 2000). However, organisms other than plants do not possess that many complicated BR signaling pathways due to specialized receptors (or close homologs nuclear steroid receptors) (McCarty & Chory, 2000). Because the non-genomic signaling of BR has not been studied in detail and there are many more things hidden than have been revealed by scientific research. Therefore, there is also a strong chance of an alternative mechanism (other than the genomic signaling and non-genomic signaling of BR) in the plant cells (Fig. 2.1).

Proteins in Brassinosteroid Signaling

Brassinosteroid signaling happens through receptor-like kinases (RLK), brassinosteroid insensitive 1 (BRI1) present in leucine-rich repeats (LRR) of transmembrane proteins. Adhesion of brassinosteroids induces a protoplasmic stimulus

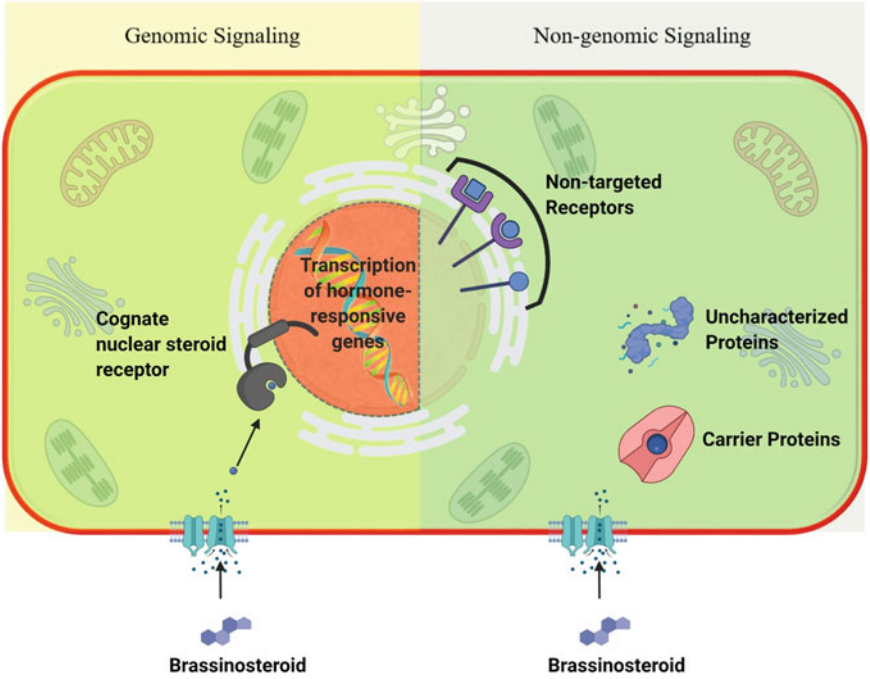

Fig. 2.1 Schematic representation of genomic and non-genomic signaling of brassinosteroids

of homodimerization and triggers intracellular phosphorylation to the inside kinase domain. BRI1 initiates a series of events comprising phosphorylation and dephosphorylation in a signal transduction cascade (He et al., 2002; Yin et al., 2002). Ultimately the prime inverse regulator of brassinosteroids pathway BIN2 is inactivated inhibiting the signal transmission but dephosphorylating the two transcriptional factors (TFs) BES1 and BZR1. The phosphorylated/activated TFs undergo translocation into the nucleus to get self-assembled with promoter E-box and BRRE elements (He et al., 2005; Yin et al., 2005; Yu et al., 2011; Ahmad et al., 2014a).

The kinase protein perceiving the BR stimulus and initiating the chain of signaling events had been studied for a number of recessive alleles (Shah et al., 2001). The findings are helpful to achieve targeted lengths of plant roots (hypocotyl elongation) and enlarged plant vigor using BR1 (Ibrahim et al., 2017). The complete process of BR signaling consists of three major domains of BRI1 protein, each playing a unique function in the perception/detection of BR and receptor-mediated activation of the downstream process. All these three domains are (i) a small domain (transmembrane), (ii) a large domain (extracellular), and (iii) a kinase domain (intracellular). An amino (N)-terminal signal peptide is found on the extracellular domain of BRI1, which 24 LRRs accompany, and a leucine-zipper motif. It also consists of an island

domain that is located between the 20th and 21st LRRs. The leucine-zipper motif is important for BRI1 along with the signal peptide.

Both of these factors are targeted to the plasma membrane for possible dimerization. It is also an idea that the LRRs may function for protein–protein interaction in the plant cells (Khan et al., 2018; Zaheer et al., 2017). Further studies related to the extracellular domain of BRI1 have concluded the involvement of at least 70-amino-acid island domain and its carboxyl (C)-terminal flanking LRR21 region as a BR-binding region. All this scheme constructs a novel binding element for steroid protein (Ahmad et al., 2014a, 2021a). Further categorization of the intracellular domain can divide it into three parts: (i) a juxtamembrane region (JM) that is small and intracellular, (ii) a kinase that acts as a catalytic domain, and (iii) a C-terminal tail. The JM domain performs the signal transduction from outside of the cell to inside. A similar pattern has been reflected in the plants lacking this region, with the overexpression of a BRI1. Such types of mutants show a dwarf phenotype of bri1-5. This type of mutant is called BR-perception mutant, and it successfully performs in vitro autophosphorylation activity along with the unaffected subcellular localization (Nazir et al., 2021). It has also been reported any mutation in the kinase domain leaves the entire receptor dead. It reveals the importance of the kinase catalytic domain of BRI1 for the death of the receptor. It means that the kinase catalytic domain is essential for the basal activity of the BRI1 (Khan et al., 2019). Recent studies have given importance to the Ser/Thr phosphorylation sites and studied them in the catalytic domain that had already been proven critical for BR signaling. The results highlighted the domain T1049, S1044, and T1045 as Ser/Thr phosphorylation sites (Yusuf et al., 2016; Khan et al., 2017a; Kataya et al., 2015).

The introduction of high throughput technologies in the elucidation of metabolic pathways has significantly broadened our knowledge about brassinosteroid signaling in Arabidopsis. Moreover, the regulatory mechanisms of BRs to influence other physiological and developmental processes of cells (e.g., cell elongation) have been discovered in detail (Wang et al., 2014a). BRs are responsible for the most complex process of plant development, seed germination. During this entire seed germination process, a quick, efficient, and precise signaling is conducted due to the intermediate protein regulated by BRs. BR has also the potential to minimize the germination inhibitory effects of abscisic acid (ABA) (Zhang et al., 2009a; Leubner-Metzger, 2001). All the scientific investigations have developed direct interrelations between BR and key genes of seed development. However, the molecular kinetics involved in these interactions is still unknown. Here, the known proteomic facts have been organized to produce a clear picture of proteins influenced by BR and playing their roles at different seed developmental stages.

Developmental Stages of the Seed

Generally, the seed developmental process consists of three distinguished stages.

First Stage of Seed Development

The rapid uptake of the available water accompanied by the onset of mRNA biosynthesis is a characteristic feature of the first stage of seed development (Howell et al., 2009).

Second Stage of Seed Development

The second stage is a compound stage of different germination events happening quickly and sometimes partially overlapping each other. The germination events include mobilization of reserves, reactivation of metabolism, cell wall loosening, coleoptile elongation, and repair of cell structure.

Third Stage of Seed Development

The third stage is cell division, radical protrusion, aerobic respiration, TCA recovery, and seedling formation, hence needs quick uptake of water (Howell et al., 2009; He & Yang, 2013). The embryo is the epicenter of genetic information to control all the developmental events in a plant's life. The seeds containing embryos are worth studied to understand the pivotal role of genes and proteins interacting with BR and controlling seed germination.

BR and Protein Controlling Seed Germination

There are 232 brassinazole (BRZ) and 608 (*OsBRI1* mutation) proteins involved in regulating the seed germination in coordination with the BR. Among all of those BR-sensitive proteins, 88 are categorized as the most sensitive proteins against BR. Another interesting fact about seed germination is that 90 percent of the cell proteins are unresponsive toward BR and take no part in the BR balance during the seed germination. Furthermore, the mutation of the BR receptor is more important in causing a significant change in cell protein profile (Li et al., 2016). The study used a mutant (*d61-125*) as a BR insensitive, and BRZ treated Nipponbare as a BR deficient germplasm to reveal the novel protein array involved in seed germination. The researchers studied the protein profiles of the germinated embryos to understand the regulatory roles of proteins (Fig. 2.2).

Proteins Involved in BR-Response Specificity

BR and its interactions stay highly specific in a plant cell. The basic mechanism involved in this specificity lies on the BRI1 receptor, for which the active site is chocked by its carboxyl terminus and by BKI1 (a negative regulator) in the absence

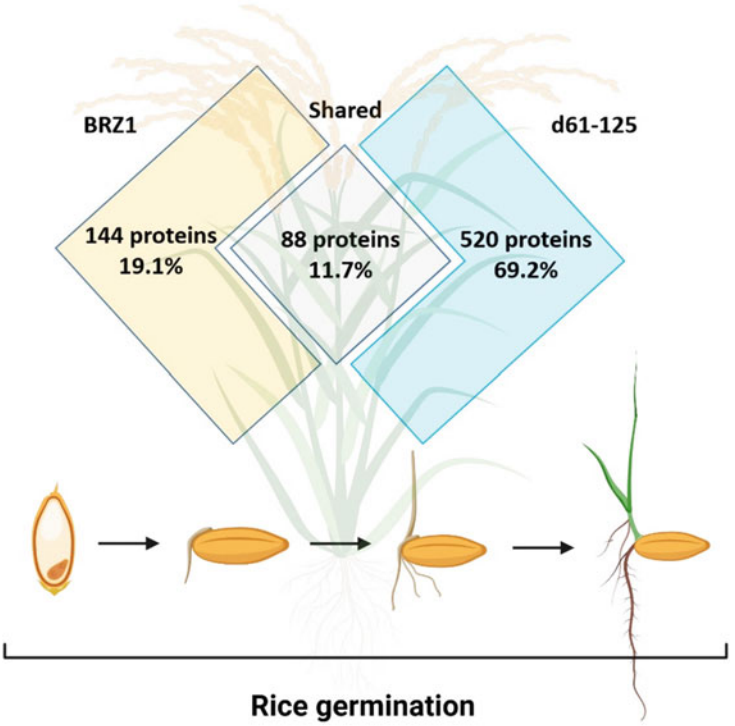

Fig. 2.2 Number of protein species involved in seed germination. The results revealed by the proteomic analysis of brassinazole (BRZ1) treated plants and a mutant d61-125 rice plant during the germination period

of a BR molecule. Both of the factors prevent the receptor from interacting with other similar substrates, e.g., BSKs and BAKI. BIN2 kinase acts as a primary kinase in the plant cell when BSU2 is inactivated. Meanwhile, 14-3-3 protein family plays a pivotal role in holding BIN2 kinase in the cytosol, which phosphorylates BZR1/BES1. However, degradation of the BIN2 kinase is carried out by 26S proteasome when required. The main factors required for proper folding and precise functioning of BRI1 are EBS1 and EBS2, which are involved in folding and targeting of plasma membrane.

Protein Involved in Activation of BR-response Genes

A plant cell in the BR environment perceives the BR signal through the BRI1 domain through the extracellular domain. It starts a chain reaction of BKI1 dissociation from the plasma membrane, which is transphosphorylated into the formation of an active BR receptor complex. The BRI1 phosphorylates BSKs that are attached to BSU1. This step initiates BSU1's activity, responsible for dephosphorylation in the

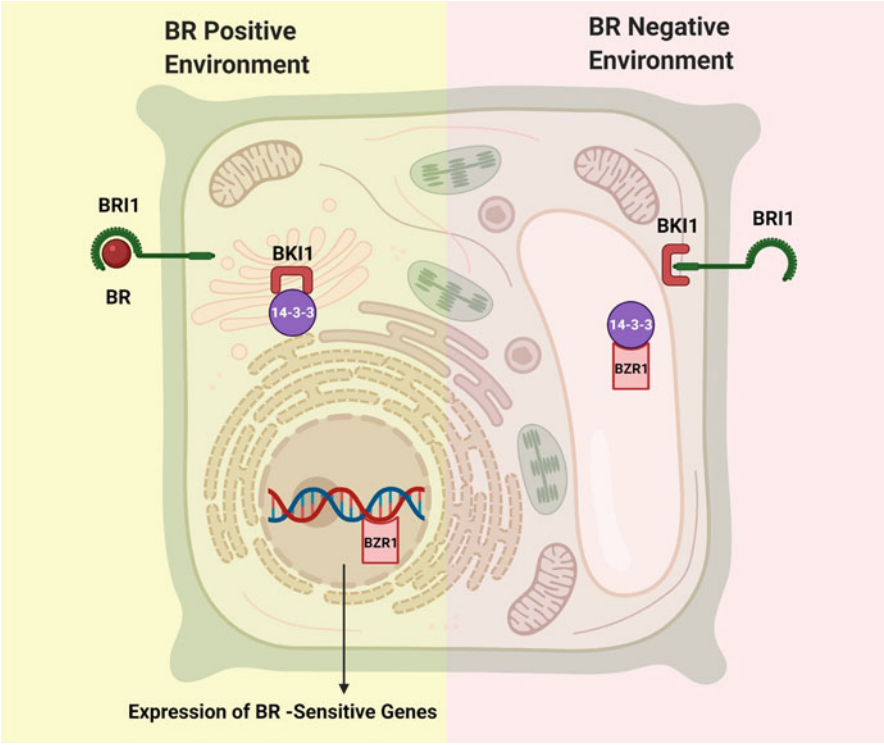

Fig. 2.3 Chain event of protein species starting from brassinosteroid receptors associated with the plasma membrane and leading to physiological responses of plant cells

cell and inhibiting BIN2's activity. The accumulation of unphosphorylated BES1/BZR1 in the plant cell nucleus triggers protein families Myb30 and BIM1. The protein families form and regulate the binding of downstream transcriptional regulators with E-boxes. The E-boxes are the key portions of the promoters to BR-response genes and centralize the expression of BR-response gene expressions. Another pathway to control the expression of BR-response genes is the bHLH pathway. However, the details of the intermediates of this pathway are yet to be explored (Fig. 2.3).

BR-Sensitive Protein Classification

Only 10 percent of the total plant cell proteins are BR-sensitive. Therefore, while talking about protein crosstalk in BR, some specific protein families can be separated. LRR protein family contains three major BR-response proteins, i.e., BRI1, BRL1, and BRL3. Similarly, a 14-3-3 protein family is a big protein family that generates a BR response in plant cells. We have individually listed BR-responsive proteins along with their genes, identifies, functions, and references (Table 2.1).

Table 2.1 List of plant proteins sensitive to brassinosteroids and taking part in cell physiology

Protein	Protein Name	Gene	Identifier	Function	
BRL3	BRI1-like 3 (receptor-like protein kinase)	AT3G13380	Q9LJF3	The protein acts as a receptor with dual-specificity kinase activity. It acts on both the substrates containing serine/threonine and tyrosine. It has the ability to bind brassinolide. Plays a role in vascular differentiation, plant development, and cell growth	Cano-Delgado (2004)
BSK3	Serine/threonine-protein kinase BSK3	AT4G00710	Q8W4L3	The protein is a serine/threonine kinase that positively regulates receptor kinase BRI1. It can also perform functions along with BSKs	Sreeramulu et al. (2013)
BRL1	Serine/threonine-protein kinase BRI1-like 1	AT1G55610	Q9ZWC8	The protein species acts as a receptor with a serine/threonine-protein kinase activity. It can bind brassinolide and differentiate vascular tissues	Cano-Delgado (2004), Zhou et al. (2004)
BRL2	Serine/threonine-protein kinase BRI1-like 2	AT2G01950	Q9ZPS9	The protein performs the receptor activity that detects the temporal and spatial signals from the extracellular environment and transduces them to provascular and procambial cells for regulating differentiation processes	Clay and Nelson (2002), Ceserani et al. (2009)
SERK1	Somatic embryogenesis receptor kinase 1	At1g71830	Q94AG2	The protein with a dual-specificity can perform kinase activity on both tyrosine and serine/threonine-based substrates. It can perform the activity with SERK2 to control male gametophyte production	Shah et al. (2001), Aker et al. (2007)
TTL3	Inactive TPR repeat-containing thioredoxin TTL3	AT2G42580	Q9SIN1	The protein has been reported for the induction of osmotic and salt stress tolerance and for the control of meristematic cell size	Ceserani et al. (2009)

(continued)

Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
WRKY54	Probable WRKY transcription factor 54	AT2G40750	Q93WU8	It is intrinsically a transcription factor that precisely interacts with the W box and inhibits senescence. It is also a famous inhibitor of the defense element salicylic acid (SA). Overall, it reduces plant defense against necrotrophic pathogens but elevates resistance against biotrophic pathogens	Li et al. (2017a), Ahmad et al. (2020a)
UVR8	Ultraviolet-B receptor UVR8	AT5G63860	Q9FN03	It is a UV-B-specific signaling protein that establishes UV-protective responses in plants.	Cloix et al. (2012)
PUB13	U-box domain-containing protein 13	AT3G46510	Q9SNC6	The protein is involved in the protein ubiquitination pathway	Samuel et al. (2008)
RGA1	DELLA protein RGA	AT2G01570	Q9SLH3	The protein is a proven inhibitor gibberellic acid (GA) pathway	Rombolá-Caldentey et al. (2014)
arf6	Auxin response factor 6	AT1G30330	Q9ZTX8	The protein lies among auxin response factors (ARFs), which are transcriptional factors	Nagpal et al. (2005)
CRY1	Cryptochrome-1	At4g08920	Q43125	The protein bears the activity of a photoreceptor. It inhibits hypocotyl elongation and regulates floral initiation based on photoperiod	Khan et al. (2019), Wu and Yang (2010), Bashir et al. (2016), Pedmale et al. (2016)
ABI5	Protein ABSCISIC ACID-INSENSITIVE 5	AT2G36270	Q9SJN0	Protein is extremely important due to its involvement in ABA-regulated gene expression	Nakashima et al. (2006), Ahmad et al. (2013)
PIF3	Transcription factor PIF3	AT1G09530	O80536	A transcription factor for the phytochrome signaling pathway	Martínez-García et al. (2000), Hafeez et al. (2019)
CRY2	Cryptochrome-2	AT1G04400	Q96524	Activities of the protein are parallel to CRY1. Additionally, it can mediate abiotic stress responses, ovule development, hypersensitive responses, and apical dominance	Yu et al. (2010), Jeong et al. (2010), Ahmad and Ashraf (2016)

DET2	Steroid 5-alpha-reductase DET2	Solyc10g086500	Q5K2N1	The protein takes part in the biosynthesis of steroids	Ahmad et al. (2014a), Rosati et al. (2005)
CBF2	Dehydration-responsive element-binding protein 1C	AT4G25470	Q9SYS6	The protein species is a transcriptional activator that can induce freezing tolerance and develops a cold acclimatized plant	Alonso-Blanco et al. (2005), Yousaf et al. (2015a), Abbas et al. (2020a)
BZR1	Protein brassinazole-resistant 1	AT1G75080	Q8S307	It is a transcriptional repressor that is responsible for ovule initiation	Huang et al. (2013)
PHYB	Phytochrome B	At2g18790	P14713	The protein is a reversibly interconvertible photoreceptor that absorbs the red and far-red region of the spectrum. It regulates most of the physiological parameters and light-regulated circadian events, i.e., CO_2 assimilation and stomatal conductance	Jung et al. (2016)
MKK4	Mitogen-activated protein kinase kinase 4	AT1G51660	O80397	The protein is related to the second phase of the biosynthesis of hydrogen peroxide and takes part in plant cell death	Meng et al. (2013), Khan et al. (2013)
MKK5	Mitogen-activated protein kinase kinase 5	AT3G21220	Q8RXG3	The protein controls the abscisic acid (ABA) responses	Li et al. (2017b)
ATG8	Autophagy-related protein 8a	AT4G21980	Q8LEM4	The protein species in autophagosome formation	Svenning et al. 2011)
TDR	Transcription factor TDR	Os02g0120500	Q6YUS3	The protein species is a transcriptional factor that takes part in tapetum programmed cell death (PCD)	Niu et al. (2013)
YODA	Mitogen-activated protein kinase kinase kinase YODA	AT1G63700	Q9CAD5	The protein triggers the MAP kinase cascade that decides the fate of the first cell of the zygote (early embryo)	Meng et al. (2013)
BES1	Protein brassinazole-resistant 2	AT1G19350	Q9LN63	The protein acts as a positive regulator of BR signaling and a transcription factor to initiate target-specific gene expression	Wang et al. (2014b)

(continued)

Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
BRI1	Protein brassinosteroid insensitive 1	AT4G39400	O22476	The protein species acts as a dual-specificity kinase that acts as a receptor and performs its activity on both serine/threonine- and tyrosine-containing substrates	Sreeramulu et al. (2013)
DSK2	Ubiquitin domain-containing protein DSK2a	AT2G17190	Q9SII9	The protein acts as a ubiquitin receptor that carries recognition of ubiquitinated substrates and is associated with the 26S proteasomal docking subunit RPN10	Fatimababy et al. (2010)
Feronia	Receptor-like protein kinase FERONIA	AT3G51550	Q9SCZ4	The protein deals with the female choice of male gamete delivery for fertilization purpose and classified as a receptor-like protein	Haruta et al. (2014)
TINY	Ethylene-responsive transcription factor TINY	AT5G25810	Q39127	The exact function of the protein is not precisely known. However, it is a putative activator of transcriptional processes	Sakuma et al. (2002)
BAK1	BRI1 kinase inhibitor 1	At5g42750	Q9FMZ0	The protein performs the activity of a negative regulator of brassinosteroid signaling	Wang and Chory (2006)
BIR3	Probable inactive receptor kinase At1g27190	At1g27190	O04567	The protein performs an ATP binding activity along with the protein kinase activity	Theologis et al. (2000)
BKI1	BRI1 kinase inhibitor 1	At5g42750	Q9FMZ0	The protein is an efficient negative regulator of BR signaling. Its mechanism of action is to get associated with the membrane and to bind with the kinase-inactive form of BRI1, hence reducing the interaction of BRI1 with BAK1	Wang and Chory (2006)
PUB12	U-box domain-containing protein 12	At2g28830	Q9ZV31	The protein species performs the activity of an E3 ubiquitin ligase	Yamada et al. (2003)
BSK1	Serine/threonine-protein kinase BSK1	At4g35230	Q944A7	The protein species is a positive regulator of BR signaling for all the steps downstream to the receptor kinase, while its function is categorized as BRI1Serine/threonine kinase	Shi et al. (2013)

CDG1	Serine/threonine-protein kinase CDG1	At3g26940	Q9LSE1	The protein functions in parallel to the BSK1along regulation of plant growth	Kim et al. (2011)
BSU1	Serine/threonine-protein phosphatase BSU1	At1g03445	Q9LR78	The protein is a positive regulator of BR signaling. However, its function is classified as a phosphatase	Kim et al. (2011), Mora-García et al. (2004)
BIN2	Shaggy-related protein kinase eta	At4g18710	Q39011	The protein species is involved in the auxin signaling pathway and BR signaling pathway, and it is classified as a negative regulator of BR that affects plant growth	Khan et al. (2013), Abbas et al. (2020b)
PP2A	Serine/threonine-protein phosphatase PP2A-5 catalytic subunit	At1g69960	O04951	The protein is associated with the serine/threonine phosphatase PP2A regulatory subunits A and B′. it is reported for the positive regulation of beta-oxidation of protoauxins and fatty acids. It has also been reported to induce salt tolerance in plants by enhancing chloride channel performance on cellular vacuoles	Kataya et al. (2015), Ahmad et al. (2014b)
KIB1	F-box/kelch-repeat protein KIB1	At4g12810	Q9SU05	The protein species has been estimated to mediate the proteasomal degradation of the proteins by ubiquitination and remains the component of E3 ubiquitin ligase complexes	Zhu et al. (2017), Ahmad et al. (2019)
OCTOPUS	Protein OCTOPUS	At3g09070	Q9SS80	The protein plays a major role in the initiation and differentiation of the primary roots protophloem	Breda et al. (2017), Ahmad et al. (2020b)
POLAR	Protein polar localization during asymmetric division and redistribution	At4g31805	Q6NQ99	The protein species is responsible for asymmetric cell division, which is imperative in the stomatal lineage cells	Pillitteri et al. (2011), Li et al. (2021)
BASL	Protein breaking of asymmetry in the stomatal lineage	At5g60880	Q5BPF3	The protein performs the functions parallel to the POLAR protein Q6NQ99, which can also recruit other proteins for the spatial recognition of MAPK signaling in the cells of the plant cortex	Zhang et al. (2016a), Ahmad et al. (2020c)

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Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
HDA6	Histone deacetylase 6	At5g63110	Q9FML2	The protein performs the deacetylation function of lysine residues on the N-terminal of the histones	Luo et al. (2012), Shah et al. (2021)
TTL1 at	TPR repeat-containing thioredoxin TTL1	1 g53300	Q9MAH1	The protein species acts in the induction of tolerance against osmotic stress and in the responses to ABA at the seedling stage	Yasin et al. (2017), Lakhssassi et al. (2012)
MAX2	F-box protein MAX2	At2g42620	Q9SIM9	Just like the Q9SU05 protein, it is a component of the complexes of E3 ubiquitin ligase. It commonly acts as a negative regulator for axillary shoots and, in this way, controls the branching.	Zhang et al. (2016b), Yasin et al. (2018a)
PUB40	U-box domain-containing protein 40	At5g40140	Q9FL17	The protein functions as an E3 ubiquitin ligase	Khan et al. (2018), Cheng et al. (2017)
COP1	E3 ubiquitin-protein ligase COP1	At2g32950	P43254	The protein functions in a parallel fashion as Q9SU05 protein. However, the repression of the photomorphogenesis in darkness is the additional function of this protein that is achieved by influencing the light-induced transcription factors, e.g., LAF1, HY5, and HYH	Zaheer et al. (2017), Srivastava et al. (2015)
SINAT2	E3 ubiquitin-protein ligase SINAT2	At3g58040	Q9M2P4	The protein species is classified among E3 ubiquitin ligases that can also accept ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester	Qi et al. (2017), Tariq et al. (2020)
TRXh5	Thioredoxin H5	At1g45145	Q39241	The protein performs its function against pathogens and other oxidative stresses and is termed thiol-disulfide oxidoreductase	Sweat and Wolpert (2007), Ahmad et al. (2021b)

BSS1/BOP1	Regulatory protein NPR6	At3g57130	Q9M1I7	The protein species is an adaptor to E3 ubiquitin-protein ligase complex that is substrate-specific and monitors the lateral organs by regulating the LOB domain-containing genes and adaxial–abaxial polarity genes	Ha et al. (2010), Yasin et al. (2018b)
BOP2	Regulatory protein NPR5	At2g41370	Q9ZVC2	It is a protein species with similar functions as Q9M1I7 protein. However, it promotes floral and leaf meristem tissues. The development of nectary, suppression of bract, and abscission zones formation are the common functions of the protein	Ha et al. (2010), Yasin et al. (2018c)
IWS1	Protein IWS1 homolog 1	At1g32130	F4ICK8	The protein plays a role in RNA polymerase II transcription factor and performs the elongation step of transcription	Widiez et al. (2011), Khan et al. (2017b)
BIM1	Transcription factor BIM1	At5g08130	Q9LEZ3	The protein positively regulates BR signaling by individually binding to the promoter as a homodimer or synergistically as a heterodimer with BZR2/BES1. It can enhance the transcription of BZR2/BES1-mediated target genes but cannot initiate it.	Yin et al. (2005), Shafique et al. (2014b)
MYB30	Transcription factor MYB30	At3g28910	Q9SCU7	The protein is categorized as a transcription factor that can positively regulate hypersensitive cell death and take part in the plant defenses by regulating salicylic acid biosynthesis	Shah et al. (2020a), Liu et al. (2014)
PIF4	Transcription factor PIF4	At2g43010	Q8W2F3	It is also a transcription factor that negatively interacts with the phytochrome B signaling pathway. It can also bind to the G-box motif and control gene expression related to cell expansion	Pedmale et al. (2016), Akram et al. (2014a)

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Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
ARF8	Auxin response factor 8	At5g37020	Q9FGV1	The protein is categorized as an auxin response factor and a transcriptional activator. The protein's maturation of both the gynoecium and stamen and seed and fruit development are all influenced by the protein	Ahmed et al. (2017), Goetz et al. (2006)
MYBL2	Proposed: At1g71030/F23N20_2	At1g71030	Q9C9A5	The protein molecule performs DNA-binding transcription factor activity and shares transcription regulatory region sequence-specific DNA binding. It has also been involved in the anthocyanin-containing compound biosynthetic process, cell differentiation, and proanthocyanidin biosynthetic process	Theologis et al. (2000), Javaid et al. (2014)
HAT1	Histone acetyltransferase GCN5	At3g54610	Q9AR19	The primary role of the protein is to perform acetylation of ADA2 and histone H3 proteins. For acetylation it targets the 14th lysine residue in histone H3. Furthermore, it takes part in DNA-binding transcriptional activation and epigenetic transcription activation. Its action needs large protein complexes that could alter the chromatin	Mao et al. (2006), Anjum et al. (2017)
HDA19	Histone deacetylase 19	At4g38130	O22446	Unlike HAT1 (Q9AR19), the protein is involved in the deacetylation of histones, including H3, H4, H2A, and H2B. It also performs deacylation of the lysine residues on the N-terminal of the histones. Thus, the protein is responsible for the successful occurrence of developmental and cell cycle events by epigenetic repression. The protein	Yousaf et al. (2015b), Wu et al. (2000)

				plays a vital part in ethylene and jasmonic acid signaling concerning plant defenses, besides repressing the floral parts differentiation genes	
TPL	Protein TOPLESS	At1g15750	Q94AI7	The most essential function of the protein species is to co-repress the transcriptional process, especially the root-promoting genes in the half part of the embryo for successful development of the root-pole during embryogenesis. It is the necessary element for the development of the ovule; however, it represses the plant defense responses (jasmonic acid) and regulates plant growth through the repression of auxin	Wei et al. (2015), Yasin et al. (2018d)
ELF6	Probable lysine-specific demethylase ELF6	At5g04240	Q6BDA0	The protein species performs demethylation of the fourth lysine residue and represses the photoperiod-based flowering pathways	Ibrahim et al. (2017), Jeong et al. (2009)
REF6	Lysine-specific demethylase REF6	At3g48430	Q9STM3	Just like the ELF6 (Q6BDA0), the protein is also a demethylase, but the target lysine residue is 27th in histone H3. Interacts with hundreds of genes and decides developmental patterns and flowering	Cui et al. (2016), Akram et al. (2013)
PICKLE	CHD3-type chromatin-remodeling factor PICKLE	At2g25170	Q9S775	The protein is mainly involved in the remodeling of chromatin and represses plant embryos' primary traits through gene regulation. It plays a vital role in the seedling establishment during post-germination stages and differentiation of carpel. Its role in the signaling of cytokinin is also well-studied.	Furuta et al. (2011), Jafari et al. (2018)

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Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
SDG8	Histone-lysine N-methyltransferase ASHH2	At1g77300	Q2LAE1	The protein functions as histone methyltransferase that could perform di- and tri-methylation of a lysine residue present at thirty-sixth number in H3. It could also prevent early flowering transition	Wang et al. (2014b), Shah et al. (2020b)
WRKY46	Probable WRKY transcription factor 46	At2g46400	Q9SKD9	The protein interacts with the W box (5′-(T)TGAC[CT]-3′) and regulates the osmotic stress response of plant cells. It is accompanied by WRKY53 and WRKY70 and takes part in plant defenses against pathogens. The protein species plays a double role in a plant's life (i) prevents the plant from drought by tuning the gene expression, (ii) promotes BR-regulated plant growth but prevents drought response by modulating gene expression	Chen et al. (2017), Akram et al. (2020a)
WRKY70	Probable WRKY transcription factor 70	At3g56400	Q9LY00	The protein regulates senescence-related gene transcription and stress responses related to abiotic and biotic stressors by modulating phytohormones signaling pathways. It also exhibits an essential role in the signaling of salicylic acid and jasmonic acid. It is imperative concerning plant diseases and environmental stressors	
RD26	NAC domain-containing protein 72	At4g27410	Q93VY3	The protein is known for its role as a transcription factor that precisely regulates the expression starts from the motif 5'--CATGTG-3'	Tran et al. (2004), Ahmad et al. (2018)

WEREWOLF	Transcription factor WER	AT5G14750	Q9SEI0	The protein species decides the fate of the epidermal cells in hypocotyl and roots. It tends to promote the formation of atrichoblasts, which are the non-hair developing cells of the roots. Furthermore, the special distribution of stomata also depends upon this protein	Ryu et al. (2005), Khan et al. (2017c), Akram et al. (2020b), Akram et al. (2019a, b)
TRANSPARENT TESTA GLABRA1	GLABRA2 expression modulator	AT2G22475	Q8S8F8	The protein performs the function of spatial cell division and cell differentiation. It is mainly taking part in defining the pattern of root epidermal cells	Caro et al. (2007), Khan et al. (2017d), Akram et al. (2020c)
SNRK2-4	Serine/threonine-protein kinase SRK2A	AT1G10940	P43291	The protein species performs ATP binding activity and protein serine/threonine kinase activity. It takes part in intracellular signal transduction and protein phosphorylation	Cheng et al. (2017), Akram et al. (2019c), Akram et al. (2020d)
DREB1A	Dehydration-responsive element-binding protein 1A	AT4G25480	Q9M0L0	The protein is a sequence-specific (5′-[AG]CCGAC-3′) transcriptional activator. It can jointly act with the C-repeat/DRE element and induce chilling-dependent transcription in the plants. It also contains CBF/DREB1 factors, which are famous for their role in tolerating freezing temperatures to acclimatize the plants against cold	Alonso-Blanco et al. (2005), Akram et al. (2019d), Ibrahim et al. (2016)
CBF1	Dehydration-responsive element-binding protein 1B	AT4G25490	P93835	The protein species is a sequence-specific (5′-[AG]CCGAC-3′) transcriptional activator and facilitates the freezing tolerance function of DREB1A	Alonso-Blanco et al. (2005), Akram et al. (2014b), Yasin et al. (2019)
DLT	Protein DWARF AND LOW-TILLERING	Os06g0127800	Q9LWU9	The protein species is well-known as a positive regulator of BR. It performs the downstream functions of GSK2 and BRI1 by being a direct target of GSK2 kinase. It is also essential in the counter BR pathway responsible for feedback inhibition of BR	Hirano et al. (2017), Zaheer et al. (2018)

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Table 2.1 (continued)

Protein	Protein Name	Gene	Identifier	Function	
OSH1	Homeobox protein knotted-1-like 6	Os03g0727000	P46609	The protein is mainly involved in the development of shoots during embryogenesis and maintains leaf arrangement on the shoots at later stages of the plant's life. Another essential function of the protein is the auxin control during the anther development	Song et al. (2018), Yasin et al. (2018e)
OsOFP19	Transcription repressor	Os12g0158300	Q2QXG3	The protein mainly functions as a repressor of the transcriptional process and is involved in many physiological processes governing the growth and development of the plant	Kawahara et al. (2013), Yasin et al. (2018f)
SDG725	Proposed: Os02g0554000 protein	Os02g0554000	A0A0P0VK92	The protein performs histone methyltransferase activity (H3-K36 specific) and zinc ion binding activity	Kawahara et al. (2013), Shafique et al. (2017)
OsBRI1	Brassinosteroid LRR receptor kinase BRI1	Os01g0718300	Q942F3	The protein species is classified as a kinase receptor and is the backbone of BR signal transduction. It takes part in all types of plant processes including plant development, flowering, and cell elongation	Zhang et al. (2016c)
DWARF11	Cytochrome P450 724B1	Os04g0469800	Q6F4F5	The protein species takes part in BR biosynthesis	Tanabe et al. (2005)
BRD1	Cytochrome P450 85A1	Os03g0602300	Cytochrome P450 85A1	Just like the DWARF11, the protein is crucial for some critical steps in the biosynthesis of BR	Mori et al. (2002)
CYP90D2/D2	Cytochrome P450 90D2	Os01g0197100	Q94IW5	The protein performs some steps in the biosynthesis of BR. Furthermore, it governs the elongation of leaf sheaths and plant stem	Hong et al. (2003)
ZmBRI1a	Proposed: Brassinosteroid insensitive1a	Zm00001d011721	K7V4X2	The protein performs ATP binding activity and protein serine/threonine kinase activity. It also takes part in the hormone-mediated signaling pathway of the cell	Schnable et al. (2009)

ZmDET2	Steroid 5-alpha-reductase DET2	Zm00001d042843	B6TIF9	The protein species takes part in the reduction step during plant steroid (brassinolide) biosynthesis	Alexandrov et al. (2009)
DWARF4	Cytochrome P450 90B2	Os03g0227700	Q5CCK3	The protein species is known for catalyzing the rate-limiting step of C22-alpha-hydroxylation during BR synthesis. It also converts campestanol to 6-deoxocathasterone and 6-oxocampestanol to cathasterone	Sakamoto et al. (2006)
ILI1	Transcription factor ILI1	Os04g0641700	Q7X742	The protein is a positive regulator of cell elongation and plant development, while it is a non-DNA-binding bHLH transcription factor. It has also been studied for its role in lamina inclination and cell elongation	Zhang et al. (2009b)
HBI1 and PRE1	Transcription factor HBI1	AT2G18300 and AT5G39860	Q9ZPW3	The protein species performs a function similar to Q7X742, while it is also an essential part of the promoters of the gene encoding cell wall loosening enzymes	Bai et al. (2013)
EMS1	Leucine-rich repeat receptor protein kinase EMS1	AT5G07280	Q9LYN8	The protein acts as a receptor with a serine/threonine-protein kinase activity. It defines the rate of embryonic development and cell enhancement in seeds	Zheng et al. (2019)
OsPIL1	Transcription factor PHYTOCHROME INTERACTING FACTOR-LIKE 13	Os03g0782500	Q10CH5	The protein takes part in the negative regulation of phyB-dependent light signal transduction. It restricts plant growth during drought stress conditions	Nakamura et al. (2007), Todaka et al. (2012)

Future Directions

There are several areas in which scientific knowledge is not enough to describe everything clearly. For example, there are several uncharacterized BR-sensitive proteins in plant cells. Furthermore, the process of non-genomic signaling is not fully known to researchers. The biochemical interactions taking place during non-genomic signaling, the role of carrier proteins, and the involvement of non-targeted receptors make the BR signaling process too complex to describe precisely. More detailed studies are imperative to reveal the answers to mechanism-related questions.

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Chapter 3
Crosstalk Between Brassinosteroids and Nitric Oxide Regulates Plant Improvement During Abiotic Stress

Fareen Sami, Shamsul Hayat, and Fariduddin Qazi

Abstract Crop productivity is declining on exposure to diverse abiotic stresses, i.e. salinity, drought, extreme temperatures, heavy metals, ultraviolet radiations, etc. that became a major restriction for agricultural production across the globe. Therefore, it is vital to equip crops with multi-stress tolerance to provide adaptation against these environmental changes to meet the demand of ever-rising population. Phytohormones play a crucial role in conferring adaptation to abiotic stress conditions either via exogenous application or by using biotechnological tools that manipulate endogenous phytohormone levels/osmotic adjustments modulating plant metabolism. Moreover, adaptive responses also involve various sensing and signaling functions where brassinosteroids and nitric oxide become a critical component mediating hormonal actions by modulating gene expression, reactive oxygen species generation, and protein activity. Earlier studies had shown that brassinosteroids (BR) are crucial for many phases of a plant's life cycle, including growth, photosynthesis, and redox balance and responses towards biotic and abiotic stress. Since last few years nitric oxide (NO) showed multifarious roles in mediating plant physiological processes. In this chapter, we summarize the effect of exogenous application of BR and NO in plant growth and development and abiotic stress tolerance in plants.

Keywords Antioxidative defense system · Abiotic stress · Reactive nitrogen species · Sodium nitroprusside · Signaling molecule

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Introduction

Agriculture is the major sector affected due to anthropogenic disorders in natural environment. In general, plant growth is affected by number of environmental stresses, such as drought, salinity, heavy metal, extreme temperatures, etc. (Cramer et al., 2011; Fancy et al., 2017; Pereira 2016; He et al., 2018). The consequences of these stresses are overproduction of reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals, superoxide radicals, and singlet oxygen that cause a number of damaging effects under stressful conditions (Gupta et al., 2016). Plant growth regulators (PGRs) are the organic compounds (other than nutrients) that affect physiological processes in plants in very small concentrations. For applied purpose, they could be exploited directly as natural or synthetic compounds to regulate life processes to improve quality and yield of plants. Among the PGRs, brassinosteroids and nitric oxide play a crucial role and regulate diverse plant responses both under natural and abiotic stress conditions.

Brassinosteroids (BR) are a class of plant steroid hormone which occur in all parts of plant (Bajguz & Tretyn, 2003) and stimulate diverse array of physiological processes in plants, such as seed germination, division and expansion of cell, stem elongation, vascular development, seedling photomorphogenesis, senescence as well as stress response (Hayat et al., 2010; Fariduddin et al., 2014). In addition, BR boost tolerance towards various abiotic stresses such as salinity, drought, heavy metal contamination, high and low temperatures and biotic stress like pathogen attack (Kagale et al., 2007; Hayat et al., 2007; Bajguz and Hayat, 2009; Soares et al., 2016, Khan et al., 2015, 2019). Previously, several studies have established that BR enhance stress tolerance by modulating the expression of diverse genes (Arfan et al., 2019). BR have been exploited primarily to improve plant growth and development under the conditions of severe stress (Soares et al., 2016).

Nitric oxide (NO) is a vital endogenous signaling molecule associated with diverse range of physiological processes including germination, transport, metabolism, photosynthesis, flowering as well as senescence in plants (Hayat et al., 2010; Saxena & Shekhawat, 2013). Additionally, recent studies have revealed that NO plays a crucial role in improving uptake of iron (Lamattina et al., 2003), stomatal movement (Sakihama et al., 2003; Neill et al., 2008; Gayatri et al., 2013; Wang et al., 2015; Laxalt et al., 2016), programmed cell death (Pedroso et al., 2000), and abiotic stress responses (Neill et al., 2003; Zhao et al., 2004). It has been shown that NO is involved in amelioration of several abiotic stresses, such as chilling (Neill et al., 2003), water stress (Garcia-Mata & Lamattina, 2001), salinity (Zhao et al., 2004), and heavy metal stress (Hsu & Kao, 2004; Wang et al., 2013). Besides, NO protects cells from oxidative damage by acting as an antioxidant agent to scavenge ROS and altering gene expression associated with antioxidant enzymes (Arasimowicz & Floryszak-Wieczorek, 2007). More recently, there is a compelling evidence that BR and NO function as signaling molecules in plants, facilitating a diverse range of plant responses (Hayat et al., 2010; Zhu et al., 2016). Moreover, NO and BR positively influence plant responses to abiotic stresses, such as temperature stress,

heavy metal stress, water stress, oxidative stress, salt stress, and UV radiation (Zou et al., 2018; Karpets & Kalupaev, 2018; Arfan et al., 2019; Kaya et al., 2019; Li et al., 2020).

The intent of the chapter is to explain that how brassinosteroids and nitric oxide interact with each other and regulate various metabolic processes in plants and improve growth, photosynthesis, antioxidative defense system, and ROS homeostasis under normal and abiotic stress conditions.

Interaction of Nitric Oxide and Brassinosteroid in Plant Physiological Processes

Plant Growth

Phytohormones play a critical role in modulating physiological and molecular responses critical for plant survival under sessile environments. Application of sodium nitroprusside (SNP) enhanced length, fresh and dry mass of shoot and root of tomato in a concentration-dependent manner (Hayat et al., 2011). In wheat, BR applied through shotgun approach improved length, fresh and dry mass of shoot, grain yield, and number of grains (Ali & Ashraf, 2008). Exogenous supplementation of 24-epibrassinolide enhanced plant height, hypocotyl diameter, root length, leaf area, root and shoot dry mass in cucumber (Anwar et al., 2019). SNP and BR had generated a visible impact on plant growth of peanut (Yuanjie et al., 2019), which could be ascribed because of their crosstalk with auxin in supporting leaf expansion and root growth (Vandenbussche et al., 2011). BR ameliorates the inhibitory effect of 1 M of SNP in tomato, where EBL was more effective than HBL (Hayat et al., 2010). In *Lycopersicon esculentum*, combination of NO and BR improved leaf number (Jangid & Dwivedi, 2017). In root cells, BR increases concentration of NO which is required for BR mediated changes in root architecture (Tossi et al., 2013). Exogenous application of 24-epibrassinolide (EBL) inhibits primary root elongation, enhances lateral root density along with a marked upsurge in accumulation of NO in Arabidopsis seedlings (Tossi et al., 2013). In *Cucumis sativus,* application of brassinolide (BR) and NO (S-nitroso-N-acetyl penicillamine, SNAP) in combination considerably promoted adventitious rooting and was found more effective than its individual application (Li et al., 2020). However, the positive effects of BR on adventitious rooting could be inhibited by scavenger of NO (c-PTIO, L-NAME, and tungstate) (Li et al., 2020). Here, BR promoted adventitious root formation by inducing the production of endogenous NO. In mustard, combined application of EBL and SNP significantly improved shoot and root length, fresh and dry mass of root and shoot (Gupta et al., 2017).

Photosynthesis

Photosynthesis is one of the crucial physiological attributes associated with plant growth and development. Application of various levels of BR enhances photosynthetic attributes in *Triticum aestivum* (Ali & Ashraf, 2008). Moreover, chlorophyll contents and maximum quantum yield of PSII were also enhanced by BR treatment (Ali & Ashraf, 2008). In tomato leaves, application of EBL enhances photosynthetic rate and PSII efficiency (Ogweno et al., 2008). In cucumber seedlings, exogenously applied BR significantly improved chlorophyll content and photosynthesis (Anwar et al., 2019). In *Lycopersicon esculentum,* NO interacts with BR to enhance photosynthesis by upregulating the activity of Rubisco (Hayat et al., 2010). In peanut seedlings, combined effect of SNP or EBL alone as well as in combination improves growth under cadmium stress (Yuanjie et al., 2019). EBL supplementation improved photosynthetic traits via elevation of endogenous NO in pepper (Kaya et al., 2019). Exogenous application of 10^{-6} M EBL and 100 μM SNP improved photosynthetic pigments, parameters of gaseous exchange, and chlorophyll fluorescence in mustard (Gupta et al., 2017). In Arabidopsis, EBL induced stomatal closure by ethylene synthesis and upregulating the expression of ACS5 and ACS9 via BRI1 dependent mechanism, thus triggering G protein to induce generation of H_2O_2 which enhances NO production and finally induces closure of stomata (Shi et al., 2015). Exogenous application of different concentrations of SNP enhanced SPAD chlorophyll and photosynthetic attributes in tomato (Hayat et al., 2011).

Oxidative Damage

Higher concentrations of reactive oxygen species (ROS) are toxic but at lower concentrations, these work as a signaling molecule (Gill & Tuteja, 2010). In peanut, SNP and BR treatment decreased the production of H_2O_2 and $O_2^{\circ -}$ generation in leaves. Furthermore, lipid peroxidation was decreased in these plants by maintaining cellular redox homeostasis and antioxidant enzymes (Yuanjie et al., 2019). In tomato, NO and BR prevent oxidative damage by boosting antioxidant machinery (Hayat et al., 2010). In mustard, combined application of 10^{-6} M EBL and 100 μM SNP decreased electrolyte leakage and lipid peroxidation (Gupta et al., 2017). Moreover, exogenous application of EBL induces the synthesis of NO to eliminate oxidative stress in pepper (Kaya et al., 2019).

Enzymatic and Non-enzymatic Antioxidants

Nitric oxide and brassinosteroids are known to regulate antioxidative system against abiotic stress (Jangid & Dwivedi, 2017). In tomato, application of SNP enhanced antioxidant enzymes (catalase, peroxidase, and superoxide dismutase) and proline content (Hayat et al., 2011). BR treatment significantly increased the activity of antioxidative enzymes in tomato leaves (Ogweno et al., 2008). Exogenous application of BR noticeably increased antioxidant enzymes in cucumber seedlings (Anwar et al., 2019). NO and EBL significantly increased the activity of antioxidative enzymes in peanut by inducing antioxidative machinery through altered gene expression (Yuanjie et al., 2019). Combined treatment of SNP and EBL enhances SOD content and activity in tomato (Jangid & Dwivedi, 2017). In pepper, application of EBL triggers production of nitric oxide to improve antioxidative defense system (Kaya et al., 2019). Application of SNP and BR significantly reduced proline content by increasing the activity of proline dehydrogenase and transcription and translation of specific genes that could be responsible for reducing the level of proline (Yuanjie et al., 2019). In tea leaves, BR enhances flavonoid concentration by upregulating NO accumulation (Li et al., 2017). Co-application of SNP and EBL enhanced proline content in mustard (Gupta et al., 2017). Tobacco seedlings, pretreated with BL had increased activity of CAT, SOD, APX, and GPX which was further enhanced by NO and corroborated the fact that NO generation plays a crucial role in BL-induced stress tolerance (Zhu et al., 2016). Exogenous application of NO enhanced proline content and antioxidative enzymes in tomato (Hayat et al., 2012). In addition, BR application also noticeably improved proline content and activity of antioxidative enzyme in rice (Farooq et al., 2009).

Interaction of Nitric Oxide and Brassinosteroid Under Abiotic Stresses

Salt Stress

Salinity is one of the main hazards to agriculture that leads to impairment of crop productivity. It is a widespread crisis in arid and semi-arid regions where groundwater quality and agricultural practices are very poor that enhance the accumulation of salt in the soil (Hasanuzzaman et al., 2013). In *Lycopersicon esculentum*, application of NO alleviates salinity stress (Hayat et al., 2012). In *Oryza sativa*, EBL improved growth, proline content, and antioxidative enzyme activities under salt stress (Sharma et al., 2013). In *Brassica juncea*, NO and BR ameliorated salinity stress through altered metabolism of nitrogen, ABA, and proline (Gupta et al., 2017). In *Brassica juncea*, 28-homobrassinolide regulates antioxidative enzyme activities and gene expression in response to salt-induced oxidative stress (Kaur et al., 2018). In *Oryza sativa* seedlings, exogenously sourced NO enhance salt tolerance by gene

modulation (Adamu et al., 2018). In cucumber roots, exogenous NO alleviates oxidative stress induced by salt through regulation of ROS metabolism in root mitochondria and ATPase, PPase activities in plasma membrane and/or tonoplast (Shi et al., 2007). In *Hordeum vulgare,* supplementation of NO ameliorates oxidative stress induced by salt stress (Li et al., 2008). In tomato, NO protects salt-induced oxidative stress in leaves of two genotypes of tomato (Wu et al., 2011). In soya bean roots, exogenous application of NO improved salt tolerance by enhancing antioxidative defense system (Egbichi et al., 2014). In chickpea, NO mitigates salt stress by regulating the level of osmolyte, antioxidative enzymes, and through gene expression of representative antioxidant enzymes (Ahmad et al., 2016). In salt-stressed wheat plants, supplementation of EBL modulates growth, photosynthetic capacity, and water relations (Ali & Ashraf, 2008). The application of NO enhances physio-morphological attributes of tomato plants by improving their tolerance to NaCl stress (Siddiqui et al., 2017). In potato, BR alleviates the toxic effect of NaCl on photosynthetic processes (Kolomeichuk et al., 2020). In *Nicotiana benthamiana* seedlings, NO is associated with BR-induced alternative respiratory pathway that plays an essential role in salt tolerance (Zhu et al., 2016).

Drought Stress

Drought stress results due to low availability of water and affects plant growth through altered physiological and biochemical processes that negatively impact final productivity of crop plants. An equilibrium between oxidative damage and antioxidative defense system is highly imperative for better survival of plants under drought stress. Foliar treatment of nitric oxide (SNP) and brassinosteroid (24-epibrassinolide) mitigates drought stress in two genotypes of tomato (Jangid & Dwivedi, 2017). In *Oryza sativa,* BR improves water relations and gas exchange under drought stress (Farooq et al., 2009). Exogenously sourced NO reduced water stress and reduced deleterious effects on yield by modulation of growth, water relations, osmotic adjustment, and regulation of antioxidative defense system (Habib et al., 2020). Exogenous application of NO alleviates oxidative damage in turfgrasses under drought stress (Boogar et al., 2014). In rice, NO alleviates drought stress by limiting transpiration in *Oryza sativa* (Xiong et al., 2012). NO improves drought tolerance in hulless barley by altering growth and physiological attributes (Gan et al., 2015). NO plays a crucial role in protecting sunflower plants by dry mass accumulation, gas exchange characteristics, and activities of antioxidant enzymes (Cechin et al., 2015). In *Capsicum annuum*, NO plays a putative role in brassinosteroid-induced antioxidative defense system under water stress (Kaya et al., 2019). In Zea mays, BR-induced NO production and NO-mediated ABA biosynthesis are crucial mechanisms to deal with water stress (Zhang et al., 2011).

Temperature Stress

In *Lycopersicon esculentum,* BR ameliorates heat-induced photosynthetic inhibition by enhancing antioxidant system and carboxylation efficiency (Ogweno et al., 2008). In cucumber seedlings, application of EBL ameliorates the detrimental effects of temperature (Anwar et al., 2019). Exogenously applied BR improved photosynthetic pigments in *Leymus chinensis* grown under high temperature (Niu et al., 2016). In pepper, BR ameliorates chilling-induced oxidative stress by enhancing antioxidative system and maintenance of photosystem II (Li et al., 2015). In mustard, supplementation of 28-homobrassinolide modulates gene expression and activity of antioxidative enzyme against temperature-induced oxidative stress (Kaur et al., 2018). Exogenous NO conferred tolerance to thermal stress in soya bean plants by maintaining metabolic homeostasis (Vital et al., 2019). Supplementation of SNP to heat-treated seedlings reduced lipid peroxidation, H_2O_2 content, and increased chlorophyll content as well as the activity of antioxidant enzymes (Hasanuzzaman et al., 2012). In Chinese cabbage, NO improves chilling tolerance by enhancing the activities of antioxidative enzymes (Fan et al., 2014). BR enhances thermotolerance through the accumulation and biosynthesis of heat shock proteins in *Brassica napus* and *Lycopersicon esculentum* (Dhaubhadel et al., 2002). In wheat coleoptiles, NO participates in EBL-induced heat resistance (Karpets & Kolupaev, 2018). In *Camellia sinensis*, BR improved tea quality by enhancing theanine biosynthesis under high temperature where NO mediates BR-induced flavonoid biosynthesis (Li et al., 2017). In *Medicago truncatula*, BR pretreatment enhanced tolerance towards cold by modulating the expression of numerous cold-related genes and activity of antioxidant enzyme (Arfan et al., 2019).

Heavy Metal Stress

In tomato, BR protects photosynthetic machinery against the cadmium-induced oxidative stress (Hasan et al., 2011). In *Zea mays* leaves, BR overcome the damages caused by manganese-induced oxidative stress (Wang et al., 2009). In radish, BR counters heavy metal induced oxidative stress by regulating the expression of key antioxidant enzyme genes (Sharma et al., 2018). In *Brassica juncea*, application of EBL improves seed germination and seedling growth under heavy metal stress (Sharma & Bhardwaj, 2007). In *Raphanus sativus*, application of 10^{-7} M EBL reduced copper toxicity and stimulated growth of root and shoot (Choudhary et al., 2012). In *Brassica juncea*, the plants treated with EBL and 28-homobrassinolide reduced the toxic effects of Ni and improved growth, photosynthetic pigments, and photosynthetic rate (Ali & Ashraf, 2008). In Cd-stressed tomato plants, BR application improved photosynthetic content and efficiency of photosynthesis (Hayat et al., 2010). In rice, NO alleviates cadmium toxicity by increasing pectin and hemicellulose contents in cell wall of root (Xiong et al., 2009). Exogenous

application of SNP alleviated cadmium toxicity by increasing chlorophyll and mineral nutrients and hence improved plant growth (Chen et al., 2018). In hydroponically grown wheat, NO reduced lead-induced oxidative damage by detoxifying reactive oxygen species through induced antioxidant system (Kaur et al., 2015). In peanut seedlings, combined application of SNP and EBL ameliorated cadmium stress through improved chlorophyll content and synthesis of antioxidant molecules and decreased translocation of Cd from root to shoot (Yuanjie et al., 2019).

Concluding Remarks and Future Prospects

Agriculture is severely affected by abiotic stress as it negatively influences the physiology and biochemistry of plants by creating nutrient disorders, ionic imbalance and toxicity, oxidative stress, and membrane disorganization altering metabolic processes. However, a well-established antioxidative defense system is present inside the plants to minimize stress-induced toxicity. But in extreme conditions plants cannot survive with stress by this self-made mechanism. So, use of exogenous protectants like plant hormones, signaling molecules, osmoprotectants, antioxidants, etc. proved highly beneficial in enhancing crop tolerance towards abiotic stress. Exogenous application of BR and SNP is popular in research aimed at enhancing abiotic stress tolerance. The mechanism by which BR and SNP regulate enzyme activities is certainly interesting and requires thorough research in crop plants. The direct mechanism of defense and signal transduction pathways is still facing darkness and needs to be discovered.

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Chapter 4
Interaction Between Brassinosteroids and Hydrogen Peroxide Networking Signal Molecules in Plants

Faroza Nazir, Fariduddin Qazi, and Mohd Tanveer Alam Khan

Abstract Brassinosteroids (BRs) and hydrogen peroxide (H_2O_2) are considered as profound signaling molecules that govern a diverse range of fundamental physiological and metabolic processes in plants from germination to senescence as well as mechanisms for adaptation to environmental changes. The physiology of H_2O_2 typically contains numerous possible mechanisms for maintaining the cellular processes of BR, which are implicated in important plant functions and stress responses. This chapter summarizes the overview of current understanding of the signaling of BRs and H_2O_2 and their interplay in modulating plant growth and development, in particular seed germination, root growth, stomatal movement, leaf senescence and fruit ripening. As well as providing an overview of their interaction under diverse abiotic stress factors. More importantly, gene expression by mitogen-activated protein kinases (MPKs), BRASSINAZOLE RESISTANT 1 (BZR1), BRI1-EMS SUPPRESSOR 1 (BES1), SlNAC2 and other transcription factors which modulate abiotic stresses in plants has also been sectioned.

Keywords Abiotic stress · Brassinosteroid · Hydrogen peroxide · Signaling · Transcription factors · Tolerance

Introduction

Plant growth and development is a complicated process, but well interconnected and managed by the intervention of small active molecules such as plant growth regulators (phytohormones). It is also well understood that phytohormones act either

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adjacently or transport to other parts from their sites of synthesis to trigger morpho-physiological, biochemical and/or molecular reactions of plants under favourable or changing environments. Brassinosteroids (BRs) and hydrogen peroxide (H_2O_2) are well defined as signaling molecules in plant cellular processes and plant responses to abiotic stresses (Nazir et al., 2019a, b, 2020a; Hussain et al., 2019). BRs constitute the polyhydroxylated sterol structures occurring in all plant species that are comparatively homologous to steroidal hormone of animals and insects. They were detected by Mitchell and his USDA colleagues in an exploration of pollen extracts of more than 30 species, which has the capability to facilitate cell elongation, but was later revealed in all growing tissues of higher plant species, with the optimal levels observed in pollen, seeds and fruit. Since this *Brassica napus* was the main source of growth-promoting extract, it was labelled as "brassins" and subsequently the biologically active form was identified as brassinolide, which is the most potent component of BRs (Grove et al., 1979). Several findings have shown that BRs have a diverse variety of impacts on morpho-physiological attributes and responses to biotic and abiotic stresses. With the innovation of technologies in functional genetic and proteomic analysis, BR signaling is now becoming one of the most widely accepted plant hormones (Lozano-Durán & Zipfel, 2015; Nolan et al., 2017).

H_2O_2 is acquiring enormously more significance in the context of molecular biology research, with its special physio-chemical attributes, such as spectacular consistency within cells (half time of 10^{-3} s), followed by gradual oxidation of specific proteins. It is a key REDOX (reduction–oxidation reaction) activator and serves as a signal transducer in cellular processes at minute levels to elicit a downstream response (Camejo et al., 2016; Saxena et al., 2016), while inducing the onset of cell death at higher concentrations (Petrov et al., 2015). Under various environmental circumstances, it acts as the principal transducer for the regulation of numerous morpho-physiological processes, such as seed germination, root surface morphology, stomatal behaviour, growth, development and senescence and adaptation to environmental stresses (Černý et al., 2018; Chen et al., 2018; Nazir et al., 2019a, b, 2020a, b).

Brassinosteroid Signaling in Plants

Brassinosteroids, a natural polyhydroxylated steroidal phytohormones, binds to BRI1 (BRASSINOSTEROID INSENSITIVE1), a plasmatic membrane leucine-rich repeat (LRR) receptor-like kinase (RLK) (Belkhadir & Chory, 2006), which interacts with its coreceptor, i.e. BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and phosphorylates many proteins such as BKI1, the BRI1 inhibitor, causing it to detach from the plasma membrane and react with 14-3-3 proteins (Wang & Chory, 2006; Sun et al., 2010; Choudhary et al., 2012). The 14-3-3 proteins further participate in the association and cytoplasmic maintenance of two major transcriptional regulators of BR signaling pathway BZR1 and BES1 (Gampala et al., 2007; Choudhary et al., 2012). Correspondingly, functionalized BRI1 also

phosphorylates BR-kinase molecules (BR SIGNALING KINASE 1) and CDG1 (CONSTITUTIVE DIFFERENTIAL GROWTH 1), both of which eventually trigger BSU1 phosphatase (BRI1 SUPPRESSOR 1), resulting in the deactivation of BIN2 (BRASSINOSTEROID INSENSITIVE 2) via dephosphorylation (Clouse, 2011). BZR1 and BES1 are quickly dephosphorylated by PP2A (PHOSPHATASE 2A) after BIN2 inactivation and consequently separated from 14-3-3 proteins, allowing them to build up into the nucleus, culminating in the modulation of many BR-biosynthetic genes (Luo et al., 2010).

In the apparent lack of BR, BKI1 interacts with the BRI1, protecting it from being associated with its coreceptor BAK1 (Wang et al., 2002; Yin et al., 2002). Additionally the activated BIN2 and 14-3-3 proteins which are associated with BZR1 and BES1 retain their deactivated form and prevent their transport between nucleus and cytoplasm, thus regulating numerous BR responsive genes (Jaillais et al., 2011). Previously, it has been reported that expression of positive regulator of BRI1 degradation, SBI1 (SUPPRESSOR OF BRI1) is increased by BR that aids in the methylation of PP2A and regulates its localization in membrane bound cell organelle. Methylated PP2A causes degeneration and dephosphorylation of BRI1 which leads to termination of BR signaling. This specifies the contribution of PP2A and SBI1 to negative feedback system that activates BRI1 turnover soon after the upregulation of BR signaling pathway is constitutively expressed (Wu et al., 2011). Brassinosteroid signaling has been depicted in Fig. 4.1.

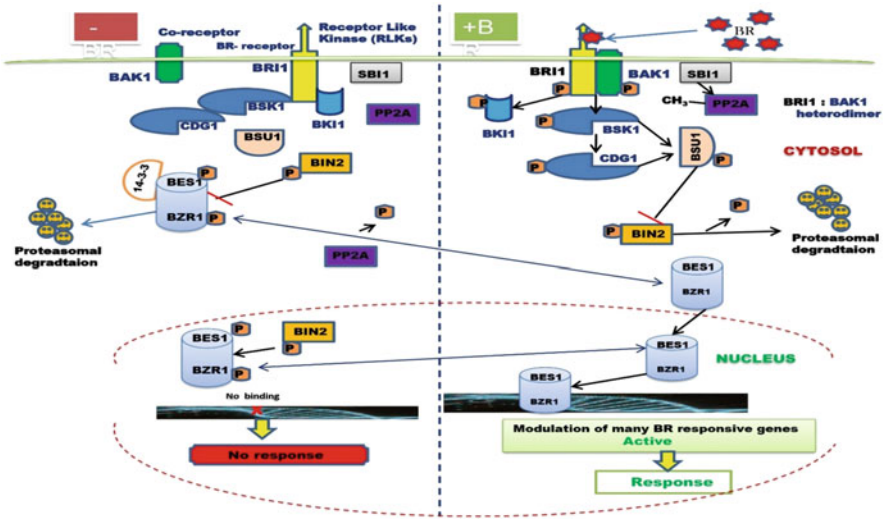

Fig. 4.1 Signaling pathway of brassinosteroid

H_2O_2 Signaling

Being relatively widely dispersed in vivo, H_2O_2 had already been identified as the most versatile signaling ROS in plants. Plants have a diverse spectrum of TFs, protein phosphatases and protein kinases in most intracellular signaling pathways, and it has been proposed that these could act as H_2O_2 transducers (Gonz_alez et al., 2012: Mullineaux et al., 2018). MAPKs are prominent tools to manage environmental hazards and allow extracellular impulses to be changed within the cell (Zhou et al., 2014; Eblen, 2018). In MAPK cascade, Nicotiana protein kinase 1 (NPK1) is amplified to transform extrinsic impulses to gene transcriptional activity for defensive purposes (Desikan et al., 2001). H_2O_2 effectively activated MAPKs in shoots even when applied to the roots (Capone et al., 2004). It was reported that the catabolic genes of ABA were facilitated by H_2O_2 facilitates to regulate and monitor germination and dormancy of seeds (Liu et al., 2010). It has also been reported that H_2O_2 maintained and stimulated the protein levels of Arabidopsis MAPK kinase kinase (MEKK1) in a proteasome reliant manner and thus affectively modulates ROS-triggered induction of the MAPK MPK4 (Nakagami et al., 2006). Deactivation of MAPK3 and MAPK6 is also reported to be induced by MAPK phosphatase (AtMKP2) and, therefore, acts as a promotor in the plants at the cellular level damage (Lee & Ellis, 2007). In response to H_2O_2, MAPK3 and MAPK6 were found to be regulated and to be a possible strategy against ecological and developmental cues that govern the behaviour of stomata (Wang & Song, 2008). Nucleoside diphosphate kinase 2 (NDPK2) can also activate the functions of MAPK3 and MAPK6. Studies of Verslues et al. (2007) revealed that NDPK2 synergizes with the salt stress signaling salt overly sensitive 2 (SOS2) kinase. NDPK2 also binds to catalase, thereby highlighting the significance of H_2O_2 signaling under salinity factor. For oxidative burst-mediated signaling, the oxidative signal-inducible 1 (OXI1) Ser/Thr kinase is needed and was first described to downregulate the signaling component of the phosphoinositide-dependent protein kinase 1 (PDK1). Multiple studies have shown the impact of MAPK signaling in a wide range of plant species in response to HM stress (Opdenakker et al., 2012). The transcriptional levels of *OXI1*, the MAPKKK, "*Arabidopsis* NPK1-like protein kinase 1" (ANP1) and the MAPK homologs MPK3 and MPK6 were adversely impacted in *Arabidopsis thaliana* plants subjected to Cu or Cd stress (Opdenakker et al., 2012). More specifically, the regulation of *CAT1* gene in *A. thaliana* was governed by MAPK signaling (Xing et al., 2007, 2008; Cuypers et al., 2011; Remans et al., 2012). In *Medicago sativa* plants subjected to either Cu or Cd stress, the efficiency of multiple MAPKs was analysed (Jonak et al., 2004). According to transcriptome study, Cu ions influenced the rapid activation of these enzymes, while Cd treatment triggered delayed activation (Opdenakker et al., 2012).

H_2O_2 Mediated Transcription Factors

The signaling cascade is revealed to be mediated by H_2O_2 by activating downstream gene expression levels by switching on or off gene regulating mechanism. H_2O_2-triggered signaling is attributed to a group of transcription factors such as NAC, Zinc Finger (ZINC FINGER OF ARABIDOPSIS THALIANA; ZAT), WRKY, ERF, MYB, DEHYDRATION RESPONSIVE ELEMENT BINDING FACTOR (DREB) and BASIC LEUCINE ZIPPER (bZIP). Many NAC TFs accompanied with H_2O_2 have been outlined in *Arabidopsis*, controlling leaf ageing processes both favourably. *Arabidopsis thaliana* Activating Factor1 (*ATAF1*), an upstream senescence regulator, is revealed to be activated by H_2O_2 and ABA. *ATAF1* mediated senescence by triggering *ANAC092* and suppressing *Golden 2-like1 (GLK1)* genes by changing the physiological stability. Some NAC TFs have been shown to slow down senescence and help to bestow tolerance during environmental stresses. One of the members of the NAC TF family, the JUNGBRUNNEN1 (JUB1) gene, exhibited slow senescence processes and resilience to environmental factors by decreasing H_2O_2 concentration at cellular level. Upregulation of AlNAC4 TF in tobacco bestows resilience to oxidative stress by lowering the content of H_2O_2 through ROS metabolism and monitoring downstream stress-related genes (Khedia et al., 2018). The involvement of *Sl*NAC2 TF in elevated abiotic stress responses was revealed by Borgohain et al. (2019) with the articulation of significant glutathione biosynthetic genes, which exhibited increased antioxidant response and impaired overproduction of ROS (H_2O_2 and $O_2^{\bullet-}$) in transgenic plants.

At transcriptional level, a member of the ZINC FINGER OF ARABIDOPSIS THALIANA (ZAT) family is also efficiently triggered by H_2O_2 (Gadjev et al., 2006). Zinc finger protein ZAT12 is one of the critical facets of cellular damage that transmits the signal to ZAT7 and WRKY25 TFs and activates cytosolic APX1 in *Arabidopsis* during H_2O_2 elevation (Rizhsky et al., 2004). Miller et al. (2008) demonstrated the involvement of AtZAT proteins (AtZAT7, AtZAT10 and AtZAT12) in signaling network of ROS and plant stress reactions. The central function of AtZAT6 in H_2O_2-induced phenolic compounds synthesis, such as anthocyanin, has been illustrated by Shi et al. (2018) via specific binding to anthocyanin synthesis regulatory proteins. PeSTZ1, a C_2H_2-type Zn finger TF in *Populus euphratica*, exhibited low-temperature resilience by direct regulation of *PeAPX2* and had an impact on ROS detoxification (He et al., 2019). Transgenics were found to be more resilient against numerous environmental stresses with improved or repressed ZAT10 levels (Mittler et al., 2006; Rossel et al., 2007). In addition, ZAT10 and ZAT6 also positively govern abiotic stress tolerance by modifying generation of ROS and expressing SA-triggered gene transcription (Shi et al., 2014).

Eulgem and Somssich (2007) have demonstrated the roles of WRKY TFs under stress conditions. The application of H_2O_2 has been revealed to enhance the activation of several WRKYs in *Arabidopsis* (Chen et al., 2010), which govern ageing and gene proliferation for defensive mechanism (Besseau et al., 2012). H_2O_2-stimulated

TFs such as WRKY52 and WRKY7 are widely known, from which WRKY52 is a senescence-related and facilitates enormous stress and defence-related gene processes. Guo et al. (2017) demonstrated the significant contribution of WRKY75 TF to the maintenance of senescence processes in *Arabidopsis* by the involvement of SA and ROS.

Model of H_2O_2 Signaling Pathway

The conceptual framework of H_2O_2 signaling pathway has been depicted in Fig. 4.2. Receptor at the cell surface could perceive H_2O_2 signal leading to an increased $[Ca^{2+}]$. Increased concentration of $[Ca^{2+}]$ cyt could facilitate phosphorylation or dephosphorylation of transcription activators by stimulating the protein signal molecules such as phosphatase or protein kinase (Fig. 4.2). Moreover, H_2O_2 can activate the expressional levels of cyt residue thiols in protein by directly oxidizing H_2O_2-responsive transcription factors. The activated transcription factor impedes with specific promoter with the adjacent cis-acting element in order to exploit expression of genes, associated with tolerance to abiotic stress in plants (Zhou et al., 2013; Eblen, 2018 and Fig. 4.2).

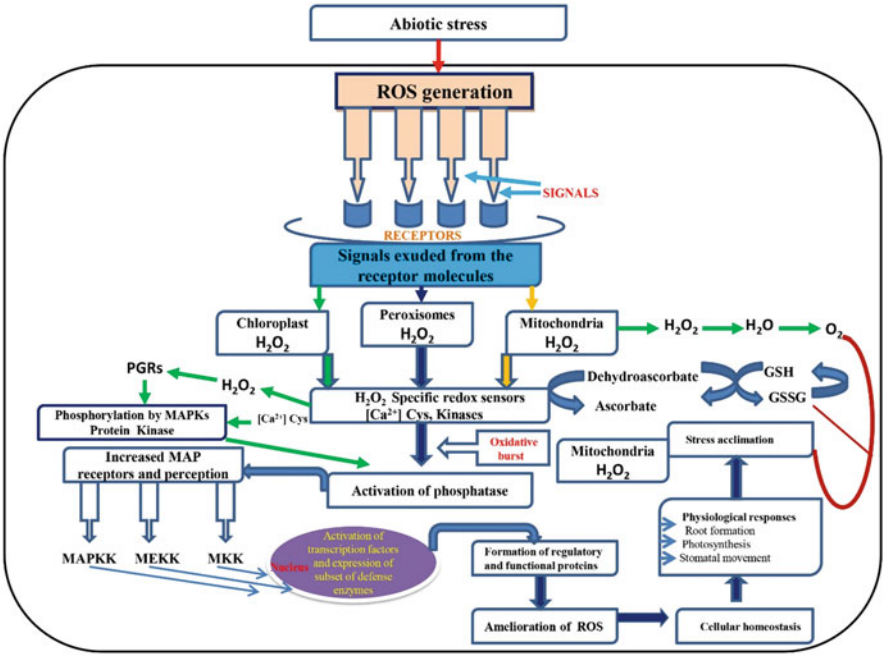

Fig. 4.2 A model of H_2O_2 signaling cascade for heavy metal stress

Interaction of BRs and H_2O_2 During Plant Development

Plant hormonal crosstalk is a challenging subject of wide and global intrigue. H_2O_2 and BRs also have a hormonal intermodulation that performs a major role in the development of plants and environmental adaptation (Zhu et al., 2018; Nazir et al., 2019a). Recent findings have revealed the functionalities of H_2O_2 in BR maintenance of plant development and stress responses. H_2O_2 effectively governs brassinosteroid signaling by triggering the BZR1, which is the key derivative of BR signaling (Tian et al., 2018). BRs have also been revealed to use pathways governed by H_2O_2 to bestow stress tolerance (Xia et al., 2009; Cui et al., 2012). ABA biosynthesis is also induced by BR-mediated transient H_2O_2 generation via NADPH oxidase, which along with enhanced H_2O_2 concentration acts as an effective and significant approach for abiotic stress resilience (Zhou et al., 2014). Oxidative modifications governed by H_2O_2 greatly improve the endogenous levels of BZR1 and facilitate its interplay with ARF6 and PIF4. Conversely, the thioredoxin TRXh5 binds with BZR1 and causes its massive decrease (Tian et al., 2018). Jiang et al. (2012) uncovered the role of H_2O_2 as an integral messenger for metabolic pathways and CO_2 fixation prompted by brassinosteroids via redox signaling in cucumber, thus enhancing the photochemical performance and effectiveness. Elevated concentration of NADPH oxidase in BR-supplied cucumber plants has also accumulated H_2O_2 (Xia et al., 2009), while improved RBOH1 activity has also achieved the same result in tomato (Nie et al., 2013). Similarly, the inhibition of RBOH in *Nicotiana benthamiana* significantly influenced the role of BR-induced AOX and therefore reduced ROS scavenging, making tobacco plants more prone to environmental variables (Deng et al., 2015).

BRs and H_2O_2 Interactions During Seed Germination

Seed germination, which begins with rapid intake of water and ends with the bursting of the seed coat, typically by radicle protuberance, is described as a three-phase process. During embryo development, BRs and H_2O_2 are well understood to facilitate germination and dormancy of seeds, which provides a defensive system under unfavourable environments (Waisi et al., 2017; Hong-juan et al., 2017 and Table 4.1). This is due to the increase in the accumulation of protein and lipid by BR in seeds, as well as the stimulation of α-amylase and the promotion of programmed cell death (PCD) by H_2O_2 in the aleurone layer, which increases the oxidation and dormancy of seeds whose germination is therefore hindered (Steber & McCourt, 2001). Likewise, BR-induced production of H_2O_2 results in BR-promoted seed germination, cellular differentiation and QC cell division in *Arabidopsis* seedling development (Tian et al., 2018).

Table 4.1 Hormonal interaction and response in different plant species

Plant development processes	Species	Hormonal interaction and their response	References
Seed germination	*Zea mays*	BRs and H_2O_2 are well known to facilitate seed germination and seed dormancy, due to the increase in the accumulation of protein and lipid by BR in seeds, as well as the stimulation of α-amylase by H_2O_2 in the aleurone layer	Waisi et al. (2017)
Root growth	*Arabidopsis*	BR-induced production of H_2O_2 results leading to BR-triggered cell differentiation and cell proliferation	Tian et al. (2018)
	Arabidopsis	Cross-regulation of hormones between BRs and ROS is implicated in regulating growth and development of roots	Lv et al. (2018)
Stomatal behaviour	*Solanum lycopersicum*	Transient production of H_2O_2 was induced by treatment with less than 0.1 μM of 24-epibrassinolid and the redox status of glutathione in guard cells changed, contributing to the opening of the stomata. Conversely, treatment with 1 μM of 24-epibrassinolide triggered H_2O_2 accumulation, which stimulated the closure of the stomata	Xia et al. (2014)
	Solanum lycopersicum	Exogenously sourced EBL and H_2O_2 improves stomatal conductivity and it is assumed to be mediated by the interplay of H_2O_2 and BR which could have resulted in osmotic adjustments that inevitably stimulated the opening of stomata	Nazir et al. (2019b, 2020b)
Leaf senescence	*Arabidopsis*	BR interacts interdependently with H_2O_2 to govern senescence processes by stimulating the calcium and calmodulin signaling and senescence-regulating mechanisms	He et al. (2001)
	Arabidopsis	BR-triggered BZR1 stimulation along with ATBS1-INTERACTING FACTOR 2 (AIF2) repression could provide valuable responses to facilitate the initial onset of dark-triggered leaf senescence	Kim et al. (2020)
Fruit ripening	*Vitis vinifera*	EBL and H_2O_2 promote colour development and trigger the early ripening due to increase in accumulation of β-carotene, ascorbic acid concentration and galactono-1,4-lactone dehydrogenase	Babalık et al. (2020)

BRs and H_2O_2 Interactions in Root Growth

Roots are a significant subterranean component of the vascular plants with two primary functions: plant soil fixation and water and nutrient uptake and are therefore important for optimal physiological processes of the entire plant. Interplay between BRs and H_2O_2 has been reviewed by Lv et al. 2018. In their work, an *Arabidopsis*

mutant (det2-9) was detected upon EMS mutant screening showing deformity in the synthesis of BR relied on its short-root phenotype and was used to explore the hormonal interplay between BRs and ROS in modulating the growth and developmental processes of roots in *Arabidopsis* (Table 4.1). In the det2-9 mutant, since both ROS and BR signalings have been enhanced, it was speculated that the short-root phenotype was caused by the hyper-accumulation of BR and ROS ions. Exogenous BR application also regulates the concentration of H_2O_2 in the root stem cell niche, leading to BR triggered cell division and cell proliferation (Tian et al., 2018 and Table 4.1). Overall, in the root growth modification, the spatial distribution of H_2O_2 is analogous to that of nuclear BZR1, compatible with H_2O_2 modifying BZR1 activity. The spatial pattern of H_2O_2 relies on the basic/helix-loop-helix transcription factor UPBEAT1 (UPB1), which controls the activity of antioxidant enzymes and growth rate in roots (Tsukagoshi et al., 2010), whereas the specific function of H_2O_2 in QC seems unknown. In response to DNA damage stress, BZR1 has been shown to facilitate the QC division (Vilarrasa-Blasi et al., 2014). Improving the transcription activity of BZR1 by H_2O_2 could effectively provide dual regulation of root growth by BR and redox signals, substantial for proper growth and development by extracellular and intracellular signals. Based on the concentration applied, exogenously sourced BRs either explicitly or implicitly monitored the biosynthesis of H_2O_2. The production of H_2O_2 was reduced slightly in plants supplied with a low level (10^{-6} M) of 28-homobrassinolide (HBL), whereas treatment with higher levels of HBL (10^{-9}, 10^{-12} M) resulted in a massive reduction (Kaur et al., 2018).

BRs and H_2O_2 Interactions in Stomatal Movement

Stomatal movement is a fundamental factor in maintaining the plant physiological processes such as photosynthesis and water use efficiency (WUE) (Antunes et al., 2017). Recent research suggests that H_2O_2 plays a role in BR regulation of stomatal responses. In *Solanum lycopersicum*, plants treated with H_2O_2 (0.1 mM) as root dipping and EBL (10^{-8} M) as foliar spray improve stomatal conductance and it is assumed to be mediated by the interplay of H_2O_2 and BR which could have resulted in osmotic adjustments that inevitably stimulated the stomatal opening (Nazir et al., 2019a, 2020b and Table 4.1). In BR-mediated stomatal movement, hydrogen peroxide functions as a rate-limiting signaling molecule.

In tomato, the transient production of H_2O_2 was induced by treatment with less than 0.1 μM of 24-epibrassinolid and the redox status of glutathione in guard cells changed, contributing to the opening of the stomata. Conversely, treatment with 1 μM of 24-epibrassinolide triggered H_2O_2 accumulation, which stimulated the closure of the stomata (Xia et al., 2014 and Table 4.1). Downregulation of H_2O_2 concentration by chemical substances, such as diphenylene iodonium (DPI) or ascorbic acid (Doussière & Vignais, 1992), restricted BR-triggered stomata closure, implying a critical role for H_2O_2 in BR-caused stomata closure. Low level of BR causes a transient increase in the production of H_2O_2 and changes the cellular redox

equilibrium in guard cells, contributing to the opening of stomata, whereas the high level of BR induces an excess concentration of H_2O_2, which allows for stress responses and stomata closure (Li et al., 2020). Zhang et al. (2001) reported the reversible effects of H_2O_2 on stomatal physiology at concentrations below 10^{-5} M, whereas those effects were irreversible at concentrations above 10^{-5} M. This implies that the impact of H_2O_2 at low concentrations may be due to the action of the signaling cascade, whereas the effects of H_2O_2 at high concentrations may be due to changes in membrane integrity. H_2O_2 and BR cumulatively elicit stomatal opening in *Arabidopsis* by facilitating the degradation of guard cell starch (Li et al., 2020).

BRs and H_2O_2 Interactions in Leaf Senescence and Fruit Ripening

Leaf senescence and fruit ripening are the fundamental processes accompanied by myriad morphological and metabolic changes attributed to effective metabolic pathways of ROS (Shi et al., 2015; Corpas et al., 2018) in which both H_2O_2 and BR act as regulators. The findings showed that during natural leaf senescence, H_2O_2 content gradually decreases (Mondal & Choudhuri, 1981), thus illustrating earlier data suggesting that anti-senescence characteristics are caused by the exogenous application of H_2O_2. Shi et al. (2015) reported opening and senescence in *Paeonia suffruticosa* plants pre-treated with 0.01 M H_2O_2 for 12 h. Furthermore, by improving BR biosynthesis and *PIF4 (PHYTOCHROME INTERACTING FACTOR 4)* gene expression, which then facilitates BR/H_2O_2 biosynthesis/signaling pathways, BR-triggered BZR1 stimulation along with ATBS1-INTERACTING FACTOR 2 (AIF2) repression could provide valuable responses to facilitate the initial onset of dark-triggered leaf senescence (Kim et al., 2020). In Arabidopsis, BR interacts interdependently with H_2O_2 to govern senescence processes (He et al., 2001 and Table 4.1) by stimulating the calcium and calmodulin signaling and senescence-regulating mechanisms (He et al., 2001: Song et al., 2016; Dai et al., 2018).

Emerging evidence suggests that the cellular metabolism of reactive oxygen and nitrogen species are triggered during the maturation of climatic and non-climatic fruits (Corpas et al., 2018; Fuentes et al., 2019). Tomatoes, typical climatic fruits, have been used as a modelling tool to analyse the influence of BRs and H_2O_2 during maturation. Modifications in the gene regulation of BR synthesis have been reported during the development of tomato fruit, implying that BRs may play a major part in this phenomenon. This was supported by other studies in which tomato plants treated with BL had lower total chlorophyll content and higher content of lycopene in their fruits, while H_2O_2-treated fruit reported lower chlorophyll degradation and lower lycopene content than non-stressed or BR-supplied tomato plants. Generally speaking, BL supplementation boosted tomato fruit ripening, whereas H_2O_2 deferred maturation (Bayoumi, 2008; Zhu et al., 2015). Extensive studies recently concentrated on non-climacteric fruits and the influence of EBL and H_2O_2 on their

maturation. In these experiments, grape berries were selected and were supplied with an exogenously sourced EBL and H_2O_2. The results imply that the EBL and H_2O_2 promote colour development and trigger the early ripening due to enhanced activity of bio-active compounds, cellulase and polygalacturonase and increased the accumulation of β-carotene and ascorbic acid concentration (Rodriguez-Ruiz et al., 2017; Guo et al., 2019; Babalık et al., 2020). As a result, during the ripening phases of grape (*Vitis vinifera* L.) fruits, a decline in concentration of NO is attributed to a high level of protein nitration, particularly catalase, which influences the concentration of H_2O_2 (Chaki et al., 2015; Rodriguez-Ruiz et al., 2017). H_2O_2 supplementations (0, 5 or 15 mM) have usually been shown to be effective in postharvest period of tomato fruits (AL-Saikhan & Shalaby, 2019). Cumulative application of H_2O_2 (0.1 mM) as root dipping treatment and foliar spray of EBL (10^{-8} M) also enhanced the content of lycopene and β-carotene in tomato fruits by modulating the antioxidant defence system, where 10^{-8} M proved best (Nazir et al., 2019a, 2020b). As a result of higher levels of antioxidant activity, the improvement of the lycopene and β-carotene through BRs and H_2O_2 boosted the profitability of the fruit.

Interactions Between BRs and H_2O_2 During Various Abiotic Stress Responses

In addition to playing a significant role in growth and development of plants, both hormones (BRs and H_2O_2) are also well understood to be implicated in the regulatory response to abiotic stress (Nazir et al., 2019a, b, 2020a, b). Table 4.2 and Fig. 4.3 highlight key interactions between BRs and H_2O_2 under different abiotic circumstances.

The upregulation of mitogen-activated protein kinases (MAPKs) is one of the examples of how these two hormones interact with each other during abiotic stress as mentioned in the work of Zhu et al. (2016). In this research, tomato plants were subjected to salt stress. Supplementation with most active BR (BL) has led to an improvement in the H_2O_2 generation, possibly triggering mitogen-activated protein kinases (MAPKs) (Fig. 4.3), which could improve ACS stabilization and thus boost the generation of aminocyclopropane-1-carboxylic acid (ACC 1) and in turn have an impact on the efficacy of ethylene in tomato environmental stresses. Further analyses were formulated to explore the role of BL and ROS in the BL-triggered AOX capability in *Nicotiana benthamiana* under cold, PEG and high-light stress. In this analysis, BRs triggered ROS production, which therefore improved the efficiency of AOX. Improved AOX activity can detoxify surplus ROS production to prevent oxidative damage to plant cells and increase their resilience to stress (Deng et al., 2015 and Fig. 4.3).

The leaves are the main blogs for agricultural products and the stomata are the main points of entry for controlling the gaseous exchange. Consequently, stomatal performance (stomatal pore opening and closing) is an essential tool in fostering

Table 4.2 Interplay of brassinosteroids and hydrogen peroxide during various abiotic stresses

Abiotic stress	Species	Applied hormones	Hormonal interaction and their response	References
Salt	*Solanum lycopersicum*	BL	Plants supplied with BL (the most active BR) has led to an improvement in the H_2O_2 generation, possibly triggering mitogen-activated protein kinases (MAPKs), which could improve ACS stabilization and thus boost the generation of ACC 1 (aminocyclopropane-1-carboxylic acid) that lead to salt tolerance	Zhu et al. (2016)
Cold, PEG and high-light	*Nicotiana benthamiana*	BL	BRs triggered H_2O_2 production, which therefore improved the efficiency of AOX. Improved AOX activity can detoxify surplus ROS production to prevent oxidative damage to plant cells and increase their resilience to stress	Deng et al. (2015)
Cu	*Solanum lycopersicum*	EBL and H_2O_2	Root dipping treatment of H_2O_2 and foliar spray of EBL resulting in stomatal opening under copper stress, which was characterized by improved stomatal size and stomatal performance	Nazir et al. (2020a)
Light	*Arabidopsis*	BR and H_2O_2	BR and redox signal H_2O_2 synergistically cause the hydrolysis of starch in guard cells, which facilitates stomatal opening	
Ni and cu	*Solanum lycopersicum*	EBL and H_2O_2	Root dipping treatment of H_2O_2 (0.1 mM) and foliar treatment of EBL (10^{-8} M) alone or in combination to strengthen root morphology, but the cumulative treatment of H_2O_2 plus EBL triggered the most favourable response in plants, suggesting that these hormones may have integrating ameliorating effects	Nazir et al. (2019b, 2020b)
Low temperature	*Lycopersicon esculentum*	EBL and H_2O_2	BR and H_2O_2 induced antioxidant activity and reduced electrolyte leakage, thus limiting the harmful effects of oxidative stress	Khan et al. (2019)

plant processes such as rate of transpiration and photosynthesis (Antunes et al., 2017). Stomatal behaviour could be affected by abiotic variables including salt, temperature and HMs (Xu et al., 2016; Sehar et al., 2019; Nazir et al., 2019a, 2020b). Numerous plant hormones that indulge in a vast network of signal transduction govern stomatal movement. ABA (abscisic acid) is the widely understood plant hormone associated with stomatal behaviour, but the latest study of Nazir et al. (2020b) has revealed that BRs and H_2O_2 also impact this phenomenon (Nazir et al., 2020b). In tomato, the application of H_2O_2 (0.1 M) as root dipping treatment and

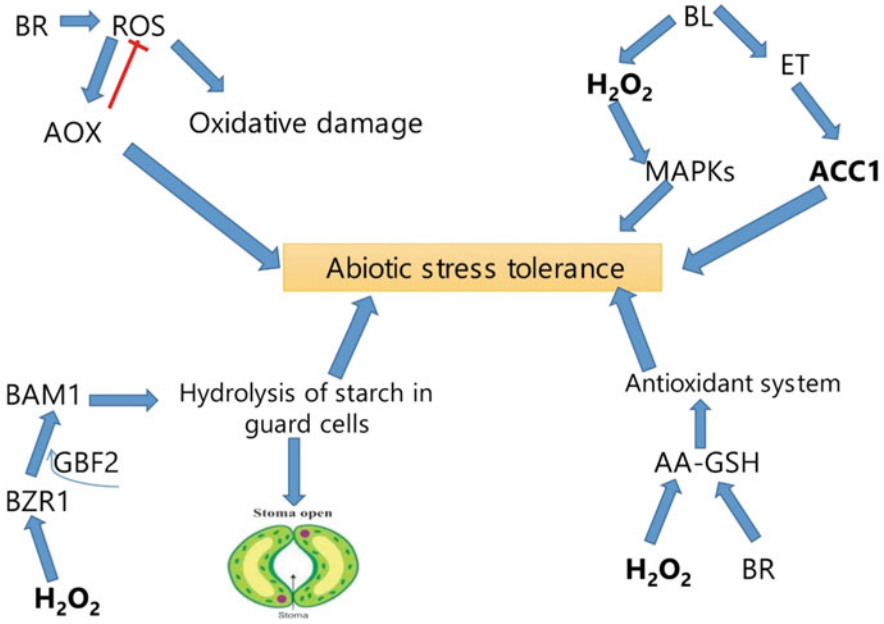

Fig. 4.3 Interactions between BRs and H_2O_2 during various abiotic stress responses

EBL (10^{-8} M) via foliar spray resulting in stomatal opening under copper stress, which was characterized by improved stomatal size and stomatal performance (Nazir et al., 2020b). Brassinosteroid and redox signal H_2O_2 synergistically cause the hydrolysis of starch in guard cells, which facilitates stomatal opening in response to light stress (Li et al., 2020). In this study, BRASSINAZOLE-RESISTANT1 (BZR1) binds with the G-BOX BINDING FACTOR2 (GBF2) basic leucine zipper transcription factor to stimulate *β-AMYLASE1 (BAM1)* expression, which is responsible for the breakdown of starch in guard cells. BZR1 oxidation is mediated by H_2O_2, which promotes the interplay between BZR1 and GBF2 to boost the expression of BAM1 (Fig. 4.3). Abnormalities in BAM1 result in the accumulation of starch and downregulate the impact of the BR and H_2O_2 on stomatal opening. In addition, the findings of Shi et al. (2015) have shown that BRI1 kinase domain mutation resulting in decreased BR responsiveness has a significant impact on the closure of the stomata and that this process requires a functional BRI1 receptor. In addition, it has been shown that H_2O_2 (a form of ROS) is involved in BR triggered stomatal closure as signaling molecules and also requires a functional BRI1 receptor in *Arabidopsis* to generate H_2O_2 stimulated by BRs in guard cells. Overall, the findings addressed that Gα-(G protein α-subunit) serves as a key factor and modulates the intervention of ethylene in BR-triggered stomatal closure. It also implied that H_2O_2 triggered NO generation in the case of BR-triggered stomatal closure. It has been reported that the closure of stomata caused by the exogenous supplementation of BR and H_2O_2 is due to the reduction in K^+ concentration and the

modulation of K^+ withdrawal at the plasma membrane of guard cell via K^+ channels (An et al., 2016). This suggests that low-dose influences of BR and H_2O_2 may be due to the activation of signaling cascade, while high-level impacts of BR and H_2O_2 may be due to altered membrane integrity.

One of the most important plant parameters, dry mass and surface root morphology, is influenced by different environmental stresses. Nazir et al. (2019b, 2020b) analysed the role of EBL and H_2O_2 on the dry mass of shoot and root and surface root morphology under Cu and Ni stress and reported that the toxic effects of Cu and Ni were significantly ameliorated by the root dipping treatment of H_2O_2 (0.1 mM) and foliar treatment of EBL (10^{-8} M) alone or in combination to strengthen root morphology, but the cumulative treatment of H_2O_2 plus EBL triggered the most favourable response in plants, implying that these growth regulators may have an integrating ameliorating impact on dry mass of shoot and root and on root surface morphology under Cu or Ni stress.

Zhu et al. (2016) recently published an extensive work on the cumulative impacts of BRs and H_2O_2 under salt stress in tomato plants. In this study, the pathway by which BRs elicit salt resilience in plants has been evaluated. Higher concentration of H_2O and ethylene in BL-treated tomato seedlings has been reported, implying that H_2O and ethylene are correlated with BR-triggered stress resilience and thereby support production of H_2O. Depending on the outcomes of the study, a model was formulated under salt stress between BRs, ethylene and ROS. The model evaluated that BRs have an adverse impact on ethylene biosynthesis and signaling by stimulating the production of ethylene synthesis hormone (ACS) and ethylene-insensitive 3-like, ethylene transcription factor family (EILs) which are at least partially prompted by BR-triggered H_2O production. Elevated concentration of both ethylene and H_2O also significantly contributes to salt stress resilience (Zhu et al., 2016).

Numerous environmental stresses in plants may lead to oxidative stress. Elevated concentrations of $O_2\bullet^-$ and H_2O_2 under abiotic stress conditions may also be induced by the upregulation of a large number of significant substrate oxidases and NAD(P)H oxidases leading to disturbances in electron transport chains of chloroplasts and mitochondria through the Mehler reaction (Halliwell, 1999). However, the application of EBL and H_2O_2 alone or in combination reduced the levels of $O_2\bullet^-$ and H_2O_2 radicals (Nazir et al., 2020b) under Cu stress. It is believed that BR and H_2O_2 induced antioxidant activity and reduced electrolyte leakage, thus limiting the harmful effects of low-temperature stress (Khan et al., 2019). Ascorbic acid also has other physiological functions, such as enhancing photosynthetic efficiency and cell proliferation in plants. Both BR and H_2O_2 have been shown to alter the levels of ascorbic acid-glutathione (AA-GSH) in tomato plants (Fig. 4.3). Nazir et al. (2020b) have revealed that during the regulation of AA content in tomato leaves, BRs and H_2O_2 signaling pathways act interdependently, i.e. both EBL and H_2O_2 decreased AA concentration in tomato leaves. However this additive effect of the AA content appears to occur via dependent manner, i.e. normal H_2O_2 signaling is needed for the BR response and intracellular BRs are also fundamental for the H_2O activity.

BR, H_2O_2 and Gene Expression

It is very important aspect to provide genome-wide analyses that recognize gene families with prominent role in oxidative stress to better understand the stress response mechanism and expedite molecular breeding. It is also understood that hormonal signaling pathways interrelate at the level of gene expression. Studies, for instance, show that there is a major similarity between the H_2O_2 and BR responsive gene sets (Tian et al., 2018). Typically, H_2O_2-repressed prevalent target genes are also suppressed by BRs, and genes triggered by H_2O_2 are also triggered by BRs, implying collaboration between signaling pathways. Taken together the results demonstrate that H_2O_2 and brassinosteroid signaling pathways often converge on a set of basic target genes. Importantly, an underlying mechanism for this convergence has been articulated in which brassinosteroid-regulated BIN2 kinase improves cellular H_2O_2levels, and elevated H_2O_2 elicits oxidative modification of BZR1 (BRASSINAZOLE-RESISTANT1) and BES1 (BRI1-EMSSUPPSSOR1), the main BR signaling transcription factors. By promoting its interaction with PIF4 (PHYTOCHROME INTERACTING FACTOR4) and ARF6 (AUXIN RESPONSE FACTOR6), the oxidative modification improves the transcriptional activity of BZR1 and promotes root meristem development (Lv et al., 2018; Tian et al., 2018). Genome-wide data found that H_2O_2-dependent BZR1 activity regulation plays a critical role in the modification of gene expression associated with several BR-mediated cellular mechanisms. Additionally, genome-wide analysis of Cucurbitaceae species was based on dehydrin genes that encode dehydrins—hydrophilic proteins behave as molecular chaperons that perform a significant role in abiotic stress response (Lee et al., 2017). Both of these analyses could have been important for the future breeding of new cultivars with stress resilience.

In addition to the transcription factors associated with plant reactions to different stressful environments, an innovative orthologue (*Sl*NAC2) of the H_2O_2 response factor gene has been detected in tomato (*Solanum lycopersicum* L.), the gene encodes a nuclear sited protein that plays a significant role as a transcription factor in stressful conditions. Although in additional experiments, transgenic Arabidopsis with the transferred *Sl*NAC2 gene showed regulated osmotic stability and antioxidant defence (glutathione metabolism) activities in response to salt and drought stress (Borgohain et al., 2019). Van Beek (2018) suggested the capability of *Sl*NAC2 in agriculture in order to ensure crop resilience for drought.

Conclusion

To summarize, the interplay between BRs and H_2O_2 plays an important role during all developmental phases of the plant life cycle, as well as during abiotic stresses. In this chapter, we reviewed the signaling of BRs and H_2O_2 and their interaction in mediating plant growth and development especially seed germination, root growth,

stomatal movement, leaf senescence and fruit ripening. As well as it summarizes their interaction during various abiotic stress conditions. More recently, gene expression by mitogen-activated protein kinases, BES1, BZR1, SlNAC2 and other transcription factors which attenuate abiotic stresses in plants has also been dissected. In conclusion, the interaction between BRs and H_2O_2 plays a significant role during all phases of development of the plant life cycle as well as during environmental stresses. In this chapter, we assessed the signaling of BRs and H_2O_2 and their interrelations in the modulation of plant growth and development, in particular, seed germination, root growth, stomatal movement, leaf senescence and fruit ripening. As well as elucidating their interactions under varying abiotic stress. More importantly, the expression level of mitogen-activated protein kinases, BES1, BZR1, *Sl*NAC2 and other transcription factors that modulate abiotic stress in plants has also been explored. Conclusively, phytohormonal crosstalk is a challenging subject in which numerous interactions remain unclear and require further investigation using innovative approaches such as genome-wide epigenetic evaluation or transcriptome analysis of plants after treatment with BR or H_2O_2 could help us understand the mechanism of interaction between these fundamental regulators of plant growth. The possibility of addressing the synergistic and antagonistic cross-talks of major plant hormones such as BRs and H_2O_2 gives us an immense contribution to enhancing stress resilience and production of fundamental agricultural crops.

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Chapter 5
Brassinosteroids and Strigolactone Signaling in Plants

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Abstract The significant versatility of plant development is influenced by hormone pathways, which not only establish developmental programs in plants, but also impart environmental inputs. Strigolactones (SLs) comprise the newly found group of plant hormones that were initially established for their function in rhizospheric parasitic and symbiotic association. SLs are recognized for their functioning in branching of shoot; however, lately, their role in various other features of plant growth has come to light. In recent years, comprehensive biosynthetic pathway of SL has been divulged and various elements of its signaling have been recognized. Although biosynthesis of SL is thoroughly described, little was revealed about the mechanisms of SL signal transduction in plants. Specific features of SL signal transduction, involving association of F-box protein and receptor by hormone, repression of protein degeneration, and stimulation of transcription factors too are noticed in plant hormones. But, several characteristics of SL signal transduction appear to be distinct for the signaling pathways of SL which involve the SL receptor enzymatic action and its SL mediated damage.

Brassinosteroids (BRs) are requisite for plant growth and reactions to several abiotic stresses. Signaling of BRs via BRI1 (plasma membrane receptor) and co-receptor BAK1 and various positive (BSK1, BSU1, PP2A) and negative (BKI1, BIN2 and 14-3-3) regulators to the transcription factors, BES1 and BZR1 actions, control several genes expressions for numerous BR responses. Recently several new signaling components in signaling pathways were identified and elaborated BR signaling regulation mechanism is being established. Identification of target genes of BES1 and BZR1 organized a transcriptional network for BR response and crosstalk with other signaling pathways. Mechanism of modulation of developmental processes and BR biosynthesis was also revealed recently. In this chapter, we provide a review and discussed some recent advances in the regulation of BR and SL signaling pathways.

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Introduction to Strigolactone and Brassinosteroid

Plants utilize chemical signals, like phytohormones, to regulate growth and other metabolic processes and also reactions to environmental cues. Besides, these signals are crucial for plant interaction with other living organisms. Strigolactones (SLs) are peculiar due to their function as both plant growth regulator and as signaling molecule (Siame et al., 1993; Gomez-Roldan et al., 2008; Umehara et al., 2008). SLs form divergent group of lactone phytohormone which are derived from carotenoid and were first perceived on the basis of their role in triggering the seed germination (Cook et al., 1966) and stimulating synergism among plants and arbuscular mycorrhizal (AM) fungi (Akiyama et al., 2005). These phytohormones are related to an expanding number of their modulating functions in the development of plants, comprising seed germination, structural patterns of root and shoot, accretion of nutrients, symbiotic and parasitic association and also regulate plant resistance to abiotic and biotic stresses (Omoarelojie et al., 2019). About 20 naturally occurring SL derivatives have been reported till date which execute their abundant role in plant growth (Al-Babili & Bouwmeester, 2015; Obando et al., 2015). SLs were first recognized as modulators of plant branching (Umehara et al., 2008); however, they are also identified as regulators of root growth (Sun et al., 2016b), reactions to nutrient deficiency (Sun et al., 2016a), and leaf senescence (Yamada & Umehara, 2015), additionally, they also participate in plant reactions to biotic stresses (Marzec & Muszynska, 2015).

Mutant analysis of *Arabidopsis thaliana* L., *Oryza sativa* L., *Pisum sativum* L., and *Petunia hybrida* L. has facilitated discovery of main proteins that participate in synthesis and signaling of SL. SL synthesis begins with the transformation of all-*trans*-b-carotene into carlactone (CL) which occurs inside plastids and requires two carotenoid cleavage dioxygenases and carotenoid isomerase (Alder et al., 2012). After its conversion, it is transported into the cytoplasm, where CL is converted into carlactonic acid by MAX1-type monooxygenases, which is then transformed into 5-deoxystrigol or orobanchol, which are predecessors of other SLs (Seto et al., 2014). SLs are composed of tricyclic lactone (ABC ring) which are associated with butenolide group (D-ring). Among all SLs, C-D part is conserved, while the A-B rings are exposed to alterations like methyl, hydroxyl, and acetyloxy group substitutions. Contrarily, information about the SL signaling remained insubstantial. However, recently various advancements were made that revealed the mechanism of SL signaling and elements involved in perception of SL, conversion of signal, and downstream responses in plants.

Brassinosteroids (BRs) polyhydroxylated plant steroid hormones playing role in various aspects of developmental processes of plants like cell multiplication and elongation, vascular differentiation, leaf senescence, and reactions to environmental cues (Clouse, 2011; Zhu et al., 2013). Light modulate functions of BR and their

responses are regulated by interaction with other plant growth regulators. Molecular studies have elucidated the BR signaling pathway from membrane bound receptor to nuclear transcription factors (TFs). In the past few years, the mechanisms involved in modulation of BR gene expression have begun to be divulged. In this chapter, we provide some of the insights of pathways of BR and SL signaling and emphasize on current discovery of the process and networks for BR and SL mediated gene expression.

Strigolactone Signaling Mechanism

Signal Perception

Studies of insensitive mutants of SL facilitated recognition of its possible receptors in several plants like D14 (rice; Arite et al., 2009), AtD14 (*Arabidopsis thaliana*; Waters et al., 2012), DAD2 (petunia; Hamiaux et al., 2012), HvD14 (*Hordeum vulgare* L.; Marzec et al., 2016), and PtD14 (*Populus trichocarpa*; Zheng et al., 2016). These belong to family a/b-hydrolase and are capable of in vitro binding and hydrolyzing SL molecules (Nakamura et al., 2013). It was revealed that GR24 (synthetic SL) can be hydrolyzed by D14 proteins into ABC- and D-ring parts (Xiong et al., 2014). It has also been revealed MeCLA, SL similar molecule, although lacking canonical four-ring structure might associate with the AtD14 and be hydrolyzed under in vitro conditions (Abe et al., 2014). Hydrolase activity of conserved catalytic triad, Ser-His-Asp (S-H-D) imparts enzymatic activity of D14 proteins. Furthermore, this conserved triad appears significant for the function of D14 proteins since its mutations at the Ser residue do not supplement mutant phenotype, as reported in petunia (Hamiaux et al., 2012). Subsequently it was suggested that during the binding or hydrolysis of SL, D14 experiences conformational changes allowing SL signaling. Supporting this view, GR24 causes thermal disintegration of intact catalytic triad of D14 (Hamiaux et al., 2012; Abe et al., 2014; Waters et al., 2015). The extent of destabilization is increased by MAX2/D3 as GR24 aids in the association between D14 and MAX2/D3 (Hamiaux et al., 2012; Zhao et al., 2014, 2015). Attachment of D14-D3 in rice is much sensitive to GR24 2'*R* stereoisomers than to 2'*S* stereoisomers (Zhao et al., 2015). Although confirmation for an allosteric signaling model is missing, as there is a considerable lack of crystal structural differences of apo-D14 and D14 in coordination with entire SL, 2,4,4,-trihydroxy-3-methyl-3-butenal, or 5-hydroxy-3-methylbutenolide (Zhao et al., 2013, 2015). Upon binding of SL molecule to the D14/DAD2, its ABC segment gets detached from the D-ring by nucleophilic attack (Scaffidi et al., 2012) and brings about the conformational change in D14/DAD2 (Nakamura et al., 2013), which takes part in its association with various elements of the SL signaling (Zhao et al., 2015). A cap established by four helicases incompletely covers the binding pocket of D14/DAD2 (Kagiyama et al., 2013). It is revealed the loss-of-function in barley mutant *hvd14.d* may be because of the decrease in gap

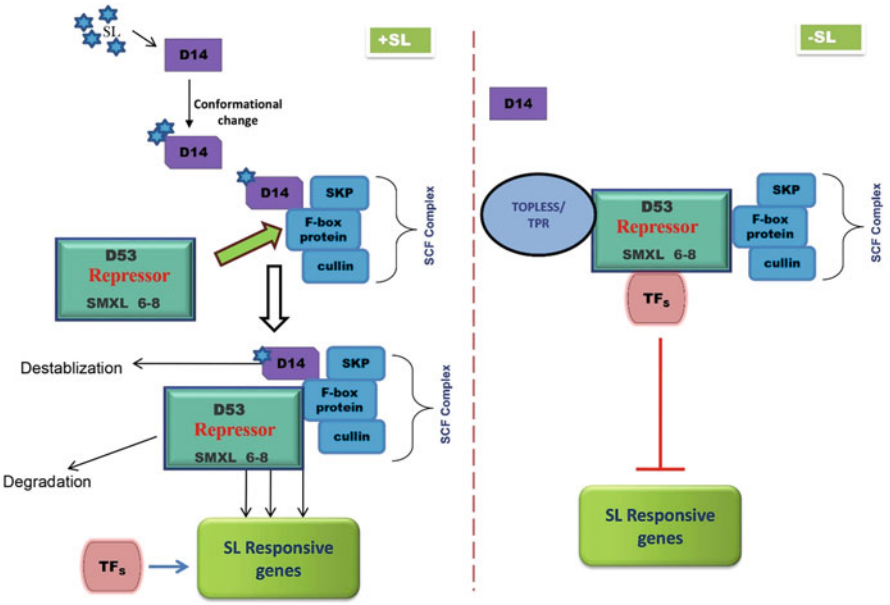

Fig. 5.1 SL Signaling pathway, SL receptor D14 recognize the SL molecules
Receptor hydrolyses SL molecules causing conformation changes of the D14 protein. In the presence of SLs, the receptor is able to bind the F-box protein (MAX2/D3) from the SCF complex and the SL repressor (D53/ SMXL6-8). The receptor is degraded in the proteasome, and receptor is destabilized because of its changed conformation. Degradation of repressor allows the expression of TFs which stimulates SL responsive genes

of bonding sac of the D14/DAD2 protein (Marzec et al., 2016). It must be emphasized that D14/DAD2 protein is a peculiar SL, as it does not recognize other structurally similar SLs (Waters et al., 2012). Characteristics by which the D14/DAD2 receptor perceives and hydrolyses various SL compounds are dependent on the stereoscopic structures of SL compounds (reviewed by Flematti et al., 2016) that play an essential part in SLs recognition and plant responses. The general account of SL signaling is summarized in Fig. 5.1.

SL Signal Transduction

Like other plant hormone signaling mechanisms, SL signaling also entails hormone-generated ubiquitination and degeneration of distinct substrate proteins (Wang et al., 2015). Notably the F-box protein MAX2, (SKP1–CULLIN–F-BOX (SCF) ubiquitin ligase protein complex), emerges as an essential player of SL-induced degeneration of proteins (Zhao et al., 2014). Each signaling molecule or hormone has its own F-box protein component because it manifests peculiarity to the entire CSF complex. F-box protein is involved in the ubiquitination and proteasomal degradation of

proteins. In *max2* and *d3* (mutants of *Arabidopsis thaliana* and rice, respectively) an F-box protein that participated in SL signaling was also recognized to be part of an SCF ubiquitin ligase protein complex (Ishikawa et al., 2005). MAX2 in *Arabidopsis thaliana* associates with SCF, AtCullin1, and ARABIDOPSIS SERINE/THREONINE KINASE 1 (ASK1), while D3 protein of rice forms complex with OsCullin1 and ORYZA SATIVA SKP1-LIKE1/5/20 (OSK1/5/20) (Zhao et al., 2014). Like other elements of the SLs signaling, MAX2/D3 is also located inside nucleus and the models of gene expression encoding this complex were alike to those detected for *D14/DAD2* (Zhao et al., 2014). MAX2/D3 and D14/DAD2 interaction was experimentally corroborated, and it was revealed to be elevated by SL presence (Zhao et al., 2014). In rice protoplasts, bimolecular fluorescence complementation analysis affirmed a GR24-interceded association between D3 and D14 in the nucleus (Zhao et al., 2014). Attributes of this association are dependent on the SL concentration and are mediated by SL and SL stereoisomers involved (Zhao et al., 2015).

Recently, various proteins were identified which serve as repressors of SL signaling, among these proteins were rice D53, belonging to class I Clp ATPase family protein, and its orthologs in *Arabidopsis*, SMXL proteins (SMXL6/7/8), located inside the nucleus (Soundappan et al., 2015; Liang et al., 2016). Ethylene-responsive element binding factor-associated amphiphilic repression (EAR), a highly conserved region of five amino acids (F/L-D-L-N-L) has been detected in the SL repressors of rice and *Arabidopsis thaliana* which is assumed to interrelate with the transcriptional co-repressors TOPLESS and TOPLESS-RELATED PROTEINS (TPR2) (Ke et al., 2015; Soundappan et al., 2015). With the utilization of a yeast-two hybrid and Co-immunoprecipitation assays, interaction between SMXL6 to 8 andTPR2 in vivo was confirmed (Wang et al., 2015). Recently, it was revealed that SMXL7, D14, and MAX2 in *Arabidopsis thaliana* interconnect in the nucleus in the presence of SL (Liang et al., 2016). Divergent modulation of SLs signaling pathway in *Arabidopsis thaliana* has been suggested due to occurrence of at least three SL repressors, increasing the diverse impact on several characteristics of plant development. This hypothesis can be substantiated by analyzing individual SMXLs and recognizing SCF modulated genes which contain different repressors. IPA1 (ideal plant architecture 1), first postulated D53-suppressed transcription factor (Song et al., 2017), was recently unveiled which belongs to the SPL (SQUAMOSA promoter binding protein-like) transcription factor family and is a fundamental modulator of plant structure in rice (Miura et al., 2010). Transcriptional activation activity of IPA1 is repressed by the interaction of D53, while SL-incited degeneration of D53 dismisses the suppression of IPA1-mediated gene expression resulting in SL-modulated plant reactions in rice (Song et al., 2017). Likewise, modulation of TaSPL13/17 by TaD53 in bread wheat (*Triticum aestivum*) was also noticed (Liu et al., 2017). It has also been shown that IPA1 also mediates the feedback regulation of SL-induced *D53* expression by directly attaching to the *D53* promoter (Song et al., 2017). The recognition of IPA1 as a straight target of D53 opens out new opportunities for advanced study on SL signal transduction.

Strigolactone Signaling and Downstream Transcription Factors

So far TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL FACTOR1 family (TCP) is the only group of transcription factors (TFs) that is reported to be downstream constituent in SL signal transduction (Braun et al., 2012). FC1, FINECULM1, and AtBRC1, BRANCHED1, are the characteristic TCPTFs that are found in rice and *Arabidopsis thaliana*, respectively, and their manifestation has been noticed in axillary buds. After treatment with GR24, both *AtBRC1* and *FC1* were upregulated, validating their function in SL-interceded plant reactions (Aguilar-Martínez et al., 2007; Minakuchi et al., 2010). In *max3* and *max2,* the SL biosynthesis and signaling mutant, it was found that *AtBRC1* expression was downregulated but in triple mutant *smxl6/7/8* its expression was upregulated (Wang et al., 2015). Comparable effects have been observed for *HB53* (target genes of *AtBRC1)* that was upraised in *smxl6/7/8* plants (Wang et al., 2015).

Ideal plant architecture 1 (IPA1), a SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factor (Wang et al., 2015), is an essential modulator of plant organization in rice which generates D53 expression (Song et al., 2017). Peculiarly, studies have revealed that there occurs physical interaction between IPA1 and D53 which represses transcriptional activity of IPA1, prompting a negative feedback loop which mediates SL regulated expression of D53 (Song et al., 2017). IPA1 is a principal model which reveals action of SMXLs/D53 transcriptional repressors instantaneously in controlling downstream transcriptional factors.

SL biosynthesis genes are the other SL responsive genes recognized that are allegedly subjected to feedback repression. This is divulged with application of GR24 and by increased magnitude of SL biosynthesis mutant expression (Mashiguchi et al., 2009).

SL Signaling and Shoot Branching

It has been recognized that SLs repress shoot branching and for this function of SLs two mechanisms are suggested. It was revealed that bud outgrowth in axil was suppressed by SLs in rice and pea by affecting TCP transcription factor OsTB1/PsBRC1 (Braun et al., 2012). This transcription factor has been found to unite various pathways, like sucrose signaling and cytokinin pathway in pea (Rameau et al., 2015). Additionally, OsTB1/PsBRC1 encoding gene of maize (the maize ortholog, TB1) appears to function independently of the SL in repressing shoot branching (Guan et al., 2012). Several transcription factors in rice, like IPA1/OsSPL14 and MADS57, taking part in branching have been found to be associated with the SL signal transduction; however, it still remains unclear whether SL signaling is regulated by these transcription factors and also their position downstream of the D14-D3-D53 axis (Lu et al., 2013). Since branching is profuse in

SL-deficient mutants than brc1 mutants (Braun et al., 2012), there occurs probably a BRC1-independent impact of SLs on branching. A non-transcriptional mechanism in *Arabidopsis* depends on SLs which activate a swift shift of the plasma membrane PIN-FORMED 1 (PIN1) of stem xylem parenchyma cells (Shinohara et al., 2013) which will enhance contention among buds to transport auxin into the main auxin stream (Waldie et al., 2014).

Regulation of Root Architecture by Strigolactone Signaling

SLs are entailed to control root organization. Under normal conditions, SLs have been found to suppress lateral root development (Ruyter-Spira et al., 2011) but contribute to root hair development (Kapulnik et al., 2011). Proof of which comes from mutants of rice SL biosynthesis (*d10*, *d17*, and *d27*) and SL-perception mutants (*d3* and *d14*), possessing smaller crown roots and less root meristem cells compared to wild-type plants (Arite et al., 2012). In the presence of adequate phosphate (Pi) nutrition, SLs were proposed to have an adverse effect on lateral root (LR) formation in *Arabidopsis*. Mutants like *max3* and *max4* and *max2* were reported to have larger number of LRs compared to wild-type (WT) (Ruyter-Spira et al., 2011). Other SL functions in *Arabidopsis* are ambiguous on the grounds that numerous studies have only essayed impact of *max2* and racemic GR24 (*rac*-GR24). In *Arabidopsis* treatment of *rac*-GR24 leads to a remarkable MAX2-dependent suppression in lateral root development, relative to this, *max2* mutants possess larger LR density (Ruyter-Spira et al., 2011). However, in *max2* mutants, *rac*-GR24 induced repressed lateral root development can be re-established by transgenic *MAX2* expression through an endodermis-specific *SCARECROW* (*SCR*) promoter, which establishes important function of the endodermis in emergence and initiation of lateral root formation (Vermeer et al., 2014). SL biosynthesis mutants of *Arabidopsis* exhibit either minute or no change in LR density phenotype corresponding to the effect of *max2* (Kapulnik et al., 2011). This depicts that *rac*-GR24 and MAX2 impact on lateral root density are not completely dependent on SL signal transduction, but appearance of roots of *Arabidopsis d14* and *kai2* also needs to be described. In *Arabidopsis* and tomato, elongated root hairs are caused by *rac*-GR24 (Kapulnik et al., 2011). But *max2*, *max3*, and *max4* do not appear to show smaller root hairs than the wild type under control conditions, demonstrating the role of endogenous SLs in this type of phenotype (Pandya-Kumar et al., 2014). In contrast, in SL-biosynthetic mutant *max4* and signaling mutant *max2*, lower root hair density appears under phosphate-limiting conditions, an impact which is balanced with a high level of exogenous *rac*-GR24 (Mayzlish-Gati et al., 2012).

Brassinosteroid Signaling

Brassinosteroids bind to membrane attached BRI1 (BRASSINOSTEROID INSENSITIVE1) which is a leucine rich repeat (LRR) receptor-like kinase (RLK) receptor (Li & Chory, 1997) and evoke signaling cascade which modulates gene expression via transcription kinases and phosphatases of cytosol and nucleus (Fig. 5.2).

Receptors for BRs at the Cell Surface

The cell surface receptor for BR is BRI1. BRI1 consists of three domains, 25 LRRs containing extracellular domain, a transmembrane domain, and serine/threonine kinase rich cytoplasmic domain (Oh et al., 2000). Various experiments like mutation in extracellular domain of BRI1, immunoprecipitation of BRI1 with BR attaching activity, and BR-induced vivo autophosphorylation of BRI1 (Wang et al., 2001), which abrogates BR of its binding and kinase activity, have established that BR signal is perceived by BRI1 via its extracellular domain and induce signaling by its cytoplasmic kinase activity (He et al., 2000; Wang et al., 2001). Three homologs of BRI1, BRI1-LIKE1 (BRL1), BRL2, and BRL3 have been identified in *Arabidopsis*.

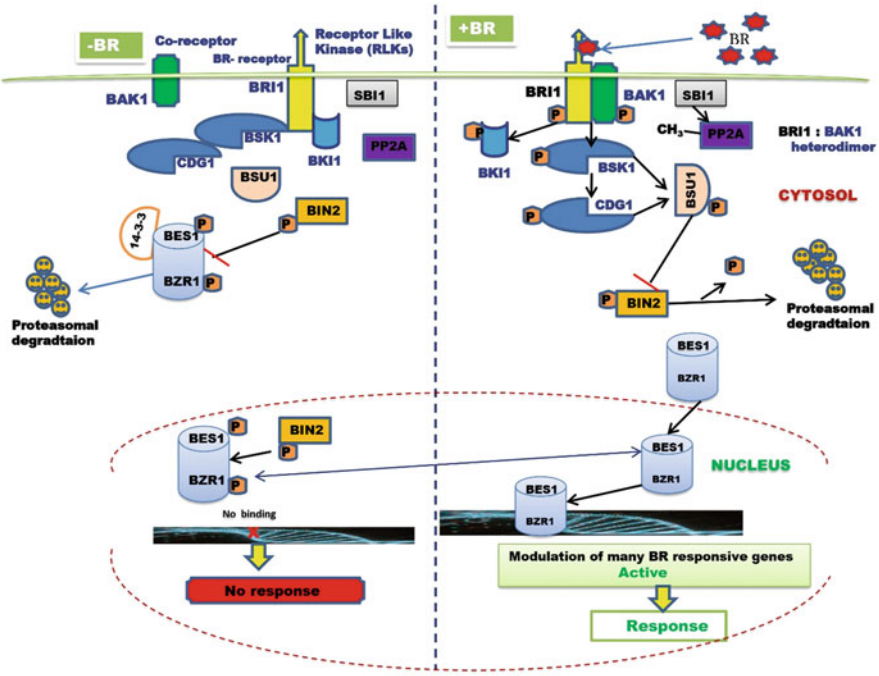

Fig. 5.2 Signaling pathway of brassinosteroid

It was revealed that only BRI1, BRL1, and BRL3 possess high affinity binding with BRs. Additionally, it was found that phenotype in the *bri1* mutant expressed in the presence of BRI1 promoter was rescued by BRL1 and BRL3, suggesting the role of BRL1 and BRL3 as functional BR receptors (Cano-Delgado et al., 2004).

Another potential constituent of BR receptor complex is BAK1 and SERKs (SOMATIC EMBRYOGENESIS RECEPTOR KINASES) (Nam & Li, 2002; Li, 2003). Interaction of BAK1 and BRI1 occurs in vitro and in vivo; however, their phosphorylation occurs in vitro. Gain-of-function and loss-of-function experimental results confirm a constructive use of BAK1 in BR signaling (Li, 2003). It was suggested that BRII and BAKI interact in a similar manner to that of animal receptor tyrosine kinases model (Nam & Li, 2002), BRn and BAKI together in their inactive monomer forms function via heterodimerization forming an active heterodimer to arbitrate BR signaling. BR attachment to BRn causes stabilization of heterodimer and leading to transphosphorylation of one another's cytoplasmic domain and then stimulates their intrinsic kinase activity to bring about a BR signaling cascade. Contrarily, Li et al. (2002) suggested other example of BRII/BAKI associations alike transforming growth factor ~ (TGF~) signaling pathway. They speculated that BR association with BRn leads to stimulation of BAKI by transphosphorylation, which then phosphorylates other downstream constituents in the pathway.

Downstream Signaling Network

The primary outcome of BR signal transduction is the BRASSINOSTEROID INSENSITIVE2 (BIN2) kinase inactivation, recognized as downstream negative regulator of BR signaling (Li et al., 2002). BIN2 inactivation results in the activation of two intimately associated transcription factors, BRASSINAZOLE-RESISTANT1 (BZR1) (He et al., 2005) and BRI1-EMS SUPPRESSOR1 (BES1) (Yin et al., 2005), also called as BZR2 (Wang et al., 2002) near the terminus of BR signaling. In signal transduction, BZR1 and BES are described as positive regulators downstream of BIN2. Application of BR leads to increased accumulation of BES1 and BZR1 (Yin et al., 2002). Most common forms of BZR1 and BES1 are phosphorylated, but are dephosphorylated and accumulated upon BR application (Wang et al., 2002; Yin et al., 2002). Biochemical studies have shown that BIN2 directly causes phosphorylation and destabilization of BZR1 and BES1 (Zhao et al., 2002; He et al., 2002). BIN2 induced phosphorylation of the nuclear BZR1 and BES1 decreases their binding to DNA, subsequently weakening their promoter attachment of target genes. Moreover, dimerization of BZR1 and BES1with other transcription factors is also hindered by phosphorylation of BZR1 and BES1 (Vert & Chory, 2006). Additionally, it was shown that BZR1 and BES1 in their phosphorylated form are attached by the 14-3-3 phosphoprotein-interacting proteins. It has been described that the 14-3-3 proteins may positively regulate BR signal by stimulating detachment of BKI1 from the plasma membrane, which results in the suppression of inhibitory effect of BKI1 on the BRI1 receptor. Therefore, taking into account the effective

function of phosphorylated BKI1 in BR signal transduction, through the attachment to 14-3-3 proteins, BR perception converts BKI1 and 14-3-3 proteins to positive regulators (Wang et al., 2011). In BR signaling, the major role of the 14-3-3 proteins is to negatively regulate BZR1 and BES1, by regulating sub-cellular confinement of these factors. It is proposed that phosphorylated forms of BZR1 and BES1/BZR2 might be retained in the cytoplasm by 14-3-3 proteins. It was also suggested the 14-3-3 protein binding to phosphorylated forms of BZR1 and BES1 could also result in their export from the nucleus, as a result, it may cause BR-dependent nucleo-cytoplasmic shuttle.

In cytosol, BIN2 kinase mediated phosphorylation of BZR1 and BES1 leads to their cytoplasmic localization that occurs by attaching 14-3-3 proteins (Kim et al., 2009). It has been revealed that BES1 phosphorylation on Ser-171 and Thr-175 and of BZR1 on Ser-171 and Thr-177 is essential for their binding with the 14-3-3 proteins and for the nuclear export, which is essential for complete suppression of BR signaling (Ryu et al., 2010; Ye et al., 2011). Eventually, BZR1 and BES1 phosphorylation regulate proteasomal degradation of these transcription factors (He et al., 2002). It has been shown the binding of BIN2 to BZR1 and BES1 occurs via 12-amino acid docking motifs located near their C-terminal ends and their interaction is essential, since their deletion causes accretion of the active, dephosphorylated BZR1 and BES1 in the nucleus (Peng et al., 2010).

Regulation of Gene Expression by BES1 and BZR1

The action of BES1 and BZR1 presents numerous aspects of crosstalk of several pathways, like morphogenesis, seed germination, cell elongation, flowering, and senescence (Zhu et al., 2013). Understanding coordination of BES1 and BZR1 and other proteins in controlling several gene expressions is essential for understanding the role of BRs regulated numerous processes at various growth and developmental stages in the presence of different environmental conditions. To deal with this question recognition and description of BES1 and BZR1 and target genes can be helpful.

ChIP-chip (chromatin immunoprecipitation coupled with Arabidopsis tiling arrays) analyses have recognized about 1609 BES1 and 3410 BZR1target genes, in which BRs regulate about 2000 genes (Yu et al., 2011). From these analyses, significant observation is that BES1 and BZR1 target genes have genes that are associated with plant development and other signal transduction mechanisms. BR-mediated BES1 and BZR1 targets have about 200 TFs (BTFs) that regulate BR-mediated genes for various responses. Functional identification of various BTFs exhibited the combination of BES1 and BZR1 and their gene products in the modulation of gene expression by BRs. BES1 associates with its induced target-MYB30 magnifying BR signal (Li et al., 2009). Recently it was revealed that association of BZR1 with its target gene products, PIF4 occurs, forming a heterodimer and binding to G-box (CACGTG, a specific E-box) promoter element

(Oh et al., 2012). PIF4 mutant and its related homologs—*pif1 pif3 pif4 pif5*—inhibit BZR1-induced elongation of hypocotyl and exhibited decreased hypocotyl elongation in darkness, suggesting the role of both BZR1 and the PIFs in the stimulation of cell elongation.

A remarkable advancement was made recently in comprehension of how BES1 and BZR1 associate with other transcription factors and regulate development modulated by GA, Aux ad light (Wang et al., 2014). BZR1 and light signaling association are also established by the observation of suppression of light signaling constituents by BZR1 (Sun et al., 2010). Correspondence among targets BZR1 and mediated transcription factors suggested the similarity of few target genes between BZR1 and HY5, a transcription factor that mediates light modulated gene expression (Lee et al., 2007). This is further established with one of the BZR1 targets (Luo et al., 2010). By genetic studies, it was suggested that GATA2 is a negatively regulated BR pathway. COP1-dependent proteasome degradation regulated the protein levels of GATA2. Hence, GATA2 is restricted at transcription by BRs and stimulated at protein level by light, thereby establishing connection among BR and light signaling pathways. Eventually, BES1 and BZR1 are involved in suppression of the expression of two linked transcription factors, GLK1 and GLK2, which aid in the development of chloroplast (Sun et al., 2010; Yu et al., 2011). It is established that there occurs premature development of chloroplast in BR loss-of-function mutants; however, the mechanisms are yet to be discovered (Chory et al., 1991). It is perceived that BRs via BES1 and BZR1 play a role to suppress GLK1 and GLK2 expression and hence development of chloroplast in the absence of light.

BES1/BZR2 stimulates expression of BR-responsive genes along with three Myc-like proteins (BIM1-3), which associate with BES1 by attaching to its HLH dimerization domain (Li, 2005; Sun et al., 2010; Ye et al., 2011). Following BRs, BZR1 and BES1 associate their own promoter sequences via positive feedback loop and stimulate their own expression (Yu et al., 2011). Apart from BZR1 and BES1, various other DNA associating proteins are also linked with BR signaling. Promoter binding peculiarity of BES1 and its degree of transcription activation are modulated by its binding with other transcription modulators, which belong to subfamilies: bHLH, MYB, IWS, and Jumonji N/C domain (Li et al., 2009, 2010). This group comprises various auxin-mediated proteins, Myb transcription factors, GRAS-family proteins, proteins regulating chromatin structure, and the family of three bHLH proteins—BRI1 enhanced expression (BEE1-3) (Wang et al., 2009). It was demonstrated that BES1 regulates gene expression by engaging transcription elongation factors and histone demethylases (Ye et al., 2011). Two factors are responsible for the increased transcriptional activity of BES1—EARLY FLOWERING6 (ELF6) and RELATIVE OF EARLY FLOWERING 6 (REF6). The factors have extremely conserved Jumonji N/C domain specific for histone H3 demethylases. Interaction of BES1 and these factors promotes BR reactions (Li, 2010; Li et al., 2010). Integration of BES1 with the interacting-with-Spt6 1 (IWS1) factor, known to modulate histone modifications, RNA polymerase II postrecruitment, transcriptional elongation, and RNA export, is necessary for the complete transcriptional activity of BES1 (Bres et al., 2008). Apart from dimerization with other transcription factors,

BZR1 and BES1 also directly bind to promoter that results in the enhanced BR-regulated transcriptional response (Sun et al., 2010; Clouse, 2011). It was revealed 1200 gene expressions are controlled by BRs. Out of which 950 genes were substantiated to be directly affected by BZR1 transcription factor (Sun et al., 2010). About 250 genes were proved to be directly affected by BES1 (Yu et al., 2011). An overlap of about 120 genes indicated the common modulation of the gene expression by BZR1 and BES1 (Clouse, 2011).

Novel Transcription Factors Modulating the BR-Dependent Gene Expression

Several other families of transcription factors are revealed to participate in BR signal transduction in rice and *Arabidopsis* (Clouse, 2011). A number of anomalous HLH (helix-loop-helix) proteins, ATBS1 (ACTIVATION TAGGED BRI1 SUPPRESSOR 1), its *Arabidopsis* homologs involving KIDARI and PRE1 (PACLOBUTRAZOL RESISTANT 1), and rice orthologs, ILI1 (INCREASED LAMINA INCLINATION 1) and BU1 (BRASSINOSTEROID UPREGULATED 1), were all recognized as positive regulators for BR because enhanced BR responses were exhibited by overexpression of these genes (Zhang et al., 2009). As ATBS1/PRE/ILI is unable to bind DNA they serve by obstructing DNA attaching capability of AIF (ATBS1-INTERACTING FACTOR)/IBH1 (ILI1–BINDING bHLH) bHLH proteins that usually are negatively regulated BR pathway. BR-like dwarf phenotype was observed in the plants that overexpressed AIF1/IBH1. It is noteworthy that BZR1 targets both AIF1/IBH1 and PRE1 and both are suppressed and stimulated by BZR1, respectively. These outcomes propose AtBS1/PRE/ILI1/BU family proteins segregate AIFs/IBH1, the negative regulators of the BR pathway and thus are positive factors for BR (Clouse, 2011).

BES1 and BZR1 homolog in rice, OsBZR1, plays a positive role in the regulation of BR response (Bai et al., 2007). Additionally, rice DLT (DWARF AND LOW TILLERING), a member of unique GRAS-family transcription factors, also functions as a positive regulator of BR reactions as BR-like dwarf phenotypes are exhibited by loss-of-function mutants and have stimulated BR biosynthesis gene expression (Tong et al., 2009). Various MADS box proteins, OsMDP1, OsMADS22, and OsMADS55 are negatively regulator BR pathway (Lee et al., 2008).

SL and BR Crosstalk

Crosstalk between SL and BR signaling pathways has been discovered recently and a complete insight of this crosstalk is still emanating. Biochemical and genetic evidences suggest that BES1 associates with MAX2 (central signaling constituent for SL) and modulate SL responsive gene expression (Wang et al., 2013) and SL receptor, AtD14, stimulates BES1 degeneration. Downregulation of *BES1* and its homologs resulted in repressed shoot branching of *max2-1* mutant. These indicate that the SL and BR signal transduction modulate the similar transcription factor, BES1, to regulate distinct processes. This breakthrough provides an understanding in a remarkable process in which BES1 is the common transcription factor of BR and SL signaling. Hence, BES1 is basic constituent for BR-SL crosstalk. BZR1 is another component which is a degeneration target of MAX2; however, it might not be a chief element to suppress shoot branching caused by SL, because *bzr1-1D* unlike *bes1D* plants grown in light have shorter hypocotyls, moderately dark green leaves, and shorter petioles compared to wild type (Wang et al., 2002) and also normal branching. Hence, BZR1 may participate in other MAX2-mediated developmental processes, which requires to be investigated.

Conclusions

The SL and BR research fields are moving rapidly and good progress is being made on SL and BR signaling pathways. Our understanding of SL signal transduction has greatly improved in recent years. SL signaling proceeds through the ubiquitin 26S proteasome based pathway used by other plant hormones also. An SCF complex (Skp, Cullin, and the F-box protein (MAX2 or D3)) in combination with the α/β-hydrolase fold receptor (D14 or DAD2) catalyzes the ubiquitination of transcriptional repressors, such as D53 and SMXL6/7/8, upon binding of the SL ligand to the receptor; this causes degradation of these repressors by the 26S proteasome and subsequent activation of the transcription of SL responsive genes. The SL receptor is different from receptors of other plant hormones, as it acts as an enzyme that hydrolyses the SL ligand and binds covalently to the released D-ring moiety. It can be presumed that the structural diversity of SLs is an outcome of an evolution toward particular functions in the communication with other organisms and in regulating plant development and stress response. Hence, the identification of this functional specificity and the elucidation of the biosynthesis routes leading to the different SLs are expected to open up new possibilities in developing crops with optimized architecture. Knowledge about particular functions of different SLs may also pave the way for designing SL analogs with specific applications. The demonstration of BR perception by BRI1 and BR-induced BRI1-BAK1dimerization, the identification of phosphorylation sites, and an autoregulatory domain of BRI1, and the discovery of DNA binding activities of BZR1 and BES1 has established the BR

pathway as one of the best-understood signal transduction pathways in plants. Yet there are still many questions to be answered. The major gap in our knowledge of the BR signaling cascade is between BRI1/BAK1 and BIN2. The mechanisms by which BR signaling regulates BIN2 kinase and the BSU1 phosphatase will be a focus of future studies.

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Chapter 6
Mechanism Associated with Brassinosteroids Crosstalk with Gibberellic Acid in Plants

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Abstract Brassinosteroids (BRs) and gibberellins (GAs) are the principal phytohormones which play their role to promote plant growth related developmental processes. Recent advancements in molecular tools have now provided a better understanding regarding the phytohormones biosynthesis, signaling, and degradation pathways. For the elaboration of signaling crosstalk and connection between BRs and GAs, different studies have been performed with the conclusion that, to control the cell elongation in *Arabidopsis*, signaling crosstalk between BRs and GAs is facilitated by the interaction between BZR1/BES1 and DELLA proteins which are the transcriptional activators from BR and GA signaling pathways. Furthermore, DELLA proteins along with restraining the plant growth also prevent the BZR1 transcriptional activities.

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Introduction

Plants being sessile organisms continually require acclimatizing their growth and development according to their external changing environment via integration of internal hormonal signals and external environmental factors. Phytohormones are the natural chemical messengers to play essential roles for plant growth and development, critical responses to the biotic and abiotic stresses, maintenance of the plant homeostasis, and adaptation to external and internal environmental factors (Vert & Chory, 2011). Until now, the characterization of eight different types of phytohormones has successfully been performed in plants including auxins, abscisic acid (ABA), brassinosteroids (BR), cytokinins (CK), ethylene (ET), gibberellins (GA), jasmonates (JA), and strigolactones (SR). Among these hormones, BRs and GAs have been designated as major growth promoting hormones. BRs were first discovered in 1970s and considered as plant-specific polyhydroxylated steroidal hormones. These hormones are engaged in the regulation of different growth and developmental processes, which include seed germination, male fertility, flowering time, cell elongation, stomatal development, and several other different types of plant growth and developmental processes (Wang et al., 2012). A typical dwarf phenotype can be observed in plants with hindered/mutated BR specific biosynthesis or signaling, which declares BRs as essential hormones for the growth and development of normal plants. GAs, the important tetracyclic diterpenoid phytohormones, are famous for their major role regarding the growth and development of plants, especially germination of seeds, hypocotyl elongation of stem, leaf and hypocotyl expansion, flowering pattern, and pollen maturation (Ragni et al., 2011; Sun, 2011). Deficiency of GA in plants exhibits the dwarfism to indicate its main role in the cell elongation mechanism and plant growth regulation.

Regardless of the significant and overlapping functions of these two phytohormones, how they perform the perfect coordination to regulate the growth and developmental functions of plants and is there any direct crosstalk/connection between their action mechanisms have been considered as main questions for deep investigation. Recent studies, related to the identification and characterization of BR and GA associated signaling pathways components, significantly advance the understanding of signaling and action mechanism of BRs and GAs. According to recent findings, BR and GA signaling pathways incorporate at transcription level facilitated by the direct interaction of several transcriptional factors including BES1, BZR1, and DELLA (Bai et al., 2012; Li et al., 2012). These advanced studies have enabled us to discuss the mechanism of action and signaling crosstalk between these two phytohormones.

Action Mechanism of BRs and GAs

During the past decade, a number of molecular, genetic, and biochemical researches have been carried out in *Arabidopsis thaliana* to identify the key factors of BR signaling pathway and to establish the complete signal transduction pathway (Fig. 6.1). Brassinosteroid-insensitive-1 (BRI1), a leucine-rich-repeat containing receptor-like-kinase (LRR-RLK), is BR receptor localized in plasma membrane which perceives the BRs (Li & Chory, 1997). Upon the binding of BRs, BRI1's intracellular kinase domain gets activated to promote its linkage with BRI1-associated receptor kinase 1 (BAK1) that ultimately boosts the actions of BRI1and

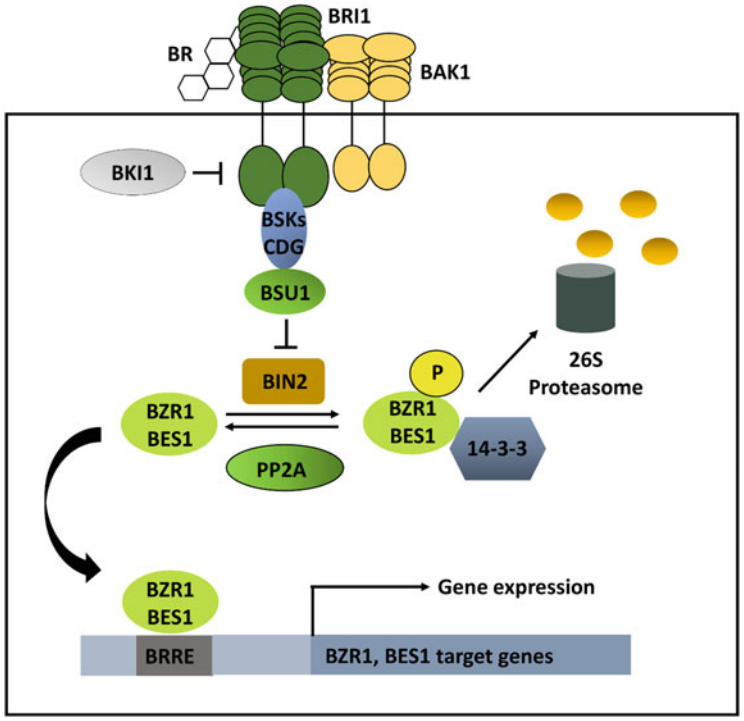

Fig. 6.1 A model to represent the brassinosteroid (BR) signaling in *Arabidopsis* (Li & He, 2013). BRs are recognized by the BR receptor, BRI1. This binding causes the activation of BRI1 via the homo-dimerization and hetero-dimerization of BAK1 and releases it from BKI1 which is an inhibitory protein. After that, BRI1 activates (through phosphorylation) BSK1 and CDG1 and BSUI (Ser/Thr phosphatase to inactivate the BIN2 kinase (negative regulator for BR signaling)). Furthermore, BR signaling stimulates the PP2A to activate BES1 and BZR1 transcription factors. BES1 and BZR1 (dephosphorylated) bind with BRRE or target gene's E-box motif to regulate their expression. During the absence of BR signal, BKI1 inhibits the interaction of BRI1 with BAK1. Cytoplasmic 14-3-3 proteins retain the phosphorylated BES1 and BZR1 and then degrade them with the help of 26S proteasome. Arrows correspond to the positive effect, while bars correspond to the negative effect

signaling of BR (Li & Jin, 2007; Oh et al., 2009; Wang et al., 2008). BRs and BRI1 binding also disassociates the BRI1 from BRI1 kinase inhibitor 1 (BKI1), an inhibitory protein, which avoids the BAK1 binding by binding the C-terminal tail of BRI1 and then stops the BR signaling in the absence of BR signal (Kim et al., 2011; Li & He, 2013). A series of phosphorylation events gets triggered upon the activation of BRI1, which include the phosphorylation and activation of BR signaling kinase 1 (BSK1) and constitutive differential growth 1 (CDG1) that ultimately phosphorylate and activate the BRI1-suppressor 1 (BSU1), Ser/Thr phosphatase (Kim et al., 2009). Then, the activation of BSU1 started the dephosphorylation and inactivation of brassinosteroid-insensitive 2 (BIN2), that is, BR signaling negative regulator (Li & Jin, 2007). BIN2 is the cytoplasmic GSK3-like protein kinase which is engaged in the inhibition of BR signaling through the phosphorylation and inactivation of BES1 and BZR1, transcription factors for the positive regulation of BR signaling (Kim & Wang, 2010; Peng et al., 2010). C-terminus site of BZR1 and BES1 was found to bind with BIN2 which is participating in the phosphorylation of these two transcription factors through the different GSK3-like phosphorylation sites (Bai et al., 2007; Vert & Chory, 2006). After phosphorylation, 14-3-3 phosphopeptide-binding proteins help in the replacement of BES1 and BZR1 in cytoplasm and then 26S-proteasome further degrades them (Tang et al., 2011). During the existence of BR signal, protein phosphatase 2A (PP2A) dephosphorylates and stimulates the BES1 and BZR1, then translocated to the nucleus and binds to particular genes through the BR-response element (BRRE) and/or E-box sequences (Sun et al., 2010; Yu et al., 2011).

During the recent years, intensive studies have been performed to get deep insights into the molecular mechanism of GA biosynthesis and signaling in plants (Fig. 6.2) (Sun, 2011; Sun et al., 2010). Gibberellin insensitive dwarf 1 (GID1), a GA receptor, perceive the GAs, and after GA binding, the receptor goes through the conformational changes to favor the DELLA proteins, cluster of nuclear-transcriptional-regulators to suppress the GA signaling and plant growth (Hirano et al., 2008; Sun, 2010). The induction of DELLAs and SLEEPY1 (SLY1)/GID2 F-box protein (SCF-type E3 ubiquitin ligase element to recruit the DELLA proteins for ubiquitination and degradation by the 26S proteasome) association depends upon the establishment of GA-GID-DELLA complex (Dill et al., 2004). Consequently, it is supposed that GAs imparts their role to improve the plant growth by eliminating the repressive DELLA proteins. For example, rice encompasses one DELLA protein named as SLENDER1 (SLR1), while in *Arabidopsis* there are five different DELLA proteins present named as repressor of GA1-3 (RGA), gibberellic acid insensitive (GAI), RGA-like 1 (RGL1), RGL2, and RGL3, which gather upon the low level of GAs and involved in the inactivation of different growth-fostering transcription factors including bHLH-like phytochrome-interacting factors (PIFs) (Bai et al., 2012).

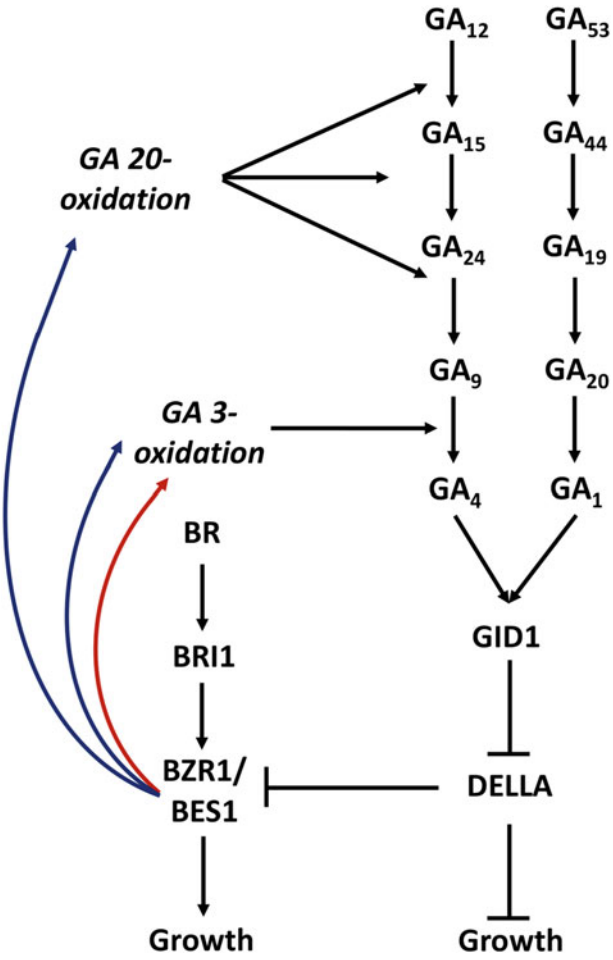

Fig. 6.2 GA biosynthesis and signaling in plants (Ross & Quittenden, 2016). BZR1 works as principle positive regulator for BR growth response. DELLA proteins, negative regulators of GA signaling, coordinate with BZR1 to lessen the BR growth response. Arrows correspond to the positive effect, while bars correspond to the negative effect. Red and blue arrows correspond to the GA signaling

BR Interactions with GA

Recent studies suggested an interaction between BRs and GAs to synchronize the various physiological processes in plants (Li et al., 2012). Nevertheless, numerous studies support the antagonistic interaction of BR-GA in plant defense associated mechanisms against *Pythium graminicola*. Moreover, severity in disease development has also been observed in GA-deficient mutants. It indicates the strong role of GA to provide the resistance against *P. graminicola*. Furthermore, susceptibility, as

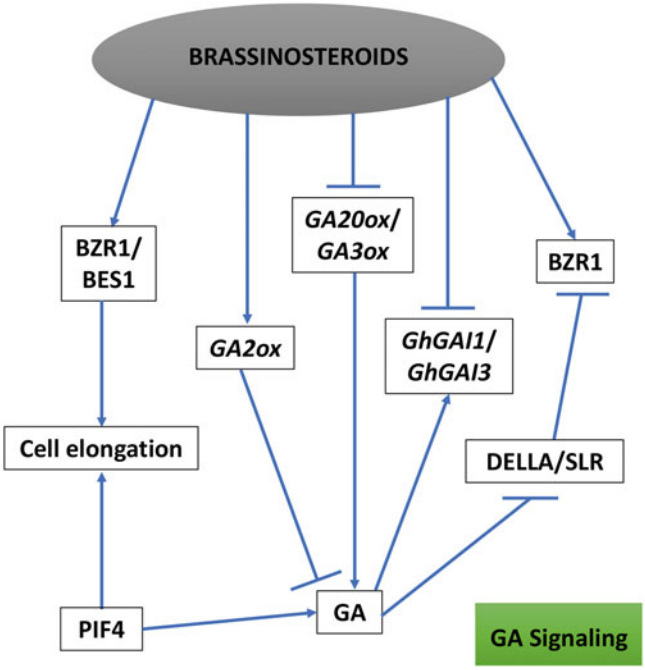

Fig. 6.3 A graphical representation of crosstalk of gibberellins (GAs) with brassinosteroids (BRs). Various key genes and transcription factors engaged in the transcriptional regulation of plant growth and development have briefly been represented here. Arrows represent the positive effect, while bars represent the negative effect

in case of BR treated plants, has been observed during interruption of endogenous GA level via GA biosynthesis blocker named as uniconazole (Bai et al., 2012; De Vleesschauwer et al., 2012). Nevertheless, no stabilizing effect was observed for the co-application of BR and uniconazole. However, application of uniconazole along with brassinazole has been observed to reduce the resistance inducing effect of brassinazole, a BR inhibitor. This observation demonstrated that immune response caused by GA was checked by the BR levels. Furthermore, it has also been observed in previous studies that GA suppressors including DELLA and SLR1 were upregulated by the application of BR. This process further directs to BR facilitated repression of GA biosynthetic genes. For example, *GA20ox* and *GA3ox3* trigger the expression of *GA2ox* (Fig. 6.3), which cause the inhibition of GA signaling and finally its inactivation (De Vleesschauwer et al., 2012).

Recent studies have evaluated the crosstalk between BR and GA in rice to regulate the elongation of plant cell. According to different researchers, BA triggers *D18*/*GA3ox-2* expression, which is a GA biosynthetic gene, to enhance the GA accumulation in cell. However, higher concentration of synthetic BR triggers the *GA2ox-3*, which is a GA inhibitor, which ultimately inhibits the plant cell elongation and further growth (Tong et al., 2014). Whereas high concentration of synthetic GA

activates the prime BR signaling pathway to help the cell elongation, however induces the inhibition of BR signaling and its biosynthesis in inhibiting loop feedback, suggesting the BR-GA crosstalk to regulate the plant cell elongation (Tong et al., 2014). The molecular networking among BR, indole-acetic acid (IAA), and GA in *Gossypium hirsutum* on cotton fiber growth has previously been investigated (Hu et al., 2011; Li et al., 2012). BR and auxin treatment downregulated the *GhGAII*, class of DELLA proteins, during the cotton fiber developmental processes especially initiation and elongation, and it also suggested its involvement in cotton fiber improvement through the genetic modification of phytohormones signaling. Though, *GhGAI1* and *GhGAI3* have shown the upregulated expression against GA treatment during cotton fiber initiation to exhibit the BR-GA interaction in cotton fiber development (Hu et al., 2011). In another study, a link, through DELLA, between BR and GA has been demonstrated to regulate the cell elongation and to promote the overall plant growth. According to literature, BZR1 interacts with repressor of *ga1-3* (RGA), member of DELLA proteins, under in vitro and in vivo environment. It has also been observed that the abnormal expression of DELLA proteins causes the reduction in BZR1 transcriptional activities which also indicates the antagonistic relationship between BZR1 and RGA transcriptional activities. Furthermore, BZR1 and RGA have also been reported as +ve and −ve regulators of BR and GA signaling, correspondingly (Li et al., 2012).

Molecular Regulation of BR and GA Pathways

In the root insusceptibility of rice, BRs and GAs function antagonistically, according to a long-term study. The pathogen *P. graminicola* was found to use BRs as destructive elements and to command the *Oryza sativa* BR apparatus for induced infection, casting doubt on the widely held belief that BRs fully regulate plant natural immunity. Furthermore, by improving the OsSLR1's steadiness, the main *O. sativa* DELLA protein that acts as a fundamental negative controller presenting defense against *P. graminicola*, this immunosuppressive effect was validated to some extent as an adversarial crosstalk with steam (Bajguz & Hayat, 2009; De Vleesschauwer et al., 2012; Nakashita et al., 2003). Vleesschauwer et al. correspondingly discovered that pathogen infection and exogenous BR treatment may well upsurge OsSLR1 expression (De Vleesschauwer et al., 2012). According to this knowledge, BRs can depreciate GAs-induced resistance reactions in rice by interfering with GA signaling, which would counteract BRs' beneficial effect in balancing out DELLA protein in rice. BRs present in fiber cells of *G. hirsutum* start to minimize four *DELLA* genes expression, *GhGAI1* as well, that is involved in fiber cell elongation. These *DELLA*-encoding genes were recognized as immediate focus *Arabidopsis* BZR1 chromatin immunoprecipitation (ChIP) study. The interpretation element in the BR pathway proposes that BRs can regulate the expression of DELLA-encoding genes directly to modify GA reactions (HU et al., 2011; Sun et al., 2010). However, findings from qRT-PCR analysis done in a study indicate that

BR treatment, which boosts BR signaling, had diminutive effect on these *DELLA* genes expression (Li et al., 2012). This suggests that BZR1 and DELLAs, rather than BZR1 regulating DELLA gene expression, facilitate collaboration among BRs and GAs in playing role in cell activation and elongation by interacting with other proteins. Other biological procedures remain a mystery, regardless of whether BRs regulate DELLA gene expression.

Functional Genes

In terms of gene expression, the GA and BR pathways are also linked. Previous research has displayed the BRs and GAs expressional control over a variety of production and growth-associated genes in *Arabidopsis* (Bouquin et al., 2001; Schünmann & Ougham, 1996). Further corporate target genes of these two paths were differentiated using high-throughput microarray approaches. For example, an investigation of expressed sequence tags (ESTs) around 4000 in *O. sativa* treated with GA and BR shows several genes that were controlled by both hormones in concert. The fact about the number of responsive genes of BR and GA in *Arabidopsis* was nominal among identified coregulated genes using relative genome expression analysis (Nemhauser et al., 2006; Yang & Komatsu, 2004). Bai et al. recently discovered that these two co-regulate several ordinary genes by comparing microarray fact collections from mutant *bri1-116* which is BR-insensitive and mutant *ga1-3* that is GA-deficient. 419 genes (35%) out of 1194 genes contrived by *ga1-3* were also influenced by *bri1-116* mutation. Furthermore, they discovered that about 30% of RGA-responsive genes are also immediately attacked by BZR1 when compared RGA-managed genes as of a distributed microarray statistic set to the distributed BZR1 target genes. All of these findings point to the possibility that BRs and GAs regulate a collective transcriptional module, which is possibly facilitated by the DELLA and BZR1 proteins (Bai et al., 2012; Sun et al., 2010; Zentella et al., 2007).

Advancements to Unveil the Molecular Mechanism of BR and GA Crosstalk

Despite the growing indication of a beneficial and mutually dependence link among BRs and GAs from previous physiological, transcriptomic, and transmissible research, an immediate crosstalk among their signaling pathways is yet to be discovered. An immediate crosstalk among two signaling pathways, in accordance with previous research, denotes the exchange of standard signaling components or partnerships among parts of their signaling pathways (Vert & Chory, 2011). For example, collaboration among the BR-controlled kinase BIN2 and the auxin-controlled ARF2 transcription factor was found to mediate the immediate signaling

crosstalk between auxin and BR signaling. BIN2 legitimately phosphorylates and inactivates ARF2, a repressor of auxin signaling, resulting in augmented auxin-responsive gene translation (Vert et al., 2008). In 2012, three autonomous research laboratories discovered that BZR1 and DELLA proteins work together to mediate the immediate signaling reaction among BRs and GAs of *Arabidopsis* to standardize cell elongation (Bai et al., 2012; De Vleesschauwer et al., 2012; Gallego-Bartolomé et al., 2012). These findings delivered a systematic context for knowing the contribution of BRs and GAs towards regulation of plant development and production.

BR Mediated GA Responses

BRs have been shown to enhance the germination of extreme GA biosynthetic and impervious mutants in previous physiological experiments. BRs were found to partially restore the developmental phenotypes of GA-impervious mutants and wild-type seedlings inoculated with the GA biosynthetic retarder, paclobutrazol (PAC), in a long-term study, however GA treatment did not reinstate the hypocotyl extension in BR-defective or -impervious mutants. It was deduced that BR signal (ing) is needed for GA work to boost hypocotyl extension after the fact. The results of microarray experiments investigating the reactions against GAs and BRs in GA- and BR-defective mutants grown under dark conditions bolstered this hypothesis (Bai et al., 2012; Steber & McCourt, 2001; Sun, 2010). Prior research has shown that BR inoculation will induce a limited or full reversion of the expression for 40% of the genes influenced by GA deficiency, while GA inoculation merely affected the expression of 16% of the genes influenced by BR deficiency. This discrepancy represents the progressive mechanism of BR and GA gene expression regulation that is in line with the findings of physiological research (Bai et al., 2012; De Vleesschauwer et al., 2012; Gallego-Bartolomé et al., 2012). Furthermore, the large number of different genes influenced by GAs and BRs found in these studies contrasts with the small number of BRs and GAs target genes found in previous studies (Nemhauser et al., 2006), which could be due to the study's varied environmental factors and plant materials. For instance, in an experiment (Nemhauser et al., 2006), microarray was conducted on BR- or GA-inoculated wild-type seedlings germinated under light, whereas in another experiment (Bai et al., 2012; Gallego-Bartolomé et al., 2012), microarray was performed on BR- or GA-defective seedlings germinated under dark conditions. This distinction depicted a difference in light's effect on GA and BR reactions.

BR and GA Control Common Genes Together

Although BZR1 interacts directly with DELLA proteins and BRs and GAs co-regulate a wide range of mutually common genes, it is yet to be determined if the BZR1-DELLA interaction module mediates the co-regulation of these genes. The antagonistic influence of RGA and BZR1 was eliminated when domains necessary for BZR1-DELLA contact (LHR1 of RGA or BIN2 of BZR1) were deleted in transcriptional transient assays (Bai et al., 2012; Li et al., 2012; Nemhauser et al., 2006). Microarray analysis confirmed that genes targeted in the GA-defective mutant *ga1-3* and the BR-impervious mutant *bri1-116* ominously overlapped (Cheminant et al., 2011). *ga1-3* had 1194 genes that were differentially expressed compared to the wild type and the *bri1-116* mutation affected 419 genes (35%). Among these co-regulated genes, *bri1-116* and *ga1-3* affected 387 genes (92.3%) in the same way. For 276 (71%) of these genes, the *bzr1-1D* mutation reversed the effects of *bri1-116*, and loss of DELLA proteins reversed the effects of *ga1-3* (Gallego-Bartolomé et al., 2012), implying that GAs and BRs have effects on most common genes through DELLA and BZR1 activities.

Direct Interaction Between BZR1 and DELLA Proteins in the Regulation of BR/GA Pathways

DELLAs may be able to closely interact with BZR1 because BRs and GAs monitor a normal transcription module by DELLA and BZR1 functions. Interaction studies in *Arabidopsis* using a variety of in vitro and in vivo methods, such as the yeast two-hybrid system, pull-down, bimolecular fluorescence complementation (BiFC), and co-immunoprecipitation (Co-IP) assays, have shown that BZR1 coordinates with GAI, RGA, and several other DELLAs (Bai et al., 2012; Gallego-Bartolomé et al., 2012). BES1 (named as BZR2), closely related to BZR1, coordinates with DELLA proteins as well. RGA or GAI domain, LHR1, and BZR1 phosphorylation domain, BIN2, are responsible for their association, according to protein domain investigations. These findings indicated that BZR1, BES1, and DELLA proteins play a role in promoting BR and GA signaling crosstalk, and further extensive studies majorly focusing on transcriptional transient tests verified that there is a partnership between BZR1 and DELLLAs that is necessary for their antagonistic impacts on transcription of growth-related genes (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012). DELLA proteins have recently converged as key regulators of crosstalk between different signaling pathways, and new evidence of their coordination with BZR1 and BES1 has bolstered this theory. BZR1 and BES1 are two primary transcriptional regulators for BR signaling, and they demonstrate their abilities by directly regulating a huge number of different target genes or by collaborating with other transcriptional factors which include DELLAs, PIFs, IWS1, BIM1, MYB30, ELF6, and REF, among others (Oh et al.,

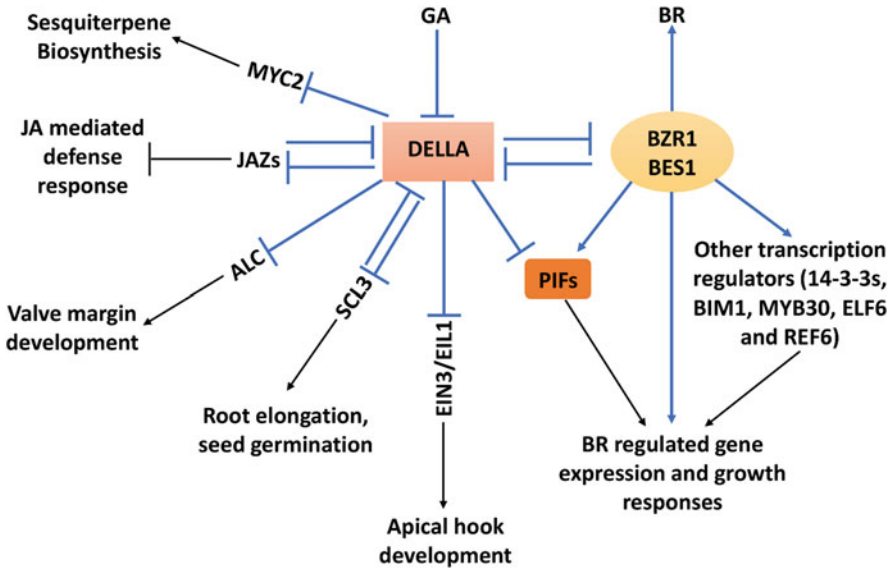

Fig. 6.4 Transcriptional network in plants regulated by the DELLA and BZR1/BES1 (Li & He, 2013). An interaction of DELLAs with different transcription factors (MYC2, JAZs, ALC, SCL3, EIN3/EIL1, PIFs, BZR1, and BES1) to control different growth and developmental processes has been represented here. BZR1 and BES1 also directly bind with their different target genes or other transcriptional regulators (14-3-3 s, BIM1, MYB30, ELF6, REF6, etc.) to regulate the plant developmental processes. Arrows represent the positive effect, while bars represent the negative effect

2012; Yin et al., 2005; Yu et al., 2008). BZR1 and BES1 can act as integrators of BR crosstalk with other pathways, similar to DELLA proteins (Fig. 6.4).

Pharmacological studies revealed that as compared to their wild forms, all mutants, *bes1-D* and *bzr1-1D*, have decreased sensitivity against GA biosynthetic inhibitor PAC, and the *rga gai* double knockout mutant has decreased sensitivity against BR biosynthetic inhibitor BRZ. Gradually, the mutant of *della* pentuple deficient with all five individuals from the DELLA family genes exhibited the significant improvement in BR response, while the mutant of GA-impervious *gai-1*, which amasses the elevated levels of GAI, showed a decrease in BR responses. BZR1 upregulates the GA pathway, while DELLAs downregulate the BR pathway, according to this information. Furthermore, evolutionary experiments backed up this theory (Bai et al., 2012). The *bzr1-1D* mutant showed the ability to marginally inhibit the GA-defective *ga1-3* mutant's short hypocotyl phenotypes, but not the GA-impervious *gai* mutant's. In the *bzr1-1D* history, overexpression of a non-degradable RGA protein also presented a dwarf phenotype, indicating that DELLAs could be epistatic to BZR1 in regulating cell extension. The reason that *bzr1-1D* masks the phenotype of *ga1-3* but not the mutant of *gai* is that BR therapy or the *bzr1-1D* mutation could induce the expression of GA biosynthetic genes,

protecting GA-deficient mutant phenotypes but not GA-impervious mutant phenotypes (Bai et al., 2012; Li et al., 2012).

Interaction Between DELLAs and BZR1

Since both the BR and GA regulation mechanisms are regulated by BZR1 and RGA, it is thought that BRs handle DELLA protein aggregation, while GAs lead BZR1 aggregation. Nonetheless, it was reported in several research papers that BR care, as well as mutations that interrupt BR biosynthesis or signaling, had little effect on DELLA protein aggregation (e.g., *det2*-1 (BR deficient mutant) and *bri1-116* (BR-impervious mutant)) (Bai et al., 2012; Li et al., 2012), BRs, unlike GAs, do not cause DELLA proteins to be degraded, according to the findings. In comparison, BZR1 protein was dephosphorylated by the GAs, while the opposite effect was observed by GA biosynthetic inhibitor PAC. Ectopic expression of undegradable DELLAs in transgenic plants (RGA or GAI deficient with 17-aa DELLA domain) indicated that the BZR1 protein was severely depleted. The GA-induced dephosphorylation of BZR1 was possibly abrogated by PP2A, a protein phosphatase that tended to dephosphorylate and relieve the BZR1, as part of the PP2A working with its inhibitor okadaic acid (OA) abrogated the GA-induced dephosphorylation of BZR1. As a result, by destabilizing the BZR1 protein, conclusion can be drawn that DELLA proteins limit plant growth and development (Li et al., 2012; Tang et al., 2011).

DELLA proteins including GAI and RGA directly interact with the dephosphorylated BZR1, apart from influencing the BZR1 stability. Since dephosphorylated BZR1 is more mobile, its association with DELLAs allows it to move more freely. RGA conjugation stopped BZR1 from bonding to its target genes, according to studies using electrophoretic mobility change assay (EMSA), protein–DNA pull-down analysis, and ChIP (Li et al., 2012), and DELLAs and BZR1 decrease each other's transcriptional events and target gene expression, according to the findings of transcriptional transient experiments, and the antagonistic effects are based on their physical activity (Bai et al., 2012). After the interaction with the complex form of BZR1 and hindering its transcriptional activities, DELLAs seem to change the yield of the BR signaling pathway, at least to a limited degree.

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Chapter 7
Brassinosteroid and Ethylene-Mediated Cross Talk in Plant Growth and Development

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Abstract Plant hormones regulate multiple physiological and metabolic systems through different signaling channels. The complex signaling network and metabolic processes play a major role in plant growth and responses to various environmental stresses. Extensive studies have unveiled most of the members of plant hormones and elucidate their principal effects on plant cell systems. Brassinosteroids (BRs) and ethylene are the two major biomolecules playing adorable roles in plant growth, physiological processes, and stress responses. Their collective interaction with each other and physiological parameters harmonize the important functions at different stages of plant growth and development. They also play a major role in biotic and abiotic stresses. This study examined the interrelation of ethylene and BRs during different developmental stages. It also highlights the two hormones' role during fruit ripening, stomatal closure, reproduction, abiotic stresses, and biotic stresses. The BRs and ethylene possess an antagonistic influence on the expansin gene AtEXPA5 expression. That antagonistic interrelation is responsible for the hook formation during the gravitropic growth of hypocotyls. The ethylene and BR cross talk comprises a complex network of signaling pathways, e.g., the ACC synthase pathway. Phytotoxins positively interact with ethylene pushing the plant into more stressed conditions. In this study, we have accounted both the hormones together to understand the plant responses better. This will help in providing knowledge of different interacting processes involved in these hormones. The cross talks of

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important plant hormones, such as BRs and ethylene, will provide us remarkable proficiency to induce stress resistance and enhance plant productivity.

Keywords Ethylene · Brassinosteroid · Plant hormones · Biotic and abiotic stress · Plant growth · Development

Introduction

Plant hormones or phytohormones are small, naturally occurring organic molecules important in plant growth, development, cellular mechanisms, physiological processes, and specific molecular activities (Akhtar et al., 2020). Classic methodologies comprising biochemistry, genetics, and physiological studies have contributed to the progress of plant hormones. These studies have identified important functions of these plant hormones in the development, growth, and subsequent plant responses to numerous abiotic and biotic stresses (Jiang & Asami, 2018). Nine classes of the phytohormones have been discovered, including gibberellins, salicylic acid, abscisic acid, cytokinins, auxins, ethylene, jasmonic acid, brassinosteroids, and strigolactones (Wang & Irving, 2011).

Brassinosteroids (BRs) are organic steroidal polyhydroxylated plant hormones that play a very diverse role in different aspects of plant growth and developmental processes (Khan et al., 2019). BRs were initially defined depending on their growth-stimulating mechanisms. Recent cellular, molecular, and physiological studies have uncovered its role in senescence, pollen development, photosynthetic performance, stem elongation, plant development, seed germination, and responses to several stresses, including extreme temperatures (Nolan et al., 2020). The development of advanced approaches plays a crucial role in providing an in-depth understanding of molecular and physiological mechanisms important in BR degradation, signaling and biosynthesis cascades, and related pathways (Ahmad et al., 2014a). Recent studies have reported BRs to positively impact the plant response to specific abiotic stresses and environmental factors, including salinity, heavy metals, heat, drought, pesticides, temperature, and cold (Khan et al., 2017a). Conversely, the definite mechanisms involved in BR signaling, which stimulate stress tolerance, are still unclear (Vardhini & Anjum, 2015).

Recent studies have revealed the interaction of BRs with other hormones, including jasmonic acid, auxin, ethylene, abscisic acid, cytokinin, salicylic acid, and gibberellin (Bashir et al., 2016; Hossain et al., 2006; Wu et al., 2019). These interactions affect the developmental, cellular, and physiological mechanisms of plants. Deficiency in BR biosynthesis can result in abnormal developmental phenotypes, highlighting the prominence of various signaling pathways. It also highlights the importance of BR biosynthesis, concentrations, and activities in regulating the cellular mechanisms (Saini et al., 2015; Ahmad et al., 2021a).

Ethylene, the first known plant hormone, is an aging hormone involved in regulating different characteristics of a plant life cycle (Yasin et al., 2018a; Iqbal

et al., 2017). The fruit ripening, germination of seed, organ longevity, root initiation, senescence, abscission, fruit ripening, root hair development, flower development, and responses toward external stressors mainly base upon ethylene biosynthesis (Schaller, 2012). It also controls various responses of the environment which are directly influencing reproduction in plants. Recently some significant advancement in understanding molecular and biochemical mechanisms involved in ethylene action and synthesis regulation has been reported (Lin et al., 2009). The ethylene hormone level changes due to environmental conditions are directly and indirectly involved in the plants' regulating lifecycle, making ethylene cross talk a major subject of interest (Iqbal et al., 2017). This analysis provides an inclusive overview of the connection between the role of BRs and ethylene in plant growth and development and the impact of biotic and biotic stress.

Root Growth

Roots are the essential plant organs responsible for structural anchorage; absorption of water and nutrients for the survival of plant by controlling its growth and development. They are also involved in the interaction with soil-living biota and serve as a symbiotic interaction site for soil-living microorganisms (Grierson et al., 2014). Root hairs and their root epidermal cells tubular extensions assist or increase subsequent functions by significantly increasing the absorptive surface. The development of root hair is persistently adjusted to alteration in the surrounding of roots, ultimately allowing root function optimizations in the soil environment (Ibrahim et al., 2017; Li et al., 2021). The interaction of plant hormones with other hormones contributes to various growth mechanisms in the plant roots. Furthermore, some important signal molecules, including reactive oxygen species (ROS), are also involved in root development (Swanson & Gilroy, 2010). The interconnection between hormone signaling and root hair signaling mechanisms with different biotic and abiotic alterations subsequently in the rhizosphere facilitates vibrant hormone-stimulated alterations in root hair growth, density, length, and morphology (Vissenberg et al., 2020).

The investigation of the connection between ethylene, BRs, and ROS has been reported. In this study, the screening of EMS mutant was carried out to identify *Arabidopsis* mutant (det2-9) with deficiency of BR synthesis, which subsequently depended on the short root phenotype. Meanwhile, the ethylene and ROS signaling cascade were increased in the Arabidopsis det2-9 mutant. It was proposed that the short root phenotype was the ultimate result of ethylene and superoxide anion (O^{2-}) accumulation. The exogenous BR application indicated that the ethylene biosynthesis regulation was carried out depending on its given concentration. The ethylene production was significantly decreased in the seedlings, which were treated with a low concentration (10–100 nM) of 24-epibrassinolide (EBL). On the other hand, the one treated with higher EBL concentrations $\geq$500 nM displayed a sharp increase (Lv et al., 2018; Shah et al., 2020).

Consequently, low concentrations of BRs result in the inhibition of ethylene response factor (ERF) expression. In contrast, when the concentration is high, it increases ERF expression, which is consistent with ethylene results after BR treatment. The study was carried out to evaluate the connection between 1-aminocyclopropane-1-carboxylic acid synthases (ACSs) enzymes by certain brassinosteroid-regulated transcription factors (BES1 and BZR1), and their role in ethylene biosynthesis was further confirmed by qRT-PCR. This study revealed that ethylene biosynthesis repression was carried out by certain transcription factors and was ultimately controlled by BR regulation. It was concluded that a high level of BRs resulted in increased production of ethylene by stimulating ACS enzymes (Lv et al., 2018). The directional growth regulation is necessary for the proper growth and development of roots and longitudinal growth (Tariq et al., 2020; Ahmad & Ashraf, 2016). Studies also proposed numerous environmental signals and factors that can further stimulate that plant root elongation and gravitropism. Previous studies showed that induced glucose stimulates root growth of the seedling, and when BRs are applied, it further increases this modulation type. Thus, the results proved that glucose increased the BR signaling by modulating BRI1 endocytosis from cell membrane to early endosomes (Singh et al., 2014a).

Singh and coworkers also evaluated the interaction of plant hormones and glucose in controlling root growth. The results suggested that the presence of cytokinins and ethylene could eradicate root growth when glucose or BRs were regulated. In this case, ethylene and cytokinins act antagonistically with BRs for subsequent growth regulation. Cytokinin pathway follows the BR signaling, which ultimately antagonizes the roots' directional growth by using ethylene-stimulated machinery (Singh et al., 2014b).

Shoot Growth and Apical Hook Development

The interaction between different plant hormones results in cell elongation, which is involved in shoot growth in plants. BRs are considered important hormone which promotes the activity of cell elongation. The experiments conducted observed that when the *AtRALF1* gene is partially silenced, the *AtEXPA5* expansin gene involved in cell expansion expression was increased. The exogenous application of BRs results in an induced AtEXPA5 level. It illuminates an antagonistic effect between BR and AtRALF1 for expansin genes. Ethylene reduces the expression of AtEXPA5 and regulates hypocotyl growth. The results from different experiments also suggested that the interaction of ethylene and AtRALF1 could achieve the same effect (Bergonci et al., 2014).

Many studies concluded that BRs and ethylene affect hypocotyl development in plants (Hoque et al., 2016; Shafique et al., 2014). The research included mutant *Arabidopsis* plant screening and identification with an improved response to acsinone7303. The acsinone7303 performs as an inhibitor for ACS enzymes. Numerous mutants of ret. with decreased sensitivity to acsinone7303 were also

investigated. Furthermore, ret41 and ret8 were characterized. The Map-based cloning results concluded that ret8 depicts a mutation in CESA6/cellulose synthase six, while ret4 represents a mutation in de-etiolated-2 (DET2). The enzyme DET2 catalyzed the campesterol to campesterol reduction process within the BR biosynthesis pathway. Whereas, CESA6 was a major part of the primary wall CESA complex (Verma et al., 2007).

Another study suggested that the mutant seedlings had short roots and hypocotyls when the mutation of eto1 was removed. That showed that the increased ethylene level did not completely affect the hypocotyl phenotype. Moreover, it was observed that the inhibitors of ethylene biosynthesis did not completely decrease the response of cesa6ret8 and det2ret41 mutants. This further suggested that mutations in DET2 and CESA6 cause short hypocotyls in mutants of cesa6ret8 and det2ret41, respectively. They play a very important role in the growth and development of seedlings in plants. The ethylene-induced level in eto1 stimulated the plant short hypocotyl phenotype in det2 and cesa6. Numerous experimental studies with subsequent EBL eto1, det2ret41, and det2-1 treatment indicated that ethylene and BR level balance is significantly important in hypocotyl growth accurate regulation (Chen et al., 2013).

The growth and development of the apical hook are very important for plant growth. This growth and development are followed by seed germination in plants (Bashir et al., 2013; Ahmad et al., 2021b). The early stages of *Arabidopsis* hypocotyl development include apical hook development, which plays an important part in protecting the apical meristem cotyledons of the shoot as the seedling growth takes place in the soil. The hook development stages include hook maintenance, the hook formation, and most importantly, the hook opening. Previous studies have reported the role of ethylene in the apical hook development phase, where BRs activate the maintenance phase, which further delays the hook opening phase. These stages of hook development are strictly controlled by a complex network of different hormones (Mazzella et al., 2014). Various experiments validated the results from these studies to investigate the BR biosynthesis role and specific signaling mutants for ethylene (Fig. 7.1).

Flowering

Flower formation and development are the most important phases in plant development, directly impacting plant reproduction and production. One of the plant families, called *Cucurbitaceae*, is known for its sex expression phenotypic variety (Abbas et al., 2020; Shafaghat, 2011). The development of plants in this family includes early male flower production followed by female flower production. Another study evaluated the role of BRs in cucurbit sex expression regulation. The experimental plant models included three different species, such as zucchini, cucumber, and melon. The cucumber plants were treated with BRs, and female buds and female flowers were observed. The ethylene level was induced simultaneously, which further concluded that the BR effect was ethylene mediated. The melon and

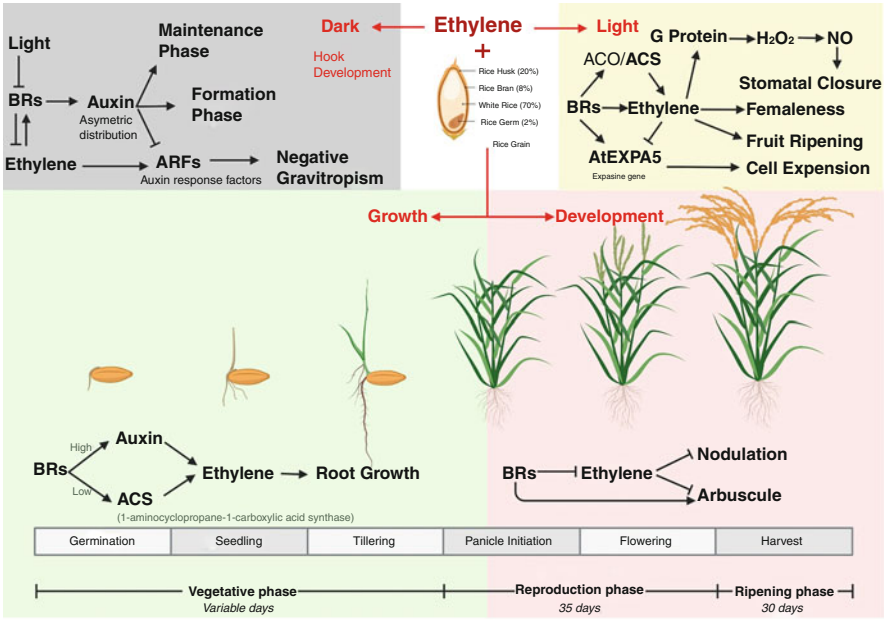

Fig. 7.1 Ethylene interaction with brassinosteroids (BRs) under light and dark conditions Furthermore, the interactions have been demonstrated on the growth and development of the plants. The arrows show positive interaction, while blunt head arrows show a negative interaction

zucchini plants also displayed the same induced level of ethylene production shown by the cucumber. Still, the induced level of femaleness was not present in melon and zucchini plants after BR treatment. This study concluded that BR-treated cucumber plants produced more ethylene than control cucumber plants. Hence, the effect of BRs on sexual expression in the cucumber plant is usually facilitated by the ethylene hormone. Different plant species have different sensitivity to ethylene. Therefore, the mechanism of interaction between ethylene and BRs in flower development BRs was found to act indirectly through ethylene increase production and increasing femaleness rate depending on specific species sensitivity to hormone ethylene (Papadopoulou & Grumet, 2005).

Ethylene and BRs mediate the sexual expression of different species of plants. Ethylene plays a vital role in regulating sex expression in plants, especially in the *Cucurbitaceae* family (Naemi et al., 2014; Hashemi et al., 2019). Its level in the buds of flowers initiates female flower development in plants. When plants were treated with ethylene biosynthesis inhibitors, such as aminoethoxyvinylglycine (AVG) or silver thiosulphate (STS), an increase in male flower development was observed. Another study compared the sensitivity of different genotypes to hormones BRs and ethylene by comprehensively working on different BRs and ethylene treatments exclusively on flower development and sex expression of different *Cucurbita pepo* genotypes, including Vegetable Spaghetti (Veg) and Bolognese (Bog). The experimental results from this study indicated that the effect of ethylene is greater as

compared to BRs on flower development and sex expression in *C. pepo*. The ethylene stimulates the female flower development and decreases the formation of male flower development. The use of ethylene inhibitors like AVG and STS decreases female flower development and, on the other hand, increases male phase development. The Bog genotype produces more ethylene, and they were more responsive to ethylene inhibitors like AVG and STS, resulting in a decreasing number of female flowers. The other Veg genotype showed lower ethylene production, reduced the male flower development, and increased female flower production by responding better to ethephon. Results showed that the development of male or female flowers was not altered or affected by the treatment of brassinazole in *C. pepo*. This showed that BRs play a significant role in ethylene production regulation. It also partially affects sexual expression and flower development in *C. pepo* and is directly involved in male and female flower development (Manzano et al., 2011).

Ripening and Postharvest Development of Fruit

Ripening of the fruits is a complex event, in which multiple phytohormones coordinate together, for normal growth, fertilization, and morphogenesis. The final fruit development involves four different stages: fruit set, fruit development, fruit maturation, or ripening phase. The last phase in plant development is fruit ripening, which plays a crucial role in making fruit attractive, edible, nutritional, and valuable agricultural commodities. This ripening process also includes physiological, cellular, and biochemical alterations such as cell wall structure modification, increased flavors and aroma, starch to sugar conversion, and changes in pigment biosynthesis (Kumar et al., 2014; McAtee et al., 2013).

The fruit ripening is classified in climacteric and non-climacteric fruit ripening depending upon its respiration and ethylene biosynthesis levels. The climacteric fruits are also called as ethylene-dependent fruits. These types of fruits can ripen once they are harvested with the ethylene production. The climacteric fruits include avocado, tomatoes, bananas, cucurbits, and apples. They are accompanied by a dramatic increase in ethylene production and respiration during their ripening process (Kumar et al., 2014; Cherian et al., 2014; Azzi et al., 2015). The non-climacteric fruits cannot ripen once they are removed from the parent plant, and ethylene is not required for their ripening. These fruits include citrus, strawberry, raspberry, and grapes (Kumar et al., 2014; Cherian et al., 2014).

BRs, a new class of plant hormones, are involved in plant growth and development and regulate ethylene production. In plant vegetative tissues, the BR exogenous application is involved in induced ethylene production and thus stimulates ethylene-mediated growth response. BRs and ethylene act together and collectively control plant metabolism (Zaheer et al., 2017; Yasin et al., 2018b). Moreover, studies have reported that BRs and ethylene hormones have antagonistic effects in the *Arabidopsis* (Deslauriers & Larsen, 2010). In fruits like strawberry and mango,

the application of endogenous BRs is present in small amounts and may not be important for fruit ripening.

In some cases, ethylene production takes place without variations of low BR levels in ripened fruit. While in other cases, the applied BRs stimulate ethylene production, signifying that ethylene production can be independent of BR (Zaharah et al., 2012; Greco et al., 2012). The role of ethylene in regulating climacteric fruit ripening, such as mango, is well-known, and numerous studies have been carried out to reveal further the mechanisms involved in it (Müller & Stummann, 2003). To further understand the role of plant hormone in regulating fruit ripening, the endogenous levels of ethylene and BRs were investigated in mango fruit ripening. The study also evaluated the effect of exogenous application of BRs and ethylene on fruit ripening. The results from this study suggested that BR endogenous level may not display an important role in the ripening of climacteric mango fruit (Zaharah et al., 2012). Recent studies have also highlighted the role of BRs in non-climacteric fruit grapes ripening (Symons et al., 2006). The exogenous BR application in climacteric fruit like tomato is involved in promoting tomato pericarp disc ripening and increased ethylene production. The endogenous BR high concentration in tomato fruit was also reported during early developmental stages. The BR-induced fruit ripening was interconnected with ethylene-increased production. The results also suggested the ability of BRs to stimulate fruit ripening and fruit senescence (Montoya et al., 2005).

Numerous studies evaluated the effects of BRs on postharvest development and fruit ripening. A recent study investigated the effects of BRs and ethylene on non-climacteric fruit ripening. In this study, strawberries were used as a study model for non-climacteric fruits. The treatment of exogenous spray of ethylene and EBL was done on this study model. The experimental results of the study showed that ethylene and BRs influence the levels of phenolic compounds in plants. The treatment of ethylene increases the level of phenolic compounds while the BR application results in reducing the level of phenolic compounds. The ethylene treatment results in high levels of phenolic compounds that result in senescence. When BR application reduces the level of the phenolic compound, the induced antioxidant activity helps in the stimulation of fruit conservation (Ayub et al., 2018).

Another study highlighted the role of ethylene and BRs in fruit ripening and postharvest development. In this study, BRs were dynamically produced during fruit ripening in the tomato plant. The transgenic lines overexpressing or silencing SlCYP90B3 were further generated. The accumulation of carotenoids and ethylene production were strongly linked with SlCYP90B3 level by the alteration in gene expression of carotenoid biosynthetic and ethylene pathway genes. The results suggested that the *SlCYP90B3* gene is involved in BR biosynthesis and fruit ripening in tomato plants, making it a gene of interest for the improvement of nutritional, visual, texture, and flavor qualities of tomato fruits (Hu et al., 2020).

BRs affect ethylene biosynthesis primarily by regulating ACC-synthase enzyme (ACS) and ACC-oxidase component activities (Ul Haq et al., 2020; Hafeez et al., 2019). The high BR levels induce ethylene biosynthesis by increasing the ACS protein stability, while the low levels of BRs decrease ethylene biosynthesis by the

activity of high expression of BZR1/BES1. These transcription factors play a major role in the BR signaling pathway and inhibit the transcription of ACS genes (Lv et al., 2018). Studies suggested that exogenous application of BRs can stimulate fruit ripening in bananas due to increased MaACO14, MaACS1, and MaACO13 expression. The exogenous application of BRs can stimulate and induce postharvest ripening, increasing the development of quality characteristics and subsequently increasing ethylene production in tomato by increasing *ACS2* and *ACS4* gene transcriptional levels.

Stress Response

The BRs and ethylene plant hormones are involved in plant growth and development and play a diverse role in plant responses to biotic and abiotic stress responses (Yasin et al., 2018a; Fariduddin et al., 2014; Ahmad et al., 2020a).

Abiotic Stresses

BRs, the natural steroid plant hormones, play a diverse role in plant growth and developmental mechanisms such as cell division, reproductive development, cell elongation, vascular differentiation, and response to abiotic stresses or tolerance. BRs play a significant role in decreasing abiotic and biotic stresses at different levels (Khan et al., 2017a; Ahmad et al., 2021b; Yasin et al., 2017). Abiotic stress factors adversely affect the plant growth, fruit yield, and agricultural productivity in plants. They interrupt the physiology and morphology of plants by different metabolic changes. This results in reducing plant growth by causing cell injury (Parvin et al., 2015). Salt affects more than 20% of cultivated land worldwide, increasing day by day, hampering crop productivity (Flowers, 2004). Plants have well-developed defense systems, including biochemical and physiological processes for protection against abiotic stress-induced injuries, including osmoregulation, antioxidant responses, and homeostasis. The plant responds to stress by stimulating antioxidant systems. These antioxidant systems can be enzymatic or non-enzymatic. The enzymatic antioxidant system includes catalase, peroxidases, superoxide dismutase, and glutathione reductase. Whereas, the non-enzymatic antioxidant system comprises carotenoids, vitamins C, vitamin E, flavonoids, and phenolic compounds. Among these, the phenolic compounds play an important role as the most dominant antioxidants (Yousaf et al., 2015; Ahmad et al., 2014b, 2020b). The study evaluated the BR effects on abiotic stress resistance in cucumber against polyethylene glycol (PEG), cold, and salt.

Previous studies have reported that BRs can increase ethylene production and induce the alternative oxidase (AOX) pathway. Results showed that the transcription levels of ethylene-mediated biosynthesis genes such as 1-aminocyclopropane-1-

carboxylate oxidase2 (CSACO2), ripening-related ACC synthase1 (CSACS1), CSAOX, ripening-related ACC synthase2 (CSACS2), 1-aminocyclopropane-1-carboxylate oxidase1 (CSACO1), and ACC synthase3 (CSACS3) were enhanced after BR treatment. Furthermore, salicylhydroxamic acid (SHAM, AOX inhibitor) and an inhibitor of ethylene biosynthesis like aminooxyacetic acid (AOA) application reduced plant tolerance to different environmental stresses and factors. This process is accomplished by blocking respiration or cellular process, which is induced by BRs. This study concluded the role of ethylene in BR-induced AOX activity, which is involved in abiotic stress resistance (Wei et al., 2015).

The transpiration rate depends on the opening and closing of stomata in plants, and stomata play a significant role in protecting the plant against stress conditions like water stress and pathogens. The stomatal movement pattern depends on different reversible alterations, including turgor pressure and water stomata flow in stomata. This stage is induced by many exogenous and endogenous stimuli. Therefore, the analysis of the opening and closing of stomata mechanism is essential to understand how the plants protect themselves against water and pathogens stress (Roelfsema & Hedrich, 2005). The opening and closing of stomata are regulated by different plant hormones involved in a complex signaling pathway network. Previously the most linked plant hormone for stomatal closure was abscisic acid only, but recent studies have suggested that BRs and ethylene affect the stomata activity (Shi et al., 2015).

Another study based on the interaction of ethylene and BRs in plants for salt stress highlighted different mechanisms. In this study, the BRs that induce salt tolerance in tomato plants were examined. In this study, the induce levels of ethylene and H2O2 in brassinolide-treated tomato seedlings were investigated. Results revealed that H2O2 and ethylene are intricate in BR-induced stress tolerance, and both BRs and ethylene could stimulate H2O2 production (Zhu et al., 2016).

The salt stress adversely affects the plant by reducing its leaf area, root and shoot length, membrane stability, accumulation of dry matter, relative water content, root weight, and reducing carbon dioxide assimilation, ultimately affecting plants' fruit production. Calcium acts as a second messenger and plays an important role in intervening mechanisms induced in response to different abiotic stresses in plants (Kader & Lindberg, 2010). It enhanced the growth of salt stress in plant and its subsequent signaling which control ion homeostasis pathways. Calcium ions restrict the entry of sodium ions in plant cells under salt stress conditions (Hussain et al., 2010). The most harmful effect of salinity stress is the accumulation of Na^+ and Cl^- ions in the plant tissues, which are highly exposed to soil with a high concentration of NaCl. When these ions enter the cell, it results in a severe ionic imbalance, which causes important physiological disorders in plants. The increased amount of calcium increases the growth and germination of the salt-stressed plant (Fig. 7.2).

Salt stress, the most adverse stress among abiotic stresses, reduces the oxidative stress, ion toxicity, and water unattainability apart from obstructing plant growth and productivity. Different other activities are involved, which ultimately leads to minimizing the plant productivity and growth (Parvin et al., 2015). The stress conditions in plants can result in oxidative damage. Therefore, the cells of plants

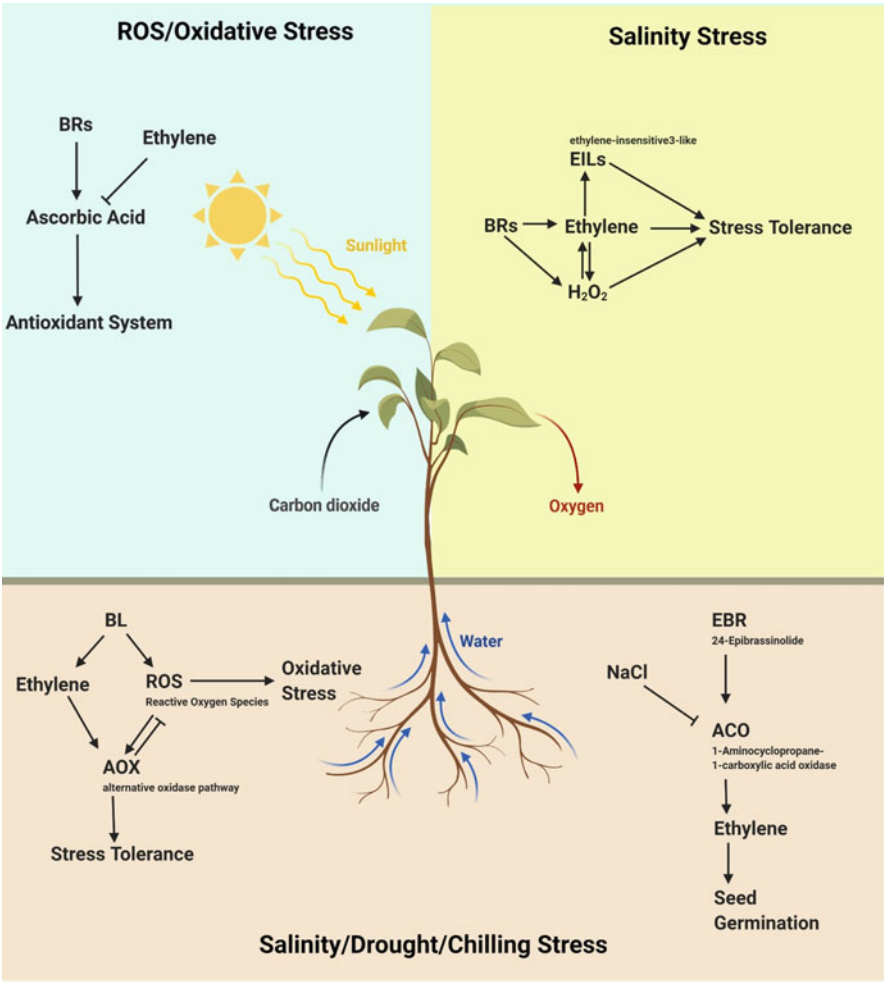

Fig. 7.2 Ethylene interaction with brassinosteroids (BRs) and abiotic stressors (i.e., salinity, drought, chilling, and oxidative stress)
Arrows show positive interaction, while blunt head arrows show negative interaction

require a sophisticated and delicate central antioxidant system. Glutathione (GSH) and ascorbic acid (AA) connection plays a significant role in this antioxidant system, which ultimately protects these plants against different oxidative damages. Ascorbic acid has various physiological functions such as photosynthesis regulation and increased cell growth in plants (Yasin et al., 2018b; Locato et al., 2013).

Different abiotic conditions, including drought or salinity, affect the symbioses relationship between plants and microorganisms. This symbioses relationship is important in the uptake of essential nutrients in plants. Studies reported ethylene signaling mutant of pea Psein2 and examined the interaction between BRs and

ethylene and its effect on mycorrhizal development (Weller et al., 2015; Khan et al., 2017b). Rice is the essential crop worldwide, and different mechanisms to induce plant resistance to stress have been discovered. The study identified a gene, *OsSta2*, which expression induces oxidative and salt stress tolerance in rice crops. The results from this study suggested that *OsSta2* gene plays a key role in the complex network of the ABA signaling pathway throughout the stress response (Kumar et al., 2017). The stress response mechanism and molecular breeding can be better understood by the genome-wide studies for different gene identification and their role in stress responses. These studies revealed that AP2/EREBP gene families were identified and classified in the *Cucurbitaceae* species. These families of genes play crucial roles in controlling different environmental stresses (Lee et al., 2017).

Biotic Stresses

Biotic stress is also one of the important constraints in plant productivity. Plants suffer from different stress conditions, which can be biotic or abiotic factors. The stress factors in plants can be abiotic like temperature or drought and biotic like pathogens and different pests like nematodes, insects, and fungi. Biotic stress is when there is damage to plants from a living organism such as parasites, bacteria, fungi, viruses, and harmful as well as beneficial insects. The defensive system of plants provides resistance to these biotic and abiotic factors. The defensive mechanism of plants includes physical or chemical barriers and functions effectively to decrease the harmful impact of biotic factors. These defensive mechanisms also involve complex pathways of complex phytohormones, including BRs, ABA, and ethylene (Ahmad et al., 2014c; Akram et al., 2014).

The involvement of these biotic and agrochemical factors is important if there is no genetically based resistance to confirm high productivity. Plants can develop morphological and physiological adaptations to survive in harsh environments. To grow in high salt stress, halophytes can excrete extra salt with the help of their secretory glands (Zaheer et al., 2017; Anjum et al., 2017; Akram et al., 2013). A previous study worked to understand the tomato plant responses like genetic control and signaling pathways to abiotic and biotic stress, including salinity and pathogen stresses (Bai et al., 2018). The research revealed that application of BR at low concentration improves the plant growth, quality, and production and induces resistance to different fungal and viral pathogens in various plants such as tomato, tobacco, and cucumber (Wang et al., 2015).

A recent study showed that in the fungal disease of cedar-apple rust, the expression levels were increased for flavonoid compounds (e.g., anthocyanin and catechin), and *MYB* genes (*MYB30*), specifically in the fungus-infected tissues. The study also suggested that plant hormones, including SA, ABA, JA, BR, and ETH, were found to be highest in infected plants of apple (Bashir et al., 2016; Lu et al., 2017). In the study on BR-treated pepper plant exposed to cold stress, the plant hormones such as SA, ETH, and JA levels were found to be significantly increased

(Li et al., 2016; Ahmad et al., 2013). The experimental work results suggested that BR functions by interacting with SA, JA, and ETH signaling hormones, especially for cold stress response. This further highlights that BRs play a crucial role in response to biotic stress tolerance by activating transcriptional factors, enzymes, hormones, biotic resistance genes, antioxidants, and signaling pathways to reduce biotic stresses of plants.

Ethylene and Pathogenesis

Ethylene biosynthesis has been reported at accelerated rates during the progressive events of pathogenicity. In this process, there is little or no discrimination of the pathogen type (e.g., bacteria, fungi, viruses, or nematodes) or the pathogenic species. Ross and Williamson first highlighted the topic during 1951 by recording the elevated ethylene contents in virus-infected plants (Ross & Williamson, 1951). The enhanced ethylene contents fall under the early biochemical communications of plant cells with the other cells in the vicinity. Generally, it is associated with the cell necrosis leaving localized lesions on the plant surfaces. Bacterial pathogens have been well investigated for the boosted ethylene contents and the characteristic lesion development. Viral pathogens also adopt the same pattern as the bacterial pathogens, but their own characteristic symptoms. Ethylene biosynthesis is increased with the viral disease progress. Fungal pathogens also drive plant cells toward an ethylene peak formation, while the height of the peak correlates with the amount of tissue damage (Ahmad et al., 2019, 2020c; Ahmed et al., 2017).

Ethylene Biosynthesis During Infections

Ethylene production does not require physical damage by the pathogens, but it is also elicited due to the pathogen-origin elicitors. The physical invasions of microbes are the secondary factors leading to the lesion formations, if detected by the hypersensitive defense systems (Khan et al., 2018). Pathogen elicitors that are difficult to be detected by the plant defense machinery cause a delayed excitation of the ethylene biosynthesis. Thus, it leads to much more damages to the photosynthetic and physiological systems of the cells. However, an interesting fact about the hormone was revealed to the researchers when some pathogenic bacteria and fungi produced ethylene by themselves under in vitro conditions. However, their ability to produce ethylene is of more assistance to trigger ACC synthase than the elicitation of the plant defense cascade. This abrupt excitation of the ACC synthase causes the stunted growth of plants, a characteristic feature of the biotic stress. Therefore, ethylene synthesis in plants is the best and the most optimized measure to alleviate plant biotic stress.

Regulation of Ethylene Under Post-Infection Conditions

A MET-ACC-independent pathway has been extensively reported for ethylene biosynthesis by the host plant during progressing disease establishment. The poor incorporation of radioactive MET derived the conclusion into plant-produced ethylene. The results proved that MET was not associated with the main C_2H_4 biosynthetic pathway in the infected plant tissues. Furthermore, the conclusion was supported by AVG application, a MET inhibitor, which could not reduce ethylene production. Another MET inhibitor Co^{+2} failed to inhibit or reduce ethylene biosynthesis in infected tissues. Similarly, ACC application, an intermediate of the MET-ACC pathway, could not enhance ethylene contents. All these facts concluded that ethylene production was involved in a mechanism other than the MET-ACC-dependent pathway.

Ethylene and Disease Spread

It is very hard to draw a generalized and precise relationship between the ethylene production and disease development. The role of ethylene during the infection process becomes more complex when it interacts with other growth hormones (e.g., auxins), pathogen-derived toxins, and arthropods associated herbivory. However, in a broader area, an interconnection between the host-derived ethylene and disease development can only be made by ignoring the other factors, that is, the negative interrelation. However, in some cases, ethylene inhibition caused a significant reduction in the disease development. On the other hand, the exogenous application of ethylene has been proved a useless strategy for plant protection programs because it promoted the disease development rather than to control the pathogen. Pathogen-derived ethylene doubles symptom severity if compared with non-ethylene-producing pathogen strains.

Ethylene Interrelation with Toxins

Toxins are classified among the plant stressors promoting plant diseases. Several phytotoxins have been reported negatively impacting plant health, causing diseases, and deteriorating the edible quality of plants. Toxins are also directly related to ethylene, which concomitantly reduces plant growth by the ACC synthase pathway. *Fusicoccum amygdali* is famous for fusicoccin production, which is responsible for developing disease symptoms on almond and peach. It stimulates the conversion of ACC to ethylene. Another example of the phytotoxin interacting with ethylene is coronatine produced by *Pseudomonas syringae*. The toxin bears the tendency to increase the ethylene release from the different plants. Similarly, *Pseudomonas*

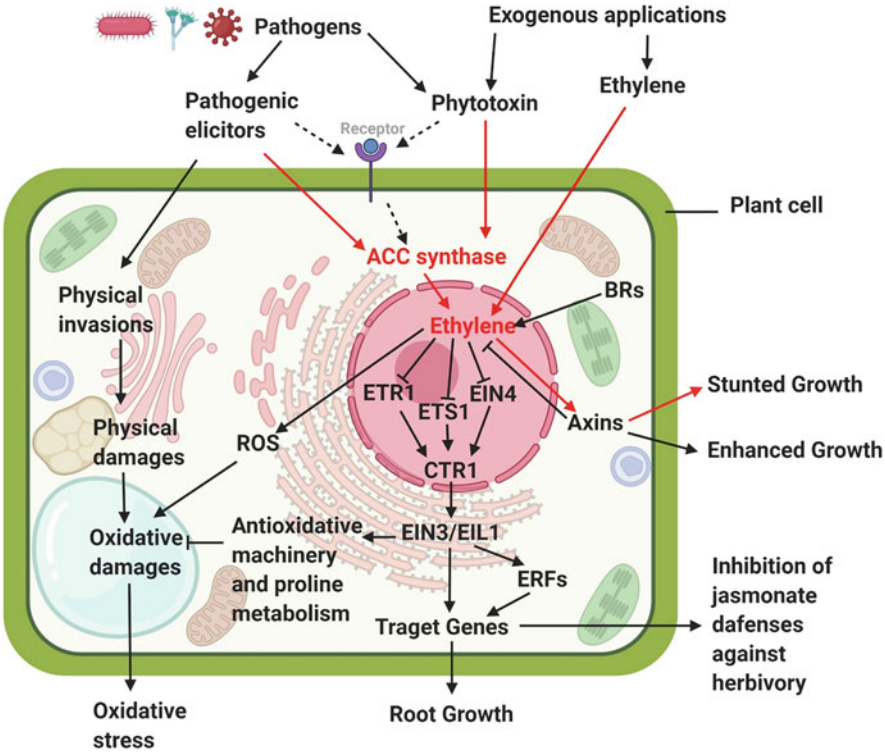

Fig. 7.3 Ethylene interaction with brassinosteroids (BRs), pathogens, and phytotoxins
The interaction leads to some physical outcome in the plant's stature. Black arrows show positive interaction, while blunt head arrows show negative interaction. Red arrows show an abrupt elevation in the contents due to some external stimuli/applications. Reception signals of pathogen or phytotoxins have been shown with dotted arrows

phaseolicola produces a toxin named phaseolotoxin in addition to the production of ethylene. This joint production of both the stressors is lethal for the plants and proves a supporting effect of toxins to the ethylene production and downstream biotic stress responses (Fig. 7.3).

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Chapter 8
Interplay of Brassinosteroids and Auxin for Understanding of Signaling Pathway

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Abstract Plant hormones play a vital role in the regulation of growth and development of plants, besides this, they also provide tolerance under different biotic and abiotic stresses. In plants, brassinosteroids (BRs) are steroidal hormone known to regulate many physiological, biochemical, and developmental processes. Recent studies showed that BRs can interplay with other plant hormones such as auxin (AUX), cytokinins (CKs), abscisic acid (ABA), ethylene (ETH), and gibberellic acid (GA) to regulate a range of growth and developmental processes in plants. Auxin and BRs are of two different groups of plant hormones which regulate many processes from seed germination to the fruit development independently. But in recent years, several studies have revealed a common link between these two hormones in the regulation of plant developmental processes. Current advancement in molecular tools has provided a better understanding toward the mechanism of signal transduction process of interplay of BRs and auxin. So, in this chapter, we discuss about the physiological responses of BRs and auxin interplay and its detail mechanism of signal transduction pathway.

Keywords Auxin · Brassinosteroids · Interplay · Signaling

Introduction

Plant hormones (phytohormones) or plant growth regulators (PGRs) regulate the growth and development of the plants at very low concentration through a definite signal transduction pathway. On basis of chemical nature, PGRs have been categorized mainly into six groups, i.e., auxin (AUX), gibberellic acid (GA), cytokinins

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(CKs), abscisic acid (ABA), ethylene (ETH), and brassinosteroids (BRs). Besides this, there are more groups of PGRs like salicylic acid (SA), jasmonic acid (JA), strigolactones (SLs), etc. (Santner & Estelle, 2009). The interaction between these PGRs is crucial for the coordinated growth and development of plants in response to various environmental stimuli (Halliday & Fankhhauser, 2003). Many workers pointed toward the interplay of BRs and auxin in the regulation of plant development and the mechanism of their interaction in these regulating processes (Vert et al., 2008; Maharjan et al., 2011; Saini et al., 2013, 2015). These scientists supports that auxin treatment stimulates the DWF4 which is responsible for the BR synthesis (Maharjan & Choe, 2011). Moreover, auxin positively regulates the *DWF4* and *CPD* gene by BREVIS RADIX (BRX) (Tanaka et al., 2005), showing direct link with the synthesis of BRs (Mouchel et al., 2006; Chung et al., 2011). Therefore, we can say that interplay between BRs and auxins are playing a key role in the regulation of growth and development of plants.

Brassinosteroids (BRs) are a group of steroidal hormone, and brassinolide was the first discovered BRs from the pollen of *Brassica napus* in 1979 (Grove et al., 1979). Brassinosteroids are well-known to control many physiological processes in plants like seed germination, cell elongation and division, cellulose biosynthesis, photo-morphogenesis, gravitropism, vasculature differentiation, flowering, male fertility, and senescence (Yang et al., 2011; Wang et al., 2012; Clouse, 2015; Ahanger et al., 2018). Moreover, BRs are also capable of mitigating the adverse effects of different biotic and abiotic stresses in plants via regulating many physiological and biochemical process like plant–water relations, osmolyte accumulation, photosynthesis, nitrogen metabolism, and antioxidant metabolism by the stimulation of genes related to a stress (Yang et al., 2004; Ali et al., 2007, 2008; Hayat et al., 2012; Krumova et al., 2013; Fariduddin et al., 2014; Ahanger et al., 2018; Ahmad et al., 2018; Tian et al., 2018). BRs signal transduction pathway was studied by many workers through the different omics tools like proteomics, transcriptomics, genomics, etc. The pathway of signal transduction of BRs was mediated by its cell surface receptor Brassinosteroid Insensitive 1 (BRI1), BRs can activate the kinase activity of BRI1 after binding with it, in this way signal transduction of BRs gets started (Li & Chory, 1997; Wang et al., 2001; Kinoshita et al., 2005; Clouse, 2011; Hothorn et al., 2011; She et al., 2011). Another kinase Brassinosteroid Insensitive-2 (BIN2) worked as negative regulator of BRs signaling pathway which act at downstream to BRI 1 action (Li & Nam, 2002). At low level of BRs, proteins belong to the family BZR1 (Brassinazole Resistant 1)/BES1 (BRI1-EMS Suppressor 1, also known as BZR2) which were hyper-phosphorylated by BIN2 and degraded by the action of proteasome (Wang et al., 2002; Mora-Garcia et al., 2004; Kim et al., 2009, 2011; Sun et al., 2010). On the other hand, after BRs perception; BIN2 is inactivated and proteins of the family of BZR1/BZR2 are dephosphorylated and collect in the nucleus to alter the expression of BR responsive genes (He et al., 2002; Yin et al., 2002; Kim & Wang, 2010; Tang et al., 2011; Yu et al., 2011; Ibañez et al., 2018). In this way, BRs regulate the number of physiological and biochemical processes in plants (Sun et al., 2010).

Auxin was the first discovered plant hormone which regulates a range of growth and developmental processes in plants from earliest embryo to fruit that includes cell enlargement by changing cell wall plasticity, tropic growth, embryogenesis, organogenesis, and vascular differentiation by stimulating cambium, formation of lateral and adventitious root, and development of shoot and fruit (Liscum & Reed, 2002). The most commonly found auxin in plants is indole-3-acetic acid (IAA). Furthermore, exogenous auxin application alleviated the different biotic and abiotic stress in plants through modulating the antioxidant defense system (Bashri & Prasad, 2015, 2016). In recent times, the molecular mechanism of auxin signal transduction pathway has been very well explored (Tian et al., 2018), and the first known nuclear receptor of auxin was Transport Inhibitor Response1 (TIR1) (Ruegger et al., 1998; Dharmasiri et al., 2005). In addition to this, many auxin-signaling f-box proteins (AFBs) recognize the auxin in cells and facilitate the signaling of auxin (Gray et al., 1998, 2001; Dharmasiri et al., 2005; Quint et al., 2005; Badescu & Napier, 2006). However, auxin signaling is negatively regulated by AUX/IAA (auxin/indole-3-acetic acid) proteins and there are 29 members of AUX/IAA encoded proteins in *Arabidopsis*. Moreover, TIR1 receptor may interact with a group of AUX/IAA proteins (Dharmasiri et al., 2003). Furthermore, AUX/IAA proteins may also interact with auxin response factors (ARF), a transcriptional regulators of auxin, to facilitate transcriptional responses for auxin. At the low level of auxin, the stability of AUX/IAA increases by Auxin-Binding Protein1 (ABP1) through hindering AUX/IAAs (Tromas et al., 2013). On the contrary, when the level of auxin was high, it resulted in the degradation of AUX/IAAs and release of ARFs+/ARFs2 by increasing the affinity of TIR1/AFBs with AUX/IAAs. Subsequently, the release of ARFs+/ARFs2 activates or represses the expression of auxin target genes as well as the responses of hormone (Guilfoyle & Hagen, 2007; Weijers & Friml, 2009). Besides this, *AUXIN1/LIKE AUXIN1* genes translate a high-affinity auxin influx carrier (Péret et al., 2012), and the level of auxin is maintained by the GRETCHEN HAGEN3 (GH3) through the conjugation of auxin with amino acids (Mashiguchi et al., 2011). In addition to auxin metabolism, its transport is critical within plants which create auxin gradients, and this transport is mediated by plasma membrane-localized PIN-FORMED (PIN) proteins (Wiśniewska et al., 2006; Mravec et al., 2009). Besides PIN proteins, researchers have also recognized the PIN-LIKES (PILS) protein family which also facilitates the transport of auxin and bears a resemblance with PIN proteins in structure. The intracellular auxin accumulation at the ER is controlled by PILS proteins which control the availability of auxin at nucleus in that way control the signaling of auxin (Feraru et al., 2012; Beziat et al., 2017). Thereby, cellular sensitivity to auxin is monitored by PILS proteins that contribute toward various growth and developmental processes of plants (Barbez et al., 2012; Feraru et al., 2019).

As we have discussed earlier that the development of plants takes place by both the independent and dependent signal transduction of different PGRs. Earlier, a number of studies have suggested that BRs and auxin can control their function independently, and several other studies have revealed a common connection between these two hormones (Mandava, 1988; Nemhauser et al., 2004; Saini

et al., 2015). Accordingly, some of the pathways are under dual control (Vert et al., 2008; Sun et al., 2020). For instance, BRs and auxin were shown to act synergistically during hypocotyl elongation in a variety of plants (Nemhauser et al., 2004). It is observed that their signaling pathways converge at the level of transcriptional regulation of same target genes (Nemhauser et al., 2004; Vert et al., 2008). For example, the expression of protein family responsible for polar transport of auxin, i.e., *PIN* genes, was regulated by BRs (Nemhauser et al., 2004). Likewise, *DWF4* genes responsible for the biosynthesis of BRs were also stimulated by auxin showing a link between BRs biosynthesis and auxin signaling (Tanaka et al., 2005; Mouchel et al., 2006). Thus, in this chapter, we will discuss the signal transduction pathway of BRs and auxin and also their interplay in regulation of physiological and biochemical processes of plants.

Physiological Role of BRs and Auxin Interplay

In plants, BRs regulates many physiological responses and their action has been influenced by the auxin. The sensitivity of any plant toward auxin has been synergistically increased by BRs, and their combined treatment increased the expression of genes (Vert et al., 2008). This interplay between these two PGRs (BRs and auxin) regulates many growth and developmental processes in plants under normal as well as in stressful conditions (Nemhauser et al., 2004; Hao et al., 2013; Chaiwanon & Wang, 2015; Yusuf et al., 2017; Ahanger et al., 2018). So, in this section, we are going to discuss the regulatory action of BRs and auxin interaction in different growth stages of plants.

Root Growth

The development of root in plant is determined by the balance of cell division and differentiation in the meristem of root. Besides this, signaling crosstalk is also present during root development of the plant which is facilitated by Brevis Radix (BRX) that also acts as a rate-limiting factor for BRs biosynthesis. In *Arabidopsis,* Mouchel et al. (2006) reported that threshold level of BRs were required for the action of auxin to determine root growth which was facilitated by *BRX* gene. The expression of *BRX* is encouraged by auxin and inhibited by BRs. Thus, biosynthesis of BRs and auxin signaling are interconnected via feedback mechanism and involves *BRX* gene in the course of root development (Mouchel et al., 2006). Moreover, exogenous treatment of BRs can restore the brx phenotype at the embryonic and post-embryonic stages, respectively. Besides this, *CPD* and *DWF4* genes responsible for the biosynthesis of BRs were also stimulated by BRX showing a link between biosynthesis of BRs and signaling of auxin (Tanaka et al., 2005; Mouchel et al., 2006). Further, Kim et al. (2006) reported BRs mediated regulation of *AXR3/IAA17*

gene expression for the development of root. Transgenic plant with overexpression of AXR3/IAA17 gene showed lesser root growth especially in the development of lateral root and root hair. Similar type of root defects were also reported in BRs treated wild-type plants. Furthermore, Kim et al. (2006) showed that BR treatment significantly stimulated the expression of *AXR3/IAA17* gene as well as several *Aux/IAA* genes such as *AXR2/IAA7*, *SLR/IAA14* and *IAA28* while BR signaling mutant bri1 and the BR biosynthesis mutant det2 showed lesser expression of *AXR3/IAA17* gene. This result provides an interplay of BRs and auxin signaling in root development as BR-induced *Aux/IAA* genes like *AXR3/IAA17* might play a role in root development (Kim et al., 2006). In another study, Chaiwanon and Wang (2015) showed that optimum expression of BZR1 necessary for root growth which maintained by the BRs signaling, and catabolism; and also by auxin biosynthesis. BZR1 stimulates the expression of target genes in the transition-elongation zone while inhibits genes in the quiescent center. However, on other hand, auxin has an opposite effect to BRs on the spatiotemporal gene expression. Thus, we can say that for optimal root growth, a balanced concentration of BRs and auxin is required (Chaiwanon & Wang, 2015). Further, Retzer et al. (2019) observed that cross talk between BRs and auxin signaling was necessary for the gravity-induced root curvature which was mediated by endocytic PIN2 through the attenuation of differential cell elongation. In *Arabidopsis*, transport of auxin from root tip to root elongation zone was mediated by PIN2 protein which determined the growth of root (Retzer et al., 2019) while BRs act as antagonists of PIN2 endocytosis and regulates sorting of PIN2. The intracellular distribution of BRs directs the formation of a lateral PIN2 gradient in gravity-induced root by auxin signaling and regulates the directional root growth (Retzer et al., 2019). Similarly, in rice, the expression of *OsIAA1* was induced by many PGRs including IAA, 2,4-D, kinetin, 24-epibrassinolide, and JA (Song et al., 2009). Overexpression of *OsIAA1* by auxin treatment led to the lesser inhibition of root elongation while showed enhanced sensitivity toward BR treatment. In addition, overexpression of OsIAA1 in plants has changed the expression patterns of few genes responsive to BRs and auxin. This result suggests that *OsIAA1* can show important role in interplay of BRs and auxin signaling pathways (Song et al., 2009).

Apart from the above mentioned role, BRs and auxin also act additively for the development of lateral root. BRs primarily act at the initiation site of lateral root primordia (LRP), whereas auxin is essential at both the stages, i.e., initiation and emergence of lateral root formation. In this phenomenon, BRs increase LRP initiation by promoting acropetal transport of auxin in the root (Casimiro et al., 2001; Bhalerao et al., 2002; Benkova et al., 2003; Bao et al., 2004). Interplay of BRs and auxin also play a significant role in the development of root apical meristem (Durbak et al., 2012). The size of root meristem was enlarged when expression of BRI1 was in the epidermis. On the contrary, bri1 mutant has small size root meristem with less expression BRI1. Moreover, BRs induced activity was mediated by auxin genes *PIN2* and *PIN4* at transcriptional and posttranscriptional level (Hacham et al., 2011, 2012). The BRs affect the PIN Aux efflux carriers, which regulate the mitotic activity and cell differentiation, indicating a possible mechanism of BR-directed

root growth by the regulation of auxin distribution. Recently, Li et al. (2020) also showed that root meristem development was promoted by cross talk between BRs and auxin in the vascular transition zone of root. They reported that BRs-mediated upregulation of *PIN7* gene expression increased the size of root meristem but the expression of *IAA3/SHORT HYPOCOTYL 2 (SHY2)* gene was downregulated in *Arabidopsis* roots. Additionally, BES1 has the ability to directly bind with the promoter regions of *PIN7* and *SHY2* genes, showing that *PIN7* and *SHY2* regulate the BR-mediated root meristem growth through BES1.

Hypocotyl Elongation

Hypocotyl (primary stem) elongation is one of the widely used assays for any physiological investigation specially to study the impact of PGRs. Photomorphogenesis controlled the growth of hypocotyl. A number of bioassays was performed by Mandava (1988), and on that basis, he suggested a synergistic cooperation between two PGRs, i.e., auxin and BRs. The study was further confirmed and extended by Nemhauser et al. (2003) in *Arabidopsis thaliana*. They used hypocotyl length to determine the growth of plant, although both PGRs are known to induce cell elongation; however, exogenously applied BRs increased the length of hypocotyl (Nemhauser et al., 2003). On the contrary, auxin treatment in the growth media slightly affects the hypocotyl elongation of *Arabidopsis thaliana* seedling. On the other hand, increase in temperature can alter the level of auxin in shoot which results in massive increase in the length of hypocotyl (Gray et al., 1998; Zhao et al., 2002). Furthermore, Nemhauser et al. (2004) examined hypocotyls of plants grown at 29 °C and compared them with plants grown at 22 °C. Plants grown at 29 °C were 1.8 times longer than those grown at 22 °C. This proves that growth of the hypocotyl also depends on the variation in the temperature apart from the hormonal gradient. This result was consistent with earlier findings (Gray et al., 1998; Zhao et al., 2002). In another study, when exogenous brassinolide (BL) was applied to hypocotyls in combination with increased temperature, a "kinked" morphology and a gravitropic growth were exhibited. This result was typically similar to saturating BL conditions (Nemhauser et al., 2004). Similarly, auxin-induced response for the hypocotyl elongation was significantly increased by the treatment of BRs (Vert et al., 2008), and BR signaling mutant bri1 does not show any sensitivity toward temperature for hypocotyl elongation which might be governed by auxin (Halliday & Fankhhauser, 2003). On the basis of this, Vert et al. (2008) suggested that auxin actions were dependent on signal transduction pathway of BRs. Moreover, Vert et al. (2008) reported a synergistic relationship between BRs and auxin and showed that gene expression was increased by the combined treatment of both the PGRs. They also reported a direct link between BIN2 and ARF2. DNA-binding repression activities of ARF2 was loosened by phosphorylation of ARF2 which was regulated by BIN2 and showed that *BIN2* gene of BRs can regulate the expression of auxin-induced genes by direct inactivation of ARF repressors. The interplay between BIN2 and

ARF2 denotes the synergistic effects of BRs and auxin in photo-morphogenesis (Vert et al., 2008). Further, Kozuka et al. (2010) showed that phytochrome-mediated stimulation of shade avoidance syndrome in the petiole of *Arabidopsis thaliana* was directly regulated by auxin/BRs response. They used auxin-deficient mutant (doc1/big) and BRs-deficient mutant (rot3/cyp90c1) to show normal petiole elongation in response to shade which was due to equal response of auxin and BRs. Similarly, Jiang et al. (2020) also reported shade-induced hypocotyl elongation in soybean by cross talk of auxin, GA, and BRs. Exogenous treatment of IAA, GA3, or 24-EBL in white light promotes the hypocotyl elongation, while the inhibitors of GA3, IAA, and BRs of these PGRs decreased the shade-induced hypocotyl elongation. Combined treatment of these biosynthesis inhibitors showed that hypocotyl elongation was fully restored by GA3 and slightly restored by EBL while repressed by IAA biosynthesis inhibitor. In a recent study, Ibañez et al. (2018) reported that growth responses of Phytochrome Interacting Factor 4 (PIF4) and auxin under high temperature were governed by BRs. Under high temperature, BZR1 transcriptional factor accumulates in nucleus and activates genes of growth responses in coordination with the PIF4.

Pattern of Vascular Bundles in Shoots

Another synergistic action of both hormones is manifested in the radial patterning of vascular bundles. Their signaling cross talk is required for the radial patterning of vascular bundles in the shoots of *Arabidopsis* (Ibanes et al., 2009). On the basis of various mathematical modeling and experiments, it was suggested that for the positioning of vascular bundles; asymmetric auxin polar transport and change in auxin level is important. Further, the treatment of BRs upregulate the expression of *PIN* and *ROP* genes which increases the polar transport and endogenous distribution of auxin (Li et al., 2005). In addition to this, many auxin-responsive genes involved in polar auxin transport such as PIN3, PIN4, and influx carriers, auxin-resistant1/like aux1 (AUX1/LAXs) were also regulated by BRs by affecting their cellular localization (Hacham et al., 2011, 2012). On the other hand, BR signal was also found to act as a stimulating signal for a number of cells of provascular ring which is coherent with auxin maxima. Hence, the creation of periodic arrangement of vascular bundles of shoot is under the controlled action of these two PGRs (Ibanes et al., 2009). Recently, Lanza et al. (2012) have showed that reconfiguration of actin cytoskeleton was mediated by BRs that causes the delocalization of the PIN2 transporters of auxin which stimulate the response of auxin.

Inclination of Leaf Lamina

Another role of BRs and auxin interplay was demonstrated by Zhang et al. (2014) on the basis of ChIP and yeast one-hybrid assay, which is inclination of leaf lamina that is associated with the architecture development of rice plant. Zhang et al. (2014) found that rice plants with overexpressing *OsGH3*.5 and *OsARF19* genes has lesser content of free IAA at lamina joint causing in modification of lamina inclination. Also, *OsARF19* binds to the promoter of *OsBRI1* and positively regulates *OsBZR1* expression that results in downstream signaling of gene for the inclination of leaf lamina (Zhang et al., 2014). The results of this study suggest that *OsARF19* gene links the signaling of BRs and auxin for the regulation of lamina inclination in rice.

Under Stress Condition

BRs play a significant role in growth and development under both normal and stressful conditions in plants. Various new researches suggested that BR-mediated stress tolerance mainly depends on the cross talk with other PGRs (Choudhary et al., 2012; Ahammed et al., 2015; Yusuf et al., 2017; Ahanger et al., 2018). The interplay between BRs and auxin for developmental and physiological processes was well documented as we have discussed earlier in this chapter; however, little attention has also been paid toward stress tolerance mechanism of BRs and auxin interplay. In this section, we discuss the role of BRs and auxin under stress tolerance. Many researchers reported cold stress-induced inhibition of intracellular-cycling of *PIN2* and *PIN3* genes of auxin that causes lesser transport of auxin toward shoot and have reduced capability to form auxin gradient in root which is a requisite for root growth and patterning (Harrison & Masson, 2008; Shibasaki et al., 2009; Sukumar et al., 2009). In rice, 12 *OsPIN* genes of auxin transport genes were found in which *OsPIN2* and *OsPIN5b* were stimulated by drought, heat, and cold stresses while the expression of other *PIN* genes remained suppressed under abiotic stress (Du et al., 2013; Saini et al., 2015). Further, Nemhauser et al. (2004) have reported the effect of BRs on auxin transporters and found lesser expression of many genes of auxin like *PIN3*, *PIN4*, *PIN7*, and *LAX* that also include auxin transporter genes. On the other hand, BR treatment upregulated the *AUX/IAA* genes which results in increased expression of ARF7 and ARF19. In *Arabidopsis* seedlings grown under both light and dark conditions, apical hook formation was governed by the antagonism between BRs and auxin (Grauwe et al., 2005) while treatment of BRs suppresses the development of apical hook by repressing the transport of auxin (Gruszka et al., 2016). Additionally, rice genome has seven *YUCCA* genes that encode for the rate limiting enzymes which governed the biosynthesis of auxin (Yamamoto et al., 2007). Du et al. (2013) reported drought stress-induced downregulation of six *OsYUCCA* genes except for *OsYUCCA4* in rice seedlings. Conversely, cold stress upregulated the transcript levels of *OsYUCCA2*, *OsYUCCA3*, *OsYUCCA6*, and *OsYUCCA7* genes and similarly heat stress also upregulated the *OsYUCCA3*,

OsYUCCA6, and *OsYUCCA7* genes up-to five times (Du et al., 2013). Further, yucca mutants of rice have alteration in the transcript levels induced by 40% through BRs, which showed YUCCA-mediated BRs and auxin interplay for abiotic stress tolerance (Nemhauser et al., 2004). Recently, Li et al. (2019) reported a cross talk of IAA, ABA, GA_3, and BRs under drought stress in tea plant. They observed 17 genes of IAA, 17 genes of ABA, 18 genes of GA_3, and 8 genes of BRs under drought stress and performed many analysis using tools like phytohormone determination, sequence analysis, gene expression profiles, Kyoto encyclopedia functional classification, and phylogenetic tree construction, and they showed that IAA, ABA, GA_3,

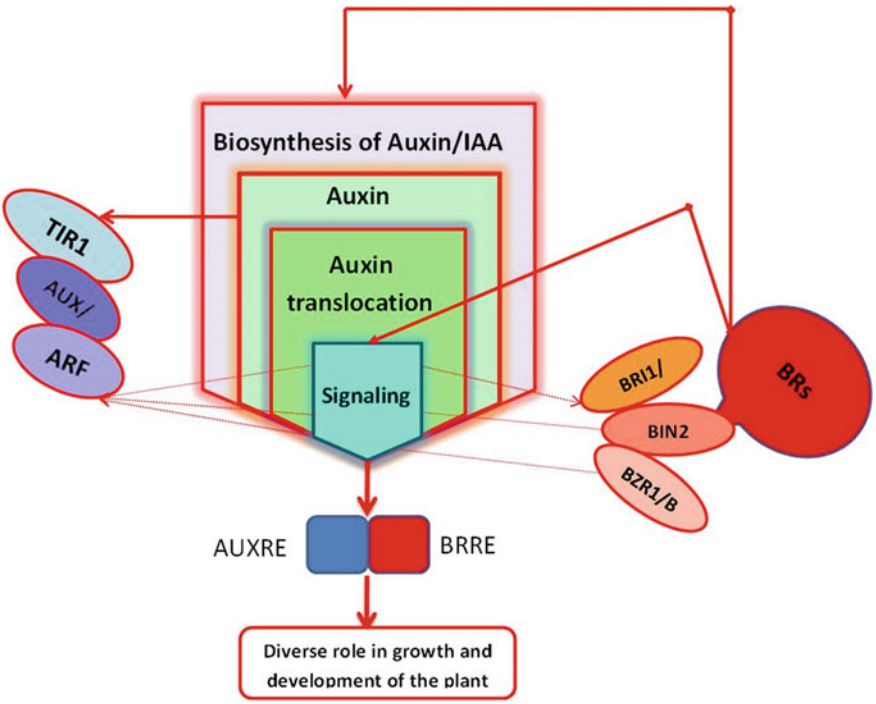

Fig. 8.1 Diagrammatic illustration of brassinosteroid (BRs) and auxin (AUX)-mediated interplay responsible for the growth and development of the plants
BRs is involved in the biosynthesis and signaling of auxin. BRI1 (BRs) and TIR1 (AUX) receptors are responsible for signal perception. When the signal is perceived, BRs binds to the extracellular domain of BRI1 and interacts with co-receptor BAK1 and forms active BRs complex. It causes inactivation of BIN2 and leads to the dephosphorylation of BZR1 and BZR2 (TF). This in turn activates transcription of genes containing BRRE in nucleus. BIN2 suppresses AUX/IAAs by phosphorylating ARF and increases transcriptional activity of the target genes. On the other hand, TIR1 interacts with AUX/IAA proteins. Then AUX/IAA is degraded, and auxin response factors (ARFs) are released. It activates the transcription of genes with auxin-responsive elements (AUXRE). ARFs bind to BRI1 and regulate its expression which activates the BRs signaling. At last the cross talk occurs by the activation of genes containing both BRRE and AUXRE and promotes integrated signaling pathways (BRs and AUX) to regulate the transcription of various target genes

and BR cross talk play an important role in the regulation of tender shoots of tea plants under drought stress (Li et al., 2019). These results suggest possible interplay of BRs and auxin under abiotic stress tolerance. Hence, it is suggested that the interplay between BRs and auxin has a vital role in the regulation of the overall growth and development of the plant (Fig. 8.1).

Signaling Pathway of BRs and Auxin Interplay

Interplay between PGRs is critical for the plant growth and development. Recent works have provided imperative understandings into the close relationship between BRs and auxin signaling pathways in the regulation of developmental processes in plants under normal as well as under stress conditions (Hao et al., 2013; Saini et al., 2013, 2015; Kissoudis et al., 2014; Chaiwanon & Wang, 2015; Yusuf et al., 2017). In *Arabidopsis*, Chung et al. (2011) found that signaling for biosynthesis of BRs was governed by auxin. Exogenous auxin-treated DWF4pro: GUS plants have enhanced expression for *DWF4* gene and concurrently increases the endogenous level of BRs. Moreover, *BRX* gene also acts as a rate-limiting factor in BRs biosynthesis and the expression of *BRX* gene was intensely stimulated by auxin while suppressed slightly by BRs, suggesting that *BRX* acts at the nexus of a feedback loop in BRs and auxin signaling (Mouchel et al., 2006). These results suggest a direct role of auxin in biosynthesis of BRs in plants (Chung et al., 2011). Besides this, the signal transduction pathways of both PGRs are linked and regulate many physiological and biochemical processes like cell elongation, vascular differentiation, and light responses as well as gene expression in plants synergistically (Nemhauser et al., 2004; Hardtke et al., 2007; Keuskamp et al., 2011). For instance, in an assay using hypocotyl elongation, researchers have shown that the auxin-responsive mutants axr1, axr2, axr3, tir1, and arf2 have less sensitivity toward BRs, suggesting the role of BRs in hypocotyl elongation which has been facilitated by auxin signal transduction pathway (Nemhauser et al., 2004; Vert et al., 2008). Another set of data further supports the interplay of BRs and auxin which suggests that auxin response in root development was mediated by BRs signal transduction pathway (Mouchel et al., 2006). Moreover, a close link between BRs and auxin has been reported through the BIN2 and ARF2. Phosphorylation of ARF2 was governed by BIN2 that leads to the loss of DNA-binding activity of ARF2 which stimulates the activity of ARF promoters (Vert et al., 2008), indicating BR–auxin synergistic interaction (Fig. 8.1). It is fascinating that the dominant bin2 mutants display BRs insensitivity and auxin hypersensitivity in the responses of root growth (Perez-Perez et al., 2002; Maharjan et al., 2011). Furthermore, AUX/IAA proteins that are crucial players in auxin signaling pathway are too involved in BRs-induced responses (Vert et al., 2008). In *Arabidopsis*, Chaiwanon and Wang (2015) witnessed that the optimum expression of BZR1 was maintained by the BR signaling, indigenous BRs catabolism, and auxin biosynthesis which is necessary for root growth. Many genes of auxin transporters were regulated by BRs that include PIN3, PIN4, PIN7, and ABCB1 (which

are repressed by BRs), and ABCB4 (which is stimulated by BRs). BRs also regulate the auxin receptor TIR1 and a numbers of ARFs and AUX/IAA transcriptional regulators (Sun et al., 2010). The expression of ARF2 was negatively regulated by BRs treatment (Vert et al., 2008; Sun et al., 2010). Similarly, BRs have been playing a key role in the regulation of many auxin-responsive genes including *PIN3* and *PIN4* genes for polar auxin transport, influx carriers, and AUX1/LAXs (Nemhauser et al., 2004; Hacham et al., 2011, 2012).

Furthermore, BRs governed localization polar transport of auxin in root through PIN2 which stimulates plant tropisms (Goda et al., 2004; Nemhauser et al., 2004). Recently, Lanza et al. (2012) have showed that reconfiguration of actin cytoskeleton was mediated by BRs which causes the delocalization of the PIN2 transporters of auxin which stimulate the response of auxin. These studies suggest interplay between BRs and auxin through auxin transporter genes. In addition to this, many genes like AXR3/IAA17 were altered by the BR treatment. For instance, Kim et al. (2006) showed that BRs treatment significantly stimulated the expression of the *AXR3/IAA17* gene as well as several *Aux/IAA* genes such as *AXR2/IAA7*, *SLR/IAA14*, and *IAA28* while BRs signaling mutant (bri1) and the BRs biosynthesis mutant (det2) showed lesser *AXR3/IAA17* gene expression. This result provides an interplay of BRs and auxin signaling in root development as BRs-induced *Aux/IAA* genes like *AXR3/IAA17* gene might play a role in root development (Kim et al., 2006). Further, as we know that auxin gradient is established by PIN auxin carriers (Ljung et al., 2005; Wiśniewska et al., 2006), however, some structurally related PIN homologs, PIN-LIKES (PILS) family proteins, are also found at the endoplasmic reticulum (ER) (Mravec et al., 2009; Barbez et al., 2012). PILS proteins check the nuclear availability and signaling of auxin apparently by seizing auxin in the ER (Barbez et al., 2012). This concept has been supported by the findings of Sun et al. (2020) after observing PILS overexpressing mutants which caused growth defects that showed similarity with auxin (signaling) deficiency. Sun et al. (2020) successfully identified the causative second-site mutation in the imp1 mutant, which is interestingly due to functional loss BRs receptor, BRI1. bri1 mutants were severely affected and showed many developmental and physiological defects. Certainly, they found that *PILS* gene activity was mediated by BRs signaling at both the transcriptional and posttranslational levels. Similarly, the promoters of *PILS2*, *PILS3*, and *PILS5* have BRs response elements which were confirmed by direct binding of BZR1 to the promoter of PILS2 (Rana & Hardtke, 2020; Sun et al., 2020). Thus, these results indicate that BRs signaling suppresses PILS availability, and in this way, auxin sequestration in the ER led to enhanced auxin availability in nucleus and signaling which has been depicted in Fig. 8.2 (Rana & Hardtke, 2020; Sun et al., 2020). Hence, the interaction of signal transduction pathway between BRs and auxin plays very important role in regulating the overall development of plants (Vert et al., 2008; Cho et al., 2014a, b; Oh et al., 2014; Rana & Hardtke, 2020; Sun et al., 2020).

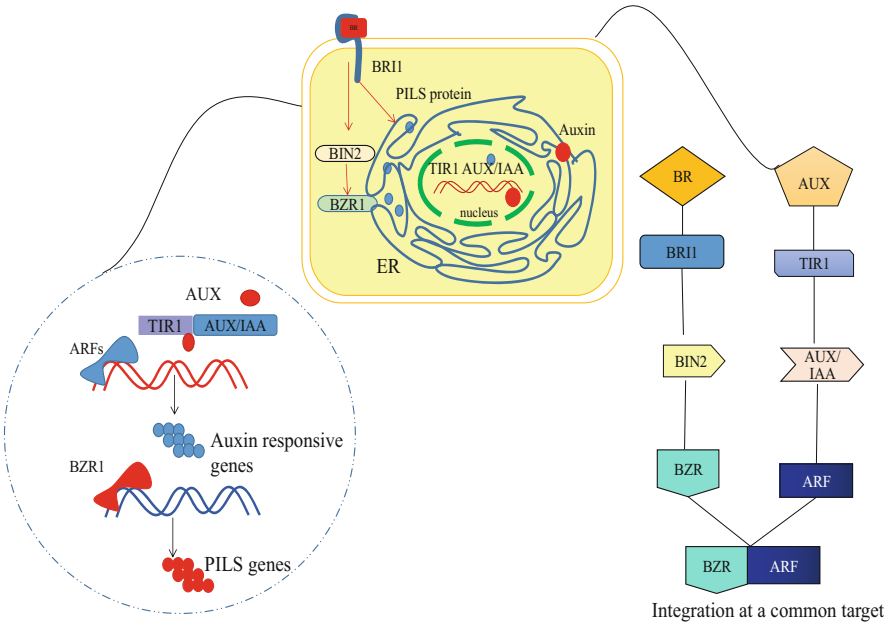

Fig. 8.2 A pictorial representation of PILS-dependent brassinosteroid and auxin cross-talk When brassinosteroid (BR) binds with its receptor BRI1, signals are transmitted to BIN2 for the stimulation of BZR1 which enter in the nucleus, where BZR1 suppresses the expression of PILS genes. In this way, auxin sequestration into the endoplasmic reticulum is decreased and nuclear auxin concentration is increased. This ultimately leads to enhanced expression of auxin-responsive genes by the release of auxin response factors (ARF) from inhibition by AUX/IAA proteins, which are degraded by auxin-regulated interaction with TRANSPORT TIR1 family of auxin receptors. (Modified from Rana & Hardtke, 2020; Sun et al., 2020)

Conclusion and Future Perspective

Brassinosteroids (BRs) have been shown to regulate several physiological and biochemical processes in plants under normal as well as in stressful conditions. But, here the interesting fact is that many BRs-induced responses are also influenced by other PGRs. Several studies have showed that BRs and auxin are involved synergistically in an array of developmental processes in plants that include root growth, hypocotyl elongation, vascular bundle development, leaf lamina inclination, etc. Besides these developmental processes, the interplay of BRs and auxin also regulates the stress responses. The synergistic actions of BRs and auxin are very complicated and contain many identical target genes which regulate each other jointly on multiple levels. Hence, we can say that a considerable interplay between these two PGRs exist which controls the overall development of the plants. However, a detailed molecular mechanism between the interplay of BRs and auxin still indefinable in plant, and further investigations are needed for the better understanding of BRs–auxin cross-talk. Using the new experimental tools, it is predictable that

in upcoming years there will be a noteworthy addition of information in the mode of BRs action with auxin in regulating plant developmental under normal as well as in stress conditions.

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Chapter 9
Brassinosteroids Cross Talk with ABA Under Stress Condition

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Introduction

Brassinosteroids (BRs) are steroid hormones and widely distributed throughout the plant kingdom. BRs were first discovered by Mitchell et al. (1970) and later purified from the *Brassicus napus* pollen by Grove et al. (1979). X-ray analysis of the purified compound revealed that this steroid hormone is similar to animal steroid hormones and was given systemic name (22R,23R,24S)-2α-3α,22,23-tetrahydroxy-24-methyl-6,7-s-5α-cholestano-6,7-lactone and common name brassinolide (BL). BL and its derivatives are called BRs and can be classified into C27, C28, or C29 according to the number of carbon in their structure (Vardhini, 2014). Now, more than 70 BR-related compounds have been identified from more than 50 species of gymnosperms, angiosperms, bryophyte, pteridophyte, and green algae (Fujioka et al., 1998). However, brassinolide, 24-epibrassinolide, and 28-homobrassinolide are the most bioactive BRs among others which are extensively used in laboratory experiments (Vardhini et al., 2006; Wang et al., 2017).

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BRs are considered ubiquitous in plant kingdom as they have been found in all tested plant organs. The higher level of BRs was found in pollen, roots, immature seeds, flowers, and young growing tissues ranging from 1 to 100 ng/g fresh weight (FW), while low amount of 0.01–0.1 ng/g FW was found in vegetative tissues, shoot and leaves (Takatsuto, 1994; Bajguz & Tretyn, 2003). Different from other hormones, BRs do not transfer between tissues but function in autocrine and paracrine manner in plants (Bishop & Yokota, 2001) and long-distance effect of BRs depends on their interaction with hormones like cytokinin, abscisic acid, auxins, ethylene, and gibberellin (Lacombe & Achard, 2016). Since the discovery of BRs, the main components of BR biosynthetic pathway have been identified through different biochemical and genetic assays (Zhu et al., 2013). Membrane-localized receptors recognize BR and send BR-mediated signals to the nucleus to activate BR-responsive gene transcription that triggers cellular growth (Zhao & Li, 2012). Plants deficient in BR biosynthesis are typically dwarf, dark green in color, exhibit epinastic leaves, and show low and no fertility with delayed development (Bishop & Koncz, 2002). BR application to BR-deficient mutant can partially and fully rescue a wild-type phenotype. Antonymously, BR biosynthesis mutants can be phenocopied by applying specific BR biosynthesis inhibitors such as brz2001 and brassinazole (Asami et al., 2000; Sekimata et al., 2001).

Brassinosteroids (BRs) as phytohormones play an important part in various plant growth and development processes and increased crop yields through both changing plant metabolism and protecting plants from environmental stresses. Like animal steroids, BRs are crucial for regular plant growth, development, and reproduction. Studies on BR biosynthetic mutants clearly demonstrated that these plant steroid hormones are essential for the regulation of a variety of physiological processes including cell division, cell elongation, vascular and stomatal differentiation, timing senescence, male fertility, seed germination, leaf development, plant immunity, and reproduction (Tang et al., 2016). Moreover, BRs are also involved in regulating hundreds of genes and plant oxidation reduction metabolism and help to control overall programs leading to morphogenesis. On the other hand, BR application in agriculture improves plant growth and yield by mediating plant responses to stress conditions including heavy metals (Cd, ZN, Ni, Cu, Al, etc.), drought, high and low temperature, salinity, and nutrient deficiency (Fariduddin et al., 2014). Despite the extensive knowledge about the BR biosynthesis components and their function in cell-specific manner, limited literature is available about the question how BR biosynthesis pathway interacts with other hormone pathways under normal and environmental stressed conditions, particularly abscisic acid hormone. In this chapter, we provide an overview of the role of BR in plant growth and development and then discuss how BR react under different environmental stress conditions. We will also highlight how BRs function with ABA to regulate plant growth and development. At the end, we review our understanding of BR cross talk with ABA and elaborate its genetic basis to overcome the gap in our knowledge related to BR cross talk with ABA.

Significance of Brassinosteroids in Plants

Previous research work on BR biosynthetic mutant of *Pisum sativum* and *Arabidopsis thaliana* has provided strong evidence that BRs are essential for plant growth and development (Nomura et al., 1997; Tao et al., 2004; Fàbregas & Caño-Delgado, 2014) and play an important role in plant metabolism regulation in a range of plant species (Ahammed et al., 2015; Çoban & Baydar, 2016) like gibberellic acid, and the role of BRs for the promotion of seed germination is well documented in literature (Leubner-Metzger, 2003). The treatment of seeds of wheat with homobrassinolide (Hayat et al., 2003), chick pea and groundnut with 28-homobrassinolide (Vardhini & Rao, 1999; Ali et al., 2005), and tobacco with brassinolide promoted germination (Leubner-Metzger, 2001). Seedling elongation and endosperm rupture are also promoted by BR treatment in tobacco seeds, and the dose of 0.01 μM of brassinolide is reported as the most effective (Leubner-Metzger, 2003). The growth of seedling is also increased by BR application which is reported in *Zea mays* (Arora et al., 2008) and *Brassica juncea* (Sirhindi et al., 2009). Study of Rao et al. (2002) revealed that BR application in late winter inhibited the flowering in grapes but regulated the number of flowering in autumn.

BR application promotes the root growth in linear fashion, and correct level of BRs is crucial of normal root growth and development. González-García et al. (2011) reported that treatment of wild-type roots with low concentration of BRs increased their length, although this enlargement is not always detectable. The excess and lack of BRs are the primary detriments in root growth and development. Mutant plants that lack BR compound synthesis exhibited short roots (Chaiwanon & Wang, 2015; Hacham et al., 2011), differently short roots were also observed in plants treated with high BR concentration (González-García et al., 2011). The treatment of low BR concentration can be used to promote the impaired BR biosynthesis in short root mutants (Chaiwanon & Wang, 2015). Application of 24-epibrassinolide and 28-homobrassinolide through root in *Cucumis sativus* (Kang et al., 2009) and 28-homobrassinolide seed soaking treatment of *Raphanus sativus* (Anuradha & Rao, 2009) and *Lycopersicon esculentum* (Hayat et al., 2010) enhanced the photosynthetic rate. It has been revealed that homobrassinolide regulates various enzyme activities that are involved in photosynthesis and reported that homobrassinolide presoaked seed improves the growth of seedling and chlorophyll a content (Hayat et al., 2007). The total chlorophyll contents increased in the leaves of *Cucumis sativus* (Yu et al., 2004) by 24-epibrassinolide, *Raphanus sativus* (Anuradha & Rao, 2009), and *Lycopersicon esculentum* (Hayat et al., 2010) by 28-homobrassinolide treatment. Exogenous application of 24-epibrassinolide was reported to promote CO_2 assimilation in cucumber plants through increasing activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and fructose-1,6-bisphosphatase (Jiang et al., 2012). In *Vigna radiata* plant (Fariduddin et al., 2006) and wheat seeds (Hayat & Ahmad, 2003), the application of BRs evidenced to increase the level of nitrate reductase enzyme activity. Application of BRs in plants increased photosynthetic potential and biomass accumulation through enhanced

regulation of Benson–Calvin cycle and sugar metabolism (Jiang et al., 2012). Improved concentration of phenols and flavonoids in tomato roots (Ahammed et al., 2013) was possible as a result of epibrassinolide-mediated increased activity of phenylalanine ammonia-lyase (PAL). Moreover, combined application of epibrassinolide and methyl jasmonic acid (MeJA) enhanced secondary metabolite concentration in sweet basil (Koca & Karaman, 2015). BR treatments play a positive role in sugar accumulation during Cabernet Sauvignon ripening and promote red color of *Vitis vinifera* fruits (Xu et al., 2015; Vergara et al., 2018). Besides, during veraison, increased sugar accumulation, total anthocyanin content, and reduced total acidity at harvest were reported by foliar application of 24-epibrassinolide in "Cabernet Sauvignon" (Luan et al., 2013; Xi et al., 2013). BRs also involved in the differentiation of vascular tissues was first reported in 1991 (Clouse & Zurek, 1991). The application of brassinolide in nanomolar concentrations into the xylem differentiation medium of *Helianthus tuberosus* exhibited ten-fold increase in the differentiation of xylem in the first 24 hours (Castle et al., 2003).

A process in which etiolated seedling grow as long hypocotyl and fail to expand cotyledons in the dark called skotomorphogenesis, is also controlled BRs and BRs biosynthesis deficient mutant fail to establish skotomorphogenesis and produced light grown plants (Clouse & Sasse, 1998). Previous researches have further uncovered a role of BRs in increased yield and yields components in plants. Application of BR under stressed condition to cucumber plants enhanced antioxidant system activity and significantly improved plant growth, yield and yield components (Anwar et al., 2018). Moreover, fruit ripening and growth of mango fruit, yield of yellow passion fruit and pear, quality of pitaya, growth, number of fruits and weight per plants was reported to improve via BRs application (Anwar et al., 2018; Zaharah et al., 2012; Thussagunpanit et al., 2015).

Brassinosteroid Under Stress Conditions

BR has greater impact on plants for the adaptation to various biotic and abiotic stresses. Metal accumulation in numerous plants has been investigated after BR application and found that BR controls defensive hormones and enzymes under stress conditions. For instance, foliar application of homo-brassinolide to Cd-exposed *Brassica juncea* alleviated oxidative damage through enhancing antioxidative enzymes activity such as POD, SOD, and CAT and osmolyte contents such as proline (Hayat et al., 2007). In Cd-exposed *Phaseolus vulgaris,* 24-epibrassinolide treatment improved Cd tolerance through increased activities of various antioxidant enzymes and proline content as well as improved relative leaf water content (RLWC) and membrane stability index (MSI) (Rady, 2011). Mitigation of toxic effects of Cd (50, 100, or 150 μM) was reported in *Cicer arietinum* as a results of 28-homobrassinolide-mediated increase in enzymes of antioxidative defense system (Hasan et al., 2008). The role of BRs to mitigate the toxic effects of Ni was also reported in different plants. Kanwar et al. (2013) demonstrated that

the pretreatment of *Brassica juncea* with 24-epibrassinolide lowered the Ni ion uptake and improved plant growth by enhancing the activities of antioxidative enzymes. Earlier these authors reported that 24-epibrassinolide application to Ni-stressed (0.2, 0.4, and 0.6 mM) *Brassica juncea* plants enhanced stress-ameliorating enzymes activities by lowering Ni ion uptake in plants (Kanwar et al., 2012). Yusuf et al. (2011) have also demonstrated that the foliar application of 28-homobrassinolide (0.01 μM) reduced the toxicity effects of Ni (50 and 100 μM) in five wheat cultivars (UP-2338, DL-LOK-01, DL-373, HD-2338, and PBW-373). Elevated seed germination and root and shoot length via the use of 28-homobrassinolide was reported in Indian mustard under Ni stress (Yusuf et al., 2011).

The use of 24-epibrassinolide in beetroots exposed to Pb content showed 50% reduction in metal uptake compared to plants treated with metal alone, indicating the role of BRs in reducing metal uptake (Khripach et al., 1998). Application of 24-epibrassinolide to Pb-stressed *Raphanus sativus* L. seedlings improved plant growth by increasing the activity of GPX, SOD, APX, CAT enzymes and reducing the activity of POD enzyme (Anuradha & Rao, 2009). Moreover, pretreatment of BRs to *Brassica juncea* seeds under Cu stress significantly alleviated metal uptake and accumulation in plants and promoted shoot generation and biomass production (Sharma & Bhardwaj, 2007). Reports are also available on the role of BRs in *Raphanus sativus* seedlings against Zn toxicity (Ramakrishna & Rao, 2013), in *Zea mays* plants against elevated levels of Mn (Wang et al., 2009) and in *Raphanus sativus* against As stress (Raghu et al., 2014). Analogously, application of BRs to Ca $(NO_3)_2$ stressed cucumber seedling increased the activity of antioxidative enzymes, rate of photosynthesis, and subsequently ultra-structure of chloroplasts (Yuan et al., 2012).

Hamada (1986) described that significantly elevated salt tolerance in *Oryza sativa* seedlings emerges from the application of brassinolide in nutritive solution under greenhouse conditions. In *Eucalyptus camaldulensis*, treatment of 24-epibrassinolide was evidence to promote seed germination when exposed to NaCl (Sasse et al., 1995). Similarly, application of brassinolide to NaCl-exposed *Hordeum vulgare* was reported to detoxify NaCl and protected nucleus and ultra-structure of chloroplast (Kulaeva et al., 1991).

Extensive studies are available on the positive role of BRs in the alleviation of toxic effect of high and low temperature in different plant species. In young seedling of two indica rice, heat-sensitive *Xieqingzao* B and heat-tolerant 082, spraying of BR (0.005 mgL^{-1}) exhibited enhanced antioxidant enzyme activities, leakage of leaf electrolytes, and decrease in MDA content (Cao & Hua, 2008). Stomatal conductance and limitation, net photosynthetic rate, and water use efficiency were improved in melon ecotypes under heat stress via pretreatment of epibrassinolide (1.0 mgL^{-1}) (Zhang et al., 2013). Numerous studies on the role of BRs in plants under low temperature stress are reported in literature (Xi et al., 2013; Liu et al., 2009; Hu et al., 2010; Kumar et al., 2010). Janeczko et al. (2007) reported reduction in ion leakage after BR treatment prior to chilling exposure. 28-Homobrassinolide (10^{-8} or 10^{-6} M) effectively reduced chilling injury in cucumber (*Cucumis sativus*) by

elevated prolein content and antioxidant enzymes like CAT, POD, and SOD (Fariduddin et al., 2011). BR-mediated reduction in MDA content and electrolyte leakage in peppermint were reported to reduced chilling injury during 18-day storage at 3 °C (Wang et al., 2012). Furthermore, pretreatment of maize seedling with 1.0 μM epibrassinolide significantly increased chlorophyll content, soluble protein and sugar content, plant height, and dry matter under chilling stress (Singh et al., 2012). Besides, the application of 1.0 μM 24-epibrassinolide promotes *Lycopersicon esculentum* growth and alleviates water stress. Similarly, treatment of 24-epibrassinolide and 28-epibrassinolide effectively increased seed germination and seedling growth of *Raphanus sativus* when exposed to water stress (Mahesh et al., 2013). Zhang et al. (2008) reported that BR spray on drought-stressed *Glycine max* elevated the POD and SOD enzyme activities, increased soluble sugar and proline content, and reduced drought toxicity effects.

Apart from the abovementioned stress factors, BRs and their analogous compounds can also play effective role in the management of numerous other abiotic stress factors such as water logging stress, photo inhibition, pesticides, etc. (Kang et al., 2009; Xia et al., 2009; Liang & Liang, 2009; Lu & Guo, 2013; Sharma et al., 2013; Vardhini & Anjum, 2015)

Brassinosteroid Cross Talk with Hormones

Previous studies revealed that BR cross talk with other plant hormones such as abscisic acid (ABA), auxin (AUX), gibberellins (GA), jasmonic acid (JA), and ethylene can regulate many plant developmental process as well as they promote stress tolerance (Peres et al., 2019; Zhang et al., 2009; Hu et al., 2017; Zheng et al., 2016), for instance, brassinolide-induced biosynthesis and accumulation of ethylene in mung bean epicotyl (Arteca et al., 1983) and tomato (Zhu et al., 2016). The BR and auxin showed synergistic interaction in stem elongation and root developmental processes (Wei & Li, 2016; Zhao, 2010), while antagonistic relationship was observed in controlling of cell elongation, stem cell maintenance, and gene expression of root tips (Chaiwanon & Wang, 2015). Enhanced auxin response in hypocotyl elongation is observed in auxin-responsive mutant after BR treatment, suggesting the existence of functional BR signal transduction pathway (Vert et al., 2008; Nemhauser et al., 2004). Consistence with these observations, researcher also found that low BR biosynthesis in de-etiolated-2 (det2) *Arabidopsis* mutant seedlings eliminated gibberellins-mediated hypocotyl elongation, indicating that cell elongation is largely dependent on both the hormones (Stewart Lilley et al., 2013). Similar interdependent relationships between gibberellins and BRs were observed in *Vigna radiate*, *Pisum sativum*, and *Oryza sativa* by metabolic, physiological, and molecular studies (Gregory & Mandava, 1982; Jager et al., 2005; Yang et al., 2004) and found that these interactions largely depend on developmental stage and physiological condition (Unterholzner et al., 2015). In growth and developmental processes, a cross talk between BRs and cytokinin demonstrated acceleration in the

growth of lateral roots in PYK10::CXK3 transgenic plants as compared to wild-type plants (Vercruyssen et al., 2011). Moreover, reduction in cytokinin-induced anthocyanin accumulation was revealed in *Arabidopsis* BR biosynthesis-deficient mutant *dwf4* and BR signaling mutant *bri1-4* as compared to wild-type plants when treated with exogenous BRs (Yuan et al., 2015).

Recent molecular studies uncovered that collateral hormone biosynthesis pathways regulate common target gene expression which provides strong evidence for BRs and other hormone interactions. Cross talk between BRs and other hormones is mainly responsible for alternation in the hormone biosynthetic genes expressions and signaling intermediates (Yi et al., 1999; Friedrichsen et al., 2002; Fang et al., 2003). Co-application of GA and BL resulted in increased *MERI5* gene expression which belongs to xyloglucan endotransglucosylase (*XET*) *gene* family involved in loosening of cell wall (Tanaka et al., 2003). Moreover, during brown planthopper (BPH) infestation in rice plants, exogenous application of BR reduced salicylic acid content by downregulating the expression of SA pathway-related genes (ICS1 and PAL), while increased jasmonic acid content by upregulating the expression of JA pathway-related genes (Pan et al., 2018). RNA sequence analysis of GA-treated plants and GA-treated plants grown on medium supplemented with BR biosynthesis inhibitor revealed that BR is required for 66.7% of GA-regulated genes (Bai et al., 2012). Besides, cross talk between BR and other plant hormones is dose dependent, where these hormones act as positive as well as negative regulator (Lv et al., 2018). High concentration of BR enhances ethylene biosynthesis by stimulating aminocyclopropane-1-carboxylate synthesis (ACS) protein stability, while low BR levels suppress ethylene biosynthesis by increasing the activity of *BZR1/BES1* transcription factors that inhibit ACS gene transcription (Lv et al., 2018). Analogously, González-García et al. (2011) reported that low concentration of BL is involved in short root phenotype of the BR-insensitive *bri1-116* mutant.

Cross talk between BRs and other plant hormones is also known to be involved in a wide range of stress responses (Larkindale et al., 2005; Bari & Jones, 2009). Application of 24-epibrassinolide showed high salt and heat tolerance in ethylene-insensitive and -deficient mutants (Divi et al., 2010). Mayak et al. (2004) demonstrated that treatment of salt-exposed lettuce plants with DI-31 brassinosteroid rescued plant from premature death and weight loss and decreased ethylene production via reducing aminocyclopropane-1-carboxylic acid (ACC) which showed good protective effects of BR against salt stress. NPR1 protein that regulates SA-mediated defense genes is an essential component of 24-epibrassinolide-induced tolerance against temperature and salt stress in *Arabidopsis thaliana* (Divi et al., 2010). Furthermore, the expression of *IPT* gene in rice transgenic lines under stress-induced promoter (*PSARK*) involved in enhanced CK content before the beginning of senescence as well as the upregulation of *BRL3*, *BRI1*, *BH1*, *BIM1*, and *SERK1* genes (responsible for BR signaling) and *DWF5* and *HYD1* (involved in BR biosynthesis) genes under water-stressed conditions (Peleg et al., 2011). In summary, the above studies suggest that BRs can cross talk with numerous other hormones in regulating many plant growth and developmental processes, as well as stress responses but have not clarified the specific role of single hormones with

BRs. In this chapter, we will mainly focus on various aspects of ABA and BR cross talk and its regulatory mechanism under stressed conditions. The use of the key points from different studies will clarify the how ABA and BR interaction increases stress tolerance.

Brassinosteroid–Abscisic Acid Crosstalk

Brassinosteroid Cross Talk with ABA in Plant Growth and Development

Abscisic acid (ABA) and BRs as phytohormones co-regulate a wide range of plant developmental processes and play an important part to overcome stress conditions (Zhang et al., 2009; Finkelstein et al., 2008). It is well established that ABA stimulates seed dormancy in maturating embryos and inhibits seed germination, whereas antagonistically, BRs promote seed germination through enhancing the potential of embryo growth (Finkelstein et al., 2008; Steber & McCourt, 2001; Wang et al., 2020).

Ephritikhine et al. (1999) screened out auxin and ABA hypersensitive mutant *sax1*, for root elongation response. Root elongation inhibition in *sax1* was two to three times more sensitive to auxin and 40 times more sensitive to ABA as compared to wild-type controls. In vitro grown Sax1 seedlings were characterized by short curled primary roots and round, small, dark green cotyledons, while greenhouse grown adult sax1 plants showed dwarf phenotype, reduced fertility, and delayed development. A wild-type size was restored after exogenous application of brassinosteroid to mutant seedling, indicating the suppression of brassinosteroid biosynthesis in sax1 plants. Besides wild-type sensitivities to other plant hormones such as ABA, auxin and gibberellins were also rescued in sax1 mutants by exogenous application of brassinosteroid (Ephritikhine et al., 1999). The *bee1 bee2 bee3 triple* mutant contains null allele of all three gene, had phenotype similar to known BR mutants in term of seedling and floral size, and was less responsive to endogenously applied brassinolide in hypocotyl growth assays. ABA treatment repressed the transcription of *BEE1, BEE2*, and *BEE3.* In addition, ABA-hypersensitive mutant, *era1*, hypocotyl was less responsive to brassinolide and 20% reduction were observed in average length of hypocotyl in the presence of brassinolide hormone as compared to wild type (Friedrichsen et al., 2002). Likewise, Steber and McCourt (2001) demonstrated the inhibition of germination in BR biosynthetic mutant *det2-1 and* BR-response mutant *bri1-1* after 0.6 μM ABA treatment, whereas the germination of WT plants *was* not inhibited by until 1.2 μM ABA treatment, suggesting that BR is required to rescue the ABA-induced dormancy and stimulate germination. Similarly, germination, root elongation, hypocotyl, and *s*tomatal apertures were severely inhibited by *det2* and *bri1-9* mutants in response to ABA treatment as compared to wild-type plants (Xue et al., 2009).

A brassinosteroid-insensitive mutant *bril* was not responsive to BRs in primary root inhibition and hypocotyl elongation assays and showed sensitivities to auxins, cytokinin, ethylene, abscisic acid, and gibberellins hormones. The mutant *bril* was characterized by dwarfed phenotype; dark green and thickened leaves, reduced male sterility, apical dominance, and de-etiolation of dark grown seedlings, which could not be reversed by brassinosteroid treatment (Clouse et al., 1996). Similarly, *bin2* mutant was insensitive to BR in root growth inhibition and feedback inhibition assays and exhibited ABA hypersensitive phenotype that was similar to *bri1* and BR-deficient mutants. *bin2* mutant had shown insensitivity only to BR but retained sensitivity to other phytohormones (Li et al., 2001). Like *bri1*, *bin5* displayed hypersensitive response to ABA treatment in root growth inhibition assay, indicating the role of *bin5* and *bin3* in BR signaling pathway (Yin et al., 2002). Based on these results, it can be concluded that BR stimulates germination and increases the expectation that BR is needed for normal germination.

Brassinosteroid Cross Talk with ABA in Stomatal Closure

The function relationship between BRs and ABA in response to stomatal closure is complex (Acharya & Assmann, 2009). On the one hand, antagonistic interaction between BR and ABA demonstrates enhanced ABA-induced stomatal closure in BR-deficient mutants *sax1* and *det2* and BR signaling mutant *bri1-9* of *Arabidopsis thaliana* (Ephritikhine et al., 1999; Xue et al., 2009). Working with *det3* mutant, Allen et al. (2000) found that guard cell can discriminate between various signals by oscillation patterns of cytosolic Ca^{2+}. However, the production of specific Ca^{2+} cyt oscillation patterns as a results of ABA stimulation in *det3* mutant leads to stomatal closure. On the other hand, very few studies on BRs and ABA cross talk to promote stomatal closure are available. BR follows similar patterns to that of ABA for the promotion of stomatal closure and inhibition of stomatal opening and inhibited the K^+ in currents involved in stomatal opening in *Vicia faba* guard cells (Haubrick et al., 2006). Similar interactions between BRs and ABA to induce stomatal closure were also reported by Xu et al. (2015). Moreover, BR treatment increased the ABA content and upregulated the expression of ABA biosynthetic gene *vp14* by increasing the NO production in mesophyll cells of maize leaves, demonstrating an important mechanism for BR-enhanced water stress tolerance (Zhang et al., 2011).

BR-induced opening and closing of stomata is a concentration dependent manner. Different levels of BR play an important role in opening and closing of stomata via kinetics and levels of ROS production. Low level of BR caused transient increase in ROS production, which leads to stomatal opening via GSH biosynthesis. In contrast, prolonged increase in ROS production by high BR concentration drives stomatal closure. These results indicated that BRs promote stomatal closure independently of ABA via ROS production (Xia et al., 2014). On the contrary, the investigation of Ha et al. (2016) revealed that BR can positively and negatively regulate ABA-induced stomatal closure. They found that ABA (*AtrbohD*, *NIA1*, and *NIA2*) induces

expression of some genes for ROS production, and the resultant ROS generation can be suppressed by BR treatment. BR application did not respond to ABA-induced stomatal closure in BR signaling mutant *bri1-301*. However, ABA hypersensitivity was observed in *BRI1* overexpressing transgenic plants during stomatal closure, and BR treatments rescued stomatal closure more completely than wild-type plants. BRI1 receptor is a leucine-rich repeat (LRR) serine/threonine receptor-like kinase (RLK) located in the plasma membrane, perceives BR to trigger BR signaling, and forms function receptor complex by inducting another type of LRR-RLK, BAK1 (Wang et al., 2005; Nam & Li, 2002). Shang et al. (2016) reported ABA insensitivity of BAK1 in stomatal closure and increased water loss was observed in BAK1 mutants as compared to WT. ABA-induced *OST1* gene expression and overexpressed *OST1* did not cure BAK1 insensitivity to ABA. BAK1 forms a function complex with *OST1* near plasma membrane, and brassinolide negatively affects the BAK1/OST1 complex. Collectively, the above studies concluded that cross talk between ABA and BR signaling is essential for the regulation of stomatal closure.

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Chapter 10
Cross Talk Between Brassinosteroids and Cytokinins in Relation to Plant Growth and Developments

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Introduction

Brassinosteroids (BRs) are steroidal phytohormones having polyhydroxylated sterol structure (Grove et al., 1979). These plant-specific growth regulators were first obtained from pollens of *Brassica napus*. These hormones are mandatory for a number of physiochemical processes in plants. Various techniques have confirmed the BR application in signaling vital biological process in plants (Divi et al., 2015). BRs have a pivotal role in cellular growth, seed germination, root growth, photomorphogenesis, shoot growth, reproduction, and stress alleviation (Clouse & Sasse, 1998; Li & Chory, 1999; Sreeramulu et al., 2013; Sharma et al., 2015). These hormones activate phosphorylation procedures in plant cells (Belkhadir & Jaillais, 2015). The multidimensional significance of BRs has attracted researchers to explore the role of these hormones in crop production (Zhu et al., 2013a, b). The endogenous concentration of these phytohormones affects growth and development phenomenon of plants (Tanaka et al., 2005). The level of BR synthesis, translocation, and breakdown regulates homeostasis and concentration of BR in plant.

Plant mutants devoid of BRs show abnormal leaf shape, stunted growth, anomalous vascular tissues, abnormal ripening, and reproduction (Clouse, 2011).

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Nevertheless, the exogenously applied BRs regulate plant growth through modulating genes involved in BR synthesis (Tanaka et al., 2005; Zhu et al., 2013a). BRs interact with other growth hormones to maintain plant growth, development, and stress tolerance (Divi et al., 2015; Yuan et al., 2014).

Cytokinins (CKs) are a group of plant hormones involved in numerous biological progressions including light reactions, organogenesis, nutrition, and stress alleviation (Vert et al., 2008; Vilarrasa-Blasi et al., 2014; Vriet et al., 2013). Isopentenyl transferases (IPTs) are the main enzymes regulating the synthesis of CKs as well as cytokinin oxidases/dehydrogenases CKXs. These CKXs modulate the activity of CKs (Vert et al., 2008).

Effect of CKs and BRs on Plant Growth

Cytokinins (CKs) and BRs have collegial interaction to stimulate cellular division and growth in plants (Sasse, 1985; Clouse & Sasse, 1998; Riou-Khamlichi et al., 1999; Hu et al., 2000; Nakaya et al., 2002). BRs regulate cellular division in applied tobacco callus by modifying the synthesis of cytokinin and auxin (Bach et al., 1991; Gaudinová et al., 1995). Application of CK in 21- to 96-h-old *Triticum vulgare* coleoptiles enhanced the effect of 24-epiBL (Sasse, 1985). The exogenously applied CK and BRs synergistically enhanced the fresh weight in *Onsoma paniculatum* culture (Yang et al., 1999).

Stress Response

Heavy metal pollution in environment have arisen due to increasing industrial manufacturing and anthropogenic activities. Multiple hazardous environmental pollutants enter the food chain, resulting in disturbance in ecosystem balance. Plants have the ability to uptake and absorb heavy metals from metal contaminated soil. Some metals are regarded as vital due to their pivotal role in regulation of redox processes. Even though, high concentration of metals may cause toxic effects on metabolomics of plants. Toxic effects of plants result in interaction of functional groups with polynucleotides and proteins (Chary et al., 2008). Ultimately, these toxic symptoms lead to reduced photosynthate accumulation, lowered pigmentation, and elevated level of malondialdehyde content. Phytohormones play a pivotal role in the regulation of oxidative damage in plants. Brassinosteroids are well known in reducing biotic and abiotic stresses in plants, along with the regulation of morphogenetic and physiochemical responses in plants (Vardhini, 2016; Bajguz & Hayat, 2009; Krishna, 2003).

The role of BRs in the accumulation of various metals like lead, zinc, copper, and cadmium in different cultivated crops (tomato, barley, and radish) has been studied (Hasan et al., 2011; Ramakrishna & Rao, 2013, 2015). Application of

24-epibrassinolide reduced lead content in beetroot as compared to metal-treated seedlings. This is due to the fact that BRs reduced metal absorption (Khripach et al., 1999). Brassinosteroids reduced the uptake and accumulation of copper in *Brassica juncea* grown in Cu-accumulated soil (Sharma et al., 2007). Brassinosteroids change the metal content in plants; however, the change depends on the application of BRs on the growth stage of plant. Recent research revealed that BRs reduced bioaccumulation of metals in cultures of *Chlorella vulgaris*. This reduced metal accumulation resulted in growth enhancement in *C. vulgaris*. BRs protected chlorophyll architecture and enhanced phytochelatin synthesis (Bajguz, 2002). Furthermore, BR application resulted in incremented growth of mung bean plants exposed to aluminum (AL) stress (Abdullahi et al., 2003). Furthermore, brassinolide enhanced chlorophyll content, root and shoot fresh weight in mung beans seedlings exposed to AL stress (Ali et al., 2008). Yusuf et al. (2011) reported that 28-homobrassinolide elevated seed germination, shoot length, root length in Indian mustard grown in Ni-contaminated soil. In another report, BR eliminated the toxic effects of cadmium on water splitting complex and reaction centers of rape cotyledons (Janeczko et al., 2005). Numerous researchers reveal that BR reduce metal toxicity in numerous plants like radish, mustard, maize, and wheat (Sharma et al., 2010; Anuradha & Rao, 2007; Bhardwaj et al., 2007; Hayat et al., 2007). Another fact is that BR enhances the activity of antioxidant enzymes related to photosynthesis and plant defense strategies in Indian mustard and wheat plants exposed to abiotic stresses (Hayat et al., 2007). It has been reported that seeds soaked with homobrassinolide enhanced chlorophyll content and seedling growth in plants. BR reduced Cr toxicity in radish and rice seedlings, thereby reducing Cr toxicity symptoms (Sharma et al., 2011, 2016).

Cytokinin increased the division of cell in root as well as shoot (Werner et al., 2010). Exogenous application of BR reduced the activity of enzymes and elevated gene expression in wheat seedlings, leading to significant increase in cytokinin level (Yuldashev et al., 2012). In case of tobacco, overexpression of brassinolide enhanced growth in tobacco plants. BR-modulated overexpression of cytokinin dehydrogenase/oxidase 3 (CKX3), linking a cross talk between brassinosteroid and cytokinin (Kim & Wang, 2010). Exogenous application of BRs enhanced salicylic acid, jasmonic acid, and ethylene content at endogenous level in plants. This proves a cross-linkage between BR and other phytohormones leading to BR-related stress tolerance (Wu et al., 2017). A positive correlation is found between exogenous application of BR and endogenous hormone quantity (Yuan et al., 2010).

Role of CKs and BRs in Stress Alleviation

CKs and BRs interact with each other to regulate plant growth and development (Wang et al., 2012). The overexpression of *CKX3* gene reduces the synthesis of CKs resulting in decrease of leaf and root growth of *Arabidopsis* (Vilarrasa-Blasi et al., 2014). However, the exogenously applied BRs enhance root and shoot growth of

these plants (Wang et al., 2012). Similarly, collegial interaction in *BRI1* and *CKX3* genes results in enhancement of plant growth. Furthermore, the interaction among CK and BRs enhances the biosynthesis of anthocyanin (Wang et al., 2011). CKs modulate expression level of BR-related genes and hence alter the source/sink relationship required for better crop production.

Regulation of Cytokinin Under Diseased Conditions

Interplay Among Brassinosteroids and Cytokinin

The indirect cross talk between BRs and CKs regulates the growth and development of lateral roots through modulating synthesis and translocation of auxins. BRs enhance root primordia by upregulating the expression level of auxin-synthesizing genes, *PIN* genes (Bao et al., 2004). Whereas, CKs reduce the expression level of *PIN* genes and obstruct the development of lateral root primordia (Benjamins & Scheres, 2008). The decreased root growth in *Arabidopsis* was attributed to the higher concentration of CKs due to elevated expression level of the *cytokinin oxidase/dehydrogenase3* (*CKX3*) gene compelled through PYK10, a root-specific promoter (Werner et al., 2010). The exogenously applied BRs improve leaves and lateral root growth in P10-CKX3 plants and confers synergistic effect between CKs and BRs to enhance plant growth (Vercruyssen et al., 2011). Yuldashev et al. (2012) also demonstrated interactive role of CKs and BRs in the improvement of growth in wheat plants. Wheat seedlings treated with BRs exhibited higher accumulation of CK derivatives including zeatin in shoots and roots (Yuldashev et al., 2012). The expression level of *isopentyl transferase* (*IPT* gene) affects the endogenous CK amount in rice plants. Upregulation of *IPT* gene alleviates drought stress by improving the synthesis of CK prior to the commencement of senescence. The higher expression of *IPT* gene enhances the expression level of genes involved in signaling and synthesis of BRs including *DWF4, DWF5, HYD1BRI1, BZR1*, *BAK1, SERK1*, and *BRH1*. Hence, the elevated level of CKs and BRs synergistically correlates to alleviate drought stress and improve grain yield in rice plants by adjusting source–sink relations (Peleg et al., 2011). The *brx-2* mutant that has restricted homoeostasis of BRs is unresponsive to the CK-induced obstruction of lateral root formation. However, the exogenously supplemented BRs reinstated this deficiency. Conversely, the BR application at post-embryonic stage did not assist in phenotypic growth of *brx-2* mutant in the presence of CK (Li et al., 2009), demonstrating independence of CKs and BRs. Hence, the inhibitory effect of CKs on the commencement of lateral roots is not directly reliant on the concentration of BRs.

The posttranscriptional interaction between CKs and BRs constantly regulate the level of ethylene in plants facing abiotic stress (Hansen et al., 2009). The *Arabidopsis* histidine kinase receptors (AHK) profess CKs in case of *Arabidopsis* plants. The autophosphorylation of AHK is responsible for the transfer of phosphoryl groups to *Arabidopsis* histidine phosphotransfer proteins (AHP). Hansen et al.

(2009) observed that AHP reduced ACS deterioration and elevated the ethylene synthesis through enhancing the activity of ARR1 which is a type B *Arabidopsis* response regulator (ARR). The quadruple mutant (*arr 1, 2, 10,* and *12*) besides *ARR* single mutants (*arr1*, *arr2*, *arr10*, and *arr12*) exhibit reduced ethylene level.

Hansen et al. (2009) reported that collegial effect between CKs and BRs enhances the stability of ACS proteins. The exogenously applied BRs enhanced the level of CK-induced anthocyanin in *Arabidopsis* plants (Yuan et al., 2014). The *dwf4* mutant is incapable to synthesize BRs, while *brassinosteroid insensitive 1-4 (bri1-4) mutant is devoid of* BR signaling. Both of these mutants synthesize less amount of anthocyanin due to lower expression level of genes involved in the synthesis of anthocyanin as compared to the BRs applied wild-type, showing a synergistic effect among CKs and BRs (Yuan et al., 2014).

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Chapter 11
Role of Brassinosteroids and Its Cross Talk with Other Phytohormone in Plant Responses to Heavy Metal Stress

Mohammad Yusuf, Mohd Tanveer Alam Khan, Mohammad Faizan, Radwan Khalil, and Fariduddin Qazi

Abstract Brassinosteroids (BRs) assume crucial part in plant development, formative cycles, and plant reaction to different abiotic stresses. From one viewpoint, plant chemicals may reserve limited assets to the most genuine burdens; then again, the cross discussions among different plant chemical signaling manage the harmony between plant development and its safeguard system under distressing conditions. It is well documented that the cross talks between brassinosteroids and various plant hormones such as auxin, cytokinin, gibberellin, abscisic acid, ethylene, salicylic acid, jasmonic acid, nitric oxide, hydrogen peroxide, and glucose are well established. Based on these studies, this chapter focusses on the cross talks between BRs and signaling of other plant hormones for the regulation of the balance between growth in plants and defensive responses under heavy metal stress. It has been observed that considerable work is required to reveal the mechanism related with BRs and other plant hormones to regulate plant growth and its metabolism under heavy metal stress. At last, it was also found that BRs act as a primary signal molecule in the phytohormone signaling network in plants under metal stress.

Keywords Brassinosteroid · Cross talk · Heavy metals · Phytohormones · Plant

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Introduction

Brassinosteroids are perceived as another gathering of polyhydroxy steroidal phytohormone with wide event in various parts of plant. The acknowledgment of this endogenous steroidal hormone was the consequence of almost 30 years of difficult work where a novel development advancing substance was distinguished in the natural dissolvable of pollen from the *Brassica napus* L. furthermore, named as Brassin (Mitchell et al., 1970). In light of its capacity to cause sensational changes in development and differentiation of plants at low concentrations, Mitchell et al. (1970) recommended that brassins established another group of plant hormone known as brassinosteroids (BRs) and brassinolide (BL) as its bioactive structure. Broad examinations identified with BR signaling pathway in *Arabidopsis* uncovered that layer-bound steroidal receptor BRI1 (Brassinosteroid Insensitive 1) connects straightforwardly with the BR ligand and prompted arrangement of record of BR-related qualities that assume imperative part in cell development and advancement (Planas-Riverola et al., 2019). Notwithstanding, in the new discoveries, essential foundation of plants showed unmistakable BR interceded cell explicit signaling pathway and its role in presenting resilience against different abiotic stresses (Planas-Riverola et al., 2019). The discoveries of Jia et al. (2019) uncovered that BR helps in controlling nitrogen scrounging movement in *Arabidopsis* during its insufficiency and furthermore distinguished BSK3 (Brassinosteroid Signaling Kinase 3) quality liable for essential root stretching during nitrogen inadequacy. A more top to bottom and subjective portrayal of BRI1 as well as BRL-related ROS/NOS creation and initiation of stress responsive record factor lead to the BR-intervened pressure insurance and improved the development execution of plants (Planas-Riverola et al., 2019). It is very much reported that BR signaling pathways showed inclusion of different phytohormones and stress reactions (Nolan et al., 2017). Also, BR signaling showed various reactions in changed cells and tissues that can be exploited to improve development resistance under distressing conditions (Fàbregas et al., 2018). Nonetheless, numerous reports unwind the potential capacities of BRs in enhancement of different ecological anxieties, yet next to no thought appeared by analysts on examining the co-operations among BRs and fundamental mineral supplements for the identification of different transporter in plant. In light of copious of proof, BRs essentially keep up legitimate development and improvement of plants under stress-free and stressful conditions, and changing BR pathway could be an amazing methodology for controlling yields for brutal climate like high and low temperature, severe salinity, dry season, and substantial metal stress. In this part, our attention will be on BR-interceded motioning under abiotic stress and its inclusion in enhancement of heavy metal stress. Furthermore, cross talk of BRs and various phytohormones under heavy metal stress will likewise be talked about. This part will offer us chance to improve the development effectiveness of plants and their transformation under heavy metal pressure through regulation in BR signaling pathway, hormone interactions, and cross talk at organ, tissue, and cell levels to more readily see the reaction of plants to heavy metal stress.

Involvement of BRs Under Heavy Metal Stress

Among the normally happening components, 53 are viewed as heavy metals; however, a couple of them have some biological importance in plants (Weast, 1984). In any case, heavy metals like cadmium, copper, nickel, aluminum, and lead, if present at raised levels in rural soils, are handily absorbed by plants and initiate genuine apparent and metabolic annoyances (Marschner, 2002). They additionally modify nitrogen digestion (Boussama et al., 1999), hinder stomatal opening (Barcelo & Poschenrieder, 2002), decline in photosynthetic rate (Yruela, 2005; Seregin & Kozhevnikova, 2006), and change numerous different elements of plant development and improvement.

BRs can deal with the take-up of particles into the plant cells, and they can be utilized to lessen the collection of heavy metals and radioactive segments in plants. Additionally, BRs likewise limit the harmful impacts and manifestations created by overabundance amount of substantial metals (Bajguz, 2010). It has been moreover settled that after the treatment of BRs, beetroot is seen to have its lead content reduced to half as compared to the plants treated only with metal, since this hormone astonishingly diminishes the osmosis of this metal (Khripach et al., 1999). When utilizing BRs for Indian mustard (*Brassica juncea*) seeds prior to germination and afterward uncovering them against copper stress, diminished take-up and amassing of copper was noticed, just as progress in shoot age and biomass creation likewise happened (Sharma et al., 2008). In addition, the way of life of *C. vulgaris* presented to metals shows that BRs forestall the deficiency of chlorophyll, sugar, and protein and improve the amalgamations of phytochelatins (Bajguz, 2000, 2002). It has been additionally announced that utilization of BRs in Indian mustard presented to nickel movement enhances seed germination just as the lengths of shoots and roots, both under Ni stress and something else (Yusuf et al., 2011). BR kills the harmful impact of cadmium on photochemical pathways in assault cotyledons, generally through reducing the harm to response focuses and O_2 advancing edifices and by guaranteeing effective electron transport (Janeczko et al., 2005). It is additionally realized that BRs direct the exercises of different enzymes associated with photosynthesis and plant safeguard mechanism in wheat and Indian mustard presented to various abiotic stresses (Hayat et al., 2007). It has been uncovered that a pre-absorbed seed with 28-homobrassinolide (HBL) improves seedling development just as chlorophyll a substance under the exposure of heavy metal. In addition, it has been confirmed that the expanded take-up of Cr^{2+} in radish or rice seedlings is reduced significantly after treatment with BR, accordingly diminishing chromium poisonousness (Sharma et al., 2011, 2016).

Ongoing examinations centering natural chemistry, proteomics, and sub-atomic hereditary qualities have uncovered that BR-intervened signaling pathways engaged with the guideline of quality articulation and plant improvement under pressure and peaceful conditions (Gallego-Bartoloméa et al., 2012; Irani et al., 2012; Zhu et al., 2015b; Wu et al., 2016). BRs tie to Brassinosteroid Insensitive1 (BRI1), a membrane surface receptor kinase restricted at the plasma layer, that phosphorylates plasma

membrane-anchored kinases like Constitutive Differential Growth1 (CDG1) and Brassinosteroid Signaling Kinase1 (BSK1) (Kim et al., 2011), bringing about the inactivation of GSK3-like kinase Brassinosteroid Insensitive2 (BIN2) by BRI1-Suppressor1 (BSU1) (Kim et al., 2009). This inactivation of BIN2 by BSU1 prompts dephosphorylation of two homologous record factors, Brassinazole Resistant1 (BZR1) and BZR2 (Wang et al., 2002; Yin et al., 2002) which at that point move to the core and result in quality enactment or suppression in the wake of restricting with the advertisers of their objective qualities (Sun et al., 2010; Zhu et al., 2013). BR-interceded physiological pathways are firmly connected with other hormonal or ecological signs (Li et al., 2012). Studies uncovered solid connections among BR and other signaling hormones like auxin, cytokinin, gibberellin, abscisic acid, ethylene, salicylic acid, and some more.

Interaction of BRs and Auxin Under Heavy Metal Stress

BRs are notable steroidal hormones that have the capacity to manage take-up of metal particles into the plant cells and can be utilized to diminish the amassing of substantial metals (Sharma & Bhardwaj, 2007). BRs can likewise invigorate the union of certain ligands, for example, the phytochelatins, that are joined with metal particle (Bajguz, 2002; Choudhary et al., 2010). They additionally increment the exercises of some cell reinforcement compounds detoxifying the expanded creation of reactive oxygen species (ROS) produced by heavy metal stress (Hasan et al., 2008; Yusuf et al., 2012), thus exogenously applied BRs improve the development and metabolic action in plants under heavy metal stress. Notwithstanding BRs, auxin signaling is likewise associated with adjusting plant development and advancement with changing conditions and under unnecessary anthropogenic exercises. It likewise assumes a significant part in the resilience system against assorted metal stress. The vaccination of auxin creating organisms in the rhizosphere of metal focused on plants improves plant profitability and flexibility (Vacheron et al., 2013). Auxin modulates metal stressed by directing the creation of ROS in different intracellular compartments of root cells, including mitochondria, plastids, peroxisome, and cytoplasm (Krishnamurthy & Rathinasabapathi, 2013; Chen et al., 2014; Kolbert et al., 2018; Piacentini et al., 2020). Upon openness to harmful level of metals, the record level of a few auxin-responsive qualities are changed, and a criticism auxin homeostasis is kept up through straightening out the dynamic pool of auxin by its debasement, inactivation, as well as transport for better flexibility during stressful conditions (Wang et al., 2015). Besides, during oxidative pressure, auxin is known to induce the action of cell reinforcement proteins by expanding their record (Agami & Mohamed, 2013; El-Gaied et al., 2013).

Over the last numerous years, an excess examination work has been directed to unravel cross talk among BRs and auxin to become familiar with the impact of one another on development and digestion of plants under pressure and peaceful conditions. Based on studies, BRs and auxin have shown synergistic reaction for

numerous plant formative qualities and physiological cycles, for example, hypocotyl stretching, vascular groups advancement, root improvement, tropisms, and some more. The interdependency and participation of auxin and BRs are confounded and include plentiful cycles on the sub-atomic level, by having a similar objective qualities, controlling each other commonly at various levels. Phosphorylation guideline assumes a pivotal part in BR signaling pathway, particularly during the discernment interaction, BRs are seen through BRI1 kinase receptor and BAK1 kinase co-receptors, and in the end control BR-controlled quality articulation through impacting downstream record factors like BES1/BZR1 exercises (He et al., 2005; Yin et al., 2005; Sun et al., 2010; Tang et al., 2011; Yu et al., 2011). Nevertheless, ubiquitination guideline appears to be fundamental for auxin signaling. When auxin ties to TIR1 receptor that goes about as a ubiquitin E3-ligase, the initiated TIR1 E3-ligase ubiquitinates AUX/IAA proteins, prompts the corruption of these repressors, and pushes down ARF record factors, and in the end causes auxin-directed quality articulation design changes and development reactions (Gray et al., 1999,, 2002; Hellmann et al., 2003; Quint et al., 2005). Besides, it has been discovered that BIN2 kinase, which is notable working in BR signaling, could phosphorylate and upgrade the exercises of ARFs, for example, ARF2 and ARF7 (Vert et al., 2008; Cho et al., 2014), and it will be intriguing to test if kinases like BIN2, which are associated with BR signaling, could likewise communicate with other auxin signaling parts like TIR1 receptor or AUX/IAA repressors and impact TIR1 E3-ligase action or AUX/IAA protein dependable qualities. Then again, the part of ubiquitination in BR signaling likewise should be tended to, particularly if TIR1 E3-ligase could straightforwardly cooperate with BR signaling components and manage their protein dependable qualities. Moreover, utilizing auxin reaction DR5 and other auxin correspondents, it has been seen that auxin controls plant development and advancement in a tissue or cell subordinate way. The assorted transcriptional yields rely upon the cell and ecological setting (Clark et al., 2014; Etchells et al., 2016; Lavy et al., 2016). Also, age of an itemized tissue or basement guide of auxin and BR disseminations is as of now conceivable, utilizing fluorescence-actuated cell arranging or laser microdissection in blend with high-goal quality articulation examination.

Interaction of BRs and Cytokinin Under Heavy Metal Stress

Cytokinins (CK) are a group of phytohormones that assume crucial parts in a few natural cycles, like the advancement of aerial and underground organs, light reactions, mineral enhancement, and reactions to abiotic stresses (Werner et al., 2010; Nishiyama et al., 2011). The key proteins associated with CK digestion are isopentenyl transferases (IPTs), which are liable for the biosynthesis of bioactive CKs and CK oxidases/dehydrogenases (CKXs), which are liable for the inactivation of bioactive CKs (Werner & Schmülling, 2009), the two focuses of BR-intervened reactions. The primary exchange among CKs and BRs is by all accounts identified

with plant development guideline (Vercruyssen et al., 2011). In wheat seedling, exogenous BRs decay the catalyst action, and encoded quality articulation of cytokinin oxidase contributes essentially to build cytokinin level, which demonstrates the association of BRs in the guideline of cytokinin (Yuldashev et al., 2012). Overexpression of BRI1 in PYK10:CKX3 expanded root and leaf development, contrasted with wild sort and comparative with a Cytokinin Dehydrogenase/Oxidase 3 (CKX3) overexpression line in tobacco, demonstrates the dynamic cross talk among cytokinin and BR, both of which assume significant parts in a few parts of plant development and advancement (Kim & Wang, 2010; Nishiyama et al., 2011; Vercruyssen et al., 2011). Then again, plants ectopically communicating both CKX3 and BRI1 present a synergistic expansion in leaf and root development. In arrangement, PYK10::CXK3 transgenic plants treated with exogenous BR showed a complemented development of horizontal roots contrasted with WT plants, unequivocally recommending a cross talk among BRs and CKs that control the development and formative cycles (Vercruyssen et al., 2011). In addition, the transaction among BR and CK can be seen in CK-initiated anthocyanin creation (Yuan et al., 2015).

Arabidopsis mutant imperfect seedlings in BR biosynthesis (dwf4, dwf4-102, and psc1) and BR signaling (bri1-4) were submitted to various preliminaries to assess the impacts of BR on CK-actuated anthocyanin accumulation. The dwf4 and bri1-4 plants introduced decreased CK-actuated collection of anthocyanin; however, when WT plants were treated with exogenous BRs, an expansion in anthocyanin levels was noticed. Also, CK-instigated articulation of anthocyanin biosynthetic qualities, for example, dihydroflavonol reductase, leucoanthocyanidin dioxygenase, and UDP-glucose: flavonoid-3-O-glucosyl transferase, introduced an emphasized decrease in the dwf4-102 and bri1-4 lines contrasted with WT. Also, WT plants treated with CK introduced higher articulation of record factors identified with anthocyanin creation, including anthocyanin color 1 (PAP1), glabra 3 (GL3), and enhancer of glabra 3 (EGL3); however, the equivalent was not seen in the bri1-4 and dwf4-102 lines. These information give proof that BR may help CK-incited anthocyanin biosynthesis by emphatically intervening the outflow of biosynthesis and signaling qualities just as record factors engaged with the two cases (Yuan et al., 2015). Therefore, the BR signal transduction pathways in relationship with CKs are associated with numerous transcriptional exercises, signal transduction, and metabolic exercises, which lead to huge protection from an assortment of stresses and shield plants from injury.

Interaction of BRs and Gibberellin Under Heavy Metal Stress

It is very much reported the presence of broad cross talk among BRs and GAs in a wide scope of natural cycles, including plant improvement and reactions to ecological boosts (Choudhary et al., 2012). GA-initiated OsGSR1, an individual from

GAST group of rice, showed basic association in GA signaling though, BRs stifled something very similar (Wang et al., 2009). RNAi plants with diminished OsGSR1 articulation showed decreased affectability to GAs, upgraded level of GAs, decreased degrees of endogenous BRs, and a bantam aggregate that could be protected by exogenous BR application. Moreover, OsGSR1 actuated BR biosynthesis through direct cooperation with DWF1, recommending that OsGSR1 is a plausible cross talk point in GA and BR signaling pathways (Choudhary et al., 2012). It was shown that 419 (35%) out of 1194 qualities differentially communicated in ga1-3 (GA-biosynthesis lacking) contrasted with wild type plants were additionally influenced in the bri1-116 (BR-obtuse) mutant, of which 387 (92.3%) of the co-managed qualities were influenced similarly by these mutants (Bai et al., 2012). Additionally, Bai et al. (2012) shown that investigation of RNA-sequencing information from GA-treated WT and GA-treated WT become on PPZ (a particular inhibitor of BR biosynthesis) medium, distinguishing 3570 and 1629 differentially controlled qualities, individually. Once more, this striking information proposed that around 66.7% of GA-controlled qualities require BR, underlining the significant part of BR in the GA guideline of genome articulation. In accordance with these information, different gatherings showed that hypocotyl prolongation advanced by GA was disposed in *Arabidopsis* seedlings with diminished BR biosynthesis (i.e., de-etiolated-2 (det2) mutants or brassinazole (BRZ) treatment), demonstrating that cell extension to a great extent depends on the suitable activity of the two chemicals (Gallego-Bartoloméa et al., 2012; Li et al., 2012). Then again, without BR, GAs could direct BZR1-subordinate quality articulation since GAs instigate dephosphorylation province of BZR1, its dynamic structure, likely through phosphatase PP2A proteins (Li et al., 2012). This activity may clarify the expanded BZR1–DNA restricting in vivo and GA-prompted regulation of BR transcriptional yields (Bai et al., 2012). In addition, DELLA proteins communicate solely with the dephosphorylated BZR1, which demonstrates that BR signaling improves GA motioning by advancing the BZR1–DELLA collaboration and, hence, the easing of DELLA's restriction forced on GA-interceded development (Li et al., 2012). This BZR1 titration may clarify why, shockingly, BR was appeared to emphatically build the wealth of the DELLA protein at the early stretching stages post germination in *Arabidopsis* (Stewart Lilley et al., 2013). Also, impact of BRs on DELLA protein steadiness may offer an unthinking clarification for the abiotic stress resilience given by BRs. The positive connection between DELLA protein levels and resistance to abiotic stresses has been ascribed to raised articulation of reactive oxygen species (ROS)-rummaging catalysts (Achard et al., 2008). In any case, the elements and security of DELLA and BZR1 protein buildings in light of microorganism and abiotic stresses stay slippery. Cross talk of BR and GA showed productive versatility of these two chemicals while mixture of their yields and signals of ecological prompts moves a harmony between plant safeguard and development reactions.

Interaction of BRs and Abscisic Acid Under Heavy Metal Stress

Abscisic acid (ABA) is a sort of plant chemical that showed wide contribution in plant reactions and is fundamental for plant advancement and endurance. This hormone goes about as a significant abiotic stress sensor, prompting defensive reactions like stomatal conclusion, seed dormancy, and restraint of development and germination (Mustilli et al., 2002; Yoshida et al., 2002; Yoshida et al., 2006; Fujii et al., 2007; Fujii & Zhu, 2009). Indeed, even in the beginning phases of plant advancement, ABA drives pressure resistance as well as evasion instruments, assisting plants with making due to unfavorable metal pressure conditions (Wang et al., 2020). Plants with surrenders in BR signaling pathways show intensified affectability to ABA during seed germination, root prolongation, and stomatal conclusion measure (Wang et al., 2020). It is additionally recorded that BR signaling likewise alienates the ABA biosynthesis measure. Erasure of the positive controller BSK5 in BR signaling fundamentally prompts ABA biosynthesis-related qualities of ABA3 and NCED3 (Li et al., 2012). Besides, a high centralization of BR restrains ABA-incited stomata conclusion through the restraint of ABA biosynthesis (Ha et al., 2016, 2018). Throughout seed germination, the protein level of the wild type of the BR positive controller BES1 is actuated by ABA treatment (Zhang et al., 2009). ABI1 and ABI2 are two negative variables in ABA signaling. In abi1 or abi2 gain-of-function mutants, dephosphorylated BES1 has been appeared to amass (Zhang et al., 2009). Notwithstanding the opposing jobs of BR and ABA, BR and ABA signaling can likewise be emphatically cross-managed. Although a high convergence of BR quells ABA biosynthesis, low degrees of ABA and BL, a functioning type of BRs, both initiate stomata conclusion (Ha et al., 2016, 2018). In *Chlorella vulgaris*, BRs upgrade the endogenous degree of ABA under abiotic stress by managing ABA biosynthesis (Bajguz, 2009; Zhang et al., 2011). In the creation of H_2O_2, BRs increment the articulation and action of NADPH oxidase in maize in an ABA-subordinate way under abiotic stress conditions (Zhu et al., 2015a). BR controls ABA upstream motioning to downstream record movement in *Arabidopsis*, BR adjusts ABA early signaling essentially by influencing the phosphorylation of SnRK2.2, SnRK2.3, and SnRK2.6, which are viewed as significant members in ABA signaling (Fujita et al., 2013; Yoshida et al., 2010). Phosphorylation is needed for the actuation of SnRK2.2, SnRK2.3, and SnRK2.6. Among them, just SnRK2.6 has solid autophosphorylation action in vitro. SnRK2.6 is confined in monitor cells and leaf vascular tissues, controlling ABA-interceded stomatal development (Mustilli et al., 2002). Curiously, BIN2 intervenes the phosphorylation of SnRK2.2 and SnRK2.3 however not SnRK2.6 (Belin et al., 2006). Bin2-1, an addition of work mutant of BIN2, shows expanded affectability to ABA treatment in essential root development. Also, SnRK2-RNAi restrains the ABA overly sensitive aggregate of bin2-1, demonstrating that BIN2 tweaks ABA motioning through the phosphorylation of SnRK2.2 and SnRK2.3 (Cai et al., 2014). Other than autophosphorylation, the phosphorylation of OST1 is

likewise intervened by the BRI1 co-receptor BAK1 (Shang et al., 2016). Like ost1, the bak1 mutant has diminished affectability to ABA-intervened stomatal development and ROS creation, which is reliant upon its kinase action. OST1 overexpression part of the way saves the diminished affectability of bak1 mutant during those cycles, proposing OST1 is epistatic to BAK1 (Mustilli et al., 2002). The collaboration somewhere in the range of BAK1 and OST1 in the plasma film of gatekeeper cells is emphatically upgraded by ABA, which mitigates ABI1 possessing OST1, permitting quick enhancement of ABA signaling transduction and enactment of target qualities in light of abiotic stress.

Under distressing environment, the stress hormone ABA aggregates in plants to threaten the capacity of BR. At the point when ABA signaling dominates, hindered by PYR/PYLs, ABI1 and ABI2 discharge SnRKs and BIN2. The enactment of a negative controller in BR signaling and a positive controller in ABA signaling by means of phosphorylation initiates ABA signaling and hinders BR signaling (Fujii & Zhu, 2009; Wang et al., 2018). Under these conditions, BR-intervened cell division and lengthening or regenerative cycles are hindered to lessen energy utilization. Directing the statement of downstream qualities, controlling exercises of anion channels and elevating the biosynthesis pathways to create optional metabolites, like proline, sugar, and anthocyanins, ABA signaling hinders seed germination, stomatal opening, and development to assist plants with promoting metal pressure conditions (Cai et al., 2014; Hu & Yu, 2014). Overexpression of ABI3 in the increase of gain-of-function mutant bes1-D reestablishes the early blooming aggregate of bes1-D, proposing that ABA can repress the BR-intervened improvement measure (Hong et al., 2019). At the point when natural conditions improve, like reasonable temperature and light thickness, the endogenous ABA level is decreased and BR biosynthesis increments, constricting the ABA pathway to change plants from safeguard over to development. The guideline of phosphorylation state and dependable qualities of significant kinases, phosphatases, or TFs through communication between two hormone signals modulates the switch between plant development or variation to natural anxieties.

Interaction of BRs and Ethylene Under Heavy Metal Stress

Ethylene is an unstable gaseous plant hormone that assumes essential part in plant development and improvement and furthermore adjusts versatile reactions to different ecological burdens (Chang, 2016) including heavy metal stress (Keunen et al., 2016). It has been accounted that substantial metal-incited ethylene creation is plant explicit and furthermore relies upon the type and grouping of heavy metal. The acceptance of ethylene by metals may cause unprofitable indications in plants and have a role in Cd-actuated cell demise. Utilization of the ethylene inhibitor, silver thio-sulfate, totally enhanced the Cd-instigated adverse consequences (Maksymiec, 2011). Transgenic tobacco plants overexpressing ethylene responsive factor 1 (ERF1) showed more noteworthy resistance to Cd pressure than the wild, which was identified with an improved articulation level of GSH biosynthesis qualities

(Guan et al., 2015). Studies utilizing mutants that are insufficient and uncaring toward ethylene showed abiotic stress resistance when treated with 24-epibrassinolide (EBL). EBL was equipped for expanding the endurance paces of the ethylene-uncaring mutant ein2 under abiotic stress in *Arabidopsis* plants. Also, the treatment of *Brassica napus* seeds with EBL decreased the restraint ein2 mutant germination under abiotic stress, returning this present line's extreme touchiness to abiotic stress to a level like those of WT plants (Divi et al., 2010).

Brassinosteroids impact ethylene biosynthesis chiefly by managing 1-Aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO) exercises (Hansen et al., 2009). The cross talk between these two phytohormones presents two situations, with BRs controlling ethylene creation at the transcriptional and posttranscriptional levels. As to guideline, past investigations in *Arabidopsis* demonstrated that seedlings treated with exogenous BRs show raised degrees of ethylene biosynthesis, at any rate halfway through an increment in ACS5 protein solidness by lifting its half-life (Hansen et al., 2009). Furthermore, different investigations have effectively discovered that BRs may likewise manage ethylene biosynthesis through the enlistment of ACS5 quality articulation in *Arabidopsis* (Zimmermann et al., 2004). The guideline of ethylene biosynthesis by BR occurs in a portion subordinate way, where BRs can be positive just as regrettable controllers, contingent upon the exogenous application portion (Lv et al., 2018). Significant degrees of BRs invigorate ethylene biosynthesis by improving the dependability of the ACS protein by forestalling its debasement by the 26S proteasome. Then again, low degrees of BRs curb ethylene biosynthesis by expanding the movement of BZR1/BES1, the two significant BR pathway record factors that repress the record of ACS qualities (Lv et al., 2018). Tests with banana natural product (*Musa acuminata* L.) showed that BZR proteins tie explicitly to BRRE components (CGTGT/CG) in any event of one ACS quality (MaACS1) and two ACO qualities (MaACO13 and MaACO14) in this species. An articulation examination showed that the statement of MaBZR1, MaBZR2, and MaBZR3 diminishes persistently during natural product aging. Additionally, MaBZR1 and MaBZR2 are equipped for stifling the record of these three ethylene biosynthetic qualities, which is expanded during the organic product maturing measure. Moreover, the exogenous use of BRs advances banana organic product aging because of the speed increase of MaACS1, MaACO13, and MaACO14 articulation, and thus, ethylene creation occurs, affirming the activity of BZR proteins as transcriptional repressors of ethylene biosynthesis (Guo et al., 2019).

Interaction of BRs and Salicylic Acid Under Heavy Metal Stress

Salicylic acid (SA) is a phenolic compound that is involved in various physiological processes (Dempsey & Klessig, 2017). It was first proved that SA plays a significant role in biotic stress responses. Before long thereafter, notwithstanding, it turned out

to be progressively evident that SA assumes a part during the plant reaction to abiotic stresses, like substantial metal harmfulness (Janda et al., 2007). Expanding proof proposes that cross talk among BRs and SA assumes a significant part in plant reaction to abiotic stresses. The probable cross talk among BRs and SA is mediated by means of non-expressor of pathogenesis-related genes 1 (NPR1) and WRKY70, encoding a transcription factor working downstream of NPR1 (Divi et al., 2010). In mutant studies, it has been seen that npr1-1 genotype was thermosensitive and furthermore showed inadequacies in the declaration of PR qualities in light of SA (Clarke et al., 2004, 2009; Larkindale et al., 2005). However, in reaction to EBL, 2.4-fold increase in percent survival of the npr1-1 seedlings as compared to ninefold increase in wild type was noted (Divi et al., 2010). The study reveals that stress tolerance is facilitated by functional NPR1 for the expression of BR effect, via governing BR signaling components such as BIN2 and BZR1 (Divi et al., 2010). Further, the presence of the cross talk among BRs and SA assumes a critical part accordingly of plants to biotic just as abiotic stresses. It has been exhibited that in tobacco just as in rice, BRs go about as an inducer of an expansive scope of illness opposition. Rather than the past see that BR decidedly manage plant intrinsic resistance, ongoing investigation gives proof that *Pythium graminicola* abuses BR as harmfulness factors and takes the prisoner of rice BR hardware to cause infection (De Vleesschauwer et al., 2012). Further, it has been recommended that the negative cross talk among SA and BR pathways prompts safe suppressive impact of BR. Additionally, upon brassinazole treatment, a diminished vulnerability toward *P. graminicola* had been seen in rice plants because of derepression of the expert safeguard controllers of SA pathway like NPR1 and OsWRKY45. The examination demonstrates that BR-interceded concealment of SA safeguard reactions happens upstream of NPR1 and OsWRKY45 however downstream of SA biosynthesis (De Vleesschauwer et al., 2012).

Interaction of BRs and Jasmonic Acid (JA) Under Heavy Metal Stress

To improve abiotic resistance, synergistic association of BRs and JA accepts crucial parts in the plant development. JA restrains plant development, while BR instigates over the ground plant development. The cross talk among JA and BR signaling pathways is engaged with the hormone concerning plant development and safeguard opposition. From one viewpoint, low convergence of BRs prompts the outflow of OsDI1 and OsDWARF at the early and late phases of BR biosynthesis, individually, and anthocyanin amassing and enacts protection reaction. Then again, high convergence of BRs initiates BR signaling falls including BR receptor BRI1, BR-related kinase BAK1, and BR-related TFs to actuate the statements of downstream qualities like BES1 and BZR1, subsequently controlling plant reactions to abiotic stresses. Strikingly, high grouping of BRs represses endogenous biosynthesis of JA and BRs,

and JA likewise hinders BR biosynthesis (Choudhary et al., 2012). Strikingly, prevention of JA prompted gathering of anthocyanins by BRs in *Arabidopsis* has been moreover definite by BR motioning on the JA pathway (Peng et al., 2011). The record levels of JA biosynthesis quality and JA-started signaling quality were down-controlled when the BR center was minimal. Nevertheless, on high BR center, the record levels of JA biosynthesis and signaling quality were up-controlled. These outcomes were, in addition, embraced through exogenous foliar application with JA which impelled the down-control of BR biosynthesis and signaling quality, OsDWF4 and OsBRI1 (Nahar et al., 2013), showing counter correspondence among BRs and JA in the rice roots.

It is all around archived that under stressful conditions, BRs upgrade JA level in rice (Kitanaga et al., 2006), which unequivocally advances the outflow of thionin qualities encoding antimicrobial peptides showing a potential cross talk point between these two phytohormones. The cross talk among BRs and JA was additionally concentrated to see how these phytohormones connect in the development of common protection in tomatoes against stresses. It has been seen that BRs and JA straightforwardly influenced trichome thickness and allelochemical content yet in an opposite way (Campos et al., 2009). The flawed mutant exams affirmed that JA advances the characteristics needed for against herbivory though BRs forestalled it. Since the BR-inadequate mutant dumpy (dpy) showed improved pubescence, zingiberene biosynthesis, and proteinase inhibitor articulation, on the contrary, inverse impacts were seen in JA-obtuse jai1-1 mutant, prompting an expanded creation of guarded attributes. Additionally, it has likewise been exhibited that BRs act upstream of the JA signaling pathway, since dpy3jai1-1 double mutant showed that jai1-1 is epistatic to dpy. Moreover, trichome number in jai1-1 mutants was seriously diminished when contrasted with dpy, which shows a high trichome thickness underscoring the significance of JA in trichome arrangement. In this manner, the cross talk among JA and BR biosynthesis might be associated with the harmony between plant development and protection opposition. Connection between abiotic stress receptive oxygen species age and plant guard framework is that BR biosynthesis is constrained by improved JA-precursor, 12-oxo-phytodienoic dangerous, and hence joining BR and JA pathway commencement (Nahar et al., 2013).

Interaction of BRs and Nitric Oxide (NO) Under Heavy Metal Stress

In the new past, different investigations showed that there is cooperation among NO and BRs during the cycles of plant development and advancement under pressure just as calm conditions (Tossi et al., 2013; Zhang et al., 2011). BRs can advance a fast expansion in NO levels in maize leaf mesophyll cells (Zhang et al., 2011). Besides, Tossi et al. (2013) likewise announced BR-prompted NO creation in

Arabidopsis root cells, where they indicated the contribution of both NR and NOS-like exercises as possible wellsprings of NO, and expansions in NO levels were recommended as the justification of BR-incited changes in root engineering. BR signaling was likewise answered to upregulate NO creation, which thus actuated ABA biosynthesis and advanced plant resilience against abiotic stress (Choudhary et al., 2012). The connection between the BR signaling pathway and AOX limit under pressure conditions showed a significantly expanded elective pathway breadth and NbAOX1 record level in BR-pretreated plants yet not in the NO-repressed plants (Zhu et al., 2016). It was tracked down that the NR-intervened NO burst chiefly adds to the increment of AOX limit and assumes a basic part in the improved cell reinforcement framework. This signaling pathway interceded by BRs because of abiotic stress adds to the comprehension of the instrument of plant reaction to ecological pressure. Further examination on the association of NR- and NOS-interceded NO age, H_2O_2 and other obscure sign particles, should reveal insight into the components of protection in plants.

Interaction of BRs and Hydrogen Peroxide (H_2O_2) Under Heavy Metal Stress

It is all around archived that H_2O_2 and BRs have a huge hormonal cross talk that assumes critical part in plant headway and physiological digestion (Nazir et al., 2019; Zhou et al., 2018). BRs are a group of plant steroid chemicals that work with plant development and control different natural impacts (Anwar et al., 2018; Planas-Riverola et al., 2019). H_2O_2 emphatically directs BR motioning by actuating the record factor Brassinazole-Resistant1 (BZR1), which goes about as a primary advertiser of BR signaling (Tian et al., 2018). In the specialty of the root immature microorganism, exogenously applied BRs likewise help the creation of H_2O_2 embroiling in BR-impacted QC division and cell flexibility (Tian et al., 2018). H_2O_2 has likewise been collected by the expanded action of NADPH oxidase in BR-executed cucumber plants (Xia et al., 2009), while a similar result is accomplished in tomato by improved RBOH1 activity (Nie et al., 2013). Similarly, the quieting of RBOH in Nicotiana benthamiana influenced the movement of AOX set off by BRs and accordingly diminished the detoxification of ROS which makes the plant more helpless against abiotic factors (Deng et al., 2015). Jiang et al. (2012) uncovered that H_2O_2 goes about as an auxiliary specialist for starch digestion and CO_2 absorption set off by brassinosteroids through redox motioning in Cucumis sativus, in this way improving the photosynthetic proficiency and efficiency. The solvency and portions of H_2O_2 are significant for managing stomatal development in light of different BR dosages in *Solanum lycopersicum* (Xia et al., 2014). A temporary ascent in H_2O_2 brought about by a little portion of BR capacities as an impetus to empower the recovery or potential biosynthesis of GSH bringing about a diminished redox status that solely manages the measure of H_2O_2 and

antagonistically impacts the ABA responsive instrument. Conversely, a raised measure of BRs can prompt an exorbitant ascent of H_2O_2 which with ABA signaling may shape a shallow intensification band bringing about stomatal conclusion (Xia et al., 2014). Furthermore, low BR levels initiate a transient H_2O_2 creation and change the cell redox status in monitor cells, hence coming about in stomata opening. High BR levels incite delayed H_2O_2 collection, which works with pressure reactions and stomata conclusion. Hindrance of H_2O_2 collection by synthetic specialists, for example, ascorbic corrosive or diphenylene iodonium, obstructed BR-actuated stomata conclusion, showing a fundamental role for H_2O_2 in BR-instigated stomata conclusion. Nonetheless, the interaction among H_2O_2 and BR motioning in plant improvement needs more examination work and discussion.

Interaction of BRs and Glucose Under Heavy Metal Stress

Plants continually sense the progressions in their current circumstance and communicate these signs as a component of ordinary turn of events. For ideal development and advancement, plants need to facilitate complex formative cycles and, simultaneously, detect and react to endogenous physiological elements and outer natural boosts. Numerous elements like light, supplements, and phytohormones are known to manage these formative cycles. Every one of these components presumably structures a perplexing sign reaction organization to achieve ideal development changes to empower better wellness in plants. Out of different phytohormones and development controllers, BRs and glucose (Glu) assume urgent part in controlling ecological prompts in plants and help in the critical development and advancement of plants. It is accounted for that BRs and Glu work synergistically just as inimically to change plant capacities. BRs altogether intervene articulation of many qualities. Out of 190 BRs instigated upregulated genes, Glu alone up-controls and downregulates 83 and 55 genes, individually (Gupta et al., 2015). Exogenous utilization of glucose to WT (7-day-old) seedlings kept in conceal progressively for the following 7 days brought about improvement of hypocotyl prolongation where Glu brings about perfectly greatest addition in hypocotyl length followed by glucose. In particular, in both light and dim developed WT seedlings, 1% Glu invigorates hypocotyl stretching while 5% Glu restrains something similar (Gupta et al., 2015). Also, joined use of various Glu concentrations (1%, 3%, and 5%) and BRs (10 nM, 100 nM, and 1 μM) prompts huge decrease in hypocotyl lengthening, underlining Glu and BR crosstalk in *Arabidopsis* (Gupta et al., 2015). Reliably, gin2-1 showcases deformity in hypocotyl prolongation, uncovering the way that HXK1 plays a critical role in hypocotyl stretching in dim (Zhang & He, 2015). The investigation likewise hurls covers where exogenous sugars upgrade Brassinazole Resistant1 (BZR1), a quality encoding BR-initiated record factor in dim. In *Arabidopsis*, *del2-1* (BR biosynthetic) mutant, and brassinazole (BRZ), BR biosynthetic inhibitor weakened sugar-incited hypocotyl stretching in dim. Besides, gin2-1 mutants of *Arabidopsis* show less affectability toward BRZ, collecting more

substantial proof that BRs are necessary for sugar-initiated hypocotyl extension (Zhang & He, 2015). Reliably, in a similar report, exogenous utilization of BRs prompts upgrade in hypocotyl proportion exhibiting that Glu-instigated hypocotyl lengthening is HXK1-subordinate where BRs act downstream in a similar pathway. Another investigation comes as an achievement where Glu and BR cross talk was accounted for the control of root development heading in *Arabidopsis* (Singh et al., 2014). A similar grouping of mannitol and sorbitol shows next to no effect on root development deviation. Conversely, 3-OMG does not infringe root development deviation to any degree, and gin2 mutant faultlessly reacts toward Glu-instigated root development deviation (Singh et al., 2014). Unquestionably, mirrors that Glu-interceded root development deviation include HXK1-reliant just as HXK1-in-subordinate signaling. Intriguingly, Glu and BRs act in a synergistic way to prompt root development deviation from vertical use of BRZ hinders Glu-instigated root development deviation. Brassinosteroid in-sensitive1-6 (bril1-6) that shows no receptivity toward BR displays no reaction toward Glu-prompted root development deviation and brassinazole-safe 1-1D (bzr1-1D) feature an overexpressed Glu-initiated root development deviation unloads the way that BR signaling upgrades the current energy Glu-intervened root development deviation (Singh et al., 2014). This investigation likewise recommends that protein phosphatase limits root deviation from vertical. In order to establish relationship between brassinosteroid insensitive1 (BRI1) and protein phosphatase 2A (PP2A) movement, a twist in naphthyl phthalamic acid1 (rcn1) mutant were revealed that shows least PP2A action. Here, both Glu and BR treatment prompts improved root development deviation from vertical when contrasted with WT plants. In any case, greatest impact on root development deviation was seen at 3% Glu and 10 nM BRs though typical root development deviation was recuperated by BRZ application uncovers that Glu improves BRI1 disguise by restricting protein phosphatase activity in *Arabidopsis* (Singh et al., 2014). Obviously, BRs likewise act downstream in Glu-instigated horizontal root development in *Arabidopsis* (Gupta et al., 2015). Exogenous utilization of various concentrations of Glu (0.5%, 1%, 3%, 4%, and 5%) to for 3-multi-day WT seedlings filled in ½ MS medium provoked parallel root improvement fundamentally in a fixation subordinate way when contrasted with their untreated controls. Likewise, comparative dosages of mannitol do not advance parallel root primordium and 3-OMG does not induce sidelong root primordium to any degree. Likewise, rgs1-1, rgs1-2, gpa1-1, gpa1-2 and thf1-1were practically unfeeling as far as sidelong root arrangement when contrasted with their WT seedlings finding out that Glu-initiated horizontal root development is HXK1 subordinate (Gupta et al., 2015). Further complexities show that exogenous utilization of 10 nM BRs and 1% and 3% Glu fundamentally incited parallel root improvement while 100 nM and 1 μM BRs appear to be inhibitory at all the dosages of Glu. Additionally, BRZ application further represses Glu-initiated parallel root improvement. In gin2-1mutants, BRs show a small reaction toward Glu-prompted horizontal root improvement bringing up the way that BR intervenes Glu-instigated sidelong root advancement by means of HXK1-dependent pathway (Gupta et al., 2015). The after effects of the exploration likewise restored that BRI1 is epistatic to HXK1 to intercede

horizontal root advancement where gin2-1bri1-6 twofold mutant shows undifferentiated from impacts asbri1-6mutant plants denoted the zenith that BR holds an advantage in Glu-prompted parallel root improvement in *Arabidopsis* (Gupta et al., 2015).

Concluding Remarks and Future Prospective

Brassinosteroids, owing to their versatility during stress-free conditions and during stressed conditions, have become a fascinating group of phytohormones. They perform a wide range of functions because of their complex and multi-target mechanisms. Laborious and contemplative efforts are being undertaken worldwide to unveil the intricate mechanisms of BR-mediated reactions during conditions of stress. These endeavors that are centered around understanding the BR homeostasis and their connections with different phytohormones will add new measurements to the continuous exploration of BRs during abiotic stress conditions. The present research has revealed that BRs play a significant role in the modulation of stress-related plant responses, but their elaborate mechanisms are yet to be understood. Advancements in the field of genomics and proteomics have helped in identifying the major genes and proteins that play a vital role in stress responses by plants, providing an appropriate means for the exploration of the role of BR signaling in stress enhancement. These studies have also explained the different components related to BR signal perceptions along with their cross talks with different phytohormones. However, cross talk between BR and other phytohormones needs to be understood at the signaling level along with their modulation by abiotic stress. Moreover, the connection of these signals that cause heavy metal stress tolerance in plants is still uncertain and requires a more comprehensive understanding about the actions of BRs at different levels. Concerning abiotic stress and other ecological conditions, there is additionally a need to investigate the interaction of BRs homeostasis which is generally reliant upon its blend, degradation, and transport. Furthermore, in-depth information on the transcriptional and posttranscriptional as well as translational and posttranslational event regulations by BRs will be essential in the modulation of the part that BRs play during heavy metal stress tolerance in plants. Moreover, with rapid development of genomics and proteomics, technologies leading to the identification of key genes and proteins related to stress responses in plants provide a suitable platform to explore the role of BR signaling in stress amelioration.

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Chapter 12
Mechanism Associated with Brassinosteroids-Mediated Detoxification of Pesticides in Plants

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Abstract Inapt usage of pesticides adversely affects the growth and development of the plants. Pesticides not only target the target species but also hampered the life cycle of nontarget species. The oxidative burst in plants with the generation of enhanced reactive oxygen species (ROS) has a detrimental effect on various physiological and biochemical mechanisms of plants which resulted in stunted growth, chlorosis, blackening of roots, accumulation of pesticides in plant parts, and decreased photosynthetic potential. Plants have the potential to withstand the stress conditions by activating different defense mechanisms like antioxidative defense system—enzymatic and nonenzymatic. Brassinosteroids (BRs) are the plant steroidal hormones known for their potential to protect and promote plant growth and development under various stressed conditions. BRs play important role in the amelioration of pesticidal stress by reducing the pesticidal generated stress on plants. BRs strengthen the plant's defense potential by stimulating the enzymatic and nonenzymatic antioxidative mechanisms which scavenge the generated ROS and activate the pesticidal detoxifying transcripts. Therefore, understanding the BRs mediated pesticide degradation process in plants is vital for global food security.

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Introduction

The worldwide increase in the size of population enhanced the continuous demand for food. It has been estimated that by 2030, the global population will reach the 8.5 billion mark (Clark & Tilman, 2017). To meet the growing need of food, utilization of pesticides acts as an important factor in repelling and controlling pests attack for quality products (Razaq et al., 2011; Yamada, 2017). However, extensive, uncontrolled, and inappropriate application of pesticides negatively affects the agricultural produce and makes them unfit to consume. Pesticides get absorbed by the plants via root system with the help of transpiration pull and shoot system through stomatal entry from leaves during the process of transpiration. Absorbed pesticides enter the plant system and get either metabolized or accumulated (Mwevura, 2000a, b). Accumulation of pesticide in the plants impairs the plant activities at the physiological and biochemical level and proved toxic to plants (Udeigwe et al., 2015; Rivera-Becerril et al., 2017). Pesticidal residue's acquisition in plants affects the plant well-being by the generation of oxidative stress in plants. Generation of oxidative stress with the overproduction of reactive oxygen species damages the various cellular components of the plants including nucleic acids, proteins, membrane integrity, hampered photosynthetic machinery, damage nucleic acids, a hormonal imbalance which eventually leads to reduced biomass (Kapoor et al., 2019). However, plants' inability to relocate has developed different mechanisms to withstand the stressed conditions. Plants have a vigorous antioxidative defense mechanism to counter against the generated reactive oxygen species. The enzymatic system which includes superoxide dismutase (SOD), peroxidase (POD), glutathione-s-transferase (GST), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APOX), and nonenzymatic system (glutathione, ascorbic acid, tocopherol, carotenoids, proline) essentially respond to the pesticidal stress (Homayoonzadeh et al., 2020).

Plant growth regulators play a key role in plant growth and development under favorable as well as nonfavorable conditions. Plant hormones act as a chemical messenger and modulate the physiological and biochemical processes under stressed conditions (Kim et al., 2019). Brassinosteroids (BRs) are a group of plant steroidal hormones that play an effective role in germination, elongation, differentiation, pollen tube formation, strengthening the photosynthesis, and mitigating the stresses (Hussain et al., 2020; Anwar et al., 2018). Several studies have shown the ameliorative role of BRs under pesticide stress in plants by boosting the antioxidative defense system (Sharma et al., 2016; Hou et al., 2019). Exogenous application of BRs also stimulates the photosynthetic efficiency of the plants, nutrient homeostasis, and secondary metabolites generation in plants under stressed conditions. Pesticides in plants get detoxified in the three-phase process—activation, conjugation, and

transportation (Jan et al., 2020). It has been well evident that P450 monooxygenase and glutathione-s-transferase (GST) involved in detoxification of pesticides by forming the conjugates and defense-related enzymes are used as a powerful weapon to protect the plant from oxidative damage (Sharma et al., 2018). Seed priming with 24-epibrassinolide regulates the expression of stress-related genes at the transcription level. Increased expression of *P450, GST,* and *GR* under chlorothalonil stress in grapevine and tomato plants has been documented (Wang et al., 2017; Hou et al., 2019). Another study by Sharma et al. (2016), on pesticide stress illustrated that plants when seeds were presoaked with 24-epibrassinolide-enhanced expression of GSH1-2 gene along with defense enzymes GST, POD, GR, APOX, GPOX, glutathione content have been observed. Concomitantly another study on castasterone pretreated *Brassica juncea* L. under imidacloprid stress showed enhanced expression of key genes: photosynthetic genes like CHLASE, PSY, CHS; Citric cycle genes, RBOH; pesticide detoxification genes CXE, P450, NADH; etc. In the present chapter, an attempt has been made to give comprehensive idea over the uptake, transportation, effect, and detoxification mechanism of pesticides in plants.

Uptake, Transport and Persistence of Pesticide

Water-soluble pesticides entered the plant through transpiration pull via the root system as well as shoot system with the stomatal opening by the process of transpiration. Plants have the ability to uptake the pesticides through both active and passive absorption method. Uptake was followed by transportation of the pesticide. However, upward movement of pesticides carried out by vascular bundles (xylem and phloem) and lateral transportation have also been observed in some cases. Transportation across membranes and translocation in the plant have also occurred with the help of some carrier systems and soil nutrients (Chen et al., 2001; Xia et al., 2014). After entering into the plant body, pesticides either get metabolized or accumulated, or compartmentalized leading to biomagnification in the environment (Mwevura, 2000a, b). Table 12.1 lists the pesticidal residue detected in various plant species. Uptake, transportation, and persistence of pesticides depend upon various environmental factors (temperature, precipitation, and humidity), chemical properties of pesticides, and soil characteristics. The rate of metabolization of absorbed pesticides in the plant depends on the amount, frequency, rate of application, pesticide degradation, biochemical properties, irrigation strategy, and physicochemical properties of the soil (Führ, 1991; Wang & Liu, 2007). A study conducted by Juraske et al. (2011), stated that application and degradation patterns of chlorpyrifos in the soil determined the uptake of the pesticide by potatoes. Uptake and translocation of nonionized pesticides are regulated by the lipophilicity of the compound which is inversely proportional to the pesticide mobility.

Table 12.1 The pesticidal residue detected in various plants

S. No.	Pesticide name	Plant name	Conc. of pesticide	Detected residue (mg/Kg)	References
1	Atrazine	*Oryza sativa* L.	0.8 mg L^{-1}	2.94 & 4.26 (leaves)	Zhang et al. (2014)
2	Chlorpyrifos	*Capsicum annuum* L.	1000 g ai ha^{-1}	1.30 & 0.47 (fruits)	Jyot et al. (2013)
3	Cypermethrin	*C. annuum* L.	100 g ai ha^{-1}	0.28 & 0.12 (fruits)	Jyot et al. (2013)
4	β-Cyfluthrin	*Solanum melongena* L.	36 g ai ha^{-1}	0.08 & 0.01 (fruits)	Mandal et al. (2010)
5	Imidacloprid	*Saccharum officinarum* L.	80 g ai ha^{-1}	12.99 & 2.37 (leaves)	Sharma and Singh (2014)
6	Imidacloprid	*C. arietinum* L.	84 g ai ha^{-1}	0.72 & 0.34 (leaves)	Chahil et al. (2014)
7	Imidacloprid	*Cucumis sativus* L.	125 g ai ha^{-1}	0.37 & 0.03 (fruits)	Nasr et al. (2014)
8	Imidacloprid	*Oryza sativa* L.	80 g ai ha^{-1}	9.40 & 0.59 (leaves)	Akoijam and Singh (2014)
9	Imidacloprid	*Punica granatum* L.	54 g ai ha^{-1}	0.33 & 0.11 (peel) 0.25 & 0.05 (whole fruit)	Kadam et al. (2014)
10	Imidacloprid (IMI), thiamethoxam (THX) and difenoconazole (DFZ)	*Oryza sativa* L.	1 & 10 mg IMI/kg soil 1 & 10 mg THX/kg soil, 2 & 20 mg DFZ/kg soil	IMI, 10.0 and 410 THX, 23.0 and 265, 0.23 and 3.4 (leaves) IMI, 1.37 and 69.3 THX, 3.19 and 30.6, 15.6 and 79.1 (roots)	Ge et al. (2017)
11	Flubendiamide	*Cucumis anguria* L.	120 g ai ha^{-1}	1.03 & 0.15 (fruit)	Paramasivam et al. (2014)
12	Fipronil	*Saccharum officinarum* L.	300 g ai ha^{-1}	0.66 & 0.16 (Leaves)	Mandal and Singh (2014)
13	Fipronil	*C. annuum* L.	80 g ai ha^{-1}	1.01 & 0.50 (Fruits)	Xavier et al. (2014)
14	Tetraconazole	*Cucumis sativus* L.	50 g ai ha^{-1}	0.10 & 0.002 (Fruits)	Nasr et al. (2014)

Effect of Pesticide

Growth Parameters

Irrational application of pesticide showed detrimental effect on germination potential of a plant. In *Zea mays* L, application of pendimethalin (10 ppm) decreased the germination percentage as well as growth of the plant (Parween et al., 2016). Root and shoot lengths were significantly reduced under high exposure of pesticides content in tomato seedlings (Rajashekar et al., 2012). The use of different pesticides like emamectin benzoate, alpha-cypermethrin, lambda-cyhalothrin, and imidacloprid at high concentrations on *Lycopersicon esculentum* hampered the shoot length which further affects the growth and yield of the plant (Shakir et al., 2016). Another study showed the toxic effect of polychlorinated biphenyl that reduced the biomass of the plant and also reduced the stomatal conductance (Ahammed et al., 2013b). Imidacloprid, a neonicotinoid pesticide downregulated the content of chlorophyll, reduced stomatal conductance and various other biological active compounds that further decreased the plant growth (Sharma et al., 2017a, b). Due to excessive use of pesticides, the roots may get flubbed resulting in the uncertain growth of the plant (Rajmohan et al., 2020). Table 12.2 summarizes the studies which showed the effect of pesticide on growth parameters of various plant species under pesticidal stress.

Pigment System

Plant photosynthetic efficiency has been enormously affected by the application of pesticides. Although pesticides incurred the crop loss by pests, their excessive and unskilled use affects nontarget plants and animals and interferes with the different physiological processes (Baig et al., 2012). Various studies reported the negative effect of pesticide on plant cell growth, photosynthesis, biosynthesis of pigments, enzyme activities, root growth, and respiration which can lead to economic losses (Sharma et al., 2017b; DeLorenzo et al., 2001). Xia et al. (2009) reported the negative effect of pesticide on photosystems which inhibit photosynthesis. Pesticides effect rate of photosynthesis by reducing the pigments and degrade the photosystems. It has been reported by Parween et al. (2016) that higher doses of captan, a fungicide, reduce the chlorophyll and carotene content. The recommended dose of the same was found to enhance the chlorophyll and carotene content. The enhancement of chlorophyll content has also been observed with the application of isoproturon and sulfosulfuron in gram plant (Khan et al., 2006). Pesticides like imidacloprid and chlorpyrifos were reported to reduce photosynthesis by degrading the chlorophyll pigments in *Brassica juncea* plant (Sharma et al., 2013). Decrease in carotene and chlorophyll content has been reported after the application of diuron

Table 12.2 Effect of pesticide on growth attributes of various plants

Plant name	Pesticide name	Conc. of pesticide	Effect of pesticide on growth parameters	References
Trigonella foenum-graecum	Organochlorine	100 mg	Decreases the root and shoot length and also alters the germinating ability of the fenugreek	Nathiya et al. (2020)
Spinacia oleracea L.	Chlorpyrifos, dieldrin, and dimethoate	100 kg N ha^{-1}	Reduce the leaf area, shoot length, root fresh weight that ultimately lowers the yield	Singh and Prasad (2018)
Brassica juncea L.	Imidacloprid	200 mg L^{-1}	Dwindling the germination, radical and hypocotyl length	Sharma et al. (2018)
Brassica juncea L.	Imidacloprid		Decreases the photosynthetic efficiency, conductance of stomata leading to downregulate the plant growth	Sharma et al. (2017a, b)
Triticum aestivum L.	TOPIK (Clodinafop-propargyl)	800 mg/L	Declined the growth efficiency and ultimately affects the yield of the plant	Lukatkin et al. (2013)
Vigna radiata L.	Chlorpyrifos	0–1.5 mM	Downregulates the overall efficiency of the plant development	Parween et al. (2012)
Zea mays L.	Pendimethalin	0–10 ppm	There is significant declined in the plumule and radical length by 77% and 90%, respectively, at 10 ppm of pesticide exposure	Rajashekar and Murthy (2012)
Cenchrus setigerus Vahl, Pennisetum pedicellatum Tan	Chlorpyrifos, cypermethrin, fenvalerate	0–100 mg Kg^{-1}	Significantly downregulates the seed germination	Dubey and Fulekar (2011)

pesticide in *Saccharina japonica* (Kumar et al., 2010). Table 12.3 shows various studies showing negative effect of pesticide on photosynthetic efficiency of plant.

Oxidative Stress Marker

Excessive application of pesticides affects the plant growth from germination to development by altered physicochemical processes which further decreased the yield of the plant. A study conducted by Zhang et al. (2014), documented that the application of pesticides increased oxidative stress via electrons leakage which reduced the various oxygen species. The increased amount of reduced oxygen species (superoxide radicals, hydrogen peroxide, hydroxyl radical, and

Table 12.3 Effect of pesticide on photosynthetic efficiency of plants

Plant name	Pesticide name	Conc. of pesticide	Effect of pesticide on photosynthetic pigments	References
Brassica juncea	Imidacloprid	250 mg/L	Total chlorophylls (chlorophyll-a and chlorophyll-b) were observed to be reduced by 53.50%, 51.21%, and 59.26%; whereas carotene, xanthophyll, and anthocyanin content were increased	Sharma et al. (2019)
Zea mays	Nicosulfuron	100 μM	Total chlorophylls (chlorophyll-a and chlorophyll-b) were observed to be reduced by 11.8% and 22.9%, respectively, as compared to the control	Liu et al. (2019)
Solanum lycopersicum	Thiram	6.6 mM	Chlorophyll content decreased	Yüzbaşıoğlu and Dalyan (2019)
Vigna radiata	Mancozeb Chlorpyrifos Metribuzin	750 g Kg^{-1} 2 mL L^{-1} 350 g L^{-1}	Reduction in chlorophyll content was reported	Fatma et al. (2018)
Zea mays	Propaquizafop	56.3 μM	Reduction in chlorophyll was reported	Rusjan et al. (2018)
Brassica juncea	Imidacloprid	200 mg_L^{-1}	Chlorophyll content was found to reduce While carotene and anthocynin were reported to increased	Sharma et al. (2018)
Spinacia oleracea L.	Chlorpyrifos	Recommended dose	The pesticide chlorpyrifos exerts negative impact on chlorophyll content (both chl a and b) reduced by −27.9% and −5.7% and carotene by −23.2%	Sing and Prasad (2018)
Spinacia oleracea L.	Dimethoate	Recommended dose	The pesticide chlorpyrifos exerts negative impact on chlorophyll content (both chl a and b) reduced by −33.0% and −7.1%, and carotene by −17.1%	Singh and Prasad (2018)
Spinacia oleracea L.	Dieldrin	Recommended dose	The pesticide chlorpyrifos exerts negative impact on chlorophyll content (both chl a and b) reduced by −37.1% and −9.7% and carotene by −26.3%	Singh and Prasad (2018)

(continued)

Table 12.3 (continued)

Plant name	Pesticide name	Conc. of pesticide	Effect of pesticide on photosynthetic pigments	References
Lycopersicon esculentum	Imidacloprid	2000 mg/L	The highest reduction in chlorophyll a and b was observed	Shakir et al. (2016)
Lycopersicon esculentum	Emamectin	60 mg/L	The total chlorophyll content (both chl a and b) and carotene found to reduce and beyond	Shakir et al. (2016)
Lycopersicon esculentum	Alpha-cypermethrin	500 mg/L	The total chlorophyll content (both chl a and b) and carotene found to reduce	Shakir et al. (2016)
Lycopersicon esculentum	Lambda-cyhalothrin	120–240 mg/L	The total chlorophyll content (both chl a and b) and carotene found to reduce	Shakir et al. (2016)
Lycopersicon esculentum	Thiamethoxam	144 mg L^{-1}	Total chlorophyll content reduction was highest at this concentration	Yildiztekin et al. (2015)
Oryza sativa	Chlorpyrifos	0.04%	Total chlorophyll content reduction was highest at this concentration	Sharma et al. (2015)
Oryza sativa	Imidacloprid	0.015%	Total chlorophyll content reduction was highest at this concentration	Sharma et al. (2015)
*O*ryza *sativa*	Atrazine	0.4 mg L^{-1}	Decrease in chlorophyll content	Zhang et al. (2014)
Arachis hypogaea	Fusilade	60 ppm	Chlorophyll content reported to be reduced	Fayez et al. (2014)
Triticum aestivum L.	Chlorotoluron	0–25 mg/kg	Chlorophyll content decreased	Song et al. (2007)
Withania somnifera L.	Triadimefon	10 mg L^{-1}	Chlorophyll content increased	Jaleel et al. (2008)
Saccharum officinarum L.	Methyl viologen	0–8 mM	Chlorophyll content was significantly reduced	Chagas et al. (2008)

thiobarbituric acidic reactive substance) interfered with the scavenging process of generated reactive oxygen species which ultimately headed to oxidative burst in the plant (Gill & Tuteja, 2010; Sheeba et al., 2011; Shahzad et al., 2018). ROS formed in various compartments of cells (Jan et al., 2012) and resulted in the disruption of lipids (polyunsaturated) leading to the production of MDA (malondialdehyde) which represents the peroxidation and electron leakage of the plant cell membrane (Srivastava et al., 2014). Another study by Song et al. (2013), reported that with the treatment of pesticides there is an elevation in the content of ROS that also enhanced the MDA content and affects the permeability of the membrane of the plant (Song et al., 2013). Application of different concentration of pesticides in tomato plants has showed enhanced MDA content and electron leakage (Yildiztekin

et al., 2015). To mitigate the pesticide toxicity, upregulation in the production of antioxidative enzymes including SOD, APX, CAT, POD, and GST has been examined that have a potent role in the homeostasis of ROS production. The toxic effect of pesticides like chlorpyrifos, dimethoate, and dieldrin showed an effective increase in the oxidative stress and lipid peroxidation via converting the superoxide radical to hydroperoxy radicals (Parween et al., 2012; Singh & Prasad, 2018). Table 12.4 summarizes the different studies on stress marker under pesticidal stress.

Antioxidative Defense System

Like other environmental stresses, pesticides are one the major stress that influences the plant growth, metabolism, and yield (Wu & Linden, 2010; Zhang et al., 2014). Unchecked use of different pesticides with high doses found to disrupt the plant biochemical parameters (Singh et al., 2014). Studies show that use of pesticides poses the risk of oxidative damage to plants due to production of reactive oxygen species at a very high rate (Zhang et al., 2014). Gill and Tuteja (2010) suggested the negative effect of pesticides on scavenging process of these harmful free radicals and their homeostasis. Damages to the cell structures and biomolecules due to stresses are considered to be caused majorly from ROS (Sheeba et al., 2011). In order to escape from these oxidative injuries, plant synthesizes both low molecular weight antioxidant compounds such as ascorbate, carotenoids, and glutathione and antioxidant enzymes which include SOD, POD, CAT, GR, and APEX. Synthesis of these compounds and enzymes plays a crucial role in containing ROS production within limit which is necessary for signaling (Zhang et al., 2014; Sereme et al., 2016). Pesticide-induced cellular antioxidant defense system has been reported by Singh et al. (2014), and later on several other workers have also reported (Table 12.5).

Detoxification of Pesticides in Plants

Applications of pesticide are believed to be an effective and easy method to control the pest attack but on the other hand they pose a serious threat to the plants and surroundings. Irrational implementation of pesticides altered the plants physiological, biochemical, and molecular processes (Zikankuba et al., 2019). Brassinosteroids combat the pesticidal toxicity in plants by scavenging ROS and maintaining hormonal homeostasis (Ahammed et al., 2012a, b, c; Lv et al., 2018; Hou et al., 2019). Meliorative role of 24-epibrassinolide in reducing 30–50% of carbamate, organophosphorus, and organochlorine residues in a wide range of plants like broccoli, strawberry, tomato, tea, cucumber, rice, and cucumber plants have been documented (Zhou et al., 2015). These studies showed that plants have the ability to detoxify the engrossed pesticide. Several studies carried out on detoxification of pesticides reported three-phase plant detoxification mechanisms. In the first phase of the

Table 12.4 Various oxidative stress markers under pesticidal stress in plant

Plant name	Pesticide name	Conc. of pesticide	Effect of pesticide on oxidative stress marker	References
Lycopersicum esculentum Mill.	Acetamiprid Imidacloprid Abamectin Thiamethoxam Abamectin + chlorantraniliprole	30 mg 100 mg 25 mg 100 mg 90 mg	Stimulate the production of H_2O_2 and MDA	Yildiztekin et al. (2019)
Brassica juncea L.	Imidacloprid	200 mgL^{-1}	Significant increase in the content superoxide anion, malondialdehyde, and hydrogen peroxide	Sharma et al. (2018)
P. sativum L.	Isoproturon	10 mM	Increases the production of H_2O_2 and MDA and also causes the leakage of electrons from cell membrane	Singh et al. (2016)
Pennisetum americanum L.	Atrazine	10 mg kg^{-1}	Upregulates the production of MDA	Jiang et al. (2016)
Nicotiana tabacum L.	Imazapic	0.12 mM	Increases the content of MDA	Kaya and Doganlar (2016)
Scirpus tabernaemontani P.	Atrazine	8 mg L^{-1}	MDA level increases	Wang et al. (2015)
Lythrum salicaria L.	Atrazine	8 mg L^{-1}	Boosts up the level of MDA content	Wang et al. (2015)
Helianthus. annuus L.	Quizalofop-*p*-ethyl	0.8 mM	MDA level rising	Bayram et al. (2015)
O. sativa L.	Chlorpyrifos	0.04%	Increases concentration of O2, H_2O_2, and MDA	Sharma et al. (2015)
O. sativa L.	Atrazine	0.4 mg L^{-1}	Stimulates the production of H_2O_2,O^{2-},and TBARS	Zhang et al. (2014)
H. annuus L.	Flurochloridone	11 mM	MDA level increases	Kaya and Yigit (2014)
T. aestivum L.	–	4 mg kg^{-1}	O^{2-}, H_2O_2, and TBARS level increases	Liang et al. (2012)
Tomato	Phenanthrene and pyrene	–	H_2O_2, OH^-, and O^{2-} production increases	Ahammed et al. (2013a, 2012a, b, c)

Table 12.5 Role of brassinosteroids on antioxidative defense system of plants under pesticide stress

Plant name	Pesticide name	Conc. of pesticide	Role of brassinosteroids in antioxidative defense system	References
Lycopersicon esculentum Mill.	Boscalid	2 g/l	Activated peroxidase, glutathione reductase and glutathione *S*-transferase	Yang et al. (2020)
Brassica juncea	Imidacloprid	150, 200, 250 mg/l	Enhanced expression of SOD, CAT, POD, DHAR, GR, and GST has been observed	Sharma et al. (2019)
Spinacia oleracea L.	Chlorpyrifos Dimethoate Dieldrin	Recommended dose	All the antioxidative enzyme (SOD, POD, APX, GR, CAT) activity found to enhance	Singh and Prasad (2018)
Solanum lycopersicum	Emamectin	40 mg/L, 80 mg/L, and 160 mg/L	At 40 mg/L, SOD was recorded highest At 80 mg/L and 160 mg/L, POD activity was highest GR was reported highest at 80 mg/L and 160 mg/L 80 mg/L exposure reported significant increase in CAT in both root and shoot And increase in APX activity was reported at 160 mg/L in root Significant increase in proline content was observed at 40, 80, and 160 mg/L	Shakir et al. (2018)
Solanum lycopersicum	Cypermethrin	125 mg/L, 250 mg/L, and 500 mg/L	SOD activity was highest at 500 mg/L POD was reported highest at 500 mg/L in shoots and at 60 mg/L in roots At 500 mg/L, GR activity was highest in shoot while in root it was dose-dependent CAT activity was highest at 250 mg/L shoot APX was at highest at 500 mg/L in root while it was dose-dependent in shoot	Shakir et al. (2018)
Solanum lycopersicum	Imidacloprid	1000 mg/L	SOD activity increased at mg/L& 1000 mg/L in both root and shoot Highest POD was recorded at 2000 mg/L in roots.	Shakir et al. (2018)

(continued)

Table 12.5 (continued)

Plant name	Pesticide name	Conc. of pesticide	Role of brassinosteroids in antioxidative defense system	References
			While in shoots, it was dose dependent manner At 1000 & 2000 mg/L both in root and shoot the activity of GR was reported highest At 1000 & 2000 mg/L CAT activity was reported highest in shoot At 2000 mg/L the APX activity was recorded highest in root	
Vitis vinifera L.	Chlorothalonil	600 times diluent	The activity of different antioxidative enzymes (SOD, APX, CAT, POD) found to enhance	Wang et al. (2017)
Oryza sativa Variety Pusa Basmati-1	Chlorpyrifos	0.04%	Significant increase of proline and various antioxidative enzymes (SOD, APX, CAT, GR, GPX) were reported	Sharma et al. (2015)
Solanum lycopersicum	Chlorothalonil	11.2 mM	Enhanced activity of glutathione *S*-transferase (GST)	Zhou et al. (2015)
Oryza sativa Variety Pusa Basmati-1	Imidacloprid	0.015%	Elevation of antioxidative enzymes (SOD, APX, CAT, GR) was observed	Sharma et al. (2015)

detoxification pathway, pesticides are absorbed and metabolically activated by the catalytic action of P450 monooxygenase, carboxylesterases, and peroxidase. Metabolically activated enzymes form a conjugation with glutathione and glucose with the help of glutathione-s-transferase and UDP-glucosyltransferase (UGT) in the second phase. ATP-dependent membrane pumps carried the conjugated products out for storage. In the third phase of the detoxification process, less toxic and soluble metabolites are confiscated and stored in the apoplast and vacuole (Fig. 12.1). The underlying mechanism of BRs mediated pesticidal stress mitigation needs more clarity (Hou et al., 2018); however, exogenous application of BRs restores the detrimental effects of pesticidal stress and residue. Application of 24-epibrassinolide in cucumber stepped up the plant's metabolism and reduced the pesticidal residues (Xia et al., 2009). Furthermore, it has been documented that the production of ROS mediated by RBOH1 gene plays important role in activating BRs-mediated detoxification of pesticides in plants (Zhou et al., 2015). A pioneering study by Hou et al. (2018), revealed that in tomato plant, BRs treatment induced a modest amount of oxidative burst which is important to enhance the glutathione-mediated detoxification of pesticide. Dietz et al. (2016) in his study recorded that

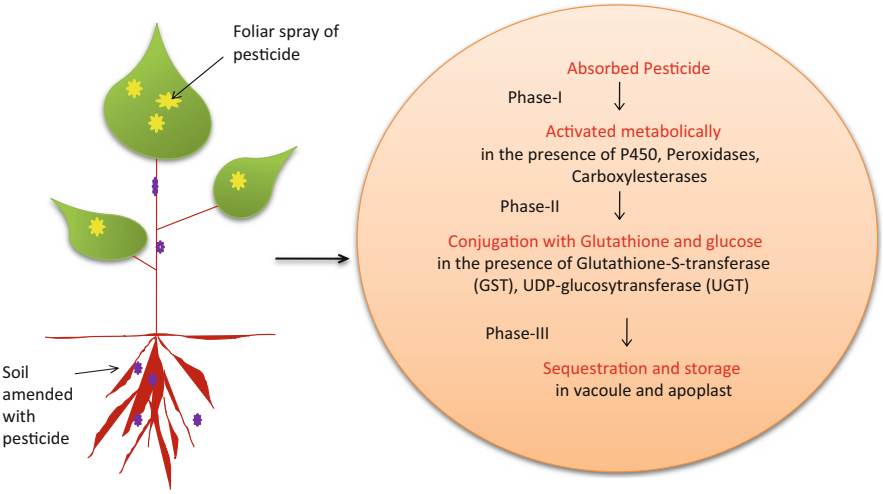

Fig. 12.1 Mechanism of pesticide detoxification in plants. (Modified from Jan et al., 2020)

glutathione, glutathione redoxins, peroxiredoxins, peroxidases, and thioredoxins have the ability to sense the generated ROS. A study by Hou et al. (2019), suggested that BR triggered the ROS metabolism in tomato for pesticide detoxification by activating the TGA2 factor that binds with motif (TGACG) through glutaredoxin S25 (GRXS25) posttranslational modification.

Conclusion and Future Prospective

Extensive and reprehensible ways of pesticide application lead to pesticidal stress in plants. Absorbed and transported residues in different parts of the plant disrupt the various physiological and biochemical mechanisms of the plants. Generation of reactive oxygen species leads to oxidative stress in plants that affect the plants at the cellular level. Plants have inbuilt several adaptations to withstand these stress conditions which include antioxidative defense mechanisms like enzymatic and nonenzymatic antioxidants. This chapter focused on the ameliorative role of brassinosteroids in the detoxification of pesticides in plants. Brassinosteroids have the potential to remediate the pesticidal stress generated toxicity as well as reduction of pesticidal residues by strengthening the defense mechanism of plants. Activation of detoxification mechanisms in plants by using BRs proves to be an effective approach for improving the contamination of agricultural produce. We anticipate that emphasis should be given on the possible process of BRs-regulated plant pesticide detoxification.

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Chapter 13
Glyphosate: Is Brassinosteroids Application a Remedy?

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Abstract Pesticides are mainly used to protect crop plants from pests and pest transmitted diseases. However, the active ingredients of the pesticides are also a source of crop toxicity and food contamination. The persistent nature of the chemicals makes them stable against environmental degradation process, and they continue to be in the form of pesticide residues in different plant tissues. In plants, glyphosate has been the best commonly used herbicide. It has a proven record of disturbing plant physiological processes and cell metabolism. Plant biological practices such as photosynthesis, carbon use, mineral diet, and oxidative trials have been exaggerated, and plant–microbe interactions have been interrupted by the pesticide. Despite the less studied detail of aminomethylphosphonic acid (AMPA), it was displayed to influence chlorophyll biosynthesis and to cause decline of plant development. In addition, brassinosteroids (BRs) are well-known for their defensive function in plants in numerous abiotic stresses, such as low temperatures, salt, heavy metals, drought, and pesticides. By triggering the antioxidant defense mechanism, BRs improve pesticide harmfulness in whole plants. In addition, BRs also increase pesticide degradation, which contributes to a decrease in residual pesticides in plant portions. Therefore, the current study offers to reveal the function of BRs in the management of glyphosate, and current research illuminates the detoxification of BR-regulated glyphosate in plants.

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Introduction

Plants are often subjected to numerous biotic and abiotic stresses during their life succession, which can decrease crop yield as a result (Yasin et al., 2018a, b, 2019; Li et al., 2021). Glyphosate [N-(phosphonomethyl) glycine] remains actually the world's best commonly used herbicide, and its agricultural science practice has been enhanced substantially after the outline of plants resistant to glyphosate (GR) (Latif et al., 2015; Mollaee et al., 2020). Glyphosate role in plant leaves as spray in agronomic fields; on the other hand, a proportion of the chemical can be placed openly on the outward of the soil or transferred by wind to adjacent soils and plants, resulting in the introduction of nontarget plants (Shafique et al., 2014a, b; Hafeez et al., 2019). In addition, several researchers have indicated that impurity of the waterway is a cause of transmission of glyphosate to neighboring cultivated fields, particularly in fields flooded by pumping into outward water bodies (Solomon & Thompson, 2003). Researchers discovered that exudation from the roots of drenched plants and its discharge from deceased plants was a critical source of glyphosate exposure (Shahid & Khan, 2018; Hage-Ahmed et al., 2019). A possibility of glyphosate harmfulness to nontarget plants owing to rhizosphere glyphosate transmission was recommended in the latest studies (Gaupp-Berghausen et al., 2015; Khan et al., 2016).

In general, glyphosate can reach plants using four possible routes when applied to foliar sections of weeds: the leaves or other green tissues, the roots, the trunk, or the shoots developing from the root or the trunk (Kanissery et al., 2019; Khan et al., 2019a; Ahmad et al., 2020a). After reaching inside the plants, glyphosate is quickly translocated to the actively growing regions. Its working principle lies on blocking the activity of the enzyme 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) which is known for catalyzing the sixth step of the shikimic acid pathway. It prevents the biosynthesis of aromatic amino acids, viz., by blocking the enzyme (Ahmad et al., 2014; Leino et al., 2020). Through the Shikimate pathway, phenylalanine, tyrosine, and tryptophan were created. Glyphosate-treated plants normally die within a span of 1–3 weeks and no plant parts can survive due to its even distribution in the plant. In addition, development and physiology of plants involving seed germination, flowering, and hypocotyl elongation are governed by brassinosteroids (BRs), a novel group of polyhydroxy steroid hormones which is capable of inducing endogenous signals required for plant growth regulation(Yusuf et al., 2017; Ullah et al., 2002; Anwar et al., 2018; Hayat et al., 2010). The abiotic stresses such as metal stress, chilling stress, low temperature stress, high temperature, drought stress, oxidative damage, and salt injury can be minimized by BRs (Fariduddin et al., 2014a; Abbas et al., 2020; Ahmad et al., 2020b). In addition, 24-epibrassinolide (EBL) analogs of BRs are capable of supporting the plant defense mechanism

against environmental evidence such as heat stress, drought, low temperature, heavy metals, and salt stresses (Rady, 2011; Yasin et al., 2018c; Hayat et al., 2007). Therefore, the present chapter offers to clarify the role of BRs in the management of glyphosate and the detoxification process of BR-regulated glyphosate in plants.

Persistence of Glyphosate in the Environment

Glyphosate, applied to combat weeds as a foliar spray, may get accumulated in various soil pools and nontarget sites (Kanissery et al., 2019). Drained from the drift of the foliage or undirected spray, death and decay of glyphosate-treated plant remains and glyphosate may be transferred to the soil by exudation from the roots (Zobiole et al., 2010). Glyphosate release can also occur in the form of exudates from intact glyphosate-resistant crop roots. This herbicide has the capability of getting adhered to soil components and is thus mainly contained in the upper part of the soil (Yunus et al., 2018; Wang et al., 2019). It may be transferred to groundwater, surface water, and water debris through activities such as surface overflow, drift, and perpendicular soil transference (Boano et al., 2014; Bhateria & Jain, 2016). Glyphosate mobility and leaching under laboratory, lysimeter, and field situations have been tested. Glyphosate has been initiated to carrying profound into the soil and leach out with drainage water in a study on glyphosate escape and association performed at a field located in Denmark, considering its great requisite tendency on soil (Kanissery et al., 2019; Saunders & Pezeshki, 2015). The concentration of glyphosate found in the sample was, however, fit under the determined level of impurity for this herbicide. Glyphosate has also been noticed in surface water beyond its occurrence in the groundwater. Glyphosate's main occurrence in surface water may theoretically be accredited to runoff of surface water. This element can carry prolonged and remote dangers to the ecological surroundings due to widespread use (Rolando et al., 2017). Microbial-facilitated deprivation or biodegradation is a big way of degrading glyphosate from the soil.

Degradation of glyphosate is a predominantly microbial-mediated approach and has been extensively planned in laboratories (Rolando et al., 2017). In most soils, it degrades at considerable rapid rates, with a half-life estimation of 7–60 days (Kanissery et al., 2019). Several researches have shown that the existence of glyphosate in the soil may improve bacterial activity, although several researches have also revealed that glyphosate has a toxic impact on soil microorganisms. As microorganisms are not capable to use it as a source of carbon, glyphosate tends to be metabolically biodegraded (Singh & Walker, 2006). The detail that glyphosate deprivation and overall bacterial movement in the soil are associated also denotes the cometabolic participation of microbes in the degradation of this product. The absence of a lag step in the soil is evidence offered for metabolic degradation of glyphosate, which means that undignified enzymes necessity by now existing in the soil earlier application of glyphosate. In comparison, a few studies have shown that

glyphosate can be used by microbes as a carbon, phosphate, or nitrogen substrate (Yunus et al., 2018; Busse et al., n.d.; Singh et al., 2020; Hove-Jensen et al., 2014).

Mainly, here are dual ways of glyphosate microbial degradation. The intermediate compound that is formed in one pathway is aminomethylphosphonic acid (AMPA), and sarcosine and glycine are made in the other (Singh et al., 2020; Huntscha et al., 2018; Reddy et al., n.d.-a). However, as it accounts for more than 90% of the recorded metabolites, AMPA is deliberated to be the utmost common glyphosate degradation metabolite. To generate AMPA and glyoxylate, the enzyme glyphosate oxidoreductase breaks the C–N bond in glyphosate. Flavine adenine dinucleotide (FAD) is used by the microbial enzyme glyphosate oxidoreductase as a cofactor that is vital in the deprivation trails of glyphosate. It is expected that the FAD is reduced by glyphosate at the vigorous site. To make glyphosate-tolerant Roundup Ready crops, the glyphosate oxidoreductase enzyme is introduced into plant genomes (Pollegioni et al., 2011; Vemanna et al., 2017).

Glyphosates and Crop Fitness

Among other issues associated to the unintentional things of glyphosate, farmers are completely worried regarding its detrimental belongings on untargeted plants. Via several pathways, glyphosate used to suppress weeds will enter the nontarget regions. The key direction is by aimless spray uses or "spray drift" that can bring the chemical herbicide directly to crops. Study has shown in crops such as soybeans and cotton that off-target effort or sense of glyphosate through application can be up to 10% of the rate useful (Kanissery et al., 2019; Reddy et al., n.d.-b). While exposure to herbicides in application drift may be measured toxic, the reply of susceptible crops could be potentially serious. For example, glyphosate drift has been found to cause warped fruits to grow at sublethal exposure rates in tomatoes (Martinez et al., 2018). The discharge of glyphosate from plant remains of glyphosate-treated weeds provides another possible way for glyphosate accumulation and maintenance in soils. Since glyphosate in many plant species is equally constant and not directly metabolized, substantial amounts, especially in young tissues, can be widely translocated to areas of vigorous growth and accumulation. Following the decay of plant pieces, it ends up in the soil after the weeds finally die. Further serious appraisals have shown that inside plants, glyphosate is translocated, stored in roots, and ultimately out into the rhizosphere (Duke et al., 2012; Mertens et al., 2018). Glyphosate can also be reabsorbed from the soil by target or nontarget plants after the early application, back through the roots. A little research has reported the effects of glyphosate root-zone contact on crops, such as cotton, maize, and rapeseed. These findings suggest that the root absorption of glyphosate into crops is probable. Maximum of the findings, however, were drawn from notes on hydroponic nutrient solutions, and later further study would be useful for deeper accepting soil absorption of glyphosate and its subsequent belongings on crop function.

By requisite and succeeding inactivation of an enzyme (EPSPS) that is crucial in the pathway of shikimate, glyphosate inhibits the production of essential amino acids (Leino et al., 2020; Schönbrunn et al., 2001; Mir et al., 2015a). The same metabolic pathway is used to derive an arrangement of phenolic compounds which show a major part in plant protection and immunity. Glyphosate predisposes crops to target soil-borne pathogens by blocking the production of such resistance compounds in plants. Therefore, it can be debated that constant acquaintance to glyphosate from crops could upsurge the vulnerability of plants to disease. In many crops, excessive application of glyphosate has been linked to disease growth. For example, the key issue in the production of diseases such as fusarium head blight in agronomic crops was found to be glyphosate applications. There are reported evidences of increased pathogen colonization in wheat and barley roots related through glyphosate burn-down uses earlier to implanting (Martinez et al., 2018; Yamada et al., 2009; Powell & Swanton, 2008). In addition, the special effects of glyphosate sublethal quantities on perennial plants often occur in a year after contact and persist for 2–3 years. By reducing the complete growth and potency of the plants, modifying soil micro flora that upsets the availability of nutrients desired for disease tolerance, and changing the biological effectiveness of plants, glyphosate may also ultimately predispose plants to diseases.

Some questions are also posed about the harmful things of glyphosate on fruit retaining, such as citrus, in tree crops. A natural phenomenon is the fruit drop in citrus, nevertheless an upsurge in the fruit droplet after use of glyphosate has been recorded, particularly in late summer stock and drop for initial period oranges and grapefruits with an effect on fruit crop (Sharma et al., 2007; Alcántara-de la Cruz et al., 2019). As it is not reliable through diverse seasons, the explanation for this glyphosate-linked drop is far from understood. However, it is known that glyphosate increases the development of ethylene in plant tissues, and exposure of complete citrus fruit to ethylene can lead to early abscission and drop in fruit. To know the reasons of this fruit fall and the exact function of glyphosate in this practice, further research is needed.

Modification of Plant Physiology by Glyphosate

Numerous plant biological practices which might be related to glyphosate-herbicidal belongings have been shown to affect glyphosate (Gaupp-Berghausen et al., 2015; Pollegioni et al., 2011; Khan et al., 2017a; Yousaf et al., 2015). Some studies have questioned the effects of glyphosate solely attributable to EPSPS inhibition, supporting this argument, as reduction of aromatic amino acids was not confirmed in glyphosate cured plants. The key biological practice that arises in photoautotrophic species is photosynthesis and reflected to be triggered by numerous anthropogenic aspects. Selected herbicides have been initiated to interfere photosynthetic electron transport directly. For instance, by competing for QB binding locations, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is recognized to stop the electron

flow between QA and QB (Fu et al., 2017). Through hindering the biosynthesis of carotenoids, chlorophylls, fatty acids, or amino acids, additional herbicides, such as glyphosate, resolve indirectly influence photosynthesis. Glyphosate blocks the shikimate pathway as an EPSPS competitive inhibitor, hindering the biosynthesis of secondary metabolites, plus photosynthesis-related compounds, such as quinones, in plants. It is uncertain, however, in what way glyphosate contributes to plant decease, and theories have been put forward, such as the degradation of protein shares and the drainage of C from other critical trails. A closer look at the effects of glyphosate on photosynthetic developments can shed light on this assumption. Many field and glasshouse experiments have indeed shown a reduced photosynthetic rate in plants after exposure to glyphosate (Khan et al., 2019b; Sharma et al., 2019, 2020; Tani et al., 2020; Zobiole et al., 2012).

Some studies have documented diminished chlorophyll content in plants following the use of glyphosate owing to chlorophyll biosynthesis degradation or embarrassment (Zobiole et al., 2012). By declining the Mg content in leaves, which hints to a reduced chlorophyll content and photosynthetic rate, glyphosate can indirectly prevent chlorophyll synthesis.

Indeed, the incorporation of Mg into the porphyrin structure by Mg chelatase is an essential stage that leads to the synthesis of chlorophyll molecules (Brzezowski et al., 2016). Studies have shown that the foliar application of glyphosate can minimize cation concentrations in GS soybean shoots and seeds (Duke et al., 2012). Similarly, glyphosate can prevent δ-aminolevulinic acid (ALA) biosynthesis, a constituent of the chlorophyll biosynthetic pathway, by inducing Fe deficiency. Both enzymes involved in ALA biosynthesis, catalase (CAT) and peroxidase, are highly responsive to Fe deprivation. Owing to its carboxyl and phosphonate groups, glyphosate is a powerful cation chelator that forms developments with nutrients in plant tissues, rendering them inaccessible for biological processes, like photosynthesis. Furthermore, by regulating the change of alpha-ketoglutarate to ALA and/or the shortening of glycine with succinyl-CoA to form ALA and CO_2, glyphosate was planned to interfere with ALA biosynthesis (Gomes et al., 2014).

Through modifying C metabolism in plants, glyphosate and AMPA similarly distressed photosynthesis (Gomes et al., 2014, 2017; Krenchinski et al., 2017). Remaining C interchange and stomatal conductance were stated to have decreased following foliar administration of glyphosate and AMPA. CO_2 assimilation ability is decreased under these conditions, prominent to an augmented intracellular CO_2 concentration. In tally to these special effects on gas interchange, after glyphosate exposure, ribulose-1,5-biphosphate (RuBP) and 3-phosphoglyceric acid (PGA) levels remain decreased. It appears that glyphosate can also decrease the activity of ribulose 1,5-biphosphate carboxylase oxygenase (Rubisco) in beet sugar. This result was also recorded in Lupinus albus leaves, where, subsequently five days of contact to 10 mM glyphosate, a 26% decrease in Rubisco activity was found. All these effects affect the efficiency of the plant in fixing and reducing atmospheric C into sugars. By interfering with sugar metabolism and translocation, glyphosate can also inhibit C metabolism. Researchers observed carbohydrate accumulation in equally the leaves and roots of glyphosate cured plants to study the belongings of

glyphosate on pea plants (Fernández-Escalada et al., 2019; Zabalza et al., 2004). Carbohydrate accumulation in roots was owing to an absence of practice of existing sugars as development was halted, which also caused soluble carbohydrate accumulation in leaves.

Most of the properties of glyphosate on nitrogen metabolism have been considered in soybeans (Leguminosae), in which synergetic N fixation denotes around 40–70% of the whole N necessity of the plant (Lindström & Mousavi, 2020; Contador et al., 2020; Zahran & Rhizobium-Legume, 1999). For gainful soybean yields and for maintaining long-standing soil efficiency, it is important to maintain this significant N input, particularly in soils with low N available concentrations wherever traditional replacements are carried out with high N-overwhelming crops such as maize. Using straight belongings on the rhizobial symbiotic or indirectly by influencing the physiology of the host plants, glyphosate can influence N metabolism. Microorganisms, apart from plants, often have EPSPS enzymes and are consequently prone to glyphosate. For instance, the soybean N-fixing symbiont *Bradyrhizobium japonicum* has a GS EPSPS and stores shikimate and hydroxybenzoic acids upon exposure to glyphosate, such as protocatechuic and/or gallic acids. This contributes to inhibition of growth and causes death at high concentrations of glyphosate. A possible translocation of the herbicide to the nodules remained designated by the gathering of protocatechuic acid in soybean nodules of glyphosate used plants. The decreased nitrogenase activity shown in B has confirmed this hypothesis. In addition, residues of glyphosate were also contained in GR soybean nodules from plants in predictable herbicide use in field situations. (i) Its detrimental role on the synthesis of aromatic amino acids; (ii) the increase of probable lethal intermediates of the shikimic acid pathway; or (iii) the extra chemical energy (ATP and PEP) expended on the shikimate pathway can be due to the lethal roles of glyphosate in the prokaryote components of bacteroides (De María et al., 2006; Samsel & Seneff, 2013, 2015).

The activity of glyphosate often contributes to plant oxidative stress, which is best, possibly an inferior result of the choked pathway of shikimate. In order to manage with oxidative stress caused by ROS accumulation by combining enzymatic and nonenzymatic antioxidants, plants have established mechanisms. Sometimes used as markers of oxidative stress in plants are enzymatic processes, the activities of ROS-scavenging enzymes, and malondialdehyde (MDA) material, a result of membrane lipid peroxidation. While there have been reports of variations in oxidative stress markers in different stress environments, there is currently less knowledge existing on the impact of glyphosate on oxidative stress (Gomes et al., 2017; Intayoung et al., 2020; Basu & Vasudeva Rao, 2020).

An improved level of lipid peroxidation, glutathione (GSH), free proline level, and ion flux were seen in maize leaves treated by glyphosate. Further, gene expression study revealed that hydrogen peroxide (H_2O_2) is manufactured by glyphosate use, resultant in peroxidation and lipid damage in rice leaves. In addition, these authors furthermore reported a reduction in the content of big Rubisco subunits and an rise in the accumulation of antioxidant enzymes, including ascorbate peroxidase (APX), glutathione-S transferase (GST), h-type thioredoxin, nucleoside diphosphate

kinase 1 (NDPK1), peroxiredoxin, and superoxide dismutase [Cu-Zn] (SOD) precursor chloroplast inside glyphosate-treated plants (Gomes et al., 2014; Kielak et al., 2011).

Physiological and Abiotic Stress Defensive Roles of BRs

In plant growth and development processes, BRs play an important role, such as increased cell division, leaf epinasty, growth of the pollen tube, stem elongation induction, proton pump activation, xylem differentiation, cell elongation, morphogenesis, tissue differentiation, and reproduction (Kondo et al., 2016; Khan et al., 2015a; Yusuf et al., 2016; Mir et al., 2015b). Several research revealed that root elongation was stimulated by uses of BRs and auxins to BRs of lacking Arabidopsis mutants (Naz et al., 2015; Nazir et al., 2020; Fariduddin et al., 2014b). Improved morphological parameters may be due to the ability of BRs to control cell elongation and division activities through xyloglucan endo-transglycosylase upregulation (Fariduddin et al., 2015; Khan et al., 2013, 2015b; Hussain et al., 2019). BRs are also recorded to boost the growth of seedlings of *Raphanus sativus*. BRs have been found to show a significant role in promoting seed sprouting, such as EBL (24-epibrssinolide) and HBL (28-homobrassinolide). When seeds were treated with HBL, the proportion of sprouting was witnessed to upsurge in *Cicer arietinum* and *Triticum aestivum*. After HBL was applied exogenously to the plants, an improve in yield, CA activity and net photosynthetic rate, and its associated traits were observed (Nazir et al., 2021; Mohammad et al., 2019). The net photosynthetic rate in diverse plant class has been renowned to be upgraded by the foliar application of BRs. In *Arabidopsis thaliana*, BRs also show a part in stimulating flowering. The researchers found that brassinosteroids play an important role in fruit maturation. Some studies have established that the treatment of BRs will contribute to the ripening of fleshy fruits during the fruit production process. The improved ripening of cucumbers, grapes, rice, tomatoes, and yellow passion fruit when the use of BRs has also been recognized by a number of investigators (Ali, 2017; Baghel et al., 2019).

It is also stated that BRs influence the expression of other genes that show an key part in plant protection and biosynthesis of other regulators of plant development (Jiroutova et al., 2018; Nolan et al., 2020; Akram et al., 2020; Shah et al., 2020). Various researcher have recorded their significant role in defending plants from harmful conditions of environmental stress, such as drought, heavy metals, pesticides, salinity, and low temperatures (Shah et al., 2021; Tariq et al., 2020; Khan et al., 2018a; Ahmad et al., 2021). The effect of EBL in variation of respiration in *Arabidopsis* in salinity stress has been demonstrated in recent studies. By stimulating the antioxidative protection mechanism, BRs assist in improving the lethal roles of several abiotic stress environments in plants (Zaheer et al., 2018; Khan et al., 2017b).

Amelioration of Pesticide Toxicity by BRs

Owing to the production of ROS, plant growth and development are adversely affected as a result of pesticide toxicity (Yasin et al., 2018c; Sharma et al., 2019). However, the plants' inner resistance mechanism is enabled to manage with pesticide noxiousness in retort to this pesticide stress (Yasin et al., 2018d, e; Khan et al., 2018b). In addition, the application of BR further activates this antioxidant plant protection mechanism, resulting in plant tolerance to pesticide toxicity being increased. Foliar application of EBL in cucumber plants improved photosynthetic rate and stomatal conductance, which were earlier adversely affected by the application of pesticides. They reported that when associated to control plants, the application of 0.48 g L-1 chlorpyrifos reduced photosynthetic rate and stomatal conductance by 81.01 and 71.97%, correspondingly. However, when compared to chlorpyrifos-treated plants, the application of EBL improved photosynthetic rate and stomatal conductance by 395 and 277%, respectively (Sharma et al., 2018). These investigators also noted that the use of EBL considerably improved the PSII's quantum efficacy and the coefficient of phytochemical quenching. The recovery of growth and photosynthetic parameters in B was also detected by scientists. Juncea plants are grown from seeds cured with EBL and grown under imidacloprid toxicity. Under pesticide threat, the antioxidative defense mechanism of plants is enabled. EBL and HBL have been documented to boost the activity of antioxidant enzymes such as SOD, CAT, APX, GPOX, GR, DHAR, MDHAR, and protein, proline, chlorpyrifos (CPF), and imidacloprid (IMI) stress pesticides in rice. They also noted the triggering effect of EBL and HBL under CPF and IMI toxicity on the overall growth of rice seedlings. After the application of BRs, the appearance and activities of enzymes intricate in the enzyme-facilitated detoxification scheme of pesticides have been documented to increase. It has also been recognized that seed treated with EBL (before sowing) suggestively upsurges the levels of several phytochemicals earlier condensed by the application of IMI pesticide in *Brassica juncea* plants (Sharma et al., 2018). In *B. juncea* plants, antioxidants such as polyphenols, ascorbic acid, tocopherol, and glutathione were too detected to increase from seeds soaked in 100 nM EBL earlier propagating in soils supplemented with IMI (250, 300, and 350 mg IMI Kg^{-1} soil).

Current findings have established that exogenous application of EBL enriched organic acid content (citric, fumaric, malic, and succinic acid) by controlling the appearance of citrate synthase (CS), fumarate hydratase (FH), succinyl Co-A ligase (SUCLG1), succinate dehydrogenase (SDH), and malate synthase (MS) genes intricate in their metabolism in *B. juncea* seedlings are diseased by pesticides (Jan & Parween, 2012; Van Hove et al., 2010; Grotjohann et al., 2001). In adding, it was also detected that the expression of PAL was controlled by the application of EBR in pesticide stress. The basic structure of Indian mustard plants under IMI toxicity is also regained by BRs. The green leaves of Indian mustard plants, which were sprouted from seeds cured with EBL and grown in soil comprising IMI, have recently registered retrieval in amino acid and protein content.

Function of BRs to Minimize Pesticide Residues

Exogenous application of BRs will decrease the remains of pesticides in plants significantly. This could be owed to the BR-controlled appearance of multiple genes, containing GST, P450 monooxygenase, POD, and carboxylesterase that encode key enzymes intricate in pesticide decontamination (Unterholzner et al., 2015; Guo et al., 2013). After presowing seed usage with EBL and cultivated in solutions/soils adjusted with IMI, a substantial decrease in IMI remains was observed in seedlings, green leaves, and pods of *Brassica juncea*. Afterward the exogenous application of EBL, a decrease in CHT remains was observed in tomato plants and grapevines. Some studies have found that the treatment of EBL decreased pesticide residues in cucumber plants by more than 30% (chlorpyrifos, carbendazim, cypermethrin, and chlorothalonil). Condensed pesticide remains have also been stated to be convoyed by improved activity of antioxidant enzymes, containing POD, GST, and GR. These investigators also renowned that the expression of the P450 (P450 monooxygenase), GST, and MRP (multidrug resistance-associated protein) genes accountable for pesticide detoxification in plants was suggestively improved by exogenous application of EBL. In intact plants, BRs induced pesticide degradation of 34–71% (chlorpyrifos in cucumber, tea, corn, broccoli, and Chinese cabbage). Tea and Chinese chives phoxim, tomato chlorothalonil, celery, strawberry and asparagus, cucumber omethoate, cucumber cypermethrin, and broccoli, garlic and Chinese chives carbofuran, and Chinese chives 3-hydroxycarbofuran). They also recorded that in tomato plants, EBL improved the appearance of genes in chlorothalonil (CHT) pesticide stress (Hongsibsong et al., 2020). Mitogen-activated protein kinase (MAPK) and nitric oxide (NO) have recently been stated to show a significant role in the BR-mediated detoxification of pesticides. They also displayed that EBL was controlled by SlMPK1 and SlMPK2, resultant in CHT pesticide metabolism. The action of GST, nitrate reductase, S-nitrosoglutathione reductase and the content of S-nitrosothiol and glutathione, alongside with the decrease of CHT remains in tomato plants, were also controlled by the EBL.

Conclusions and Future Prospects

It is normally appealed that glyphosate destroys unwanted plants by disturbing the EPSPS synthase enzyme, troubling the synthesis of aromatic amino acids (Gomes et al., 2014; Fartyal et al., 2018). On the other hand, glyphosate, which can also justify its herbicidal effects, has many secondary or unintended effects on plant physiology. Glyphosate's toxicity may be attributed to its effects on additional biological practices, such as mineral nutrition and photosynthesis, and to the hormone and oxidative status of the plant. The harmful roles of glyphosate detected on plant growth and development may be specifically associated with the alteration of these cellular processes. As a metal chelator, glyphosate may remove plants of

significant nutrients such as enzymatic cofactors, biomolecular constituents, and antioxidant systems that have important roles (Mollaee et al., 2020; Shahid & Khan, 2018; Sharma et al., 2018). However, it is concluded, on the basis of numerous studies clarifying the role of BRs in the detoxification of pesticides and improvement of toxicity, that BRs have good forthcoming predictions for crop safety and can reduce the amount of pesticide residues in food crops. Moreover, after the treatment of BRs in plants in pesticide toxicity, complete transcriptome sequencing/genome-wide expression studies will add new knowledge to improve understand the defensive functions of BRs. In addition, researching significant secondary metabolites and pathways for stress signaling may help to explain the precise mechanism behind plant retorts to pesticide stress. In addition, crosstalk studies may add extra data to pesticide stress organization in plants among various plant growth regulators under pesticide stress.

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Chapter 14
The Production of High-Value Secondary Metabolites Through Hairy Root Transformation in the Presence of Brassinosteroids

Taiba Saeed, Anwar Shahzad, and Vikas Yadav

Abstract Plants are the key source of value-added bioactive compounds of medicinal repute. Extended usage of these secondary metabolites (SM) in several industrial areas has necessitated researches on growing their production by exploiting various plant tissue culture (PTC) approaches. PTC technologies have proved to be efficient implements for both studying and generating plant secondary metabolites under in vitro conditions. SM production is under severe metabolic regulation and tissue-specific localization. Hence, the use of differentiated cultures like hairy root cultures is a method of choice. These transgenic roots are known to produce SM at high or comparable amounts to that of intact plants. Enhanced SM biosynthesis by elicitation in transgenic root cultures has become widely employed biotechnological strategy for commercial production of desired product. PGRs have been exploited as efficient elicitors in hairy root cultures of different plant species. Brassinosteroids (BRs) are steroidal lactones that form a new group of PGRs with pleiotropic effects and are found crucial for normal growth and enlargement of plants. However, accumulation of SM in response to BR application has been observed in numerous plant species under ex vitro conditions. Moreover, very little is reported up to date about the outcome of BRs on secondary metabolism in cultured plant cells or hairy root cultures. This chapter focuses on the basic information regarding delivery of important SM and in vitro strategies involved for optimal metabolite production with special reference to the use of BR as abiotic elicitor in improving metabolite yields in hairy root cultures.

Keywords Brassinosteroids, Hairy root, Secondary metabolites, Tissue culture

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Introduction

Plants have been the source of secondary metabolites (SM) that are utilized in numerous significant traits of human being since ancient eras for various purposes. Due to the huge molecular assortment and wide bioactivities of these compounds, they are being exploited in various areas such as medicine, pharmaceuticals, make-ups, and nutraceuticals (Chandran et al., 2010). Many of these natural products such as artemisinin and paclitaxel have been used to treat specific ailments and diseases in humans (Miller & Su, 2011; Demain & Vaishnav, 2011) resulting in the increasing demand for such products. However, the amount of such plant metabolites has been intensely restricted by the lack of active methods for their production. Majority of secondary metabolites have very complicated chemical structures that mark their chemical creations rather incompetent, complex, and expensive (Kotopka et al., 2018). Even though common profitable causes that depend on extraction from natural resource exist, such production approaches are challenging in terms of their long-term sustainability and low overall richness in their hosts (Chandran et al., 2010). Therefore, the present scenario has sparked the necessity to discover strategies to augment the manufacture of such valuable plant products without intervening in their natural habitat.

Various plant biotechnological approaches are being utilized as possible tools for big scales manufacture of SM. Plant tissue culture (PTC), an extremely vital facet of plant biotechnology, proved to be a continuous, sustainable, economical, and viable cause for the production of valued plant products (Shahzad et al., 2017). Other biotechnological approaches like screening and assortment of elite lines, optimization of nutrient media arrangement and physical conditions, use of bioreactors, hairy root culture, elicitation, precursor feeding, metabolic engineering, plant cell immobilization, biotransformation have been employed to assess their efficacy to increase in vitro production of SM in different plant species (Halder et al., 2019).

Enhanced SM biosynthesis by elicitation in transgenic hairy root cultures has become top active and extensively working biotechnological strategy for commercial production of such important compounds under in vitro situations (Halder et al., 2019). Numerous researches demonstrated improved accumulation of SM on exogenous use of growth regulators to the transformed root cultures (Bais et al., 2001a, b; Liang et al., 2013; Kastell et al., 2013; Jamwal et al., 2018). Brassinosteroids are a group of steroidal hormones with high and diverse phyto-physiological activities (Sasse, 1997). The role of brassinosteroids on SM accumulation has been testified in various in vivo grown plants (Çoban & Baydar, 2016a, b; Asci et al., 2019a, b). However, little is reported up to date about its effect on cultured plant tissues, especially for the plant secondary metabolism. The present chapter summarizes the advances made in the field of enhanced SM production through hairy root transformation in the occurrence of plant growth regulators (PGRs) with special reference to brassinosteroids (BRs).

Plant Secondary Metabolites: Classification, Application, and Production Strategies

The term "metabolites" refers to the intermediate products of metabolism. Metabolites have known to possess several roles, comprising fuel, structure, signaling, stimulatory and inhibitory belongings on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme), resistance, and connections with further organisms. Plant produces a heterogeneous group of composites, the excessive mainstream of which are not essentially required for growth and development. These ingredients, conventionally stated to as secondary metabolites, often are differentially dispersed among restricted taxonomic groups within the plant kingdom (Demain & Fang, 2000).

Classification of Plant Secondary Metabolites

Classification of plant secondary metabolites is built on their chemical structure, biosynthesis, and utilities. They are biosynthesized from acetyl coenzyme A, mevalonic acid, shikimic acid, deoxyxylulose 5-phosphate, or collective ways. They are primarily categorized into three main classes viz. terpenoids, alkaloids, and phenolics as shown in Table 14.1 (Kabera et al., 2014; Pusztahelyi et al., 2015).

Applications of Plant Secondary Metabolites

Plant secondary metabolites are a significant source of drug contestants in pharmaceutical manufacturing. People such as scientist and herbalist around the globe are focused on acquiring deep knowledge of specific classes of SM in direction to preview the viewpoints in new drugs research and development (Guerriero et al., 2018). Few commercially important SM along with their source plants have been summarized in Table 14.2.

In Vitro Strategies for Improved Plant Secondary Metabolites Production

In vitro induced increased production of SM took place in two different stages. The first stage involves aggregation of biomass wherein explants under the influence of PGRs dedifferentiate to form unorganized mass of cells known as callus. Accumulation of SM occurs in second stage where calli are utilized either for the regeneration routes producing multiple clones or can be exploited for the production of

Table 14.1 Classification of plant secondary metabolites

Class	Subclass	Examples
Terpenoids	Hemiterpene (C5)	Isoprene, prenol, isovaleric acid
	Sesquiterpene (C15)	ABA (abscisic acid)
	Diterpene (C20)	Gibberellin
	Sesterterpenes (C25)	
	Triterpene (C30)	Brassinosteroids, squalene, lanosterol
	Tetraterpene (C40)	Carotenoids, lycopene
	Polyterpenes (C>40)	Ubiquinones, rubber, cytokines, vitamin E
Alkaloids	Non-heterocyclic	Hordenine, colchicine, taxol
	Heterocyclic	Quinine, caffeine, nicotine
Phenolics	Phenolics with one aromatic ring	
		C6: Phenols, hydroquinones, pyrogallol C6-C1: Gallic acid, salicylic acid C6-C2: Acetophenones C6-C3: Hydroxycinnamic acid, ferulic acid, coumaric acid, eugenol, zosteric acid
	Phenolics with two aromatic rings	
		C6-C1-C6 Xanthones: Mangosteen C6-C2-C6 Stilbenes: Resveratrol C6-C3-C6 Flavonoids: Quercetin
	Quinones	Naphthoquinones, anthraquinones benzoquinones
	Flavonoid polymers and non-flavonoid polymers	Tannins
Glycosides	Glucosinolates Cyanogenic glycosides	Sinigrin, glucobrassicin Amygdalin, sambunigrin, linamarin

single-cell suspension cultures using batch or continuous fermentation to synthesize the preferred SM. Organized cultures like shoots or roots culture are also the method of choice for metabolite production when its synthesis is restricted to specialized part of the host plant (Murthy et al., 2014; Fig. 14.1).

Hairy Root Cultures: Natural Factories for Enhanced SM Production

In some cases, the production of SM is under firm metabolic regulation and tissue-specific localization. The undifferentiated cultures like cell suspension cultures did not acquire impetus due to the lack of stability and uniformity in the formation of desired products. Hence, differentiated organ cultures such as hairy root cultures are

Table 14.2 Therapeutic metabolites, their plant source, and applications

S. No.	Therapeutic metabolites	Class	Plant source	Pharmacological activity
1	Ajmaline, ajmalicine	Alkaloid	*Rauwolfia species*	Ajmaline possesses antihypertensive and antiarrhythmic activity, whereas ajmalicine is useful in circulatory disorders (Phillipson & Zenk, 1980)
2	Artemisinin	Terpenoid	*Artemisia annua*	Antimalarial (Rai et al., 2021)
3	Atropine	Alkaloid	*Atropa belladonna*	Anticholinergic, antispasmodic (Holmstedt et al., 1963; McBrien et al., 2013)
4	Bacosides	Triterpenoid saponins	*Bacopa monnieri*	Defensive activities alongside morphine-induced cerebral toxicity, chemical-induced liver toxicity, and wound healing activity (Russo & Borrelli, 2005)
5	Berberine	Alkaloid	*Coptis species*	Anti-inflammatory, antibacterial/viral, antidiabetic, anticancer (Kim et al., 2010; Agyapong et al., 2013)
6	Camptothecin	Alkaloid	*Camptotheca acuminata*	Anticancer (Takimoto, 2002)
7	Codeine and morphine	Alkaloid	*Papaver somniferum*	Codeine has analgesic, antitussive, antidiarrheal, antidepressant, sedative and hypnotic properties (Smith et al., 2006; Simera et al., 2010) Morphine has strong analgesic effects and used to treat shortness of breath (Takita et al., 2000)
8	Diosgenin	Steroidal sapogenin	*Dioscorea doryphora*	Antidiabetic (Bhaskarachary & Joshi, 2018)
9	Digitoxin, digoxin	Steroids	*Digitalis lanata*	Used to treat heart conditions such as atrial fibrillation, atrial flutter or heart failure (Øiestad et al., 2009)
10	Forskolin	Labdane diterpene	*Coleus forskohlii*	Stimulates the enzyme adenylate cyclase; used for glaucoma treatment (Morrone et al., 2015)
11	Ginsenosides (dammarane and oleanane)	Terpenoids	*Panax ginseng*	Vasorelaxation, antioxidation, anti-inflammation, and anticancer (Lü et al., 2009)
12	Gymnemic acid	Triterpenoid glycosides	*Gymnema sylvestre*	Antidiabetic (Spasov et al., 2008)
13	Paclitaxel	Terpenoid	*Taxus species*	Anticarcinogenic (Barbuti & Chen, 2015)
14	Rosmarinic acid	KOf6	*Coleus blumei, Thymus species*	Antioxidant (Munoz-Munoz et al., 2013)
15	Resveratrol	Stilbenes	*Vitis vinifera*	Phytoestrogenic, antioxidant, antitumor, antidiabetic, increases life span (Gambini et al., 2015)

(continued)

Table 14.2 (continued)

S. No.	Therapeutic metabolites	Class	Plant source	Pharmacological activity
16	Shikonin	Quinones	*Arnebia euchroma*	Antimicrobial, anticancer, antipyretic, anti-inflammatory (Chandran et al., 2010)
17	Stevioside, steviol rebaudioside	Glycosides	*Stevia rebaudiana*	Low calorie sweeteners (Brahmachari et al., 2011)
18	Vincristine, vinblastine	Alkaloid	*Catharanthus roseus*	Antitumoral (Fernández-Pérez et al., 2013)

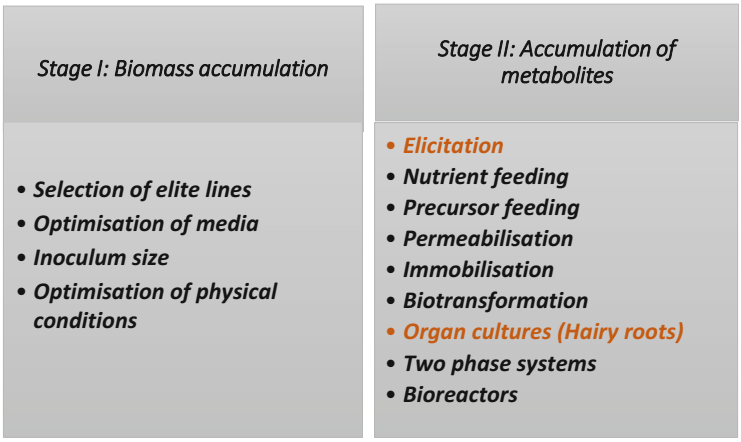

Fig. 14.1 Strategies employed for improved in vitro production of bioactive compounds

being extensively used in such plant species as another strategy for improved SM production. Hairy roots are formed through genetic transformation using Gram-negative soil bacterium known as *Rhizobium rhizogenes* (previously referred to as *Agrobacterium rhizogenes*). Hairy roots show profuse growth, even in hormone-free media, negatively geotropic and are genetically stable. The products being expressed by them can match the specific metabolites, produced by the plant naturally, or recombinant, heterologous proteins. They are capable of producing metabolites at an amount comparable to that of intact plants (Chandra & Chandra, 2011).

There is a lack of knowledge regarding the understanding of molecular events that took place during hairy root syndrome. However, the whole procedure of formation of genetically transformed roots can be separated into the following steps: (a) acetosyringone, a phenolic compound, is secreted by the explants after wounding which promote the adherence of agrobacteria to explant/root cells; (b) T-DNA processing in bacterial cells and formation of T complexes (T strands and other associated proteins); (c) transferring of T complexes from the agrobacteria

to the host plant genome; (d) incorporation and expression of T-DNA in the plant genome; and (e) formation of HRs at the infection site (Guillon et al., 2006).

The two primary criteria for choosing the best plant species for hairy root cultures are its capacity to produce and secrete large quantities of the target compound and its biomass production ability. After the development and selection of high yielding hairy root strains, they go through the diverse maintenance events according to monetary and practical constraints. Presently, the most exploited preservation technique is monthly subculture of individualized hairy roots on solid and/or liquid media. However, this procedure is costly, laborious, time taking, and prone to contamination and loss of true strains. Cryopreservation presents an alternative technique for the maintenance of hairy root clones thus avoiding the abovementioned problems (Lambert et al., 2009; Häkkinen et al., 2016).

Hairy roots are used in conjunction with other in vitro strategies for improved SM production. Elicitation of hairy roots leads to enhanced production of important metabolites in many plant species (Wang et al., 2009; Rhee et al., 2010; Srivastava et al., 2019; Gharari et al., 2020). Elicitors can be abiotic or biotic agents, which induce metabolite production by controlling the biosynthesis rate, accumulation and/or vacuolar transit, turnover, and degradation (Barz et al., 1990). Transformed hairy roots allow scheming of metabolic setups for maximum product adsorption, avoiding feedback inhibition as well as metabolite degradation in the culture media. T-DNA activation tagging in transformed roots can be utilized to unravel new genes that take part in the metabolite pathways (Rischer et al., 2006). Large-scale bioreactors are used to scale-up production of plant-expressed compounds of interest in hairy root cultures of many plant species (Georgiev et al., 2013).

Apart from elicitation, hairy roots can be used synergistically with other approaches for significant production of bioactive compounds which are stored intracellular and secreted minimally in the culture medium. Enhanced yields of such hydrophobic metabolites can be obtained by exploiting cell permeabilization technique in hairy root cultures (Boitel-Conti et al., 1996). Boitel-Conti et al. (1996) found that Tween 20 treatment to hairy roots of *Datura innoxia* caused movement of substantial quantities of alkaloids from cells into the culture medium and higher accumulation compared to untreated roots. The accumulation of metabolites to the toxic level results in inhibited cell growth as well as rapid degradation of desired products. To elevate such problems, use of polymeric adsorbents is an attractive technique for efficient and effective product recovery. Yan et al. (2005) reported that addition of hydrophobic polymeric resin (X-5) in the culture medium of *Salvia miltiorrhiza* transformed roots trapped 80% of the secreted diterpenoid tanshinones. Use of an artificial phase can be another active method for retrieving desired product (s) that are prone to feedback inhibition or degradation. The two-liquid-phase bioreactor was designed and studied by Tikhomiroff et al. (2002) in *Catharanthus roseus* transformed root cultures for successful extraction of two important alkaloids, tabersonine and lochnericine, using silicon oil. This study showed that silicon oil did not hamper nutrient availability to the roots, and the affinity of alkaloids for silicon oil was about nine times greater than for the aqueous phase. Moreover, an overall

increase in the specific yields of tabersonine and lochnericine were noticed with the usage of silicon oil in control cultures.

Application of transformed roots obtained from different medicinal plants as extremely active biotransformation schemes has well exploited for the synthesis of valuable compounds such as anabasine, digitoxin, hyoscyamine, nicotine, quinine, scopolamine, stilbene quinines, glycosylated phenolic compounds, and so on (Peng et al., 2008). Faria et al. (2009) reported that *Anethum graveolens* hairy root cultures displayed effective biotransformation capacity with regard to two oxygen-containing monoterpene substrates, i.e., menthol and geraniol, ensuing modification of menthol to menthyl acetate and geraniol to ten new products in the form of alcohols, aldehydes, esters, and oxides.

Recent advancement in transgenic research has paved a way to the metabolic engineering of biosynthetic pathways for production of high value SM. To increase the level of valuable desired product through metabolic engineering, strategies are focused on enhancing flux to the target molecule, overcoming rate limiting steps, decreasing flux through competing pathway, overexpressing regulatory gene or transcription factors to induce the pathways, inhibiting, or limiting catabolism of the molecule (Chandra & Chandra, 2011). Moyano et al. (2003) reported improved productions of hyoscyamine and scopolamine in hairy root cultures of *Datura metel* by pmt. gene (codes for enzyme putrescine: SAM N-methyl transferase capable of catalyzing the first step of tropane alkaloid pathway) overexpression. On the other hand, only hyoscyamine yield was increased by pmt. gene overexpression in *Hyoscyamus muticus* hairy root cultures. Recently, transgenic root system has also been successfully utilized for the production of recombinant proteins such as the green fluorescent protein (GFP) (Medina-Bolívar & Cramer, 2004), human acetyl cholinesterase (Woods et al., 2008), murine interleukin (Liu et al., 2009), thaumatin sweetener (Pham et al., 2012), human interferon alpha-2b (Luchakivskaia et al., 2012), and recombinant alpha-L-iduronidase (Cardon et al., 2019). Even complex glycosylated proteins can be synthesized utilizing transformed roots with extremely homogeneous posttranslational profiles (Cardon et al., 2019).

Various other issues such as light, carbon source and its concentration, the ionic concentration and pH of the medium, PGRs, temperature, and light quality are recognized to effect the production of secondary metabolites in hairy root cultures (Gutierrez-Valdes et al., 2020). As this chapter deals with the brassinosteroid (a phytohormone), here we will emphasis on the role of phytohormones on enhanced secondary metabolite production in hairy roots.

Effect of Exogenous Phytohormones on Metabolite Production in Hairy Roots

Plant growth regulators (PGRs) comprise phytohormones (hormonal substances synthesized in plants) as well their synthetic analogues (Basra, 2000). PGRs must exploit as effective elicitors to encourage the synthesis of bioactive compounds in plants. Numerous studies demonstrated impact of PGRs on increased SM production in hairy root cultures of diverse plant species depending upon the type and concentration used (Bais et al., 2001a, b; Gangopadhyay et al., 2011; Božić et al., 2015). Bais et al. (2001a, b) studied the effect of exogenous use of three different hormones on the coumarin production in hairy root cultures of *Cichorium intybus*. Auxin in mixture with lower concentrations of kinetin resulted in rapid disorganization of hairy roots and ultimately reduced levels of coumarin. While application of gibberellic acid (GA_3) stimulated both hairy root growth, branching and coumarin content over the respective control. Weathers et al. (2005) showed that different hormones (auxins, cytokinins, ethylene, abscisic acid (ABA), and GA_3) produced varied growth and yielded different levels of artemisinin in *Artemisia annua* hairy root cultures. 2-isopentenyladenine (2-iP) was found to be the best type that stimulated maximum artemisinin production more than any other hormone.

The efficiency of exogenous hormones in combination for improved plumbagin production in *Plumbago indica* hairy roots was described by Gangopadhyay et al. (2011). This study showed that among the various hormones used individually, GA_3 resulted in highest root growth while the maximum plumbagin accumulation occurred on α-naphthalene acetic acid (NAA) treatment. 2,4-Dichlorophenoxyacetic acid (2,4-D)-treated hairy root cultures exhibited reduced root growth as well as plumbagin production. They reported combination of GA_3 and NAA (0.5 mg/L, each) to be the optimal one stimulating maximum root biomass and plumbagin production. GA_3 on specific concentration has also proved to be an excellent PGR to optimize SM production in hairy root cultures of *Echinacea purpurea* (Abbasi et al., 2012). Liang et al. (2013) stated that the interaction of different hormones played important roles in the biosynthesis of phenolic acids (caffeic acid, rosmarinic acid, and salvianolic acid B) in *Salvia miltiorrhiza* hairy roots. They included three important phytohormones viz. abscisic acid (ABA), GA_3, and ethylene in their study, wherein all three were effective in improving phenolic acids production. Regarding interacting pathways, they found that GA_3 signaling was essential for ABA and ethylene improved phenolic production but ABA and ethylene signaling was not imperative for GA_3-induced phenolic production.

Kastell et al. (2013) described the diverse role of kinetin on glucosinolates accumulation in two brassicaceous plant species viz. *Sinapis alba* and *Brassica rapa*. It was found that kinetin enhanced glucosinolates accumulation in *B. rapa* hairy roots, but failed to stimulate glucosinolates synthesis in *S. alba*. Huang et al. (2014) reported the varied effect of PGRs on the production of three different metabolites viz. gentiopicroside, swertiamarin, and loganic acid in hairy root

cultures of *Gentiana scabra*. Zeatin induced improved accumulation of loganic acid while gentiopicroside and swertiamarin accumulations were found higher in the presence of NAA. Greater accumulation of metabolites on PGR (cytokinins) action in a different *Gentiana* species was also stated by Božić et al. (2015). They found stimulatory effect of lower concentrations of kinetin and 6-benzylaminopurine (BA) on gentiopicrin and sweroside contents in hairy roots of *G. pneumonanthe*, and further increase in the concentrations of both cytokinins decreased the content of these metabolites.

Brassinosteroids: Role in Plant Tissue Culture and Production of Secondary Metabolites

Brassinosteriods (BRs) form a unique class of naturally occurring steroidal lactones being broadly dispersed in the plant kingdom (Sasse, 1997; Clouse & Sasse, 1998a). These compounds represent a new group of PGRs with pleiotropic effects and are found vital for normal growth and development of plants. BR was first revealed by Grove et al. in 1979 from rape (*Brassica napus*) pollens. It comprises of the highly bioactive brassinolide and its analogues (Mandava, 1988). Physiological retorts of BR comprise effects on elongation, bending, cell division and vascular development, pollen tube growth, reproduction, photomorphogenesis, and resistance to various biotic and abiotic stresses (Kang & Guo, 2011). While, countless works have established the potential of BRs to increase various plant performances under field conditions, fewer reports throw light on the impact of BRs under the in vitro condition.

Role of Brassinosteroids in In Vitro Regeneration

24-epibrassinolide (EBL) encouraged adventitious shoot regeneration from hypocotyl explants in cauliflower (Sasaki, 2002) and shoot tip explants in *Cymbidium elegans* (Malabadi & Nataraja, 2007a). Franck-Duchenne et al. (1998) obtained healthy shoots by transferring adventitious shoot buds to media containing EBL in sweet pepper. EBL successfully reported to induce somatic embryogenesis in many conifers such as *Pinus taeda*, *Pseudotsuga menziesii*, *Picea abies*, *Pinus wallichiana* (Pullman et al., 2003; Malabadi & Nataraja, 2007b), rice (Pullman et al., 2003), and cotton (Wang et al., 1992). Nakajima et al. (1996) showed increased cell division and callus formation rates from protoplast on EBL, 2,4-D and kinetin combination media in Chinese cabbage.

Lu et al. (2003) showed that brassinolide in combination with indole-3-acetic acid (IAA) and BA stimulated regeneration in *Spartina patens*. EBL was noticed to increase the rate of cell division in isolated leaf protoplasts of *Petunia hybrida*

(Oh & Clouse, 1998). Many studies have proved brassinolide to be more active than, or synergistic with, auxins such as IAA or NAA (Brosa, 1999). Hu et al. (2000) advocated that EBL might stimulate cell division through a D-type plant cyclin gene known as Cyc D3, the same gene through which cytokinin activates cell division. The study also revealed that EBL can be successfully used in the place of cytokinin in *Arabidopsis* callus and suspension cultures. Exposure of cultured calli to exogenous brassinolide stimulated successful differentiation of somatic embryos and transition to maturation phases in *Cocos nucifera* (Azpetia et al. 2003) and *Gossypium hirsutum* (Aydin et al., 2006). Brassinolide significantly increases the shoot regeneration rate and shoot regeneration index of callus induced from young leaves and stems in *Populus euphratica* and shorten the time for adventitious bud/shoot formation as well (Cai et al., 2015).

Homobrassinolide (HBL) has been reported to improve rooting efficacy and survival of Norway spruce seedlings (Ronsch et al., 1993). HBL in combination with 2-iP had a noticeable effect on enhanced shoot proliferation and the subsequent increased length of regenerated shoots from apical meristems of banana (Nassar, 2004). Induction of embryogenic callus in coffee and potato was noticed through the use of spirostane analogues of BRs in the culture medium as a cytokinin substitute or complement (García, 2000; Moré et al., 2001). Moreover, callus induction and shoot restoration from cotyledon explants was improved in lettuce by these spirostane analogues at determined concentrations in mixture with BA (Nuñez et al., 2004). Verma et al. (2012) reported in vitro shoot multiplication and flowering on BR and BA combination treatment in groundnut using cotyledonary node as explants. They further noticed that BR alone could induce in vitro root formation in regenerated shoots of groundnut.

Secondary Metabolites Production in Presence of Brassinosteroids

The influence of BRs on secondary metabolism in various plants has incited numerous investigations. Accumulation of SM in response to BR application has been observed in several plant species under ex vitro conditions, and few important works in this field have been discussed here. Xi et al. (2013) reported that exogenous application of EBL significantly induced greater production of overall phenols, tannins, flavonoids, anthocyanins, and specific anthocyanin in Cabernet Sauvignon and Yan73 grape skins. Among all the treatments, 0.40 mg/l EBL proved to be the optimal concentration. The enhanced contents may be due to the BR-promoted increased activities of the key enzymes, PAL (phenylalanine ammonia-lyase) and UFGT (UDP-glucose: flavonoid 3-O-glucosyltransferase), involved in phenylpropanoid and flavonoids pathway. In another attempt in the same plant, the increased proanthocyanidins accumulation in seeds and skin of Cabernet Sauvignon was noticed in retort to EBL treatment. Changes in the expression arrangements of

structural genes (VvLAR1, VvLAR2, and VvANS) and a transcriptional regulator (VvMYBPA1) of proanthocyanidin production varied to diverse points under the influence of EBL (Xu et al., 2014). The foliar spray of BRs at different concentrations resulted in increased vincristine content at all concentrations as compared to control in *Catharanthus roseus* (Muthulakshmi & Pandiyarajan, 2015). Similarly, the foliar application of EBL improved the overall phenolic and essential oil (linalyl acetate and 1,8-cineol) contents in *Lavandula intermedia* (Asci et al., 2019a, b).

Fujioka and Yokota (2003) found that BR-induced disease resistance in cucumber plants is mainly due to the improved accomplishments of peroxidase and polyphenoloxidase, key enzymes intricate in the metabolism of polyphenols. Ahammed et al. (2013a, b) reported greater activity of secondary metabolism-associated enzymes as well as improved production of SM in response to EBL application under phenanthrene individually and in combination with cadmium-induced stress in tomato. Their observations suggested that EBL regulates secondary metabolism in tomato which might augment tolerance to phenanthrene and cadmium-induced stresses either individually or in combination. Farooq et al. (2009) noticed significant upsurges in phenolic contents in rice plants treated with BRs in water stress. Moreover, EBL application increased the phenolics and essential oil content in NaCl-stressed plants of *Mentha piperita* (Çoban & Baydar, 2016a, b). The significance of their results lies in the fact that peppermints plants can be developed in salt enrich soils when given appropriate BRs treatments.

However, very little is reported till date about the outcome of BRs on secondary metabolism in cultured plant cells or hairy root cultures. Early in 1991, Ikekawa and Zhao showed that BR-treatment improved nicotine levels in tobacco cultures. Later, Yang et al. in 1999 reported that BR in combination with BA and IAA induced increase in shikonin production by 31% as compared to control (BA + IAA containing media) in *Onosma paniculatum* cell culture. This significant increase has been attributed to enhanced activities of PAL and PHB-geranyl transferase, two important enzymes involved in shikonin biosynthesis. Moreover, BR significantly decreased PHB-O-glucosyltransferase activity, which regulates shikonin synthesis through the supply and storage of the precursor PHB, thus inhibiting shikonin formation by consuming its intermediate PHB (Yang et al., 2003). Zang et al. (2001) found that optimized concentration of BR exhibited concurrently enhancing effects on both cell development and paclitaxel biosynthesis in *Taxus chinensis* cell suspension cultures.

In hairy root cultures, elicitation of secondary metabolites through exogenous application of BR is limited to two reports. Wang et al., in 2002, reported that the use of (22S, 23S)-homobrassinolide (HBL) concurrently augmented the biomass and artemisinin production in hairy root cultures of *Artemisia annua*. Their data showed that HBL at a range between 0.1 μg/l and 10 μg/l can significantly stimulate artemisinin production, 1.0 μg/l being the optimal concentration resulting in 57% increase in artemisinin over the control cultures without HBL treatment. Moreover, improved synthesis of nucleic acid and soluble protein content in hairy roots was also observed which displayed that their diverse levels may be concerned directly, or indirectly, with artemisinin accumulation convinced by HBL. Recently, Demirci

et al. (2020) explored the effects of EBL and L-phenylalanine (L-phy) on the root development, total phenolics, total flavonoids, and caffeic acid derivatives (CADs) accumulation in hairy root cultures of *Echinacea purpurea*. They found EBL application to be other effective approach as compared to L-phy treatment. Among the EBL treatments, 1.0 mg/L EBL was reported to be the optimal concentration, resulting in the utmost total phenolics, total flavonoids, cichoric acid, caftaric acid, echinacoside, and p-coumaric acid contents. Earlier studies have exposed that EBL improves root growth and development of not only stressed but also nonstressed plants when applied at the suitable concentration and development period (Bao et al., 2004; Rady, 2011).

Conclusions and Perspectives

Growing demand for sustainable and cost-effective bioactive molecules of plant origin necessitates their bulk production via plant tissue culture approaches. Numerous in vitro approaches have been employed to improve the yields of secondary metabolites in short time period as related to conventional methods. The exploitation of novel genetic outfits and regulation of biosynthetic trails involved in secondary metabolism will deliver the base for the profitable production of the desired bioactive product. The biotechnological potential of transgenic roots as a genetic factory for biosynthesizing medicinally important metabolites has been well documented. The studies on the use of brassinosteroids as an abiotic elicitor in improving the amounts of valued secondary metabolites in hairy root cultures are very limited. More attempts are required in order to gain optimal utilization of brassinosteroids for greater production of desired metabolites under in vitro environments especially in transgenic roots. Application of knowledge related to brassinosteroid induced regulation of metabolites biosynthetic pathways would provide us powerful tools for exploiting this new class of PGR in improved accumulation of secondary metabolites. The ongoing research on genomics, proteomics, and metabolomics remain helping us to widen our understanding of metabolic trails while progress made in systems biology would give us ideas on the influence of diverse modifications more accurately.

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Chapter 15
Role of Brassinosteroids in Protein Folding Under High-Temperature Stress

Mohammad Faizan, Fangyuan Yu, Vishnu D. Rajput, Tatiana Minkina, and Shamsul Hayat

Abstract Brassinosteroids (BRs) are a cluster of naturally up plant steroidal compounds with extensive range of biological action that proffer the exclusive opportunity of growing crop productivity through both altering plant metabolism and defensive plants from environmental cues. Research on BRs, assisted by the new progress in knowledge, has interpreted their function not only in crop development but also in crop adaptation under heat stress conditions. Existing reports point out that BRs play important functioning in plant's tolerance against heat stress, resultant in proficient stress supervision under unfavorable conditions. Due to their characteristic and resourceful purpose, BRs are usually used to enhance plant value and productivity. However, how heat stress could function in protein folding throughout BR act is badly tacit. This chapter focuses on the present position of our considerate about the function of BRs in protein folding in elevated temperature stress.

Keywords Brassinosteroids · Heat stress · Crop yield · Developmental processes

Introduction

Brassinosteroids (BRs) are steroid plant hormones, displaying shaping resemblance with mammal's steroid hormones. Their construction participates a series of rejoinders constitute a dense biosynthetic passageway. Almost 50 different BRs have been recognized in crop plants, with number of intermediary and brassinolide (BL), the

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concluded result of the process, which is generated through transversion as of castasterone (Shimada et al., 2001; Bajguz & Tretyn, 2003).

Participation of BRs in plants is very important for growth and yield. They control gene appearance and handle a large number of functions such as cell division, plant development, vascular demarcation, and reproductive progress (Clouse, 2011). They are also participated in seed germination, leaf angle, flowering time, and seed yield, which are of important impacts (Divi & Krishna, 2009; Vriet et al., 2012; Faizan et al., 2018). Due to all these properties, BRs gain attention since many decades, and more advancement has been finished in considerate the omics mechanisms participated in BR metabolism and signaling.

Protein role is reliant on its precise three-dimensional organization that is assumed by the early ruin of the polypeptide manacles after translation. Fixed with DNA and formed on ribosomes as a row of several amino acids, every protein must fold correctly with its characteristics, relatively numerous alternatives in order to proper functioning (Pain, 2000). Folding into its local and vigorous structure may engage one or more partially folded in-between states. It is not astonishing that stress persuaded variations in physiological activity might alter the ruin procedure and increase protein misfolding and accumulation (Sadana & Vo-Dinh, 2001). Number of processes with the cell is integrated with protein folding and unfolding, and it is a very unique process to monitor environmental stresses like concentration of denaturation, temperature, and pH. Internal dynamics take part in protein functioning.

Brassinosteroid Signaling

Brassinosteroids are participated in controlling various physiological and growth processes in crop plants. The BR ligand attached to its sense organ, BRI1 turns on BR reliant signaling functions in plasma membrane and permits the removal of BRI1 kinase inhibitor, BKI1 from the BRI1 sense organ (Wang & Chory, 2006; Kim et al., 2009). Separation of BKI1 from the BRI1 guides to the creation of BRI1-BAK1 compounds that trigger several phosphor/dephosphorylation signaling procedures. This finally guides to the dephosphorylation of BR INSENSITIVE2 (BIN2) kinase that adversely leads to the BR signaling procedures (Li & Nam, 2002; Russinova et al., 2004). Dephosphorylation of BIN2 stops its function and increases its deprivation consequently; turn on transcription units, BZR1/BES1 (Wang et al., 2002). The oxidative state of BZR1 increases its activity and stimulates its contact with major genes participated in developmental and signaling functions (Tian et al., 2018).

Recently, two Jumonji domain-containing proteins ELF6 and REF6 were identified, along with BZR2/BES1 cooperating proteins which are originated to manage flowering (Yu et al., 2008). Also, BIM1 be exposed to interrelate with AP2/ERF record factors, DORNROSCHEN and DORNROSCHEN-LIKE, accounted to be concerned in prototyping through embryo expansion, and the BIM1 mutant too demonstrated embryo-patterning imperfection at low down penetrance. These

consequences suggested that BZR2/BES1 recruits additional transcriptional controller to adapt the look of subsets of goal genes and exact life gaining functions in plants (Kim & Wang, 2010).

BRs in the Directive of Plant Development

BRs play vital role in plants growth enhancement. Biosynthetic procedures of BRs have been clearly portrayed in *Arabidopsis thaliana* that speed-up our projections about the controlling mechanisms of BRs (Nolan et al., 2019). In plants, deficiency of BRs leads to consequences in less seed germination, shortening of plant, senescence, hinder male fertility, and de-etiolation (Clouse, 2015). In plants, BR's synthesizing location is not yet recognized, but it produces by all the tissues of the plant. Exogenous application of BRs significantly increased the lengths of hypocotyls, epicotyls, mesocotyls, and mesocotyls of the plants (Mandava, 1988; Clouse et al., 1996). Enhancement in the growth after BRs application is accompanied by proton extraction and hyperpolarization of cell membranes. Brassinosteroids increased length of several crop plants such as soybean (Yopp et al., 1981), bean (Mandava et al., 1981), mung bean (Gregory & Mandava, 1982), and wheat (Sasse, 1985). Concentration of BRs plays important role to its effects, and nano to micrometer concentration is very effective for their impacts on the crop plants. Circumlocutory modulation of ATPase activity had also been participated to portray BR-induced impacts on sucrose transport. Along this, BRs stimulated the cell division rate in Petunia. Brassinosteroids encourage plant enlargement through cell elongation through straight intentioning expression of CELLULOSE SYNTHASE (CESA) genes in *A. thaliana*, thereby rising cellulose amount and biomass accretion. Escalating granule yield is strongly linked with growing biomass (Evans & Fischer, 1999).

High Temperature Stress

Heat stress is one of the major abiotic stresses and caused very severe impacts on crop plants (Nolan et al., 2019). It caused leaf burning, abscission, senescence, fruit injuries, reduced plant growth, and productivity (Bita & Gerats, 2013). Heat stress drastically reduced cell elongation and causes cell cycle arrest via downregulation of genes such as CESA and certain cyclins (Xie et al., 2011; Guerriero et al., 2014). Apart from CESA, BR also triggers the appearance of cell wall extension and relaxing enzymes such as expansins, xyloglucan, endotransglucocylase, and pectin-lyase (Uozu et al., 2000). It is vital to message that cell increment is susceptible to heat stress; however, it can be corrected by BRs application (Hatfield & Prueger, 2015). Because of targetting CESA, BR supplementation increases

morphological attributes and finally crop yield under normal as well as stressful environments.

Heat stress modifies plant omics profiling to alleviate injuries for endurance. It is very important to note that it significantly increased heat shock proteins (HSPs). Several functions of HSPs appear in downfall, intracellular allocation, and dilapidation of proteins under stress as well as stressful condition (Qu et al., 2013). Therefore, HSPs give heat lenience by steady proteins important to photosynthesis, transpiration, and membrane stability (Momcilovic & Ristic, 2007). Moreover, HSPs trigger the expression of various HSPs and play the defensive role in heat tolerance. Representing the vital function of BR in correct HSP induction, wild-type *Brassica juncea* displays an augment in HSPs when plants were adjusted to high temperature, whereas BR mutant varieties show abridged raise (Sadura et al., 2020). Several HSPs play important protective functions in specific organelles vital for cellular functioning from heat stress. It is demonstrated from previous studies that HSPs give tolerance from heat by modifying electron transport chain compound and save translational machines from heat stress (McLoughlin et al., 2016). However, application of BRs plays defensive function in heat stress by enhancing the accretion of HSPs in *Solanum lycopersicum* (Singh & Shono, 2005). Photosynthesis included one of the first metabolic processes to in affect by stress. Heat stress retards photosynthesis by disturbing chloroplast activity and photosynthetic machinery (Al-Khatib & Paulsen, 1989). However, chloroplast HSPs play protective function for photosynthesis under heat stress. Application of BRs is famous to manage the damaging effects caused by high-temperature stress after modification in HSPs of mitochondria. Various researches demonstrated that BRs play important role in the production of HSPs under high-temperature stress.

Protein Folding and High-Temperature Stress

The protein functional model has been reassessed with the detection of in part extended messy proteins to be entirely efficient. These proteins are extensively spread in eukaryotes and accomplish vital meaning like transcriptional directive, signal transduction (Kjaergaard et al., 2010), enzyme catalysis, and protein ligand associations. They hold local like secondary structure rudiments but lack the tertiary connections of folded proteins. To hunt for relationship among purpose, organization, and dynamics, it is necessary to comprise all situation fashioned at equilibrium (Zhang et al., 2005) in order to characterize protein dynamics under adverse environmental conditions. Proteins have copious sheets of structure every one of which is major in course of protein folding. The early level of this arrangement is the series of amino acids themselves (primary structure). Secondary structure contains α-helixes and β-sheets. Tertiary structure takes the α-helixes and β-sheets and allows them to fold into a three-dimensional construction. The protein has folded and detained jointly by numerous structures of molecular relations.

Role of BRs in Abiotic Stresses

Apart from the developmental processes, BRs obstruct the negative impacts of environmental cues. With a minute exception, BRs have been revealed to advanced plant altered to various stresses (Xia et al., 2018; Fig. 15.1).

The action mechanism of BRs in improving the plant tolerance to environmental cues is still not clear properly. In *Lycopersicum esculentum,* mutants of BRs biosynthesis (dwf) demonstrate compassion to chilling stress, whereas overexpression of DWF consequences in an enhanced cold tolerance (Fang et al., 2019). BRs have been exposed to be anxious in plant respond to nitrogen malnourishment during lilt of autophagy, a self-destructive apparatus of cells, which is worn by plants to adjudicate reply to stresses (Wang et al., 2018). Application of BR increases the transcription plane of autophagy-related genes and the arrangement of autophagosomes.

BRs Effects in Thermotolerance

BRs contribute in excess of a number of developments and regulate them as to the atmosphere and assist plants in adaptation and modification of their progress as to the situation. BRs sharing in instruction of plenty of physiological procedures happening in plants is glowing familiar, like cell division and root shoot growth (Kumar

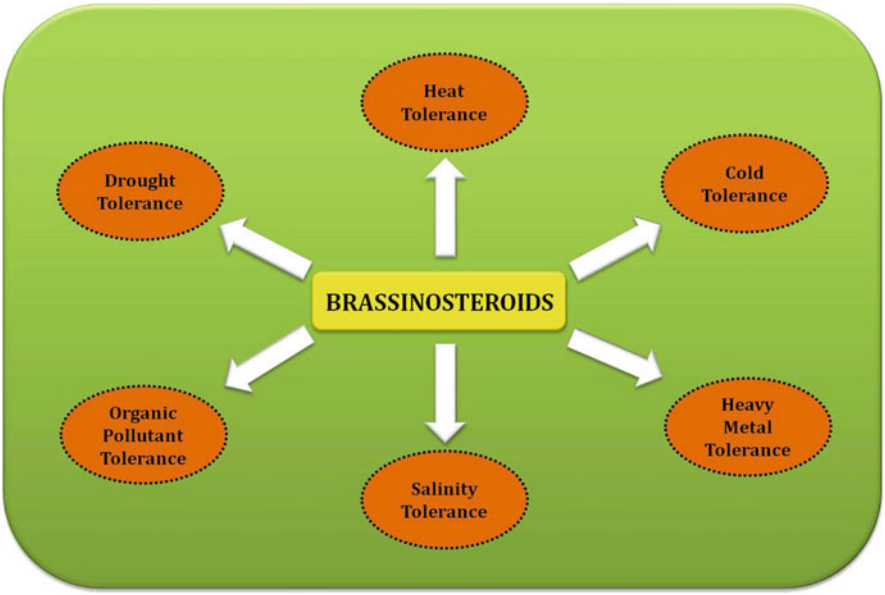

Fig. 15.1 Brassinosteroids recover plant tolerance to abiotic stresses

et al., 2010); synthesis of cell wall parts; combination of DNA, RNA, and a variety of proteins; and total carbohydrate increase (Sirhindi et al., 2011). *Brassica juncea* L. plantlet exposed with 24-EBL and 28-HBL enhanced germination, growth and protein content over untreated seedlings (Sirhindi et al., 2009). It was also observed in the same study that before sowing seed soaking with 24-EBL and 28-HBL enhanced activities of several enzymes like auxinase, polyphenol oxidase, SOD, CAT, and POX, which assist in growing the developmental latent of plants and strengthening the homeostasis. Apart from the function of developmental processes, BRs confined plants from number of abiotic and biotic stresses, suggesting lenience as an effect of change in cellular level ROS construction and appearance of genes programming, both proteins such as structural and regulatory (Kagale et al., 2007). BRs are responsible to activate the production of H_2O_2 in plants uncovered to diverse stresses, which perform as a indication particle for initializing cellular and molecular transformation to persuade lenience in plants. According to Cui et al. (2011), BRs generated abiotic stress tolerance in cucumber through the modulation in the formation of H_2O_2. In several crops, BR appliance enhanced the basic thermotolerance through rising formation of HSPs and parts of translational machinery (Dhaubhadel et al., 2002).

Heat is the primary abiotic issue preventing the expansion and yield of crop. Using of plant growth controller is frequently followed currently all over the world to boost plant productivity under normal as well as stressful environmental conditions. *Brassica juncea* L. exposed to BRs significantly enhanced the stress tolerance against the chilling stress and H_2O_2 (Sirhindi et al., 2011). Exogenous application of EBL increased ascorbic acid and GR amount in the presence of chilling stress. They concluded from these outcomes that BRs significantly mitigated the oxidative stress by enhancing the performance of antioxidative enzymes ensuing against chilling tolerance in *C. bungeana*. BRs abridged MDA and ROS construction to crucial levels thus shielding membranes and assisting in preserve the structural veracity of the membranes and increase the tolerance against chilling stress.

Stress Mechanism

Plants have several acclimatization characteristics to encounter the adverse conditions (Chu et al., 2015). At omics level, the stress signaling conduit participates in important functions in plant abiotic stress tolerance through connecting the sensing mechanism and the genetic response. Principally, three steps are included in stress transduction pathways such as perception, transduction, and response (Fig. 15.2).

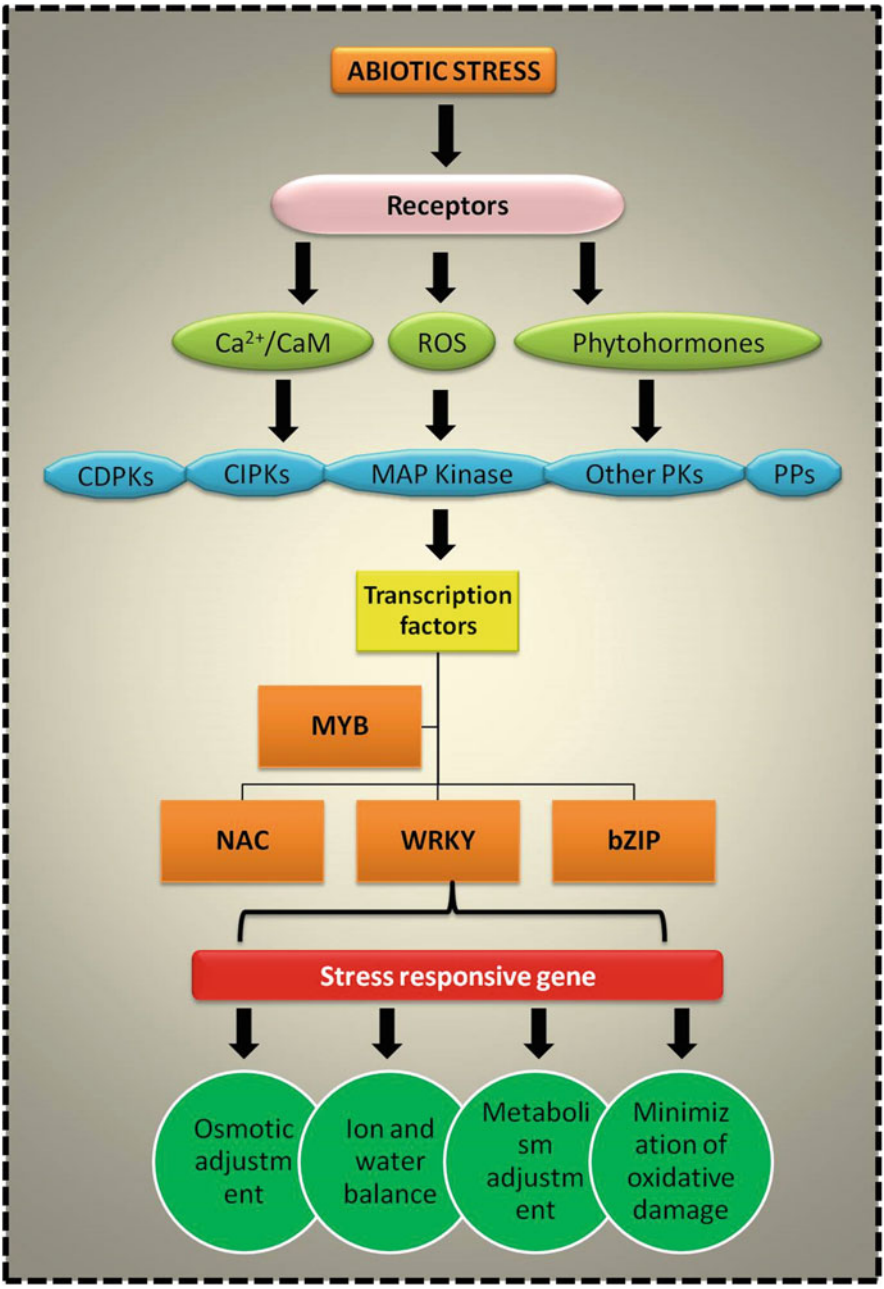

Fig. 15.2 Transcription factor model for regulating abiotic stress signaling pathways

Conclusions

The application of BRs has been rising for the improvement of agricultural methods because of its beneficiary impacts. The present chapter could help to increase the scientific understanding of BRs tolerance against abiotic stress and protein folding under heat stress. It is proposed that abiotic stress harmfully influences plant growth and development including protein folding. However, application of BRs could cover the injuries caused by abiotic stress in plants. Cellular modifications, detoxification, and regaining growth capacity play major role to get plants normal functioning under stress.

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Chapter 16
Molecular Mechanism of Brassinosteroids in Boosting Crop Yield

Reena Dubey and Deepti Tiwari

Abstract Increasing population leads to accelerated demand for food and fodder for fulfilling the needs of generation. Upturn in production by agronomic practices and mechanization is reaching to the plateau demanding more innovative techniques in crop production. Biotechnological approaches in crop plants can serve us with numerous avenues for enhancing crop-related traits. Brassinosteroids (BRs), which are found naturally in plants, can serve as potential regulators in crop production. They act as vital part in regulating plant metabolism related to development, differentiation, and stress retort. However, the mechanism to control/modify the BR signal is difficult. BR application for agricultural application is quite limited. BRs are known to regulate several processes in different plant parts, leading to some side effects. Therefore, efficacious strategies are needed to manipulate BR signals and avoid side effects during the process. To implement such model, there is necessity of creating molecular design of the crops to understand and employ the technique in smooth manner. In this chapter, we focused in representing the molecular mechanism, genes and cascades in plants (both *Arabidopsis,* and crop plants) for controlling growth-related factors. These techniques upon allocation in crops can set out perceptible biological and cellular BR mechanism and its future application in controlling traits that can serve as approaching tool for enhancing yield and quality.

Keywords Biotechnological approaches · Brassinosteroids · Crops · Regulators · Signaling · Yield

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Introduction

Improving agricultural productivity and sustainability has become essential of burgeoning debates and policies associated with the agricultural sector around the world. The increase in population, climate change, and demand for food is pushing the agriculturist and plant scientists to devise innovative solution in this regard. Different scientific strategies are being harnessed using biotechnology, breeding, physiology, etc. to optimize yield, plant architecture, response to biotic and abiotic stress and other plant traits. Here in this chapter, we will mainly focus on the role of brassinosteroids for increasing yield and metabolic mechanism behind it. Plants have its intricate mechanism of communication more precisely in practice of chemicals called plant growth regulators (PGRs). The inherent capability of these hormones to attenuate different biochemical processes can be of great importance in the current situation when we have a responsibility to feed 690 million starving population and fulfil Sustainable Development Goals (SDGs) laid down by UNDP (United Nations Development Programme). In addition to their regulatory function PGRs can be harnessed for modulating the plant response towards the directed trait of interest.

The basic phytohormones found in plants are auxin, ABA (abscisic acid), cytokinin, ethylene, and gibberellins. They play active role in plant response, development and growth. Several studies has been conducted towards understanding the molecular and genetic mechanism involved in the plant metabolism, its action in planta, and development of novel techniques (Bleecker & Kende, 2000; McCourt, 1999). Advancement in genomics, provided us whole plant genome for many crops, which enabled us with an opportunity to understand metabolic mechanism and its interlinked genomic responses with greater depth; receptors and its underlying mechanisms are studied extensively based on comparisons with model plant *Arabidopsis*. The optimized understanding of phytohormones assisted us in manipulating it towards indispensable application technology in vitro and in vivo. In addition to plant hormones there are some additional chemical compounds known to regulate physiology-related traits like growth and development. One of the major class belongs brassinosteroids others include polyamines, jasmonates, systemins, and strigolactones (Fig. 16.1). Each of them has specific function in plant system, but here we are going to discuss about the brassinosteroids.

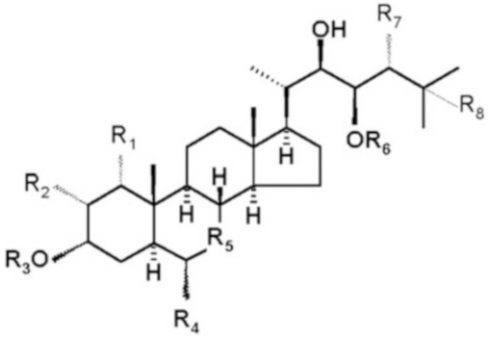

Fig. 16.1 Brassinosteroid structure, class of polyhydroxy steroids categorized as C27, C28, or C29 (C = dissimilar alkyl-substitution outlines of the side chains)

Brassinosteroids (BR) is ubiquitous to plant kingdom. It mimics animal steroidal lactones which is known for regulating various spectrum of physiological roles like organ elongation, epinasty, stimulation of ethylene biosynthesis, synthesis of proteins and nucleic acids, regulation of carbohydrate absorption and distribution, and photosynthesis initiation (Khan et al., 2019). Due to its extensive role, BRs are often referred as "sixth group of phytohormone." In addition, BRs are reported to have vital role in pesticide application therefore offering a promising green technology for pesticide degradation (Zhou et al., 2015). The molecular modulation of BR associated genes and signaling pathway will open avenues to more resilient agriculture, and furthermore it has potential qualities to emerge as "the hormone of twenty-first century."

Endogenous Mechanism of BR Signaling

The intensive studies determine the picture of molecular signaling mechanism of BR in plants (Nolan et al., 2020). It initiates with perception of BR on plasma membrane, followed by signal transduction cascade in cytoplasm. This final relay of signal goes to nucleus leading to gene expression regulation in nucleus. The gene expression and the related crosstalk involved in the signaling can serve as potential to control essential yield and architecture-related traits in plants. To take an overview, let us take a look on how this molecular signaling component works in the plants.

Perception and BR Signal Induction

Signaling of BR starts with the receptor kinases like BRASSINOSTEROIDINSENSITIVE1 (BRI1), present outside the cell at plasma membrane (Wang et al., 2002). The *bri1* mutants of Arabidopsis appear like BR biosynthetic mutants but they are not liberated by application of BR, representing its crucial role as a receptor. BRI1 gene encodes a protein, which includes an extracellular leucine-rich repeat (LRR) domain, a cytoplasmic serine/threonine kinase and a single transmembrane domain (Li & Chory, 1997). With more advancement in research and surge of scientific interest for brassinosteroids, many homologs of BRI1 have been identified in Arabidopsis so far like BRI1-LIKE1 (BRL1), BRL2, and BRL3. Studies in Arabidopsis showed that BRI1 is extensively expressed, whereas arrival of BRL1 and BRL3 is in vascular matters for the most part. Among all the homologs, high-affinity BR-binding capacity is found in BRI1, BRL1, and BRL3, then again not in BRL2. The phenotypic faults of the *bri1* mutant can be overcome by the application of only BRL1 and BRL3 when expressed with BRI1 promoter, providing them prime position of functional receptors (Fig. 16.2).

An LRR receptor kinase BRI1-ASSOCIATED KINASE1 (BAK1), similarly recognized as SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (SERK3),

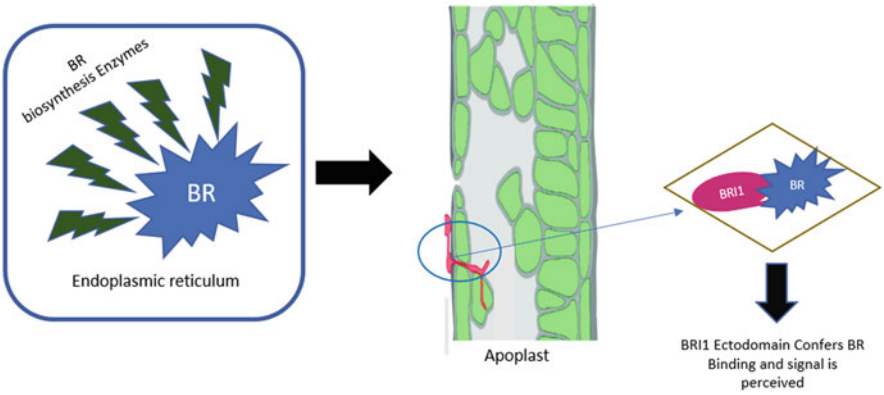

Fig. 16.2 BRs are recognized at apoplast of cell membrane with co-receptor intricate made of BRI1 and BAK1, in exhibition of BR, BKI1 form BRI1 and BRI1:BAK1 complex is generated. (This intricate is involved in inactivation of BIN2 (BR binds to the BRI1:BAK1 composite, BKI1 is released, and a phosphorylation cascade is activated resulting in the deactivation of additional kinase, brassinosteroid insensitive 2 (BIN2). BIN2 and its adjacent homologues prevent numerous transcription factors) and transcription factors can then exert their effects. BRI1 activity get blocked in lack of BR, and BKI1 and BIN2 constrain the transcription factors. The box represents the synthesis of BR in the endoplasmic reticulum from which it gets activated upon getting the perception and binding with BR biosynthesis enzymes)

interacts with BRI1 for modulating brassinosteroid signaling (Li et al., 2002). The interesting role of BAK1 can be observed by studying its loss-of-function mutants which resemble weak *bri1* mutants, and it suppresses the bri1 phenotype upon overexpression.

Hothorn et al. (2011) in their crystal structure study of BRI1 reported that the ectodomain of BRI1 forms superhelix (right-handed) composed of 25 LRRs. BL binding takes place in the hydrophobic groove of BRI1 formed by the 70-amino acid "island domain" situated among LRRs 21 and 22 and inner surface of the helical LRR. This mechanism is specially conserved for BRL1. BL causes heterodimerization of BRI1 and BAK1 or SERK1, which indicates that BAK1 and SERK1 function as co-receptors of BRI1 and are responsible for BL recognition. Triggered BRI1–BAK1 receptor complex leads to further downstream signaling cascade for BR-induced expressions.

Signal Cascading

There are two different mechanisms for signal flow in the lack and presence of BR. When BRs are not present, BRI1 stays in inactive state. This inactivation may be because of multiple mechanisms like:

1. Auto-inhibitory carboxyl terminus.
2. Auto-phosphorylation.

3. PROTEIN PHOSPHATASE 2A (PP2A)-facilitated dephosphorylation of the kinase domain.
4. Communication with the inhibitory protein BRI1 KINASE INHIBITOR1 (BKI1).

Inactive BRI1 leads to constitutive expression of BIN2 (BRASSINOSTEROID INSENSITIVE2), which results in phosphorylation of BZR1 and BES1 transcription factors. Phosphorylated BZR1 and BES1 fix to 14-3-3 and transfer to deprivation by several ligases, for example, MAX2, COP1, SINAT, and PUB40 (Wang et al., 2011). In this case, BR-responsive gene expression is repressed.

When BRs are present in the cell, phosphorylation of BKI1 through BRI1 dissociates it from BRI1. Now, BRI1 becomes fully active through trans-phosphorylation among BRI1, BAK1, or SERK members. BRI1 further phosphorylates the BSKs and CDG1 proteins (Kim et al., 2012). BSKs on phosphorylation through BRI1 interact with BSU1/BSLs (BRI1 SUPPRESSOR1/BSU-LIKEs) which belongs to family of phosphatases and activate them (Tang et al., 2008).

Triggered BSU1 or BSLs block the expression of BIN2 protein by dephosphorylation of a conserved tyrosine residue and inactivate its kinase activity. BIN2 negatively regulates the signaling responses of BR and dephosphorylation of BIN2 leading to its degradation by utilizing E3 ligase KIB1. With the inactivation of BIN2, BZR1 and BES1 are characterized by PP2A for dephosphorylation (Tang et al., 2011) which further get activated. Activated BZR1 and BES1 move to nucleus and lead to further gene manifestation, which is BR responsive (Wang et al., 2002).

BR-Regulated Transcription Response

Previously, researchers came across the numerous major BR signaling components using *Arabidopsis* as model plant, and outline of BR signaling pathway has been exposed (Wang et al., 2012). In this model, the protein kinase BIN2 phosphorylates two homologous transcription factors, BZR1 and BES1, and inhibits BZR1/BES1 from controlling the expression of their target genes when the BR level is down (Vert & Chory, 2006). When the BR level rises, it triggers plasma membrane-localized receptor BRI1 and co-receptor BAK1 (Li et al., 2002; Li & Chory, 1997; Nam & Li, 2002).

BRASSINAZOLE-RESISTANT 1 (BZRs) proteins remain as major transcription factors that control BR-regulated plant growth and gene expression. BES1 stores in nucleus in response to BL application. Many investigations show that BES1 is negatively controlled by BRASSINOSTEROID INSENSITIVE2 (BIN2). BES1 accumulates in nucleus in response to BR to control gene expression (Yin et al., 2002).

Most importantly, BZR and BES1 are major transcription factors of BR signaling which serves as a node of various signaling cascades. Some of the direct target genes are experimentally verified, like BZR/BES1-DNA interactions. BZR/BES1 also integrates with different growth and development events like protein networks

making it centrally regulated complex to coordinate different functions *in planta*. In addition, there are some epigenetic adaptation mechanisms intricate in BZR1/BES1-mediated gene expression. Previous studies demonstrate that activities of BES1 and BZR1 in the activation of target genes can be altered by a constituent in pathway of light signaling. There are confirmations regarding directive of BR levels by the phototransduction pathways (Kang et al., 2001; Neff et al., 1999). Potential targets for BR signaling pathways are delivered by BES1 and BZR1 (Fig. 16.3).

BR Signaling and Its Target Genes

The signal cascade induced by BRI1 at cell surface regulates the transcription mechanism. BZR1 and BZR2 are also accountable for control and response of the expression of genes encoding BR biosynthetic enzymes and upstream BR signaling modules (He et al., 2005; Sun et al., 2010; Yu et al., 2011). Further, BR similarly prompts the manifestation of the SUPPRESSOR OF BRI1 (SBI1) leucine carboxyl methyl transferase, which further methylates PP2A and promotes PP2A localization to membranes, where it dephosphorylates and inactivates the internalized BRI1 and provides alternative mechanism of feedback regulation (Wu et al., 2011). BR-responsive gene expression is modulated by BZR1 and BZR2, with other interrelating transcription factors. BIM1 (Yin et al., 2005) and BZR2 interact with the transcription factors MYB30 (Li et al., 2009; Yin et al., 2005), INTERACTS WITH SPT6 1 (IWS1) (Li et al., 2010), EARLY FLOWERING6 (ELF6), and the histone H3 lysine 27 demethylase RELATIVE OF ELF6 (REF6) (Yu et al., 2011). Various transgenic and genetic experiments pointed out those BZR2-interacting proteins have minor effects on BR-regulated growth response like hypocotyl elongation, and their interaction with BZR1 still remains unidentified. Both BZR1 and BZR2 interact with the PIF (phytochrome-interacting factor) family of bHLH factors and the GA signaling DELLA proteins to coregulate expression of a large number of genes, cell elongation, and photomorphogenesis as described in Table 16.1 (Bai et al., 2012a; Gallego-Bartolomé et al., 2012; Li et al., 2012; Oh et al., 2012).

Role of BRs *In Planta*

BRs were initially known for their utility in cell development (Nolan et al., 2020) but extensive studies conducted show numerous portrayals of BR during the physiological and the developmental phases *in planta* including plant architecture and yield. Below-mentioned compilation shows some belongings of BR on different developmental aspects in plants. These primary elucidations are in relation to its studies in *Arabidopsis thaliana* as a model organism.

Pollen and Anther Developments Pollen and anther development were greatly reduced in BR mutants bri1-116 and cpd (Ye et al., 2010). In addition, they had abnormal exine pattern and tapetal development in mutants, giving description in place of irregular deposition of pollen wall constituents and irregular pollen exine pattern (Ye et al., 2010). Also, upon external application, *Arabidopsis* pollen responded in dosage-dependent manner. The pollen tube growth showed five to

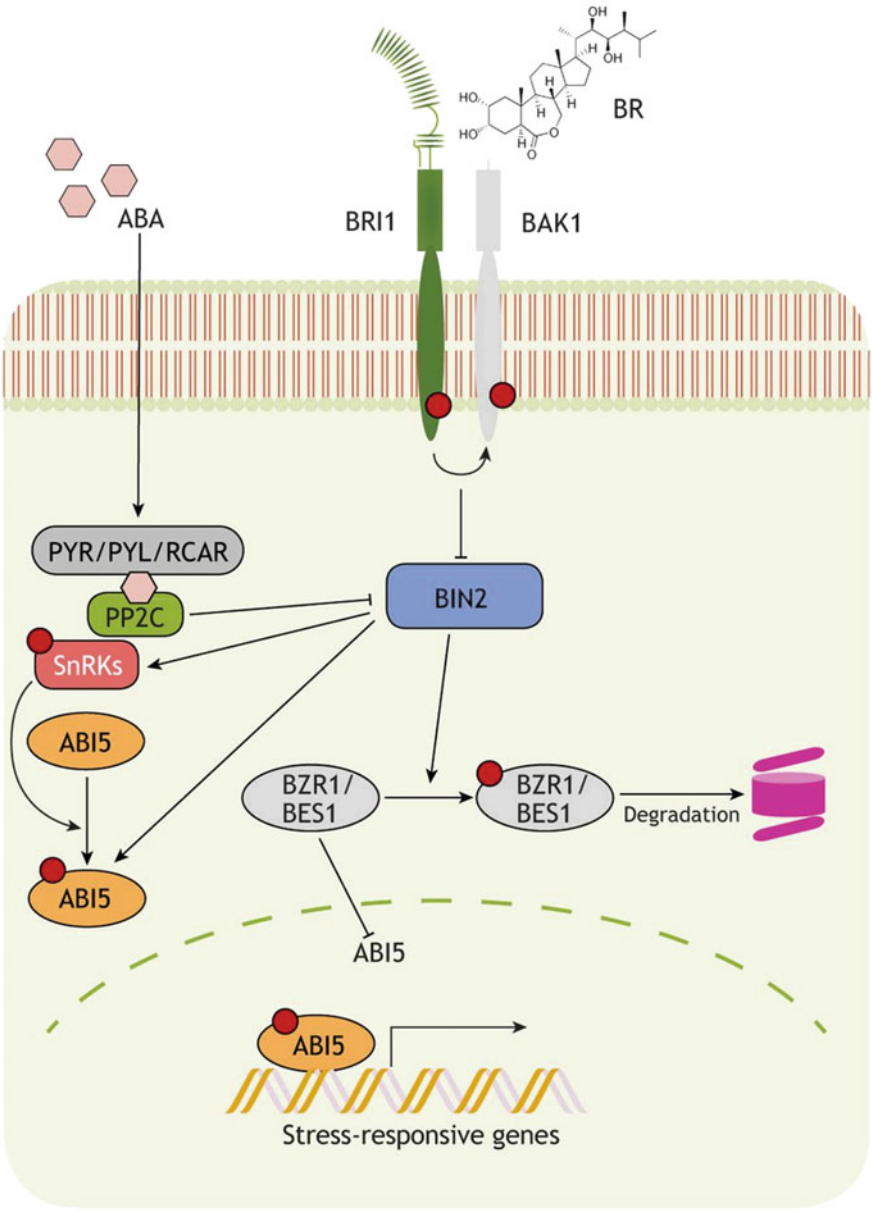

Fig. 16.3 Regulation of stress retorts via BR-ABA crosstalk
ABA is documented by PYR/PYL/RCAR receptors and improves the phosphorylation and activation of SnRKs, therefore alleviating them from PP2C-mediated constraint. SnRKs, thusly, phosphorylate downstream transcription factors, for example, ABI5 that modulates the transcription of several stress-responsive genes. BIN2, which is a negative modulator of BR signaling, can openly phosphorylate and trigger SnRKs and ABI5, while PP2C can deactivate BIN2. ABI5 is additionally an immediate target of BZR1, which inhibits its transcription to regulate the stress-responsive genes negatively (Planas-Riverola et al., 2019)

Table 16.1 Key genes in BR Signaling (Nolan et al., 2020)

Functional classification	Gene name	AGI	Function	References
BR perception	BRI1	At4g39400	BR receptors	Caño-Delgado et al. (2004), Li and Chory (1997)
	BRL1	At1g55610 At3g13380		
	BRL3			
	SERK3/ BAK1	At4g33430	Functions as a co receptor of BRI1 along with homologs SERK1, SERK2, and SERK4	Gou et al. (2012), Nam and Li (2002)
	BKI1	At5g42750	BRI1 kinase inhibitor hinders BRI1/BAK1 interaction	Wang and Chory (2006), Belkhadir et al. (2006)
	BIR3	At1g27190	Prevents BRI1/BAK1 interaction	Hohmann et al. (2018)
	PUB12	At2g28830 At3g46510	Ubiquitinates BRI1 after BR perception	Zhou et al. (2018)
	PUB13			
Phosphorylation and dephosphorylation cascade	BSK1	At4g35230 At4g00710 At3g26940	Together with their homologous proteins, phosphorylate and trigger BSU1; BSK3 acts as a scaffolding protein to control BR signaling	Tang et al. (2008), Kim et al. (2011), Ren et al. (2019)
	BSK3			
	CDG1			
	BSU1	At1g03445	Dephosphorylates and inactivates BIN2	Kim et al. (2009)
	BIN2	At4g18710	Together with other GSK family members, phosphorylates and inactivates BES1 and BZR1	Li and Nam (2002), Kim et al. (2009)
	PP2A	At1g69960	Dephosphorylates and activates BES1 and BZR1	Tang et al. (2011)
	BES1	At1g19350 At1g75080	Control BR-controlled gene expression along with homologs BEH1-4	Wang et al. (2002), Yin et al. (2002, 2005), He et al. (2005)
	BZR1			
BIN2 interactors that modulate BIN2 activity	KIB1	At4g12810	Facilitates BIN2 ubiquitination and subsequent degradation	Zhu et al. (2017)
	OCTOPUS	At3g09070	Limits BIN2 to the PM, blocking its interaction with BES1/BZR1	Anne et al. (2015)
	POLAR	At4g31805 At5g60880	Regulate the nuclear versus cytosolic and PM localization of BIN2	Houbaert et al. (2018)
	BASL			
	HDA6	At5g63110	Deacetylates BIN2 and represses BIN2 kinase activity	Hao et al. (2016)

(continued)

Table 16.1 (continued)

Functional classification	Gene name	AGI	Function	References
	TTL1	At1g53300	Together with its homologs TTL3/4, acts to scaffold BR signaling components at the PM	Amorim-Silva et al. (2019)
Modulators of BES1/BZR1 degradation and activation	MAX2	At2g42620 At5g40140 At2g32950 At3g58040	Mediate BES1/BZR1 ubiquitination and degradation	Wang et al. (2013), Kim et al. (2014, 2019), Yang et al. (2017)
	PUB40			
	COP1			
	SINAT2			
	DSK2	At2g17200	Autophagy receptor for BES1 degradation	Nolan et al. (2017)
	14-3-3λ	At5g10450	Together with other 143-3 proteins, retains phosphorylated BES1 and BZR1 in the cytoplasm	Gampala et al. (2007), Ryu et al. (2007)
	TRXh5	At1g45145	Interacts with BZR1 to stimulate its reduction and inactivation	Tian et al. (2018)
	RGA1	At2g01570	Together with new DELLA proteins, inhibits BES1, BZR1, PIF4, and ARF6 in low GA conditions	Bai et al. (2012b), Gallego-Bartolomé et al. (2012)
	BSS1/BOP1	At3g57130 At2g41370	Sequesters BES1 and BZR1 in the cytoplasm in the absence of BRs	Shimada et al. (2015)
	BOP2			
	UVR8	At5g63860	UV light receptor, prevents DNA binding activity of BES1	Liang et al. (2018)
	CRY1	At4g08920 At1g04400	Interrelate with BES1, BZR1, and BIM1 in retort to blue light to hinder their activity	Wang et al. (2018), He et al. (2019)
	CRY2			
	PHYB	At2g18790	Obstructs the transcriptional activity of BES1 in response to red light	Wu et al. (2008)
Transcriptional regulators involved in BR-mediated gene expression	IWS1	At1g32130	Interrelates with BES1 to promote BR-regulated gene expression	Li et al. (2010)
	BIM1	At5g08130	Together with its homologs BIM2 and BIM3, relates with BES1 to activate the expression of BR-induced genes	Yin et al. (2005)
	MYB30	At3g28910	Collaborates with BES1 to stimulate BR-induced gene expression	Li et al. (2009)

(continued)

Table 16.1 (continued)

Functional classification	Gene name	AGI	Function	References
	PIF4	At2g43010	Relates with BES1 and BZR1 to regulate BR-induced gene expression	Oh et al. (2012), Martínez et al. (2018)
	ARF6 ARF8	At1g30330 At5g37020	Interacts with both PIFs and BZR1 to regulate gene expression	Oh et al. (2014a)
	MYBL2 HAT1	At1g71030 At3g54610	BES1/BZR1 target transcription factors, assist BES1 in BR-repressed gene expression	Ye et al. (2012), Zhang et al. (2014b)
	HDA19	At4g38130	Facilitates histone deacetylation for BES1 and BZR1-repressed genes	Oh et al. (2014b), Ryu et al. (2014)
	TPL	At1g15750	Networks with BES1/BZR1 and recruits HDA19	Oh et al. (2014b), Ryu et al. (2014)
	ELF6 REF6	At5g04240 At3g48430	Eliminate repressive H3K27me2/H3K27me3 marks, permitting BES1 to activate gene expression	Yu et al. (2008), Lu et al. (2011)
	PICKLE	At2g25170	Inhibits H3K27me3 marks for BR-induced genes	Zhang et al. (2014a)
	SDG8	At1g77300	Improves H3K36me2/3 levels for BR-induced gene expression	Wang et al. (2014)
	WRKY46 WRKY54 WRKY70	At2g46400 At2g40750 At3g56400	Cooperate with BES1 to inhibit drought-responsive gene expression	Chen et al. (2017)
	RD26	At4g27410	Prevents BES1 and promotes drought responses	Jiang et al. (2019)
	TINY	At5g25810	Interaction with TINY2/3, controls drought replies through an antagonistic communication with BES1	Xie et al. (2019)

ninefold increase when treated with 10 μM EBL, resultant growth in vivo (Vogler et al., 2014).

Flowering Induction Flowering induction in Arabidopsis biosynthetic *det2* mutants was delayed by more than a week (≈10 days) related to wild type. The flowering time was overdue in the BR biosynthetic dwf4, cpd, and in bri1 mutants.

The endogenous BL content and the level of diverse BL precursors affect flowering time in *Arabidopsis* (Clouse, 2011a, b).

Stomatal Enlargement Stomatal enlargement in plants is controlled by BR via GSK facilitated embarrassment of MAPK pathway (Kim et al., 2012). *Arabidopsis* studies show that BR controls stomatal improvement by triggering the MAPKKK (MAPK kinase) YDA (also known as YODA). Genomic studies show that receptor kinase-mediated BR signaling hinders stomatal development through the GSK3 (glycogen synthase kinase 3)-like kinase BIN2, and BIN2 acts upstream of YDA but downstream of the ERECTA family of receptor kinases (Kim et al., 2012).

Aging or Leaf Senescence Aging or leaf senescence is marked as the ultimate process of the leaf expansion. It involves various physiochemical changes like chlorophyll deprivation, protein degradation, restructuring of nutrients, improve reactive oxygen species (ROS), improved programmed cell death/necrosis, membrane ion leakage, and differential expression of numerous senescence-associated genes (SAGs) (Fischer, 2012; Sarwat et al., 2013; Havé et al., 2017). In *Arabidopsis*, AIF2 (ATBS1-INTERACTING FACTOR 2) is a non-DNA-binding basic helix–loop–helix transcription factor which interrelates with ICE1 (INDUCER OF CBF EXPRESSION 1) via their C-termini. The coordination of AIF2 and ICE1 functions in maintaining stay-green traits (Kim et al., 2020).

Root Development The controller of root growth by BR signaling is also segregated spatially. BZR1 is more powerfully triggered at the transition (meristem to the elongation zones) and in the elongation zone itself (Chaiwanon & Wang, 2015; Fig. 16.4). BR signaling is not homogeneous throughout the root.

Environmental Stress

The ability of plants to adjust among growth activation and suppression in the critical conditions like variable water availability, temperature gradients, and soil salinity is governed as plants potential to deal with plant stress (Bechtold & Field, 2018; Feng et al., 2016). Abscisic acid (ABA) signaling pathway is the main mechanism to deal with these circumstances in plants (Yoshida et al., 2014; Zhu et al., 2017). However, extensive studies conducted on BRs show that BRs similarly show imperative role in monitoring the balance among normal growth and adaptation for environmental offensives, either through crosstalk with ABA pathway or independent manner. These mechanisms are known to regulate BR-mediated variation to drought, cold, heat, and salinity in plants.

A. Improvement of stress-responsive transcript mechanisms (Ye et al., 2017).
B. Triggering antioxidant mechanisms (Kim et al., 2012; Lima & Lobato, 2017; Tunc-Ozdemir & Jones, 2017; Xia et al., 2009; Zou et al., 2018).
C. The production of osmoprotectants (Fàbregas et al., 2018).

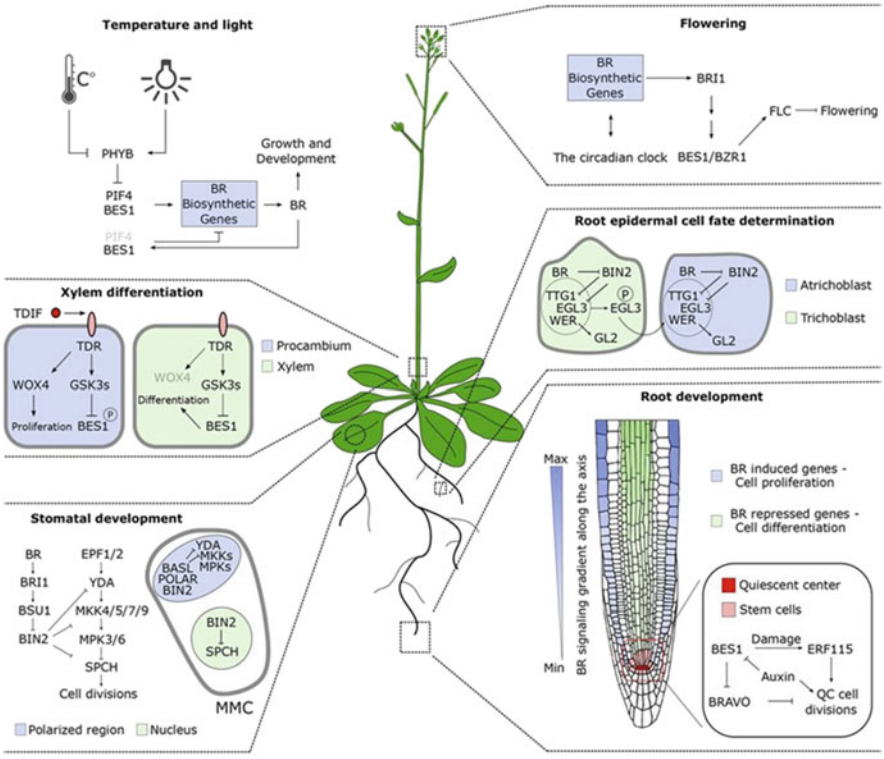

Fig. 16.4 BR regulated developing model in Arabidopsis
Temperature and light regulate PHYB activity, harmonize concentration of PIF4, and determine the levels of PIF4–BES1 heterodimerization. These interactions dictate the gene targets and lead to variable cellular responses. TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR signaling pathway determines the xylem differentiation. In addition, GSK3s act as negative controllers of xylem differentiation and allow the crosstalk with signaling pathway, thereby acting as crucial component. BIN2 is responsible for the controlling the stomatal development. In nucleus, BIN2 negatively regulates the SPCH activity while in complex with BASL and POLAR, it rearranges the PM polarized region of MMC and acts as a negative regulator of YDA and MKKs, which leads to SPCH activation. BRs prevent flowering by expressing FLC, a flowering inhibitor.In addition, the articulation BR biosynthetic genes show diurnal changes. During the root epidermal cell determination step, BIN2 phosphorylates EGL3, prompting its dealing from the nucleus to cytosol in trichoblast cells, which facilitates its transfer from trichoblast to atrichoblast cells. BIN2 can similarly phosphorylate TTG1 to repress the action of the WER–GL3/EGL3–TTG1 transcriptional complex. In the root apical meristem, BRs control the size of the stem cell by adjusting the outflow of BRAVO, which contrarily directs cell divisions in the quiescent center. BR signaling levels increase along the longitudinal axis, with more elevated levels present in cells nearer to the differentiation/elongation zone. BRAVO, BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER; BSU1, BRI1 SUPPRESSOR1; EGL3, ENHANCER OF GLABRA3; EPF1/2, EPIDERMAL PATTERNING FACTOR 1/2; FLC, FLOWERING LOCUS C; GL2, GLABRA2; MKK4/5/7/9, MITOGEN-ACTIVATED PROTEIN KINASE KINASE4/5/7/9; MMC, Meristemoid mother cell; P, phosphorylation; PHYB, PHYTOCHROME B; QC, Quiescent center; TDIF, TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR; TDR, TDIF RECEPTOR; TTG1, TRANSPARENT TESTA GLABRA1; WER, WEREWOLF; WOX4, WUSCHEL RELATED HOMEOBOX4; YDA, YODA (Nolan et al., 2020)

In addition, importance of BR in model plant Arabidopsis and the need for extensive studies in crop plants in order to harness its various roles. There are some fascinating molecular stories in crop plants which are being studied in order to optimize the demand-driven agriculture production. The vast networking in case of BR signaling involved in metabolic cascade, for better understanding, and devoted research towards it can help us to browse the hidden tales in BR-mediated signaling. In nutshell, BRs are responsible for varied array of activities in the plant. All these activities combined together are responsible for yield management. All the crops have different criteria for measuring the yield, for case in cereals grain content is major target, however in fodder crops more biomass is the aim, in dissimilar to the crops like potato or sugar beet, radish, carrot where the root is the main harvest.

Effect of BR-Mediated Regulations in Crops

Due to biosynthesis of BRs in plants, it is quite likely that the signaling mechanism of BR is conserved across the species. Several homologues of BR biosynthetic pathway are found in crops. For instance, DWARF4 (encodes 22α hydroxylase in the BR biosynthesis) in Arabidopsis has homologs with similar jobs in rice (Sakamoto et al., 2006) and maize (Makarevitch et al., 2012; Zea mays; Liu et al., 2007). Similarly, BRI1 homologs have been identified in rice (Yamamuro et al., 2000), maize (Kir et al., 2015), and tomato (Holton et al., 2007) that probably function as BR receptors established on mutant phenotypes and BL binding activity (Holton et al., 2007). In addition, the substantial effect of BR is also noted upon its exogenous application. However, more studies and research work are needed to define plant-specific molecular mechanism. Following section gives an overview on exogenous application technology and its effects in plants. The availability of BR by external medium is responsible for inducing the relay of cascade in specific crops.

Yield Enhancement Yield enhancement through BR application has been reported in many cereals, horticultural, ornamental as well as fiber crops. Upsurge plant height of maize (Holá et al., 2010), accelerated fiber growth in cotton (Shi et al., 2006), increase in the seed yield, number, and protein contents of pea (Shahid et al., 2011), noteworthy increase in growth parameters and yield-related traits of tomato plants (Varduini et al., 2001), and increase in yield parameters like seed weight in soybean (Prochazka et al., 2019) are some classic results indicating the potential of exogenous BR application in transforming conventional farming into resilient agriculture.

In commercial crops like lettuce, an increase in all desired traits like weight, diameter, and length was observed when treated with BR (Zhang et al., 2007). Drenching in BR in pepper augmented the number of fruits/plant (Serna et al., 2012) and in fenugreek, seed yield increased by 14.6% (Godara et al., 2017). The fruits production increased by 9%– 34% in strawberry when treated with BR (Salazar-Henao et al., 2016).

BR plays a vital role in **fruit development** and induces cell division and parthenocarpic growth. In addition, inhibitory outcome was also seen by BRZ treatment (Fu et al., 2008). BR application results in increasing levels of carbohydrates, soluble proteins, and essential vitamins like niacin and ascorbic acid in radish (Vardhini et al., 2011). Increased levels of protein content were also found in *Brassica juncea* plantlets with exogenous application of EBL and HBL (Sirhindi et al., 2009). Basera et al. (2018) obtained magical results of increased tuber growth by treating potato with 0.5 μM GA (gibberellic acid), 0.1 μM NAA (naphthalene acetic acid), and 0.1 μM EBL. In watermelon, 0.1 ppm BR spray at second and fourth leaf stage significantly increases TSS, total sugars, and lycopene content (Susila et al., 2012).

Advanced Maturity and Early Ripening Advanced maturity and early ripening are some of the economical features of BR. In winter rapeseed, advanced maturity by 4–8 days was seen due to BR application (Wan et al., 2017). Symons et al. (2006), on the basis of their experiments on Grape berry, reported that ripening can be promoted by BR and delayed by BRZ (BR inhibitor) application. BR promotes invertases and sucrose synthase activities at various stages, which lead to increase in the soluble sugars content and early ripening of berries (Xu et al., 2015).

Apart from increasing various yield parameters, BR also plays essential role in **disease and stress** tolerance. Seed priming of lucerne with BL is reported to enhance seed sprouting and seedling vigor in saline soils (Zhang et al., 2007). In water-deficit conditions, treatment of onion with synthetic BL increases bulb weight and hence provides more yield (Dolež alová et al., 2016). BR action in jujube fruits consequences in overdue senescence and disease resistance (Zhu et al., 2010).

Through more advanced studies, knowledge about BR signaling pathway is emergent. Mechanisms of BR perception on plasma membrane, transduction in cytoplasm, and gene expression regulation in nucleus are well known now. All of the accomplishments possibly enlighten research in signal transduction to study crosstalk among phytohormone signaling pathways and feature mechanisms of BR regulating plant development. The positive results gained after exogenous application of BRs are directing the researchers for understanding endogenous BR signaling mechanism in crop plants.

BR Story in Crops

In order to study the molecular mechanism, several studies are conducted in *Arabidopsis thaliana* to unravel the mechanism behind BR signaling and synthesis. To understand the role of BR in different crops is still a big thing to browse, but to some extent elucidation of the BR-associated mutants in *Arabidopsis* plant model aided the fundamental research to know the important component in signaling cascade like BR synthesis, metabolism, signaling, and response. Developments in cloning of BR-related genes and employing them into suitable pathways are

potential breakthrough for BR modulation in crop plants. Typically, there are two types of BR mutant, first are the biosynthesis mutants which respond to the exogenous BR application (they have less amount of BR content) and second are BR signaling mutants with abundant BR content (Clouse et al., 1996; Szekeres et al., 1996). For endogenous modulation, genetic approaches would be significant.

The increasing range of BR biosynthesis and signaling genes has been identified using Arabidopsis, which can be utilized further for crop improvement. Previous studies showed that overexpression BR-related genes like dwarf increases plant height in tomato (Bishop, 2003) and DWARF4 (gene encoding the Arabidopsis BR C-22 hydroxylase) increases plant height and seed yield individually in Arabidopsis and tobacco (Choe et al., 2001). Overexpression of several genes intricate in BR signaling can be utilized to improve plant growth, for example, BRI1 (Wang et al., 2001) BAK1 (Li et al., 2002; Nam & Li, 2002), BZR1 (Wang et al., 2002), and BES1 (Yin et al., 2002). In addition, several orthologs genes are also identified in crops upon simulation with *Arabidopsis thaliana* mutants.

The stress tolerance due to BRs has been reported frequently (Fariduddin et al., 2018; Khan et al., 2015; Khan et al., 2018; Nazir et al., 2020; Yusuf et al., 2017). Kagale showed that treatment of seedlings with 24-epibrassinolide (24-epi-BL) can improve drought tolerance in both *Arabidopsis thaliana* and *B. napus* (Sahni et al., 2016). Modulating antioxidants activity and the leaf gas exchange system in maize improves drought tolerance upon BR application (Chen et al., 2019). Studies showed capacity of BRs in improving oxidative stress tolerance convinced by polyethylene glycol (PEG) management. BR induces NO production, and NO further activates ABA biosynthesis in maize leaves, resulting in improved stress management (Zhang et al., 2011). BR-induced tolerance to stresses, such as photo-oxidative stress, cold stress, and cucumber mosaic virus infection, is facilitated by improved H_2O_2 due to raised NADPH oxidase activity (Xia et al., 2009). BR-deficient *Arabidopsis* mutant det2–1 was found more sensitive to salt stress in comparison to wild types (Zeng et al., 2010). In addition, overexpression of the BR biosynthesis gene AtDWF4 rises the cold tolerance of transgenic seedlings in *Arabidopsis* (Divi & Krishna, 2010). BRs participate in cold tolerance by regulating pectin methylesterase (PME) activity (Qu et al., 2011). Moreover, overexpression of AtDWF4 increases seed yield as well as improves stress tolerance in *B. napus*. The transcriptome analysis has shown the integrated effects of BRs on growth as well as in stress retorts (Sahni et al., 2016). The BR-deficient mutant in tomato (Micro-Tom) is hypersensitive to drought stress (Lee et al., 2018). It is not the end of story, there are dozens of BR-sensitive and -insensitive mutants identified in Arabidopsis and other crops (Clouse, 2011a, b), identifying the suitable ortholog and its cloning in different crops can help to regulate the genes related to BR response and induce numerous biological responses in plants. For example, BRs control male fertility by regulating the genes intricate in anther and pollen development in *Arabidopsis*.

Brassinosteroid and Crop Yield: Future Outlook

BRs are known to regulate plant height, leaf angle, and inflorescence architecture, the three important traits which determine yield in almost all agricultural crops (Morinaka et al., 2006; Wu et al., 2008; Yamamuro et al., 2000; Zhang et al., 2014a, b). Both BR exogenous application and endogenous cellular modulation can aid in manipulating/regulating the yield according to trait-specific requirements. But definitely we need to understand the cellular mechanism in more depth and analyze the cause-and-effect relation in different crops. On observing the bigger picture of BR story and its role in plant architecture and stress responses, it seems quite promising in boosting the yield-related traits in plants.

In agronomy, there are two basic pillars, first is to modify the crop (plant architecture/metabolism) and second is to opt for better cultivation techniques while farming (exogenous application/spraying). BR looks promising for both, but controlling genetic/molecular mechanism can serve as further sustainable way and definitely help the breeders and agronomists in the long run and avoid the use of heavy machinery and labor-intensive activities. With advancing research in BR and associated mutants in crops, it is quite evident that we will develop some path breaking strategies in the future to utilize BR-related traits for better quality and productivity. Altering BR biosynthesis levels and regulating its metabolism can be a potential source in forthcoming to efficiently regulate the BR-related traits in crops to boost crop yield.

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