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[Barbara Sawicka](#)<sup>\*</sup>, [Piotr Barbaś](#), Bernadetta Bienia, [Dominika Skiba](#), [Piotr Pszczółkowski](#),  
[Barbara Krochmal-Marczak](#)

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*Review*

# Toward Enhanced Plant Immunity: Unveiling the Role of Brassinosteroids in Abiotic and Biotic Stress Re-Response

Barbara Sawicka <sup>1,\*</sup>, Piotr Barbaś <sup>2</sup>, Bernadetta Bienia <sup>3</sup>, Dominika Skiba <sup>1</sup>, Piotr Pszczółkowski <sup>4</sup> and Barbara Krochmal-Marczak

<sup>1</sup> Department of Plant Production Technology and Commodities Science, University of Life Science, Akademicka 15 str., 20-950 Lublin, Poland; barbara.sawicka@up.lublin.pl; (BS); dominika.skiba@up.lublin.pl, (D.S.)

<sup>2</sup> Potato Agronomy Department, IHAR – PIB Branch in Jadwisin, 15 Szaniawskiego Street, 05-140 Serock, e-mail: p.barbas@ihar.edu.pl; (P.B.)

<sup>3</sup> Department of Plant Production and Food Safety, National Academy of Applied Sciences in Krosno, Rynek 1, 38-400 Krosno, Poland, bernadetta.bienia@pans.krosno.pl (B.B.), barbara.marczak@pans.krosno.pl (B.K-M.)

<sup>4</sup> Experimental Station for Cultivar Assessment of Central Crop Research Center, 21-211 Dębowa Kłoda, Poland; p.pszczolkowski.inspektor@coboru.gov.pl (P.P.)

\* Correspondence: barbara.sawicka@up.lublin.pl (B.S.)

**Abstract:** Brassinosteroids (BR) are essential plant hormones that play a crucial role in regulating plant growth and development, as well as their resistance to abiotic and biotic stresses, including pathogen infections. In the context of plant immunity, BR influence various processes such as the expression of genes related to pathogen defense, the production of antimicrobial compounds, and the re-inforcement of the plant cell structure. The aim of work is understand the role of BR in plant defense against abiotic and biotic stresses. BR are involved in the regulation of metabolic processes, water economy, and photosynthesis, contributing to increased plant resistance to abiotic stresses. The study discusses various mechanisms of BR action on plant immunity, including the regulation of gene expression related to defense, activation of the plant defense system, interaction with other plant hormones, regulation of water and salt balance, and influence on photosynthetic processes. In the context of plant immunity, BR influence various processes, including the expression of genes related to pathogen defense, the production of antimicrobial compounds, and the reinforcement of the plant cell structure. Additionally, BR are involved in the regulation of metabolic processes, water economy, and photosynthesis, contributing to increased plant resistance to abiotic and biotic stresses. The paper emphasizes the importance of further research on the role and mechanisms of action of BR in plant immunity, which may contribute to the development of more effective strategies for the use of BR in agriculture, horticulture, and plant protection.

**Keywords:** plant hormones; gene expression; BR functions; antimicrobial actions; immunity mechanisms; plant stress

## 1. Introduction

BR constitute a novel group of steroid hormones that share structural similarities with hormones found in animals and insects. Initially identified in rapeseed pollen, their presence has been documented across various plant species [1]. Despite their low concentrations in plants, BR profoundly influence numerous cellular and molecular functions [2]. They play a pivotal role in regulating physiological processes such as cell division, cell expansion, seed germination, development of conductive tissues, growth differentiation, reproduction, and responses to light (photomorphogenesis and scotomorphogenesis). Notably, inappropriate levels of BR can result in phenotypic abnormalities in plants. BR act as key growth regulators, offering plants the ability to counteract abiotic stresses such as drought, salinity, extreme temperatures, flooding, exposure to

toxic heavy metals/metalloids, and other contaminants, all of which can lead to substantial agricultural crop losses.

Plants have evolved effective defense mechanisms, including the neutralization of Reactive Oxygen Species (ROS) generated under stress conditions. Over the last two decades, extensive research has enhanced our understanding of the complex of BR signaling pathway in response to various stresses. This knowledge has paved the way for the development of strategies to improve crop performance by manipulating BR signaling, biosynthesis, or perception [1, 2, 3].

BR, as phytohormones, play a crucial role in regulating plant growth and development, aiding plants in adapting to changing environmental conditions [3]. They are found in various plant organs and tissues, including roots, stems, leaves, pollen, anthers, and fruits [4, 5]. The highest concentrations of BR, as reported by Li and He [6], are found in seeds, pollen, and fruits. Young plant tissues (1-100 ng/g fresh weight) generally contain higher BR concentrations than mature tissues (0.01-0.1 ng/g fresh weight) [7]. BR play a crucial role in various developmental and physiological processes, including cell expansion, cell division, maintenance of stem cells, vascular development, elongation of different cell types [8, 9]. They also influence hypocotyl growth [10], root and shoot development [11], stomatal regulation [12], seed germination [13], xylem shaping and differentiation [14], photomorphogenesis, plant reproduction [15], and aging processes [16].

In the realm of plant immunity, BR regulate processes such as the expression of defense-associated genes, production of antimicrobial compounds, and reinforcement of plant cell structures. In response to pathogen infection, BR can enhance the production of lignin, phenolic compounds, and glycosides, fortifying cell walls against bacterial, viral, or fungal infections [2, 17, 18]. Regarding plant resilience to abiotic stresses, BR are involved in regulating metabolic processes, including the accumulation of osmotic substances and the adaptation of chloroplasts to light stress. Additionally, BR contribute to increasing the stability of cell membranes, protecting them against oxidative stress [18-20]. Furthermore, BR stimulate root growth, aiding plants in water and mineral nutrient absorption from the soil, thereby enhancing resilience to abiotic stresses [17, 20, 21].

Manipulating the levels of BR in plants can impact their ability to adapt to both abiotic and biotic stress. For instance, increasing BR levels can enhance resistance to drought and cold but may simultaneously reduce resistance to pathogen infections [22-26]. The adjustment of BR signaling, biosynthesis pathways, or perception holds significant potential for improving crop yields [23, 27]. Therefore, the objective of this study was to investigate and analyze the actual role of BR in signaling cascades, emphasizing their positive contribution to biotic and abiotic stress tolerance.

## 2. . Structure and Functions of BR

### 2.1. Structure and Biosynthesis of BR

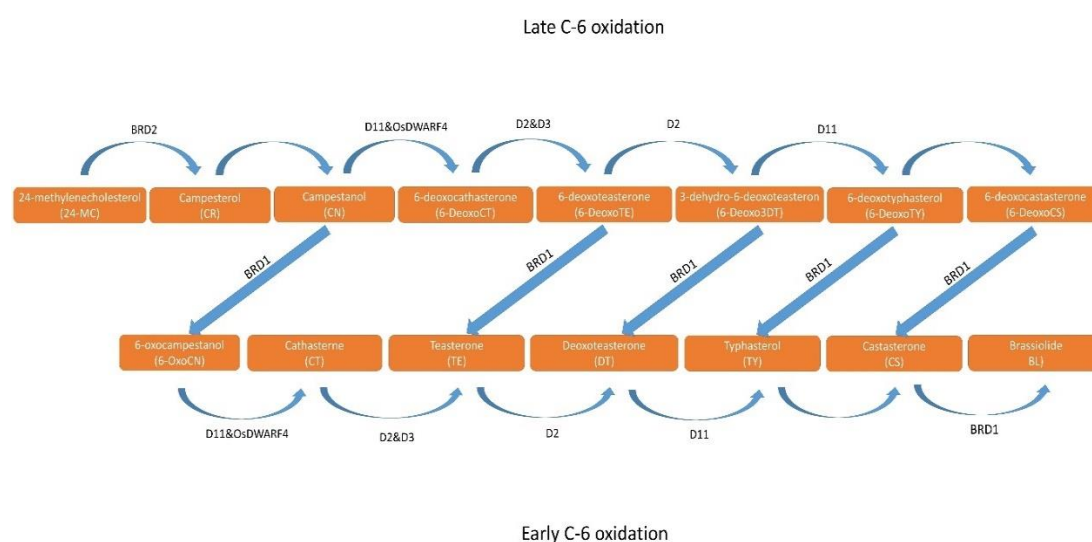
**Structure and Biosynthesis:** BR are steroid compounds characterized by a complex structure, featuring a steroid ring with two side chains. Their structure is based on triterpenoids and includes several distinctive elements [14, 23, 28]. The general chemical structure of BR can be outlined as follows:

- Triterpenoid system: BR possesses a triterpenoid ring with a total carbon atom count of 30 or 32. It consists of several rings, including rings A, B, C, D, and E [22, 29].
- Tetracyclic ring: A and B form a tetracyclic ring, which is characteristic of BR [14, 30].
- Functional groups such as hydroxyl group [-OH], ketone group [-C=O], alkoxy group [-OR], methoxymethyl group [-O-CH<sub>2</sub>-], etc. These functional groups are crucial for the biological activity of BR [29,30].
- Substituents: Various substituents may be present on the triterpenoid rings of BR, influencing their biological properties [4, 22, 28].
- Biological activity sites: The biological activity of BR is often associated with specific functional groups and particular substituents on the triterpenoid rings [14, 28, 31].

Examples of BR include brassinolide (BL), castasterone (CS), and teasterone (TZ), which differ in chemical structure but exhibit similar functions as phytohormones influencing plant development and responses to environmental stresses. BL and CS are considered the most important BR due to

their significant biological activity and widespread occurrence in plants. However, due to commercial availability, 24-epibrassinolide (EBR) is the most commonly used BR in studies on exogenous steroid phytohormones in plants [31]. Since the discovery of BL, approximately 70 different BR have been identified in 64 plant species, including 6 gymnosperms, 53 angiosperms, one horsetail species (*Equisetum arvense*), three algae species (*Hydrodictyon reticulatum*, *Cystoseira myrica*, and *Chlorella vulgaris*), one moss species (*Marchantia polymorpha*), one moss species (*Physcomitrella patens*), lycopods (*Selaginella moellendorffii* and *S. uncinata*), and thirteen fern species [6, 32,33]. It has been observed that in *Cupressus arizonica* pollen, the concentration of 6-deoxytyphasterol (6-deoxyTY) was 6400 times higher than BL. The highest BR concentration, amounting to 6.4 mg 6-deoxyTY per kilogram (kg) of pollen, was found in *C. arizonica* [22]. To date, only fifty-two BR have been detailed in terms of their biological activity in plants [31]. CS, BL, TY, 6-deoxyCS, TE, and 28-norCS are the most common BR in plants [33]. The greatest diversity of BR (2 conjugated forms and 25 free forms) was found in immature common bean (*Phaseolus vulgaris*) seeds [34]. Various BR have also been identified in shoots, seeds, and pollen of other leguminous plants, with their content ranging from 0.007 to 628 µg/g fresh weight [35].

According to Chaudhuri et al. [27], BRs are a group of naturally occurring polyhydroxylated phytosterols that have at least one oxygen group at position C3, as well as additional groups at one or more carbon atoms, such as C2, C6, C22, and C23 [36]. Their chemical structure can be schematically represented (Figure 1), [28].



**Figure 1.** The brassinosteroid biosynthesis pathway in rice and the enzymes that catalyze them. Abbreviations for each compound are given in parentheses below the full name. Source: own.

The transport and metabolism of brassinosteroids (BRs) in plants are complex processes with significant implications for growth, development, and stress responses. More than 70 BR derivatives can be produced through various metabolic transformations, including acylation, demethylation, epimerization, glucosylation, hydroxylation, side chain cleavage, sulfonation, and oxidation [22, 28, 30, 31, 37-42].

Exogenous BR transport in plants occurs in a directed manner, known as basipetal transport, from apices to the base. This process, independent of vessel movement, involves energy consumption and specific carrier proteins in the cell membrane. Calcium ion levels may influence this process. When applied to roots, BR transport becomes acropetal, moving toward leaves. Endogenous BR transport remains unclear [14,15,22].

BR perception occurs on the cell surface through receptors, leading to a phosphorylation cascade activating the transcription factor BZR1. BZR1 regulates BR-responsive gene transcription, influencing photosynthesis efficiency and plant biomass accumulation. Plant responses to BR vary,

and genetic studies suggest a positive correlation between endogenous BR levels and abiotic stress tolerance [36-43].

Genetic studies show a positive correlation between the level of endogenous BR and tolerance to abiotic stress, although this assumption contradicts the behavior of some BR mutants under stressful conditions. It is worth noting that plant responses to BR vary significantly depending on the plant variety and species, developmental stages, and environmental conditions. Additionally, other hormones and signaling molecules participating in fine-tuning BR action also play a significant role in plant adaptation to stress [15, 22,36,40]. An example is cucumber plants, where the application of exogenous 24-epibrassinolide (24-epiBL) to the roots causes acropetal transport of this compound in the plant. It has been shown that 24-epiBL applied to cucumber roots was metabolized only in the leaves, transforming into 2,24-diepiBL [35,41]. Fatty acids and/or sugars may also be attached to the hydroxyl groups of the A ring or the side chain of BR. Exogenous BRs seem to move very slowly or not at all within plants after application to leaves. Even when BR-deficient plant mutants are grafted onto wild-type plants, it does not lead to changes in the plant's phenotype, according to Bajguz & Hayat [36]. Removing the growth tip or mature leaves also does not lower the BR levels in the remaining parts of the plant. For example, in tomatoes, the presence of wild forms does not significantly affect the growth of BR-deficient forms when both types are present in the same leaf. Despite the fact that BRs do not undergo long-distance movements, they can still influence signaling over greater distances by modifying the transport of, for example, IAA produced in growth cones. Enzymes involved in BR biosynthesis are located inside cells, especially in the endoplasmic reticulum. BR reception occurs on the external surface of the cell, so BRs must move from inside the cell to the outside, where they are recognized by the same cell or neighboring cells [36,38,43-44].

Metabolic transformations of exogenous BR, such as in cucumber plants, result in acropetal transport, producing different derivatives like 2,24-diepiBL [37]. Despite limited long-distance movement, BRs influence signaling over greater distances by modifying the transport of other compounds. Enzymes involved in BR biosynthesis are located intracellularly, while reception occurs externally [14,45,46].

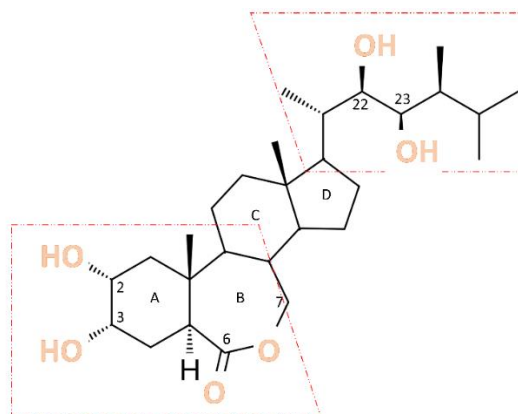
A feedback mechanism suggests cells can perceive and produce BRs, indicating complex transport and perception processes. The biosynthesis of BR involves sterol processing, hydroxylation, reduction, C-22 hydroxylation, cyclopropylation, C-23 hydroxylation, and the final reduction to form BR. BRs act as steroid hormones influencing plant growth, development, and stress responses [30, 33].

Recent research by Aitken et al. [30] explores the synthesis and evaluation of brassinosteroid (BR) analogs with modified alkyl side chains, particularly incorporating phenyl or benzoate groups. Their study indicates that introducing fluorine atoms (F) enhances the bioactivity of these analogs. Building on this, a series of 23,24-bisnorcholesterol analogs with benzoate groups substituted with F atoms in ortho or para positions was synthesized and assessed for their impact on plant growth stimulation. Results demonstrate that analogs with an F atom in the para position show the highest activity, often exceeding that of brassinolide. Docking studies reveal that these F-containing compounds adopt a similar orientation to brassinolide, with the F atom in the para position forming an additional hydrogen bond in the binding site. Overall, these findings suggest that BR analogs with fluorine substitutions exhibit promising biological activity, offering potential for enhancing plant growth under stressful conditions [47-51].

Exogenous BR application or manipulation of biosynthetic pathways can enhance yields. Signaling involving BR, IAA, and GA plays a crucial role in regulating various processes, including leaf angle development. A phosphorelay signaling system governs BR signal transduction, regulating plant growth. BR binding initiates phosphorylation events, activating downstream kinases and transcription factors, ultimately influencing gene expression [48,52,53]. Jo et al. [54] conducted a comprehensive study on cucumber metabolomics and gene expression to understand the metabolism of bioactive compounds. They found consistent expression patterns between carotenoid and chlorophyll-related genes and the metabolome analysis. The upregulation of carotenoid and chlorophyll metabolism in fruit peel and flesh, exhibiting the highest antioxidant activity, suggests

their contribution to fruit quality. The identified bioactive compounds can serve as biomarkers for commercial fruit and vegetable quality.

The application of exogenous BR or manipulation of biosynthetic and signaling pathways can potentially increase yields. BR, IAA, and GA signaling plays a crucial role in regulating leaf blade bending in rice, and their manipulation could enhance crop yields [44,53,61]. A complex phosphorelay signaling system governs BR signal transduction, regulating plant growth and development through the activation of transcription factors like BZR1/2 (Figure 2).



**Figure 2.** Chemical structure of BL with steroid rings (A, B, C and D). Parts within the dashed lines can be replaced with different groups. Source: own.

BR binding phosphorylates BKI1, relieving BRI1 receptor inhibition. BRI1 activation involves mutual phosphorylation with BAK1 or other SERK family members. BRI1 then phosphorylates BSK1 and CDG1, activating BSU1 and PP2A. These dephosphorylate and deactivate BIN2, allowing BR response transcription factors like BZR1/2 to accumulate in the nucleus and bind to DNA, along with other transcription factors [48]. In summary, the transport, metabolism, and perception of BR in plants are intricate processes with significant implications for plant physiology, growth, and stress responses, necessitating further research in this area.

The biosynthetic pathway of BRs in rice involves enzymatic reactions:

- a) Squalene → campesterol by SQE.
- b) Campesterol → obtusifoliol by CYP90B2.
- c) Obtusifoliol → epicastasterone by CYP90D1.
- d) Epicastasterone → teasterone by CYP85A2.
- e) Teasterone → typhasterone by CYP95A3 [44, 49-53].

Enzymes in the phytosterol biosynthetic pathway include squalene epoxidase (SQE), cycloartenol synthase, C24 methylation enzymes, cyclopropylsterol isomerase, CYP51, Fackel/Hydra2/Extra-Long-Lifespan 1 (Fk/Hyd2/Ell1), and  $\Delta 8$ - $\Delta 7$  sterol isomerase (HYDRA1/HYD1). They convert precursor molecules into phytosterols, serving as intermediates for BR biosynthesis [46, 47, 50, 55, 56]. Typhasterone is the final product in the BR biosynthetic pathway, further modified into active forms like brassinolide (BL) and castasterone (CS). BR biosynthesis is a complex process occurring in different cellular compartments and regulates various physiological processes in plants, including growth, cell expansion, and their stress responses [4, 47, 54].

Receptor-like kinases (RLKs) are vital surface receptors in plants, crucial for signaling pathways. RLK structure includes three domains: extracellular for ligand binding, transmembrane for membrane anchoring, and cytoplasmic kinase for initiating intracellular signaling (Figure 3) [4, 12, 22, 35, 36].



**Figure 3.** RKL responses to abiotic stress. Source: own.

RLKs play diverse roles in plant signaling, influencing defense responses, hormone perception, and development. They mediate extracellular signals, regulating growth, immunity, and environmental adaptation [51,57-59]. Recent research highlights their involvement in complex signaling networks, interacting with other proteins dynamically [57]. Understanding RLK-mediated pathways is crucial for enhancing crop performance amid changing conditions. Some RLKs share signaling cascades, like BRI1, EMS1, and NILR1, suggesting intricate signaling mechanisms [4,39,51,57]. Advanced techniques such as domain swapping and single-cell omics analysis can deepen our understanding of RLK pathways with precision.

New techniques are needed to identify RLK-binding ligands, complementing existing proteomic and genetic approaches. Functional redundancy among RLKs complicates their identification; for instance, BAK1 serves as a co-receptor for BRI1 and regulates immune responses through FLS2 and EFR pathways. Advanced methods like CRISPR and sophisticated phenotypic analysis can address this redundancy [4, 57].

RLKs often participate in multiple pathways, posing challenges in understanding their specific functions. Technologies such as genetics, structural biology, and proteomics will be crucial for unraveling these complexities, ultimately improving crop yields and developing stress-tolerant varieties through bioengineering and breeding approaches [58-60].

BRI1 is a pivotal receptor for BR, crucial in plant physiology and development. Mutations in its gene can disrupt plant growth. Other BR signaling components include BAK1, BIN2, BSU1 phosphatase, and BZR transcription factors [40]. Despite progress, uncertainties remain in BRI1 kinase activation. Continuous discovery of new signaling components highlights the complexity of BR pathways and their integration with other signaling networks, essential for plant development and defense. Understanding these mechanisms may unlock new agricultural applications [35,60].

## 2.2. Physiological Functions of BR in Plant Growth and Development

Research on BR-deficient mutants has identified them as a novel class of phytohormones, yet many of their functions remain undiscovered due to limitations in analyzing plant species. Specific inhibitors of BR biosynthesis can be valuable tools for studying their role in various plant developmental stages, such as germination, leaf development, pollen formation, and flowering. BR influences plant growth, development, and responses to abiotic and biotic stresses, including stomatal apparatus formation, sexual plant development, etiolation, elongation of the pistil, leaf morphology, responses to atmospheric pollution, and thermotolerance [27, 28, 61].

Manipulating BR levels, both through exogenous application and changes in biosynthesis and signaling pathways, holds the potential to increase crop yields. Studies on growth regulation in *Arabidopsis thaliana* have highlighted the significant role of factors like WRKY46, WRKY54, and

WRKY70, activated by BR. The histone methyltransferase SDG8 plays a crucial role in regulating the expression of BR-related genes, affecting various aspects of plant development, yield, and quality. Inhibitors of BR biosynthesis, used to study BR-deficient mutants, can effectively elucidate the functions of these phytohormones [46, 47, 61-63].

**Impact on Cell Growth:** BR serves as a key regulator of cell growth, especially elongation, leading to increased shoot and root length. Approximately 70 different BRs have been identified, and they play essential roles in reactions such as stem growth, pollen tube growth, leaf bending, leaf unfolding, root growth inhibition, proton pump activation, ethylene production, and cell elongation [4, 14, 22, 25, 35, 62-64].

**Role of BR in Shaping Plant Morphology:** BR influences various aspects of plant morphology, including leaf, bud, root, and flower shape. They are crucial for the development of cultivated plants and the production of secondary metabolites. BR also affects cell elongation, which is pivotal in the initial growth stages. Studies on BR-deficient mutants in cereal plants underscore the importance of these phytohormones in regulating plant morphology and architecture [15, 40, 65].

BR also influences cell elongation, crucial in the initial growth stages of plants. Research confirms the positive effect of BR on the formation of root nodules in pea plants (*Pisum sativum*) and the shaping of lateral roots, often regulated through synergistic interactions with auxins and other key plant hormones. Changes in signal transduction pathways and BR synthesis at the molecular level can lead to metabolic defects, influencing plant morphology and architecture, particularly in cereals. For example, dwarf cereal plants show resistance to lodging, enabling increased fertilizer doses and higher yields [9,10, 66].

**BR in Root Growth:** Exogenous phytohormones like MRW or EBL promote marigold's lateral root growth, suggesting that methane (CH<sub>4</sub>) induces lateral roots, possibly by increasing endogenous BR levels. CH<sub>4</sub> affects cell wall components and enzyme activity during root formation, with BR potentially involved, highlighting BR's role in CH<sub>4</sub>-induced lateral root growth and cell wall regulation [60].

**BR in Xylem Development:** BR influences xylem development in woody plants via the BR signaling pathway. Exogenous BR increases stem length and diameter, enhancing xylem area in stems and roots. Gene expression changes, including upregulation of *EgrBRI1*, *EgrBZR1*, and *EgrBZR2*, along with alterations in *HDZIPIII* and cellulose synthase genes, were observed. Six-month-old trees treated with BR showed increased xylem area, fiber cell length, and cell number, along with elevated expression of BR signaling and xylem-related transcription factor genes [69, 70].

BR significantly impacts plant photomorphogenesis, influencing various morphological and physiological processes in response to light. Key aspects include:

- Regulation of developmental processes such as embryogenesis, seed germination, cell division, elongation, pollen development, pollen tube growth, tracheal element differentiation, leaf aging, and senescence [20, 28, 43, 69].
- Control of proton pumping through stimulated action of transmembrane H<sup>+</sup> ATPases, affecting physiological processes in plant cells [22, 35, 69]. Yoshizawa et al. [70] demonstrated the light-driven proton pump activity of diatom rhodopsin, mainly localized in plastid membranes.
- Impact on photosynthesis by enhancing CO<sub>2</sub> assimilation and Rubisco enzyme activity, thus increasing photosynthetic efficiency [43, 71-75].
- Regulation of water transport via aquaporins, crucial for plant water homeostasis [38, 41]. Positive effects on embryogenesis, observed in somatic and microspore embryogenesis, especially in Brassica species [27-28, 31].
- Influence on gene expression and cell wall structural organization by affecting alpha- and beta-tubulin gene expression, microtubule reorientation, and cellulose microfibril spatial organization [20, 43, 69].

These mechanisms underscore BR's role in plant adaptation to environmental conditions and the efficiency of growth and development.

### 3. Mechanisms of Defense Related to BR

### 3.1. Impact of BR on the Expression of Genes Related to Plant Defense

Regulation of defense-related gene expression: BR can influence the expression of genes related to plant defense, such as genes encoding antioxidant enzymes, anti-inflammatory proteins, or proteins involved in the synthesis of secondary compounds that play a role in plant defense against biotic and abiotic stresses [35, 42, 65].

BR can stimulate the production of secondary compounds, such as alkaloids, flavonoids, or phytoalexins, which play a role in plant resistance to pathogen attacks [65]. BR influences the activation of signaling pathways that regulate the plant's defense response. They can activate kinases and transcription factors involved in the regulation of the expression of defense genes [14, 76-77]. BR also influences the growth and development of plants, which may increase their resistance to mechanical stresses, such as damage caused by herbivores or wind. They can also regulate interactions with other plant hormones, such as jasmonates or salicylates, which are involved in plant defense responses. This can affect the complex regulation of plant defense [78-79].

### 3.2. Interactions between Electrical Signals and Plant Hormones

Interactions between electrical signals and plant hormones are vital for plant responses to environmental stresses. Plants employ a three-stage strategy involving cellular homeostasis, detoxification, and restoring growth processes [78, 80-81]. Brassinosteroids (BR) become more active under stress, aiding in scavenging reactive oxygen species (ROS), maintaining redox balance, improving photosynthesis, and enhancing antioxidant systems [5, 80, 82-83]. Local stimuli trigger long-distance electrical signals that influence hormone levels in distant plant parts, mediating important integration points involving changes in pH, calcium ion levels, and ROS [5, 83-85].

Research by Ladeynova et al. [47] sheds light on intricate interactions between electrical signals and plant hormones, elucidating systemic responses. Further studies are crucial to deepen our understanding of these interactions, particularly in managing environmental stress [47, 80, 86]. Action potentials (AP) and other electrical activity patterns influence physiological processes like photosynthesis and plant reactions to environmental stimuli [78].

Plant electrical activity is vital for understanding adaptive strategies with implications for agriculture and environmental conservation. Despite progress, unanswered questions persist, necessitating ongoing research [47,86, 87].

Plants utilize a three-stage strategy to cope with environmental stresses: maintaining cellular homeostasis, detoxification, and restoring growth processes. Brassinosteroids (BR) play a significant role in stress alleviation by scavenging reactive oxygen species (ROS), enhancing photosynthesis, and strengthening the antioxidant system [88,89].

Local stimuli can trigger long-distance signals that affect hormone levels throughout the plant. Electrical signals, pH changes, and ROS are crucial in mediating these responses. Further research is needed to fully understand these interactions and their applications in agriculture, horticulture, forestry and plants protection [90-94].

Research highlights BR's role in plant defense mechanisms, including the induction of pathogenesis-related genes (PR), increased expression of defense-related transcription factors, and regulation of the JA signaling pathway. BR also enhances plant tolerance to oxidative stress and influences plant-microbe interactions, improving overall plant health and productivity [2,17,80].

In summary, understanding the interplay between electrical signals, hormones, and defense mechanisms is crucial for developing effective stress management strategies in plants, with BR emerging as a key player in this process.

### 3.3. Regulation of Gene Expression Related to Immunity

Brassinosteroids (BRs) exert a significant influence on the expression of genes associated with plant defense against both biotic and abiotic stresses [3,6,70,94-97]. Here, we delve into the mechanisms underlying this regulation:

Gene Responses to Stresses: Genes modulated by BR respond to diverse stressors, encompassing those encoding antioxidant enzymes, anti-inflammatory proteins, signal receptors, and other factors implicated in plant defense [45, 98-100].

Signaling Mechanisms Involved in Gene Expression Regulation via BR Signaling Pathways:

- BR Receptor Activation: The initiation of BR signaling cascades hinges upon hormone recognition by membrane-bound receptors. These receptors, positioned on the cell surface, serve as the primary sensors for BRs [28, 45].
- Activation of MAP Kinases: Following BR binding to receptors, mitogen-activated protein kinases (MAP kinases) or other protein kinases undergo activation. These kinases act as pivotal signaling intermediates, transmitting the BR signal to the nucleus [10,45].
- Activation of Transcription Factors: Phosphorylation of transcription factors such as BZR1 (Brassinazole-Resistant a) or BES1 (BRI1-EMS-SUPPRESSOR; b) by activated MAP kinases or other protein kinases modifies their activity, facilitating their translocation into the nucleus [45,58].
- Expression of Defense Genes: Transcription factors like BZR1 and BES1 wield influence over the expression of defense-related genes. They orchestrate the activation of genes involved in plant defense against a spectrum of stresses, both biotic and abiotic. These genes encompass those encoding enzymes for synthesizing chemical compounds, receptors for pathogen recognition, and factors pivotal for the growth and development of defense cells. Consequently, BR-mediated signaling pathways modulate the expression of defense genes, bolstering plants' resilience against stressors and pathogens. Nonetheless, the specific targets and mechanisms underlying these processes may vary contingent upon the plant species and the nature of the stressors [14-15,58].

Pathogen Resistance: A plethora of studies underscores the pivotal role of BR in enhancing plant resistance to pathogen assaults, including those by fungi or bacteria. Alterations in the expression of defense-related genes significantly contribute to this heightened resistance [14, 39, 90]. Research conducted on model plants has unveiled the intricate interplay between signaling pathways involving salicylic acid (SA), jasmonic acid (JA), and ethylene (Et) in modulating plant defense specificity. Investigations into the biosynthesis and signaling of these compounds during infections have corroborated SA's protective role against biotrophic pathogens, which thrive in living cells, while JA and Et synergize to combat necrotrophic organisms that feed on dead or dying cells [17,91, 101].

### 3.4. Regulation of Water and Salt Economy

#### 3.4.1. Drought Response

Brassinosteroids (BRs), a class of plant steroid hormones, play pivotal roles in modulating plant growth, development, and responses to abiotic stresses like drought. Recent research has shed light on several mechanisms through which BR influences plant responses to drought [92-94]:

- Stimulation of Root Growth: BRs have been found to stimulate root growth, which is particularly crucial during drought conditions. A denser root system enhances the plant's ability to absorb water efficiently from the soil, thereby mitigating the effects of water scarcity [11, 98].
- Regulation of Stomatal Dynamics: BRs are involved in the regulation of stomatal opening and closing on leaf surfaces. This regulation is vital for managing water loss through transpiration and optimizing gas exchange. By modulating stomatal behavior, BRs help plants conserve water during drought stress [12, 94-95].
- Protection Against Oxidative Stress: Acting as antioxidants, BRs play a role in safeguarding plant cells from oxidative stress induced by drought. Oxidative damage often arises from an imbalance between the production of reactive oxygen species and the cell's antioxidant defense mechanisms. BRs contribute to maintaining cellular redox homeostasis, thereby protecting plants from oxidative injury [23-24, 63, 71].
- Regulation of Drought-Responsive Gene Expression: BRs are implicated in the regulation of gene expression associated with drought response. Studies have identified genetic links between

BR signaling and the activation of drought-responsive genes, highlighting the involvement of BRs in orchestrating molecular responses to water scarcity [56, 99].

- **Enhanced Water Use Efficiency:** Emerging evidence suggests that BR-treated plants may exhibit improved water use efficiency, allowing them to utilize available water resources more effectively. This enhanced efficiency can confer a competitive advantage to plants facing drought stress, enabling them to maintain essential physiological processes despite limited water availability [55, 96-97].

These insights into the multifaceted roles of BRs in drought response underscore their significance as potential targets for enhancing crop resilience to water scarcity and advancing sustainable agricultural practices in the face of changing climatic conditions.

### 3.4.2. Salinity Protection

Soil salinity, affecting approximately 6% of the Earth's land area, poses a significant challenge to plants and crop productivity worldwide. Saline soils are prevalent in dry and semi-arid climates, but anthropogenic activities such as excessive fertilization, pesticide use, and the application of de-icing salts contribute to salt accumulation even in humid regions. Soil salinity imposes both ionic and osmotic stresses on plants, disrupting metabolic processes, inducing nutrient deficiencies, and exacerbating oxidative stress. Plants respond to salinity through mechanisms aimed at minimizing salt entry and reducing salt concentrations within cells. These adaptive responses encompass a wide range of strategies, from morphological adaptations to alterations in gene expression, underscoring the complexity of plant responses to salinity stress. Understanding these mechanisms is crucial for developing strategies in plant breeding and genetic engineering to enhance salt tolerance in crops [95-96].

BRs play a significant role in plant defense against salinity stress through a variety of adaptive mechanisms, including:

- **Regulation of Stomatal Aperture:** BRs participate in the regulation of stomatal openings on leaf surfaces, thereby facilitating gas exchange and modulating transpiration rates, which helps plants conserve water under saline conditions [94-95].
- **Stimulation of Root Development:** BRs promote increased root development, enhancing the plant's capacity to absorb water and nutrients from the soil, particularly crucial in saline environments where water availability is limited [23].
- **Enhancement of Cellular Structure:** BRs can stimulate the synthesis of lignin, a structural component of cell walls, thereby fortifying cell walls and enhancing resistance to stress induced by salinity [66, 98].
- **Reduction of Oxidative Stress:** BRs mitigate oxidative stress by activating antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase, which scavenge reactive oxygen species and help maintain cellular redox balance under salinity-induced oxidative stress conditions [23, 69, 95].
- **Osmotic Adjustment:** BRs influence the accumulation of osmoprotectants such as proline and polyols, aiding in the maintenance of osmotic balance within cells under saline conditions, thus minimizing cellular damage [99].
- **Regulation of Gene Expression:** BRs modulate the expression of stress-responsive genes, including those involved in salinity defense mechanisms, orchestrating molecular responses to mitigate the adverse effects of salinity stress [56].
- **Promotion of General Growth:** By stimulating overall plant growth and development, BRs enable plants to adapt more effectively to changing environmental conditions, including salinity stress [28, 68].
- **Ion Transport Regulation:** BRs influence ion transport processes, including the uptake and translocation of ions such as sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>), crucial for maintaining ionic balance within plant cells and mitigating the harmful effects of excess salt accumulation [100].

In summary, BRs play a multifaceted role in enhancing plant tolerance to salinity stress, integrating various physiological and molecular processes to bolster plant resilience against this environmental challenge. Understanding the mechanisms underlying BR-mediated salinity

protection holds promise for the development of strategies aimed at improving salt tolerance in crops, thereby contributing to sustainable agriculture in saline-affected regions.

### 3.5. Activation of Plant Defense System by BR

BRs play a pivotal role in activating the plant defense system against both abiotic and biotic stresses by regulating various metabolic and signaling processes. The mechanisms through which BR influences plant resistance to stressors are multifaceted and involve intricate interactions with various defense-related pathways and molecules [37, 65, 88, 101].

**Regulation of Phytohormone Production:** BRs can stimulate the production of phytohormones such as jasmonic acid (JA) and salicylic acid (SA), which are essential for inducing plant defense responses. JA is primarily associated with defenses against pests, while SA is crucial for defense against pathogens [22, 35, 38, 47]. By regulating the expression of defense-related genes, BRs activate signaling pathways that induce the synthesis of defense proteins like Pathogenesis-Related (PR) and Resistance (R) proteins, aiding the plant in combatting pathogens [78, 91].

**Enhancement of Surface Barriers:** BRs may reinforce the plant's surface barriers, making it more challenging for pests and pathogens to attack. This reinforcement could involve the production of physical deterrents such as trichomes or the secretion of chemicals that deter pathogens [91-92].

**Stimulation of ROS Production:** BRs stimulate the production of ROS, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which serve as signaling molecules to activate defense responses and exhibit toxic effects on pathogens [80]. Additionally, ROS influence the regulation of autophagy, a process in which plant cells eliminate damaged organelles and pathogens, aiding in the plant's self-cleansing of infections [22].

**Reinforcement of Cell and Tissue Structures:** BRs may strengthen plant cell and tissue structures, limiting pathogen access to sensitive plant parts [43, 69].

**Control of Photosynthesis Process:** BRs enhance the efficiency of the photosynthesis process, conserving energy and resources that can be allocated towards defense reactions [22, 35, 69, 73-74].

Overall, BRs play a significant role in reinforcing plant resistance to biotic stresses, aiding in the fight against pathogens and pests, and stimulating defense responses [28, 93]. However, the specific mechanisms and effects of BR-mediated defense activation may vary depending on factors such as the type of pathogen, plant variety, and species.

#### 3.5.1. Mechanisms of Phytoalexin Induction by BR

**Transcription Factor Activation:** BR activates transcription factors like BZR1 and BES1, which regulate gene expression. These factors directly or indirectly influence genes involved in phytoalexin production by binding to their regulatory sites [88].

**Interactions with Other Hormones:** BR interacts with other plant hormones such as salicylic acid (SA) or jasmonic acid (JA), which play roles in regulating the plant's defense response. By influencing interactions between different hormonal pathways, BR can activate genes encoding enzymes involved in phytoalexin biosynthesis [78, 81].

**MAPK Signaling Pathway Activation:** BR may activate key MAPK enzymes associated with stress responses, thereby influencing the expression of genes involved in phytoalexin production [78, 81].

**Regulation of Phytoalexin Biosynthetic Enzymes:** BR can affect the activity of enzymes responsible for phytoalexin biosynthesis, such as chalcone synthase and stilbene synthase [81].

#### 3.5.2. BR Impact on Plant Defense Response

**Pattern Recognition and PTI:** BR influences the plant's first line of defense, known as pattern-triggered immunity (PTI), by modulating gene expression related to defense proteins, cell wall reinforcement, and PTI-associated signaling pathways [65].

**Induction of Phytoalexin Production:** BR triggers the production of phytoalexins, antimicrobial compounds, through activated signaling pathways, bolstering the plant's defense against pathogens [22, 28, 65, 102].

**Transcription Factors Involvement:** BR activates transcription factors like BZR1 and BES1, which regulate gene expression involved in biotic stress responses, including the production of defense proteins [78, 81].

**Regulation of MAPK Signaling:** BR influences MAPK kinase activity, essential for relaying stress signals, thereby regulating the expression of genes related to phytoalexin production and other defense mechanisms [58, 81].

**Phytoalexin Biosynthetic Enzyme Regulation:** BR modulates the activity of enzymes like chalcone synthase and stilbene synthase, boosting phytoalexin production upon pathogen detection [53, 55].

**Cell Wall Reinforcement:** BR affects cell wall structure, impeding pathogen penetration and reinforcing the plant's defense mechanisms [48, 88].

These findings underscore BR's intricate role in plant defense responses against pathogens. However, further research is needed to fully elucidate the mechanisms underlying BR-mediated defense regulation [4, 37, 65, 88], as highlighted in recent studies such as those by Sirangelo et al. [103], which delve into the molecular intricacies of plant immunity against diverse pathogens.

### 3.5.3. BR Modulation of Pathogen-Responsive Gene Expression

BR influences gene expression associated with pathogen response, including the induction of phytoalexin production, through interactions with other hormones and signaling pathways [88]. Recent research by Sirangelo et al. [103] sheds light on the molecular mechanisms underlying potential pathogen resistance in *Cannabis sativa*.

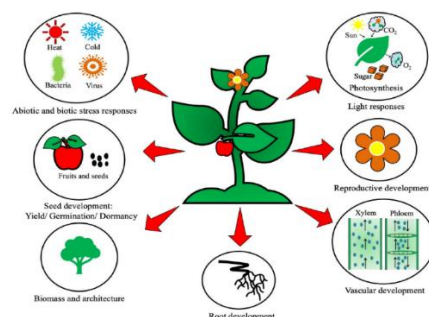
The initial defense response in *C. sativa* involves recognition of pathogen-associated molecular patterns (PAMP) by plant pattern recognition receptors (PRR), activating PAMP-triggered immunity (PTI) against nonspecific pathogens. Two key molecules, Harpin and Flg22, induce genes like FRK1 and PR1, with Harpin additionally activating ERF1, a transcription factor crucial for ethylene-mediated defense responses. Ethylene response factor 1 (ERF1) plays a pivotal role in regulating gene expression in response to ethylene signals, essential for plant defense mechanisms. Furthermore, receptor-like kinases (RLK) participate in defense against *Fusarium*, while reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) activation contribute to PTI [103].

These findings underscore BR's comprehensive role in enhancing plant defense against pathogens and biotic stresses, emphasizing the intricate interactions within the signaling network that warrant further investigation for a complete understanding of BR's mechanisms of action.

## BR and Abiotic Stresses

Plants respond differently to temperature changes, with mild to moderate increases leading to thermomorphogenesis, influencing plant development. However, heat stress negatively affects growth and can cause cellular damage. Plants have sophisticated mechanisms to cope with heat stress, including thermosensors, heat shock proteins, and systemic responses, crucial for agricultural adaptation [104,105].

**BR Responses to Abiotic Stress:** BR aids plants in adapting to various stresses like drought, extreme temperatures, salinity, UV radiation, and heavy metal pollution. It regulates processes such as stomatal aperture control and osmotic compound accumulation, influencing the plant's defense system. BR's role in activating the plant defense system is vital, as these hormones play a key role in plant responses to diverse threats and their ability to survive in changing environmental conditions (Figure 4) [22, 35].



**Figure 4.** Regulation of plant development processes by BR. Source: own adapted to Chaudhuri et al. [27].

#### 4.1. Role of BR in Abiotic Stress Responses

Plants rely on receptor-like kinases (RLKs) to perceive stress signals and initiate intracellular responses. While RLKs are well-known for their role in defense responses to biotic stresses, their involvement in abiotic stress responses is less documented. RLKs consist of extracellular, transmembrane, and intracellular kinase domains, enabling them to respond to various stimuli and regulate growth, reproduction, hormone perception, and defense responses [45]. The functions of RLKs in various abiotic stress responses are discussed in Table 1S.

Polyhydroxylated steroidal phytohormones, such as BR, are essential for regulating plant growth, reproduction, and stress responses throughout the plant's life cycle. BRs impact various physiological processes, including stomatal regulation, photosynthesis, biomass increase, and gene expression stimulation, thereby enhancing stress tolerance [27, 56, 79, 93].

BRs play a crucial role in helping plants combat abiotic stresses induced by factors like drought, temperature extremes, salinity, heavy metals, and air pollution. They influence stomatal regulation, photosynthetic efficiency, antioxidant defense mechanisms, and gene expression, enhancing stress tolerance [28, 40, 55-56, 106-107]. Moreover, BRs interact with other hormones to modulate stress responses, such as increasing ABA content to protect against drought [108].

Temperature fluctuations profoundly affect plant growth and development, with heat stress causing cellular damage and inhibiting photosynthesis. BRs improve thermal tolerance by enhancing antioxidant enzyme levels, mitigating lipid peroxidation, and preventing leaf necrosis and chlorosis under high-temperature conditions [71, 109-111]. Additionally, BR application in heat-sensitive plants increases photosynthetic pigments, assimilation rates, and water-use efficiency [43].

Cold stress negatively impacts plant growth, but BRs alleviate oxidative damage, maintain photosynthetic efficiency, and activate antioxidant enzymes to enhance cold tolerance. BR-induced cold tolerance involves signaling cascades that regulate gene expression, antioxidant capacity, and cellular redox homeostasis [89].



**Figure 5.** Multidirectional effects of BR. Source: own.

BRs play a multifaceted role in plant responses to abiotic stresses, influencing various physiological and molecular processes to enhance stress tolerance and improve plant resilience in changing environmental conditions (Figure 5).

Water stress in plants, caused by soil water deficiency, results in osmotic stress, disrupting plant homeostasis and leading to developmental changes like inhibited shoot growth and intensified root growth [66, 82, 109]. Plant hormones play a crucial role in long-distance communication within plants, with recent research highlighting the significance of hormone movement in the vascular system [9, 38, 78, 103].

Heavy metals, such as chromium, cadmium, nickel, and lead, induce oxidative stress in plants, decreasing photosynthetic efficiency and limiting growth. Excessive accumulation of these metals in plant tissues elevates reactive oxygen species levels, exacerbating oxidative stress [92, 112-115].

Brassinosteroids (BR) alleviate heavy metal stress in plants by promoting phytochelatin synthesis, facilitating metal ion complexation, and enhancing antioxidant enzyme activity to mitigate reactive oxygen species accumulation [116]. Similar benefits are observed in plants exposed to cobalt salts, where BR application enhances antioxidant systems and promotes growth [24]. Additionally, BR application to tomato seedlings growing in cadmium-contaminated medium improves fruiting and fruit antioxidant capacity [117].

Salt stress affects plant growth through ionic and osmotic stress components, leading to inhibited cell growth and stomatal closure. Excessive Na<sup>+</sup> ions cause further defense responses, reducing sodium transport and storing ions in roots [31, 76, 82].

Irfan et al. [68] found that tomato seeds germinated under salt stress and treated with exogenous BR (0.25 and 0.5 ppm via roots and foliar spray) showed improved growth compared to salt-stressed controls. Plants treated with BR exhibited increased plant height, root length, leaf and fruit number, and biomass, mitigating typical salt stress symptoms.

BR, as internal regulators, play a crucial role in plant responses to environmental stresses and developmental processes. They regulate physiological and metabolic processes, acting in a paracrine or autocrine manner. BR biosynthesis genes are widely distributed, and their long-term effects depend on interactions with other hormones [2, 20, 36, 38, 118].

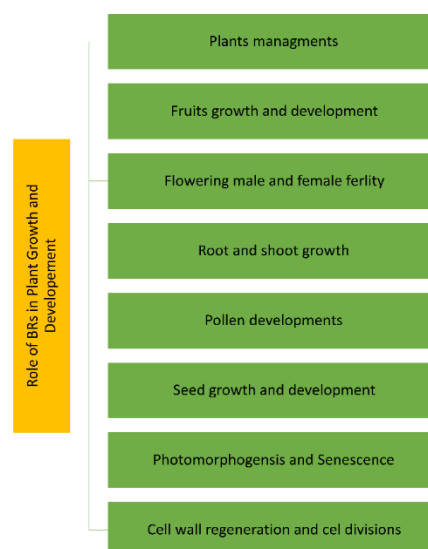
BR contribute to plant protection by enhancing physiological processes, such as growth, organogenesis, and adaptation to environmental changes. They interact with other hormones like auxins and gibberellins and are transported intracellularly from synthesis sites to cell membranes and endosomal compartments [38,118]. However, BR transport occurs from their synthesis sites in the endoplasmic reticulum to the cell membrane and early endosomal compartments, where they are received through passive or active intracellular transport. Here are several ways in which BR can contribute to ensuring plant protection against environmental stresses:

- Growth stimulation: BR promotes plant growth and aids in faster recovery from stress-induced damage by increasing cell length and numbers [38].
- Drought tolerance enhancement: BR helps plants cope with drought by regulating stomatal closure, reducing water loss [93, 97].
- Vascular tissue structure improvement: BR stimulates the formation and maintenance of vascular tissue, enhancing plant resistance to stresses like drought and facilitating water and nutrient transport [38, 41].
- Regulation of stress-related gene expression: BR influences gene expression related to environmental stress responses, activating or inhibiting relevant pathways to help plants adapt [15, 79, 102].
- Increased salt stress resistance: BR enhances plant tolerance to salt stress by maintaining ionic homeostasis and regulating gene expression involved in ion transport [38, 85].
- Oxidative stress prevention: Acting as antioxidants, BR helps plants protect against oxidative damage caused by environmental stresses like UV radiation or air pollution [24, 36, 113, 118].

#### 4.1.1. Leaf Rust Kinase (LRK 10-like)

The LRK10 protein, found in wheat (TaLRK10), and its homolog AtLRK10L1 in Arabidopsis are associated with resistance to leaf rust caused by *Puccinia recondita*. AtLRK10L1.2, a transcript of

AtLRK10L1, plays a crucial role in regulating flowering time, pathogen defense, and ABA signaling during seed germination, especially in drought responses by aiding stomatal closure [25,100,119-120]. Ongoing research investigates LRK10's domains in response to various stresses [25, 100, 120-121]. BR are pivotal in enhancing plant resilience to environmental stresses by regulating growth, adaptive mechanisms, and damage prevention [65,79,100,112,120], influencing developmental processes and gene expression [23, 28, 43]. See Figure 6 for a schematic diagram illustrating BR's key roles in plant life.



**Figure 6.** Selected key roles of BR in plants. Source: own.

BR plays a crucial role in plant responses to abiotic stresses by:

- Enhancing drought tolerance by stimulating the formation of a more effective wax layer on leaf surfaces and regulating drought-responsive genes [23, 43, 68].
- Improving cold resistance through the regulation of genes for cold-resistant proteins and protective enzymes [6, 12, 22, 68, 112].
- Mitigating salt and extreme soil pH stress by maintaining ion homeostasis, regulating enzyme activity, and enhancing adaptability [28, 31, 68, 122-123].
- Reducing oxidative stress by influencing antioxidant enzyme activity to neutralize reactive oxygen species [38].
- Regulating root growth to enhance water and nutrient absorption, particularly crucial in drought conditions [11, 53].
- Controlling stomatal opening and closing to manage transpiration and water loss, especially significant during drought [12, 69].

Overall, BR plays a pivotal role in regulating plant responses to diverse abiotic stresses, aiding plants in survival and adaptation to challenging environmental conditions.

#### 4.2. BR and Plant Immune Activation through Antimicrobial Production

BR influences the production of antimicrobial substances and activates the plant immune system through various mechanisms:

- Stimulation of Phytohormone Production: BR stimulates the production of phytohormones like JA and SA, crucial for activating the plant immune system against pests and pathogens [124].
- Regulation of Defensive Gene Expression: BR regulates the expression of genes encoding defensive proteins, including Pathogenesis-Related (PR) proteins and Resistance (R) proteins, aiding in combating pathogens [90].
- Stimulation of Chemical Substance Production: BR induces the production of antimicrobial compounds like phytoalexins, cardenolide glycosides, and alkaloids, assisting in plant defense [38, 69, 125].

- Regulation of Plant-Pathogen Interactions: BR influences interactions between plants and pathogens, affecting pathogen recognition mechanisms and intercellular communication [125].
  - Enhancement of Cell Cleansing Mechanisms: BR impacts autolysis and autophagy processes, aiding in the elimination of infected or damaged cells to limit pathogen spread [22, 35, 92, 114, 126].
  - Regulation of Nonspecific Defense: BR reinforces cell and tissue structures and stimulates the production of reactive oxygen species (ROS) involved in defense against pathogens [24, 63, 113].
- BR's influence on antimicrobial substance production and plant immune activation varies based on factors like pathogen type, plant species, and ecological context. Despite ongoing research, the precise molecular mechanisms underlying plant tolerance to abiotic stresses remain incompletely understood. Understanding these mechanisms is crucial for addressing global food production challenges caused by various stressors like drought, temperature fluctuations, salinity, and more, impacting plant growth, physiology, and photosynthesis [63, 113, 124].

#### 4.3. Role of BR in Biotic Stresses

BR plays a crucial role in regulating defense processes against pathogens, including the production of antimicrobial compounds like phytoalexins and the activation of the plant's immune response. Manipulating BR levels can impact a plant's ability to combat pathogens, with excessive production potentially reducing resistance to diseases and deficiencies increasing susceptibility to attacks [21, 33, 42, 115, 127].

BR significantly contributes to plant defense against various pathogens and pests. It influences plant responses to biotic stresses through several mechanisms:

1. Enhanced Pathogen Resistance: BR stimulates the production of phytoalexins, strengthening the plant's defense system against infections [127].
2. Activation of Defense Signaling Pathways: BR activates defense signaling pathways, leading to the expression of genes related to pathogen defense and the production of defense proteins [21, 43, 75, 128].
3. Increased Phytoalexin Production: BR enhances the production of phytoalexins, acting as a defensive barrier for the plant [127, 129].
4. Regulation of Insect Pest Responses: BR influences plant responses to pests, affecting the expression of defense-related genes and deterring or harming insects [36, 127].
5. Reinforcement of Immunity: BR stimulates the production of chemical substances with anti-parasitic and antibacterial properties, enhancing overall plant immunity.
6. Regulation of Signaling Pathways: BR strengthens signaling pathways involved in responding to biotic stress, enhancing plant defenses [1, 127, 130].
7. Increased Resistance to Oxidative Stress: BR helps plants withstand oxidative stress induced by pathogens by promoting the production of antioxidant enzymes [36, 80].
8. Regulation of Defensive Cell Development: BR influences the development of defense cells like trichomes, aiding in defense against pests and pathogens [36].
9. Chitin-Chitosan Interaction: BR interacts with chitosan, a natural biopolymer, which can be effective in plant defense when appropriately applied [78, 81, 127].

Overall, BR's role in plant defense against pathogens and pests is crucial, although its effects can vary depending on factors such as the type of pathogen, plant species, and ecological context.

### 5. Interactions Between Plant Hormones in Immunity

Plant responses to stress involve intricate interactions between hormones and signaling pathways, particularly those involving jasmonic acid (JA) and salicylic acid (SA) production. These pathways regulate various stages, including transcription, protein-protein interaction, and targeted protein degradation [36]. Additionally, numerous phytohormones influence plant growth and development, with brassinosteroids (BR) notably enhancing resistance to biotic stresses. BR's impact involves regulating protein and nucleic acid production, influencing metabolic processes like photosynthesis, and modulating enzymatic reactions crucial for stress resilience [30, 33, 42, 92]. Through complex biochemical reactions, BR acts as immunomodulators, strengthening plant ability

to combat pathogens by regulating enzymatic reactions, protein production, and gene expression [33, 42]. Examples include their cross-talk with JA and SA, modulation of enzymatic reactions, induction of defense-related protein production, and modification of gene expression. In summary, BR orchestrates interactions between plant hormones, ensuring a coordinated response to environmental stresses and bolstering plant immunity against pathogens [102, 124].

### 5.1. Absciscic Acid and Salicylic Acid

Interactions between ABA and SA are complex and context-dependent. In some cases, ABA may suppress defense responses triggered by SA, while in others, it may act synergistically or independently. JA is an important hormone in defense against pests and plant pathogens, such as feeding insects and fungi. SA is often involved in defense against pathogens, especially in infections by viruses, fungi, and bacteria. Interactions between JA and SA are complex and depend on the type of pathogen and plant. Antagonism often occurs between these hormones, meaning that an increase in one may inhibit responses induced by the other hormone [124,127].

### 5.2. Interaction of ABA and JA

Interactions between BR and other plant hormones, ABA, and JA, play a significant role in regulating various growth and development processes in response to plant stresses. Key interactions of BR-ABA include:

- Synergism: There is evidence of synergistic interactions between BR and ABA in certain developmental and stress processes. For example, in drought situations, both BR and ABA may be involved in regulating stomatal closure, helping the plant reduce transpiration and water loss [68-69, 84].
- Antagonism: In other cases, BR and ABA may act antagonistically. For instance, BR may stimulate shoot growth, while ABA can inhibit this process. This antagonism may be crucial in balancing plant growth and resilience to abiotic stresses [69, 84].
- Gene Expression Regulation: Both BR and ABA influence gene expression in response to various stresses. In drought conditions, BR and ABA may regulate different sets of genes related to their absence. During drought, ABA levels increase, leading to the activation of signaling pathways controlling the expression of genes responsible for adapting to water deficiency. These genes may be associated with stomatal closure, activation of genes involved in osmotic compound synthesis, or maintaining water homeostasis. On the other hand, BR may be involved in regulating genes related to long-term stress adaptation, such as controlling the expression of genes associated with processes like stomatal closure, root growth, or activation of signaling pathways related to plant defense against stress, while ABA focuses on faster adaptive responses [69, 81, 84].

In summary, the interplay between BR and ABA involves complex synergistic and antagonistic interactions, influencing various aspects of plant growth and stress responses. These interactions are context-dependent, and their understanding is crucial for unraveling the intricacies of plant hormone signaling.

### 5.3. BR-JA Interactions

BR and JA often act antagonistically in regulating various processes. For instance, BR may stimulate shoot and flower growth, while JA is known for its role in defense against pests and immune responses against pathogens. In some cases, acting antagonistically, BR may suppress JA activity, and vice versa, helping plants adapt to changing conditions. Hayat et al. demonstrated the influence of rootstocks on scion vigor and hormonal mechanisms related to dwarfing, such as IAA, CK, ABA, GA, and BR. Both BR and ABA play a crucial role in regulating gene expression in response to drought, adapting plants to challenging environmental conditions. Together and individually, they influence various aspects of responses to water deficiency, adjusting plant growth, development, and defense reactions [69, 81, 102].

The interaction of BR-JA may be crucial in plant defense against pathogens and pests. JA is often involved in defensive responses against these threats, while BR may assist the plant in maintaining growth and development in the face of attacks, simultaneously stimulating defense by regulating the expression of defense genes [69, 84, 102, 124].

Both BR and JA can influence the production of phytoalexins, chemical substances with antibacterial and antiparasitic properties, crucial components of plant defense against pathogens [69, 84, 102].

Interactions between BR, ABA, and JA are context-dependent and may vary depending on the plant variety and species, the type of stress, and the plant's developmental stage. These plant hormones collaborate to provide a response to changing environmental conditions, balancing growth, development, and defense as needed. BR can interact with both ABA and JA, influencing plant growth and development processes as well as the regulation of defense responses [36,81,84].

Interactions between BR and other plant hormones are complex and context-dependent. They can act synergistically, antagonistically, or independently concerning other hormones, depending on the type of stress and the plant. For instance, in the case of CK and GA, interactions between CK and GA are essential for regulating plant growth and development processes, impacting the plant's ability to counteract biotic and abiotic stresses through changes in plant architecture. Interactions between plant hormones are dynamic and depend on many factors [69,78,81,93, 102]. Plants adjust their defense responses based on the type of threat and hormonal signals they receive. Therefore, researchers continue to delve into these complex mechanisms to better understand how plants regulate their immunity and growth in changing environmental conditions.

6. Practical Applications of BR

BR enhances plant resistance to both biotic and abiotic stresses, such as drought, extreme temperatures, salinity, oxidative stress, toxic heavy metals, UV radiation, and attacks by pathogens. Their ability to defend against stress and regulate development is associated with the regulation of specific proteins and nucleic acids, as well as their influence on metabolic processes related to photosynthesis [21, 30, 33, 42, 92].

6.1. Potential Applications of BR in Agriculture and Horticulture

BR has several practical applications in agriculture and plant protection, although its use is relatively new and still under investigation [130-132]. The potential applications of BR in these fields are illustrated in Figure 7.

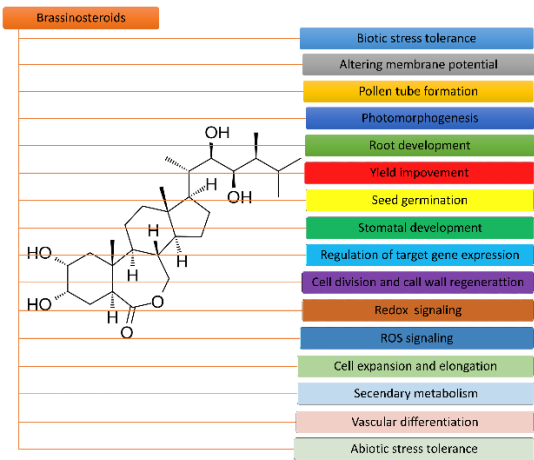


Figure 7. The importance of BR in agriculture, horticulture and plant protection. Source: own.

Enhancing Crop Yield and Quality: BR stimulates plant growth and development, potentially increasing crop yield and improving characteristics like size, shape, texture, taste, and color of fruits or flowers [68, 128, 132-133].

**Protection Against Abiotic Stresses:** BR aids plants in coping with extreme environmental conditions like drought, low temperatures, and soil salinity by enhancing plant tolerance to these stresses. They regulate stomatal opening and closing, controlling transpiration and water loss [44, 93, 97].

**Protection Against Pathogens and Pests:** BR stimulates the plant's immune system, aiding defense against fungal, bacterial, and viral infections. They influence the production of antimicrobial substances and activate defense reactions against pests, affecting plant development and reducing pest and disease damage [69, 130].

**Root System Stimulation:** BR impacts root development, increasing the plant's ability to absorb water and nutrients, especially under abiotic stress conditions [53].

**Acceleration of Maturation and Fruiting Processes:** BR can accelerate fruit maturation, benefiting agricultural and horticultural production and fruit availability in the market [60, 86].

**Mitigation of Oxidative Stress Effects:** BR helps plants protect against oxidative stress by neutralizing reactive oxygen species and preventing cell damage [24, 118].

**Applications in Plant Breeding and Biotechnology:** BR can be used in breeding programs to obtain varieties more resistant to stresses and in genetic engineering to modify plants for improved tolerance to environmental conditions. Studies on *Arabidopsis* mutants affecting the BR biosynthesis pathway have contributed to understanding BR's role in regulating plant growth and the role of BR receptors in this signaling pathway [20-21, 134].

While BR shows potential for improving plant productivity and resilience, further research optimizing doses, application timing, and considering ecological and environmental safety is necessary for effective application in agriculture, horticulture, and plant protection.

## 6.2. Significance of BR in Cell Division and Elongation

BRs participate in the regulation of plant growth by influencing two cell processes: cell expansion and division [9, 28]. They impact the elongation of hypocotyls, epicotyls, and stems in dicot plants, as well as coleoptiles and mesocotyls in monocot plants [26, 135]. These hormones play a role in cell enlargement, involving gene expression and enzymatic activity [78]. In *Arabidopsis*, leaves emerge on the sides of the shoot apical meristem through cell division, followed by a gradual maturation characterized by the completion of the cell cycle and cell expansion [59]. Genetic, hormonal, and environmental factors determine leaf growth and development [96]. Das et al. [131] demonstrated the beneficial impact of BR on cell elongation and division in studies on the second internode of beans. Stimulating effects of BR, auxin, and cytokinin on cell division in bean parenchyma cells were also shown by Bariş and Sağlam-Çağ [25], Li et al. [136], as well as in cabbage protoplasts by Cimino et al. [137] and petunia by Cui et al. [138].

### 6.2.1. BR in Seed Development and Germination

Seed germination in plants like *Arabidopsis thaliana* and *Nicotiana tabacum* relies on a delicate balance between the growth potential of the embryo and the mechanical constraints imposed by the micropylar endosperm [129, 139]. BR application has been shown to promote seed germination and counteract the dormancy-inducing effects of abscisic acid (ABA), particularly in stressful conditions [64].

Chakma et al. [64] studied cotton seed germination under various hormone treatments and found that BR facilitated germination even under stress conditions like heat and salinity. It also promoted cotyledon opening and lateral root development. Similarly, research on rice seeds by Xiong et al. [13] demonstrated that both GA and BR enhanced germination and post-germination growth, with GA compensating for BR deficiency or insensitivity. Baset et al. [63] observed increased germination parameters in rice with BR treatment, despite the presence of toxic aluminum.

Thongsri et al. [140] investigated the effects of BR and GA3 on soybean seed germination at low temperatures, where BR and GA3 application helped overcome delayed germination due to cold stress. Additionally, Hayat and Ahmad [141] showed positive effects of 28-homobrassinolide (HBR) treatment on wheat seed germination and enzyme activity.

In another study, Kumar et al. [133] examined mung bean response to zinc stress and BR treatment, noting significant improvements in germination percentage and seedling growth with BR application, albeit with reduced antioxidant enzyme activity.

Finally, Kiliç et al. [98] found that 24-epibrassinolide (EBR) positively impacted barley seed germination and root elongation under saline stress conditions.

In summary, BR application shows promise in enhancing seed germination and seedling growth, particularly under adverse environmental conditions. However, further research is needed to optimize its application for maximum benefit while considering potential trade-offs in antioxidant activity.

#### 6.2.2. BR in Root and Shoot Growth

The impact of BR on root and shoot growth is influenced by factors such as concentration, treatment duration, plant stage, and application method. Lower concentrations of BR typically stimulate root growth, while higher concentrations can inhibit it [7].

In a study on *Eucalyptus grandis* Hill, Chinese researchers investigated the effects of arbuscular mycorrhizal fungi symbiosis and BR application on root growth. They found that increasing concentrations of exogenous 24-epibrassinolide initially increased the mass and length of fresh taproots but eventually led to a decrease in growth [142].

Liu et al. [57] studied the effects of homobrassinolide (HBR) on barley root growth and observed enhanced mitotic activity and significant root tip enlargement compared to the control group.

Meanwhile, research by Nováková et al. [143] focused on the application of BR and its effects on Scots pine seedlings. They noted that BR treatment slowed down both the height and radial growth of the seedlings. Specifically, the height increment of BR-treated seedlings was 11% lower, and the diameter increment at the root collar was 13% lower compared to the control. Additionally, BR-treated pine trees exhibited higher mortality rates compared to the control group.

Overall, while BR can have varying effects on root and shoot growth depending on concentration and application method, it is crucial to consider its impact on overall plant health and survival when applying it in agricultural or horticultural settings.

#### 6.2.3. BR Effects on Plant Growth, Photosynthesis, and Aging

The influence of BR on root and shoot growth is concentration-dependent, with lower concentrations stimulating root growth and higher concentrations inhibiting it [7]. Studies on *Eucalyptus grandis* Hill roots showed increased mass and length with higher exogenous BR concentration [142]. Homobrassinolide (HBR) positively affected barley root growth, demonstrating higher mitotic activity compared to the control [57]. However, BR application in Scots pine seedlings slowed down height and radial growth, with increased mortality compared to the control [143].

BR plays a crucial role in maintaining photosystem II (PSII) performance, especially under stress conditions. Exogenous BR application in rice under heat stress helped maintain net photosynthetic rates and improved water use efficiency [128]. Similar positive effects on rice photosynthesis were observed with the application of HBL under cold stress [72].

BL, including 24-epibrassinolide (EBR), proved effective in alleviating growth inhibition in melon under high-temperature conditions [43]. Cucumber seedlings treated with 28-homobrassinolide showed higher antioxidant enzyme activity and proline content under chilling stress [72].

The impact of BR analogs on leaf aging varied among plants. In papaya, BR maintained the greenness of older leaves, while in soybean, aging was accelerated after epibrassinolide (EBL) application [25, 61, 144-146]. Sunflower leaf aging, influenced by EBL, showed differences between upper and lower leaves, possibly linked to auxin distribution [147].

In summary, BR plays a multifaceted role in regulating plant growth, photosynthesis, and aging under various environmental conditions.

#### 6.3. BR under Salinity and Heavy Metal Concentrations

Soil salinity poses a significant challenge to plant growth, often leading to toxic metal ion accumulation and nutrient imbalances. Studies have shown that the application of brassinosteroids (BRs) can mitigate the negative effects of salinity stress on plants.

In research conducted by Pakistani scientists, *Lycopersicon esculentum* (tomato) plants treated with NaCl experienced decreased growth. However, the application of BR enhanced various plant parameters, both in stressed and non-stressed soil conditions [68].

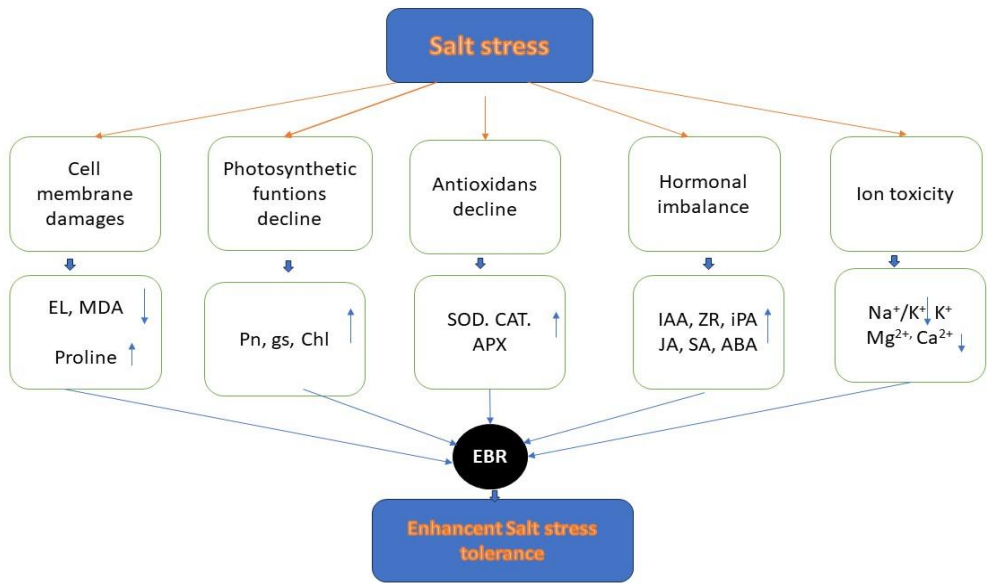
Optimizing mineral nutrient levels and the application of plant growth regulators are crucial strategies for enhancing crop yield under stress conditions. Abbas et al. [17] suggested potassium and jasmonic acid supplementation for crops irrigated with water containing heavy metals, providing a potential solution to mitigate the adverse effects of heavy metal stress.

Rice plants subjected to salt stress (100 and 200 mM NaCl) showed improved germination, growth, and photosynthesis when treated with BR. Additionally, BR treatment enhanced the activities of antioxidant enzymes, helping to counteract the oxidative stress induced by salinity [100].

Pea seeds soaked in 24-epibrassinolide (EBL) along with sodium chloride (NaCl) exhibited increased germination rates and growth parameters, as well as enhanced photosynthesis and antioxidant activities, indicating the protective role of BR against salt stress in pea plants [122].

In rapeseed plants subjected to salt stress, exogenous application of 24-EBL mitigated the negative effects of salinity, resulting in improved growth, photosynthesis, and nutrient content in both roots and shoots [31]. Additionally, research by Efimova et al. [148] confirmed the protective action of EBL on rapeseed, highlighting its role in restoring growth and preventing lipid peroxidation. Furthermore, investigations by Houimli et al. [149] explored the role of 24-EBL in enhancing salt tolerance in pepper plants. Their findings indicated improved growth and maintenance of cell membrane integrity in pepper plants treated with BR under salt stress conditions [149].

- A mechanism depicting the involvement of 24-epibrassinolide (EBR) application in conferring salt stress tolerance in perennial ryegrass can be outlined as follows [150] (Figure 8):
- Activation of Stress Signaling Pathways: Upon exposure to salt stress, perennial ryegrass plants perceive the stress signals, leading to the activation of stress signaling pathways.
  - Recognition and Uptake of EBR: Exogenous application of EBR to the plants allows for its recognition and uptake, either through the roots or foliar application.



**Figure 8.** This mechanism illustrates how exogenous application of 24-epibrassinolide (EBR) facilitates the adaptation of perennial ryegrass to salt stress by modulating multiple physiological and molecular processes involved in stress tolerance. Source: own.

- **Brassinosteroid Signaling Cascade Activation:** EBR binds to its receptor, initiating a signaling cascade that involves various downstream components, such as BRASSINAZOLE-RESISTANT (BZR) transcription factors and BRI1-EMS-SUPPRESSOR 1 (BES1), leading to the activation of stress-responsive genes.
- **Enhanced Ion Homeostasis:** EBR application modulates ion homeostasis by regulating the uptake and compartmentalization of ions, particularly sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ), thus preventing their toxic accumulation in plant tissues.
- **Osmotic Adjustment:** EBR promotes osmotic adjustment by enhancing the accumulation of compatible solutes such as proline and sugars, which help maintain cellular turgor pressure and osmotic balance under salt stress conditions.
- **Antioxidant Defense Activation:** EBR treatment boosts the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), scavenging reactive oxygen species (ROS) and minimizing oxidative damage to cellular components.
- **Maintenance of Photosynthetic Machinery:** EBR application preserves the integrity and functionality of the photosynthetic apparatus by mitigating the adverse effects of salt stress on chlorophyll content, photosystem efficiency, and carbon assimilation rates.
- **Stimulation of Growth and Development:** Despite salt stress, EBR promotes plant growth and development by modulating hormone signaling pathways, particularly auxins, cytokinin's, and gibberellins, which contribute to enhanced root and shoot growth and biomass accumulation.
- **Regulation of Stress-Responsive Gene Expression:** EBR-mediated regulation of stress-responsive genes involved in various physiological and biochemical processes helps orchestrate adaptive responses to salt stress, thereby improving overall stress tolerance in perennial ryegrass.
- **Overall Improvement in Salt Stress Tolerance:** Through these mechanisms, EBR application confers enhanced salt stress tolerance in perennial ryegrass, enabling the plant to withstand adverse environmental conditions and maintain optimal growth and productivity [150].

As a result, exogenous 24-epibrassinolide (EBR) may: improve the integrity and stability of the cell membrane (lower MDA, EL, higher proline), enhance photosynthetic function (higher  $\text{Pn}$ ,  $\text{gs}$ , and chlorophyll), enhance antioxidant defense (resulting in higher activity of SOD, CAT, and APX), increase hormonal metabolism (higher levels of IAA, ZR, iPA, JA, SA, and ABA), and reduce ion toxicity (lower  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$ , higher  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$ ), leading to increased tolerance to salt stress and visual quality of plants [151] (Figure 8).

These studies collectively demonstrate the potential of BRs to alleviate the adverse effects of salinity and heavy metal stress on plants, highlighting their significance in enhancing plant resilience to environmental challenges.

#### 6.4. Enhancing Crop Yield and Resilience with BR: Potential Strategies and Benefits

BR manipulation holds promise for enhancing crop yield and resilience against various stresses in agriculture. Key strategies and potential benefits include:

- **Growth Promotion:** Elevating BR levels stimulates the growth of stems, leaves, and roots, potentially leading to increased yield mass [51].
- **Abiotic Stress Tolerance:** Manipulating BR levels can enhance plant tolerance to stresses like drought, low temperatures, and soil salinity, aiding in maintaining better homeostasis and reducing damage [73, 84].
- **Defense Against Pathogens and Pests:** BR manipulation may bolster plant resistance to pathogen infections and pest attacks by stimulating the immune system [28, 93].
- **Fruit Ripening Control:** Controlled BR application can accelerate fruit ripening, benefiting agricultural production and marketing [60, 127].
- **Plant Breeding Support:** BR manipulation can expedite the development of productive and stress-resistant plant varieties, facilitating selection processes [56, 127].
- **Improved Water and Nutrient Use Efficiency:** Increased BR levels influence root development, enhancing a plant's ability to extract water and nutrients from the soil, especially under stressful conditions [23, 28, 44-45, 56, 95].

It's important to acknowledge that manipulating BR levels is nuanced and context-dependent, requiring precise control of doses and application timing. Environmental and safety considerations are paramount when using these substances in agriculture. Ongoing research aims to fully grasp the potential benefits and complexities of BR manipulation for agriculture and plant protection [149-151].

## 7. Towards the Future

In the face of climate change challenges, leveraging BR offers promising strategies to improve plant tolerance to abiotic stresses. Key approaches include:

- Advanced Research Tools: Precision translomics and fluorescence-activated cell sorting (FACS) enable a deeper understanding of how plants respond to BR under stress, facilitating the mapping of BR interactions with other hormones.
- Exogenous BR Application: Utilizing BR application can boost plant resilience, particularly in cereal crops. Genetic manipulation of BR-related genes can pave the way for developing transgenic varieties with heightened stress resistance.
- Exploring Hormone Interactions: Investigating how exogenous BR impacts the synthesis and signaling of other hormones under stress conditions is crucial. Combining different plant hormones may offer synergistic strategies to enhance stress tolerance.
- Genetic Engineering: Manipulating BR-related genes through genetic engineering, coupled with advanced research tools, holds promise for increasing crop yields by bolstering tolerance to abiotic stresses.

While these strategies require further research, their potential to address global food demand by bolstering plant resilience underscores the importance of BR in agriculture.

## 8. Conclusions

Understanding the complex interactions between BR and other plant hormones is crucial for harnessing their potential in agriculture.

Manipulating BR levels shows promising results in increasing yields, enhancing resistance to abiotic stresses, and defending against pathogens and pests. Advanced research tools, such as precision translomics and genetic engineering, offer opportunities for deeper understanding of BR mechanisms and their applications in agriculture.

Further research and experiments are necessary to optimize strategies for manipulating BR levels and ensure their effectiveness across different plant species and environmental conditions.

Despite challenges, the potential of utilizing BR in agriculture is significant and offers promising solutions that may contribute to addressing global food security issues in the face of climate change.

BR plays a crucial role as plant hormones influencing various aspects of plant development and adaptation to changing environmental conditions. Conscious manipulation of BR levels can significantly impact crop productivity and their resilience to stresses and pathogens.

Understanding these mechanisms can contribute to the development of more effective strategies to improve the resilience of cultivated plants to various environmental challenges.

## List abbreviations

ABA – Absciscic Acid  
 BL – Brassinolide  
 BR – Brassinosteroid  
 BZR – Brasinol  
 CAT – Catalase  
 CH<sub>4</sub> - methane  
 CK – Cytokinins  
 CS – Castasterone  
 EBL – benzylaminopurine  
 EBL – Epibrassinolide  
 EBR – 24-epibrassinolide  
 ERF – Ethylene Response Factor  
 FRK1 - Fusarium-Induced Receptor Kinase  
 GA – Gibberelins  
 HBR – Homobrassinolide  
 IAA – indolyl-3-acetic acid  
 IAN – indolylacetonitrile  
 IBA – Indolyl-3-butyric acid

IBA – indolyl-3-butyric acid  
 IPA – indolyl-3-propionic acid  
 JA – jasmonate  
 MAPK – Mitogen-Activated Protein Kinase  
 MRW - indole-3-butyric acid  
 NiRA – Nitrate Reductase  
 PAA – phenylacetic acid,  
 PAMPs – Pathogen-associated molecular proteins  
 POD – Peroxidase  
 PR - Pathogenesis-Related Genes  
 PRRs – Pattern Recognition Receptors,  
 PSII – photosystem II  
 PTI – Pattern Triggered Immunity  
 RLK – Receptor-Like Kinases  
 ROS – Production of reactive oxygen species  
 ROS – Reactive Oxygen Species  
 SA – salicylic acid  
 SERK – Somatic Embryogenesis Receptor-Like Kinase  
 SOD – Superoxide Dismutase  
 SQE – Squalene Monooxygenase Enzyme  
 TZ – Tezasterone  
 WRKY46, WRKY54, WRKY70 – regulators of proteins from the WRKY family activated by BR

**Supplementary Materials:** Table S1: title: Functions of RLKs in different abiotic stress responses.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization: B.S., B.B., and P.B.; methodology: B.B., B.S., P.P.; software: D.S., P.P.; validation: D.S.; formal analysis: P.B., B. K-M., investigation: P.P.; resources, B.B., P.B., B. K-M.; data curation: D.S., P.P.; B.K-M.; writing—original draft preparation: B.B., B.S., P.B., D.S.; writing—review and editing, B.S., P.P., visualization: D.S., supervision, B.S.; B.K-M.; project administration: P.B.; funding acquisition: B.S. All authors have read and agreed to the published version of the manuscript.”.

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