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BRASSINOSTEROIDS AS BIOACTIVE STEROIDS: A REVIEW OF STRUCTURE–ACTIVITY RELATIONSHIPS AND BIOCHEMICAL MECHANISMS OF ABIOTIC STRESS MITIGATION

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Brassinosteroids (BRs) are a class of polyhydroxylated steroid plant hormones that participate in a wide range of physiological, biochemical, and molecular processes, including seed germination, cell division, and elongation, vascular differentiation, photomorphogenesis, photosynthesis, enzyme activation, and senescence. Adaptation to fluctuating environmental conditions is a fundamental feature of plant life, regulated by complex mechanisms that optimize resource allocation. Brassinosteroids enhance plant tolerance to numerous abiotic stresses. In this review, we focus on the ability of BRs to improve plant adaptability under abiotic stress. Abiotic stress represents a major constraint on agricultural productivity, as it substantially reduces crop yield. The effects of abiotic stressors on plants are complex, and many underlying mechanisms have only recently been elucidated. Nevertheless, the mitigation of abiotic stress through the application of plant hormones continues to attract considerable research interest. Plants adapt to different environmental stresses by altering physiological and molecular processes that are co-regulated with changes in the levels of phytohormones, including BRs. Most studies have focused on the external application of BRs to stimulate growth, increase crop yields, and enhance tolerance to abiotic stresses. However, plant growth and development are inseparably linked to the coordinated interaction between endogenous phytohormones and environmental signals. Overall, this review discusses brassinosteroid biosynthesis, BR-mediated signaling, and the role of endogenous BRs in regulating plant responses to abiotic stressors. Based on the available research, it is expected that the practical use of BRs to alleviate the negative impacts of abiotic stress on plant vitality will continue to expand. Furthermore, a deeper understanding of their mechanisms of action will bring undeniable benefits to both basic and applied plant science. Ultimately, understanding the mechanisms by which BRs regulate stress responses in crops will likely evolve into an independent direction of research.

Keywords: brassinosteroids, abiotic stress, plant adaptation, structure–activity relationships, biochemical mechanisms.

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Functional role of brassinosteroids in the formation of plants' resistance

Brassinosteroids (BRs) are a class of polyhydroxylated steroid plant hormones. Although BRs belong to the class of steroid compounds based on their structural features, they possess distinctive characteristics that are critical for their biological activity, particularly in plant systems. These features will be examined in detail in the present review.

BRs participate in numerous physiological, biochemical, and molecular processes such as seed germination, cell division and elongation, vascular differentiation, photomorphogenesis, photosynthesis, enzyme activation, and senescence. They are also known to protect plants from various abiotic and biotic stressors, including salinity, temperature extremes, drought, heavy metals, and pathogens. BRs enhance the yield of cereals, legumes, and oilseed crops and improve both productivity and fruit quality in horticultural species [1,2].

Disruption of BR signal transduction negatively affects multiple developmental processes such as seed formation, flowering time, and pollen maturation. BRs coordinate tropic responses of plant organs by regulating polar auxin transport. Given their pivotal role in plant growth, development, and stress responses, BRs are considered promising targets for manipulation to improve agronomic performance [3].

While BRs control several key agronomic traits-flowering time, plant architecture, seed yield, and stress tolerance-genetic modification of BR biosynthesis, transformation, or uptake offers an extraordinary opportunity to significantly increase yield by modulating plant metabolism and protecting plants from adverse factors. Leaf angle, plant height, and inflorescence architecture are three crucial yield determinants potentially regulated by BRs [4–7]. BRs play a critical role in controlling leaf erectness by inhibiting the division of abaxial sclerenchyma cells, which provide mechanical support to lamina joints in Oryza sativa under clustering conditions [8].

Adaptation to changing environmental conditions is a universal feature of plant life, regulated by fundamental mechanisms that optimize resource allocation. This balance is achieved through tightly coordinated communication between growth and stress-response signaling pathways. Understanding the interactions between BRs-key hormones that control growth and stress adaptation; general stress responses provide a valuable framework for discovering new aspects of plant resilience.

BRs induce plant tolerance to a wide range of stresses. Rapidly changing environmental conditions can severely damage plants, and the modern stress-laden environment poses significant challenges to plant growth and yield formation [9]. Notably, plant responses to BRs vary depending on species, developmental stage, and environmental conditions. Moreover, other hormones and signaling molecules that fine-tune BR effects also contribute significantly to stress adaptation [10,11].

Molecular genetic analyses of Arabidopsis thaliana by Hardtke et al. revealed that BRs regulate physiological and developmental processes not independently but in conjunction with another plant hormone, auxin [12]. Together, these hormones modulate cell expansion and proliferation, exhibiting overlapping and synergistic effects. Such interactions likely occur through the combinatorial regulation of shared target genes by auxin- and BR-responsive transcription factors. Furthermore, auxin and BR signaling – as well as auxin biosynthesis and transport – may be interconnected through upstream components involving calcium-calmodulin and phosphoinositide signaling.

In this review, we focus on the ability of BRs to improve plant adaptability to abiotic agents. Abiotic stress is a major factor hindering agricultural development and significantly reducing crop yields. Although the effects of abiotic stressors on plants are complex and only partially elucidated, the use of phytohormones to mitigate these effects remains an active area of research. Plants have evolved to withstand environmental stresses through physiological and molecular adjustments co-regulated by intrinsic and extrinsic phytohormone levels, including BRs [13]. Studies using *Solanum lycopersicum* demonstrated that BR signaling pathways participate in mediating responses to abiotic stress by fine-tuning stress-related gene networks [14].

Most studies have focused on exogenous BR application to promote growth, enhance yield, and strengthen tolerance to abiotic stress. However, plant growth and development cannot be separated from the coordinated interaction between endogenous phytohormones and environmental signals. This paper reviews BR biosynthesis, BR-mediated signaling, and the roles of endogenous BRs in regulating plant responses to abiotic stressors [15].

Contribution of structure of BRs to their functional properties and activity

Regio- and stereospecificity are essential for the activity of BRs. This is realized through shape compementarity. For example, BSs are known to bind with BRI1 receptor in narrow hydrophobic pocket, where the orientation of hydroxylic groups and the configuration of steroid skeleton provide the stabilization of complex by the mechanism, described by induced fit model [16]. Even minor changes in stereostructure (e.g., epimerization of individual centers or alternations of the configuration of rings) can considerably reduce the affinity for receptors and consequently biological activity, as it was demonstrated on various natural and synthetic BSs [17]. Steroid functional activity, in the sense of selective interaction with specific receptors in plant and animal cells, is characteristic across biological kingdoms.

Despite sharing common structural and functional features with other biogenic steroids, BSs exhibit several distinctive characteristics:

– *chemical specificity*: BSs are characterized by distinct structural motifs, including a 2α,3α-diol in ring A, oxygenated groups in ring B (often in the form of a lactone), and a side chain specifically modified at the C-24 position.

– *biologic importance*: the presence of a specific receptor system in plants, together with its absence in other biological kingdoms, supports the classification of BSs as a distinct group within steroid compounds.

Therefore, the classification of brassinosteroids as a distinct group of steroids is determined less by their origin than by the unique combination of their chemical structure and functional properties. Indeed, BSs constitute a distinct subclass characterized by a well-defined relationship between structure and function. This relationship is observed predominantly, if not exclusively, in plants [18]. Researchers foreground the following structural features of BSs:

– the existence of 2α,3α-diol in ring A, as a characteristic feature of active BSs [18,19];

– typical modifications of B ring: 6-ketone or 7-oxolactone) [20];

– variations in side chain (C-24-alkilation, C-22- and C-23-diols) [21].

Within the subclass of BSs, further classification exists based on a set of structural features directly related to the ability to interact with the BRI1 receptor. The most acceptable is the division by the oxygenation of steroid skeleton, whereby C-27-, C-28-, and C-29-BSs are identified. This classification reflects the variations in side chain (particularly, C24-alkilation), which affect on spatial organization of molecule as well as on its affinity for receptor. C-28-BSs, including brassinolide (the derivates of campesterol) tend to exhibit the higher biological activity, because of favorable optimal combination of hydrophilic (polar) and hydrophobic interactions in binding pocket of BRI [22,23].

The structure of ring B is another important criterion that allows discrimination between 6-oxo-BSs and 7-oxolactones. The presence of 7-oxolactonic structure (e.g., in brassinolide) is associated with maximal bioactivity, since this functional group is essential for the formation of specific hydrogen bonds with amino acid residues of receptor. The transition from a ketonic to a lactonic structure is accompanied by a considerable increase in activity. This was supported by both biological assays and structural analyses of the ligand–receptor complex [24,25].

In addition, the stereochemistry of the hydroxyl groups at the C-2, C-3, C-22, and C-23 positions, which constitute the BSs' pharmacophore, is of great importance. The most active compounds are characterized by 2α,3α-diol and 22R,23R-diol configurations. Even slight deviations, such as epimerization or changes in relative configuration, result in a drastic loss of activity. Distortion of the geometry of hydrogen bonds and hydrophobic interactions within the receptor binding pocket explains this loss and indicates the strict stereospecificity of these interactions [25,26].

In light of the foregoing, the diversity of BS variants serves to fine-tune ligand-receptor interactions.

Temperature stress

Plants respond to heat stress primarily through the down-regulation of leaf transpiration, which leads to water deficit and, consequently, further reduction in transpiration. Heat stress also causes damage to cell membranes and protein denaturation. As a result, the consistency of metabolic processes is disrupted: cells die due to the accumulation of toxic intermediate products of metabolism, such as soluble nitrogenous compounds. High temperatures additionally impair cell membrane permeability and intracellular exudation of primary substances, induce a surge in reactive oxygen species (ROS) levels, radicals, and oxidative stress, and inhibit photosynthesis, plant respiration, and phytohormonal activity [27,28].

To minimize heat damage, plants accumulate osmoregulators. The accumulation of endogenous hormones stabilizes plant cell membranes under hyperthermal stress and enhances the productivity of the photosynthetic apparatus, thereby increasing plants' tolerance to high temperatures [15].

Heat shock tolerance is also induced by exogenous brassinosteroids (BRs). For instance, [29] demonstrated that BRs mediated the effects of high temperature stress on pistil activity in *Oryza sativa* L. during ovary formation, alleviating the adverse effects of extreme heat by increasing antioxidant levels and inhibiting ROS production. Similarly, Yang et al. showed that the combined application of BRs and jasmonates mitigated disorders in spinose structure opening and improved pistil receptivity under hyperthermal stress [30]. The main physiological mechanisms through which BRs and jasmonates alleviate heat stress include activation of the defense system, strengthening of osmotic regulation, protection of photosynthesis, and interaction with other phytohormones, particularly ethylene and abscisic acid. Sonjaroon et al. [31] reported that the brassinosteroidal mimetic 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD) enhanced photosynthesis and mitigated hyperthermal stress by increasing net photosynthetic and transpiration rates, stomatal conductance, and stomatal limitation, while down-regulating intercellular CO_2 concentration and water use efficiency. αDHECD also increased total soluble sugar content in *O. sativa* leaves at both normal and elevated temperatures and improved yield parameters, including kernel number, weight, and sugar and starch content in both straw and seeds.

Hypothermal stress is another major factor limiting plant growth, yield, and vegetation coverage globally. Low temperatures inhibit plant growth, disrupt photosynthesis, reduce chlorophyll content, cause flower bud abortion, and result in significant yield losses [32]. Cold stress affects membrane fluidity, macromolecular interactions, cell osmotic pressure, and imposes mechanical restrictions [33]. Photosynthetic activity is compromised through reduced CO_2 assimilation, photo-inhibition in photosystems I and II, and inhibition of enzymatic activity [34].

Enhancing frost resistance by even 2–3^{0}C can significantly improve crop yields, especially in areas previously unsuitable for cultivation due to low temperatures. Such regions, termed «hazard agriculture zones», cover much of Ukraine. Therefore, studying plant adaptation to low temperatures is critical for developing methods to enhance crop cold and frost tolerance.

Brassinosteroids have been shown to mitigate low-temperature and chilling stress by increasing chlorophyll content, maintaining photosynthetic activity and carbohydrate metabolism, modulating defense enzymes, reducing toxic ion levels, activating gene expression, and up-regulating signal transduction pathways and endogenous plant hormones [35–38]. BRs also increase endogenous salicylic acid and jasmonic acid levels and up-regulate ethylene biosynthesis, indicating their role in synergistic crosstalk with these hormones to counter chilling stress [36].

BRs enhance cold resistance by inducing genes associated with antioxidant defense and proline accumulation [39]. In experiments on Lycopersicon esculentum, gradual exposure to decreasing temperatures (from 20/14^{0}C to 12/7^{0}C and then 10/3^{0}C) caused a decline in chlorophyll content, net photosynthetic rate, and carbonic anhydrase activity; treatment with BRs combined with hydrogen peroxide improved these parameters and up-regulated antioxidant enzyme activities (catalase, superoxide dismutase) and proline accumulation [40].

Studies on winter wheat seedlings revealed that combining BRs with progesterone increased the lipid area in monolayers, creating more flexible surface structures, whereas 24-epicastasterone had the opposite effect. Lipids from frost-resistant wheat husks had higher fatty acid unsaturation, with steroids showing relatively weak effects on monolayer structure [41]. Combining melatonin with BRs improved cold and drought resistance in *Lolium perenne* depending on stress duration and intensity, whereas chemical removal of H_2O_2 impaired seed germination and root growth [42]. BRs also prolong post-harvest shelf life under low-temperature stress by reducing chilling-induced damage in harvested fruits, such as tomatoes [43–45].

Water stress

Prolonged and severe water stress adversely affects crop growth and development, reduces yield, and can disrupt metabolic processes to the point of plant death. Reactive oxygen species (ROS) are relatively stable under normal conditions; however, drought stress disturbs the balance between ROS accumulation and the plant's antioxidant defense system. To mitigate ROS-induced cellular or tissue damage, the antioxidant system is activated. Overall, protection against oxidative damage depends on the equilibrium between these opposing processes [46].

The accumulation of osmoregulatory substances increases as drought stress intensifies [47,48]. Additionally, drought conditions often reduce chlorophyll content and photosynthetic capacity, sometimes accompanied by leaf wilting [49,50]. If the stress does not lead to plant death, more resilient plants can restore their vital functions and form reproductive organs. Nevertheless, even mild stress of varying nature and duration can reduce the intensity of physiological processes, activate some enzyme systems while inhibiting others, impair metabolism, and ultimately reduce productivity. Endogenous hormones play a critical role in drought response: drought generally decreases the levels of growth-promoting hormones while increasing the levels of growth-inhibiting ones [51,52].

The application of brassinosteroids (BRs) represents a promising strategy to mitigate the long-term effects of drought [53]. Exogenous BR treatment increases abscisic acid (ABA) levels and alleviates the negative impacts of drought. ABA accumulation is closely associated with drought resistance. Treatment with epibrassinolide (EBR) enhances drought tolerance in tomato plants by improving photosynthetic apparatus status, leaf hydration, and antioxidant defense under stress [54]. BR treatment also increases relative water content and net photosynthetic rate in *Solanum lycopersicum* while upregulating antioxidant enzyme activity (catalase, ascorbate peroxidase, and superoxide dismutase) and reducing stomatal conductance, intercellular CO_2, H_2O_2, and malondialdehyde levels. Recovery from drought stress is therefore attributed to the BR-induced increase in endogenous ABA concentration and antioxidant enzyme activity [55].

Nie et al. [56] reported a negative correlation between BR signaling via the BRASSINOSTEROID-INSENSITIVE 1 (BRI1) receptor and drought resistance, suggesting that enhanced drought tolerance represents a BR signaling branch downstream of BR but independent of BRI1. It was demonstrated that BRI1 overexpression negatively affects tomato drought tolerance, highlighting the complexity of BR-related stress responses [57].

Kaya et al. [54] showed that treatment of water-deprived tomato plants with 1.0 μM EBR restored growth and reduced oxidative stress, coinciding with increased endogenous nitric oxide (NO) levels and antioxidant activity. These results suggest that BR-induced NO generation and NO-mediated antioxidant defense are crucial mechanisms underlying BR-enhanced drought tolerance. Thus, BR and NO act synergistically to improve water stress resilience.

Feng et al. [58] reported that BR signaling and biosynthesis are highly expressed in *Arabidopsis*, *Oryza*, and *Brachypodium*, and that BR signaling directly regulates key drought-responsive genes, contributing to drought tolerance. Zhang et al. [59] suggested that BRs mediate the effect of soil desiccation on spikelet degeneration in *Oryza sativa* L., with elevated BR levels in panicles reducing degeneration under moderate drought by enhancing antioxidant capacity and energy status. Castaneda-Murillo et al. [60] demonstrated that foliar application of the BR analog DI-31 improved photosynthesis, photochemical efficiency, and pigment concentration in *Solanum quitoense* under drought, while enhancing growth and suppressing membrane lipid peroxidation. Similarly, Salah et al. [61] found that BR combined with exogenous spermidine increased maize seedling tolerance to waterlogging by optimizing root anatomy, photosynthetic performance, and antioxidant defense. Wang et al. [62] showed that BR application alleviates water stress symptoms in *Vitis vinifera* L., reducing H_2O_2 and O_2-levels while increasing antioxidant content and activity, including components of the ascorbate-glutathione cycle. BR treatment also enhanced the transcription of genes involved in ABA biosynthesis, supporting the existence of a complex crosstalk between BRs and ABA in drought-induced antioxidant defense.

Salt stress

Salinity is a major contributor to osmotic stress, also referred to as physiological drought [63]. The detrimental effects of increased salt content in soil or irrigation water are manifested as disturbances in cellular metabolism, imbalances in endogenous regulators, and disruption of basic physiological processes, ultimately reducing plant productivity and nutritional value [64]. Under salt stress, plants generate large amounts of reactive oxygen species (ROS), which can continuously induce lipid peroxidation, cause protein denaturation, impair cell membrane integrity, and even lead to cell damage or death [65,66]. Elevated salt concentrations also trigger changes in the plant photosynthetic apparatus, including degradation of chloroplast structure, reduction of photosynthetic pigment content, and decreased activity of photosynthetic enzymes, leading to suppressed plant growth [67].

This environmental issue is further exacerbated by the difficulty of preventing the penetration of industry-derived salts into the soil. Soil self-purification is a slow process, and current soil remediation technologies are characterized by high energy requirements, low efficiency, and considerable environmental challenges [68]. Therefore, the effective utilization of saline soils for agriculture necessitates increasing the salt tolerance of economically valuable plant varieties.

Brassinosteroids (BRs) have been shown to mitigate the harmful effects of salinity in numerous plant species, including arabidopsis, pepper, cucumber, common bean, and black locust [69]. In eggplants, BR treatment increased the activity of antioxidant enzymes, decreased Na^+ and Cl^- levels, and elevated K^+ and Ca^{2+} concentrations, demonstrating the potential for enhancing salt tolerance [70]. Exogenous application of 24-epibrassinolide (EBR) improved net photosynthesis rate, stomatal regulation, evapotranspiration, and the maximal quantum yield of PSII under saline conditions by reducing leaf Na^+ accumulation and membrane permeability [71].

BR treatment also prevented the death of Mentha piperita L. plants at 150 mM NaCl, alleviating salt stress effects through up-regulation of antioxidant enzyme activity, reduced membrane permeability, and decreased lipid peroxidation. Additionally, BR significantly increased essential oil content under saline conditions [72]. In lettuce, BR DI-31 mitigated the negative effects of NaCl on both shoots and roots, although spermine levels in shoots remained unchanged [13]. Ahanger et al. [73] reported that combined EBR and kinetin (Kn) treatment in *Solanum lycopersicum* increased ascorbic acid content, reduced glutathione and secondary metabolites, and promoted redox homeostasis, thereby enhancing the enzymatic antioxidant system's ability to cope with salt stress. The synergistic application of Kn with EBR regulated growth and photosynthesis more effectively than individual treatments, likely due to cross-regulatory interactions. Similarly, in cucumber, BR-induced salt resistance improved photosynthesis, nitrogen use efficiency, and total polyamine levels [36].

Rice seedlings subjected to NaCl exhibited stunted growth and altered antioxidant enzyme levels; however, EBR treatment restored growth, protein and proline content, and enhanced antioxidant enzyme activity. The elevated malondialdehyde levels observed under salt stress were reduced by EBR. Furthermore, EBR promoted expression of genes associated with oxidative stress at varying levels. Specifically, OsDWF4 expression was down-regulated, while SalT was up-regulated under salt stress with EBR treatment. Notably, combined salt and EBR treatment resulted in a significant down-regulation of SalT compared with salt stress alone, while OsBRI1 expression increased under both EBR and combined treatments, confirming the effectiveness of EBR in mitigating salt stress [74].

Kong et al. [75] studied the physiological differences between salt-sensitive and salt-tolerant *Allium sativum* L. genotypes and found systematic regulation of BR biosynthesis-related transcripts in sensitive genotypes, suggesting a role for BR-mediated lignin accumulation in salt stress adaptation. In *Populus euphratica* Oliv., overexpression of the BR biosynthesis gene PeCPD under salt stress modified BR biosynthesis and regulated genes controlling metabolic processes, enhancing BR responses. This modification improved growth, wood quality, and salt tolerance in woody plants [76]. Additionally, BRs regulate DNA methylation, which is crucial for salt tolerance; seed priming with EBR increases global DNA methylation and enhances salinity tolerance [77].

Pollution stress

The BR-facilitated tolerance of plants to organic pollutants merits particular attention. Economic activity is inevitably accompanied by environmental degradation. The normal development of industrial production is inextricably linked with the biosphere, which provides raw materials, energy, and resources. At the same time, waste streams from production return to the biosphere, affecting the human environment. The scale of pollutant discharge, as well as the potential for adverse impacts on ecosystems, is substantial. The increasing anthropogenic load on natural ecosystems-including contamination with heavy metals and persistent organic pollutants-leads to irreversible degradation or destruction of natural habitats, with adverse consequences for human health and limitations on resource consumption. Heavy metal-induced stress negatively affects both the quantity and quality of crops cultivated in contaminated soils. Furthermore, the migration of heavy metals through food chains poses a risk of human exposure [78].

Heavy metal-induced stress can be mitigated through the application of growth regulators, bioactive substances, and manipulation of endogenous hormones and signaling pathways [33]. Brassinosteroids have been reported to alleviate the effects of heavy metal stress in a variety of plant species [79]. For example, BR application mitigated cadmium (Cd) toxicity in grapevine seedlings through the ascorbate-glutathione cycle, facilitating phytoremediation [80]. Hayat et al. [81,82]demonstrated that BR treatment neutralized the harmful effects of Cd on *Brassica juncea* and *Solanum lycopersicum*, accompanied by increased activity of antioxidant enzymes (catalase, peroxidase, and superoxide dismutase) and upregulation of proline levels. Exogenous BR treatment promoted the activity of the photosynthetic apparatus and the antioxidant defense system, thereby eliminating the harmful effects of Cd [83,84].

Similarly, 24-epibrassinolide (EBR) decreased Cd levels and oxidative stress parameters in *Capsicum annuum* leaves, improved plant growth, regulated water balance, increased proline content, and enhanced the activity of enzymes involved in the ascorbate-glutathione cycle, antioxidant defense, nitrate reductase, and endogenous nitric oxide (NO) production. However, the beneficial effects of EBR were suppressed in the presence of tungstate, an inhibitor of nitrate reductase, which also reduced NO levels, suggesting nitrate reductase as a key contributor to EBR-induced NO generation, critical for Cd tolerance in pepper plants [85]. Activation of BR-pathway components, including H^+-ATPase and NADPH oxidase, has also been reported [86]. Spraying with 28-homobrassinolide or 24-epibrassinolide further amplified Cd stress-induced increases in proline content and antioxidant enzyme activity [87].

Wu et al. [87] reported that foliar EBR application in *Arabidopsis thaliana* significantly reduced antimony (Sb) accumulation and membrane lipid peroxidation, increased chlorophyll and proline content, and enhanced antioxidant enzyme activity, indicating a critical role of BRs in heavy metal stress regulation. Similarly, EBR treatment mitigated lead (Pb) toxicity in *Brassica juncea* by upregulating catalase and peroxidase activity, although 10^{-8} M EBL increased Pb accumulation without affecting growth rate [88]. Under Pb stress, EBR enhanced biomass and photosynthetic pigment levels in *Festuca* species, decreased reactive oxygen species accumulation, increased antioxidant enzyme activities, and elevated osmoprotectant levels, reducing lipid peroxidation [89].

In maize (*Zea mays*), Mn overload induced oxidative stress, evidenced by increased malondialdehyde and lipoxygenase activity, and accumulation of superoxide radicals and H_2O_2. EBR treatment significantly alleviated these symptoms and enhanced the activities of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase, alongside increased levels of reduced ascorbate and glutathione [90]. In *Solanum lycopersicum*, combined treatment with EBR and H_2O_2 improved photosynthesis efficiency, chloroplast ultrastructure, stomatal behavior, root structure, cell viability, and antioxidant and proline production, enhancing tolerance to Cu stress [91].

BR treatment also mitigates the detrimental effects of organic pollutants. For instance, EBR enhanced detoxification of benzene in *Chlorophytum comosum* by upregulating antioxidants, benzene detoxification-related genes, and glutathione (GSH) conjugation products, leading to increased removal of gaseous benzene [92]. Foliar EBR application increased biomass, net CO_2 assimilation, and antioxidant enzyme activity in *Solanum lycopersicum*, while reducing malondialdehyde and H_2O_2 levels, thus protecting root cells from severe damage. Detoxification gene expression (CYP90b3, GSH1, GST1) was modulated, with higher glutathione-S-transferase activity and lower residues of polychlorinated biphenyls, phenanthrene and pyrene observed in roots [93–95].

Treatment with EBR also alleviated mycotoxin stress. In wheat, both pure and zearalenone-supplemented EBR increased homocastasterone levels, particularly in chloroplasts, reduced zearalenone accumulation, and restored photosystem II activity, mitigating oxidative stress and modulating antioxidant enzyme activity [96]. EBR pretreatment upregulated transcription of antioxidant, detoxification, and defense genes in *Solanum lycopersicum*, improving tolerance to pesticides and reducing phytotoxicity, with concomitant upregulation of photosynthetic gene mRNA [97]. CO_2 enrichment produced similar or even more pronounced effects.

Finally, the combined application of *Sinorhizobium meliloti* NM strain and brassinosteroids enhanced endogenous BR accumulation and the antioxidant network in Medicago model plants. Inhibition of BR biosynthesis abolished these rhizobia-induced responses, indicating the key role of BR-dependent signaling pathways in PCB detoxification and systemic antioxidant defense [98]. These findings highlight the potential of BRs and rhizobia in phytoremediation strategies for contaminated soils.

Conclusions

Exogenous application of brassinosteroids holds promise for enhancing crop tolerance to various abiotic stresses. However, its large-scale agricultural use is currently limited by the high cost of BR synthesis and a lack of knowledge regarding hormone dynamics under field conditions.

Manipulation of the BR pathway for crop improvement appears highly attractive. In the long term, however, it requires a deeper, more integrated understanding of how BR signaling operates across different species, environmental conditions, and developmental stages [3]. Despite significant progress, many questions remain unresolved. In particular, the molecular mechanisms controlling gene expression, including tissue and cell-type specificity, remain insufficiently elucidated. Further insights are also needed regarding BR biosynthesis, degradation, and modes of transport, which determine their spatial and temporal activity within plant tissues [99].

Profound brassinosteroids investigations conducted at the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine focus on the regulatory role of BRs in plant adaptation to abiotic stress, particularly in relation to changes in primary and secondary metabolism. These studies complement global efforts to elucidate the molecular mechanisms of BR signaling and its potential applications in crop improvement.

In summary, expanding our knowledge of BR signaling and metabolism will not only enhance the theoretical understanding of plant stress physiology but also open new prospects for sustainable agriculture. Ultimately, the comprehensive elucidation of BR-mediated stress adaptation mechanisms is expected to emerge as a distinct and rapidly developing field within plant science.

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БРАСИНОСТЕРОЇДИ ЯК БІОАКТИВНІ СТЕРОЇДИ: ОГЛЯД ВЗАЄМОЗВ'ЯЗКУ СТРУКТУРИ ТА АКТИВНОСТІ І БІОХІМІЧНИХ МЕХАНІЗМІВ ПОМ'ЯКШЕННЯ АБІОТИЧНОГО СТРЕСУ

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Брасиностероїди – це клас полігідроксильованих стероїдних рослинних гормонів, які беруть участь у широкому спектрі фізіологічних, біохімічних і молекулярних процесів рослин: проростанні насіння, поділі та подовженні клітин, диференціації провідних тканин, фотоморфогенезі, фотосинтезі, активації ферментів і старінні. Пристосування до мінливих умов навколишнього середовища є фундаментальною властивістю рослинного життя, що регулюється складними механізмами оптимізації розподілу ресурсів. Брасиностероїди підвищують толерантність рослин до широкого спектра абіотичних стресів, зокрема температурного, водного, сольового та стресу, спричиненого забрудненням важкими металами й органічними полютантами. В умовах стресу вони стабілізують фотосинтез, регулюють антиоксидантний захист шляхом індукції відповідних ферментів, стимулюють накопичення захисних сполук вторинного метаболізму та взаємодіють з іншими фітогормональними сигнальними шляхами, включаючи абсцизову кислоту й ауксини. Вплив абіотичних стресорів на рослини є складним процесом, і деякі його механізми були з'ясовані лише нещодавно. Незважаючи на значний прогрес, механізми біосинтезу, транспорту та перехресної взаємодії брасиностероїдів з іншими сигнальними мережами залишаються недостатньо вивченими, що робить їх перспективним об'єктом як фундаментальних, так і прикладних досліджень.

Ключові слова: брасиностероїди; абіотичний стрес; адаптація рослин; взаємозв'язок структури та активності; біохімічні механізми.

BRASSINOSTEROIDS AS BIOACTIVE STEROIDS: A REVIEW OF STRUCTURE–ACTIVITY RELATIONSHIPS AND BIOCHEMICAL MECHANISMS OF ABIOTIC STRESS MITIGATION

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Brassinosteroids (BRs) are a class of polyhydroxylated steroid plant hormones that participate in a wide range of physiological, biochemical, and molecular processes, including seed germination, cell division, and elongation, vascular differentiation, photomorphogenesis, photosynthesis, enzyme activation, and senescence. Adaptation to fluctuating environmental conditions is a fundamental feature of plant life, regulated by complex mechanisms that optimize resource allocation. Brassinosteroids enhance plant tolerance to numerous abiotic stresses. In this review, we focus on the ability of BRs to improve plant adaptability under abiotic stress. Abiotic stress represents a major constraint on agricultural productivity, as it substantially reduces crop yield. The effects of abiotic stressors on plants are complex, and many underlying mechanisms have only recently been elucidated. Nevertheless, the mitigation of abiotic stress through the application of plant hormones continues to attract considerable research interest. Plants adapt to different environmental stresses by altering physiological and molecular processes that are co-regulated with changes in the levels of phytohormones, including BRs. Most studies have focused on the external application of BRs to stimulate growth, increase crop yields, and enhance tolerance to abiotic stresses. However, plant growth and development are inseparably linked to the coordinated interaction between endogenous phytohormones and environmental signals. Overall, this review discusses brassinosteroid biosynthesis, BR-mediated signaling, and the role of endogenous BRs in regulating plant responses to abiotic stressors. Based on the available research, it is expected that the practical use of BRs to alleviate the negative impacts of abiotic stress on plant vitality will continue to expand. Furthermore, a deeper understanding of their mechanisms of action will bring undeniable benefits to both basic and applied plant science. Ultimately, understanding the mechanisms by which BRs regulate stress responses in crops will likely evolve into an independent direction of research.

Keywords: brassinosteroids; abiotic stress; plant adaptation; structure–activity relationships; biochemical mechanisms.

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