Brassinosteroids in Plants

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Abstract

Brassinosteroids (BRs) are a unique class of plant hormones, that are essential for normal plant growth. Brassinosteroids are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Since their discovery more than 30 years ago, extensive research on the mechanisms of BR action using biochemistry, mutant studies, proteomics and genome-wide transcriptome analyses, has helped refine the brassinosteroid biosynthetic pathway, identify the basic molecular components required to relay the brassinosteroid signal from perception to gene regulation, and expand the known physiological responses influenced by brassinosteroids. These mechanistic advances have helped answer the intriguing question of how brassinosteroids can have such dramatic pleiotropic effects on a broad range of diverse developmental pathways and have further pointed to brassinosteroid interactions with other plant hormones and environmental cues. Moreover, brassinosteroids physiological responses including cell elongation and division, vascular differentiation, flowering, pollen development and photomorphogenesis.

Keywords: Brassinosteroids, Photosystem, Phytohormones, Signaling.

Chapter 8: Brassinosteroids in Plants

1. Introduction

Brassinosteroids (BRs), inherent plant hormones with widespread presence, play a pivotal role in promoting growth by impacting cell expansion and proliferation. Brassinosteroids are known to be naturally occurring polyhydroxylated plant steroids that showed diverse roles in regulation of various physiological and developmental processes in plants under both natural and stressful conditions. In the recent past brassinosteroids have shown the ability to cross talk with other phytohormones such as auxin, polyamines, cytokinin, ethylene, and jasmonic acid in regulating varied ranges of physiological and biochemical processes in plants. On the other hand, the exogenous application of brassinosteroids in agriculture to improve growth and yield under various stress conditions including drought, salinity, extreme temperatures, and heavy metal toxicity is of immense significance as these stresses severely hamper the normal metabolism of plants.

The information available till date regarding brassinosteroids will definitely help in establishing various mechanisms which modulate various processes in plants and overcome the future challenges in agriculture. Brassinosteroid also plays pivotal role in promotion of cell expansion, cell elongation, cell division, and vascular differentiation, and provides protection against various abiotic and biotic stresses. Mutant plants lacking proper brassinosteroid biosynthesis and signaling display distinct phenotypes, underscoring the crucial role of these hormones as regulators in fundamental physiological processes. This includes organ elongation, vascular differentiation, male fertility, senescence timing, and leaf development (Fridman and Savaldi-Goldstein 2013).

2. Structure of brassinosteroids

Brassinosteroids is a polyhydroxylated derivative of 5α-cholestan, namely (22*R*, 23*R*, 24S)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (Fig. 1). Thus, plants possess a growth-promoting steroid with structural similarity to cholesterol-derived animal steroid hormones such as androgens, estrogens and corticosteroids from vertebrates, and ecdysteroids from insects and Crustacea. The brassinosteroid family consists of BL and about 68 other free brassinosteroids plus several conjugates (Fujioka and Yokota 2003). These differ from BL by variations at C-2 and C-3 in the A ring; the presence of a lactone, ketone, or de-oxo function at C-6 in the B ring; the stereochemistry of the hydroxyl groups in the side chain, and the presence or absence of a methyl (methylene) or ethyl (ethylene) group at C-24. The conjugates are glycosylated, meristylated and laurylated derivatives of the hydroxyls in ring A or in the side chain. Many of the known brassinosteroids are biosynthetic precursors or metabolic products of BL, although castasterone, the immediate precursor of BL, is believed to have independent biological activity in some plants. The optimal structure for highest brassinosteroid activity normally is that found in BL, consisting of a lactone function at C-6/C-7, cis-vicinal hydroxyls at C-2 and C-3, *R* configuration of the hydroxyls at C-22/C-23 and a methyl substitution at C-24 (Mandava 1988).

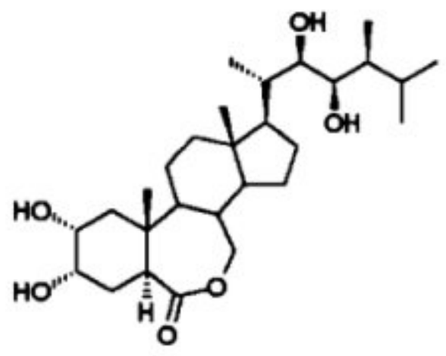

Figure 1. Chemical structure of brassinosteroids.

3. Biosynthesis of brassinosteroids

Brassinosteroids, like animal and insect steroids originating from cholesterol, undergo transformation from plant sterols. Teasterone, typhasterol, and castasterone, among natural BRs, are considered precursors of brassinolide. These analogs, including brassinolide, are metabolized from campesterol, a common plant sterol with a similar carbon skeleton in the side chain (Akira et al. 1997). Brassinosteroids are naturally occurring polyhydroxylated steroidal phytohormones crucial for normal plant growth and development. They fall into three categories (C27, C28, or C29 steroids) based on their C-24 alkyl substituents. Among the identified BRs, brassinolide (BL) stands out as the most biologically active compound, widely present in numerous plant species. BL, a 28-carbon compound with an S-methyl group at C24, is a key focus in brassinosteroid research. Other brassinosteroids typically represent intermediates or inactivated products resulting from various catabolic reactions.

The biosynthesis of BL Involves two parallel pathways: the early and late C-6 oxidation pathways. These pathways start with the brassinosteroid-specific biosynthetic precursor campesterol (CR). In the early C-6 oxidation pathway, CN (campestanol) undergoes C-6 oxidation before DWF4-mediated C-22 hydroxylation, leading to intermediates like 6-oxocampestanol (6-oxoCN), cathasterone (CT), teasterone (TE), 3-dehydroteasterone (3DT), typhasterol (TY), and ultimately castasterone (CS). In contrast, the late C-6 oxidation pathway involves C-22 hydroxylation prior to C-6 oxidation. DWF4 hydroxylates CR at C-22, forming intermediates like 6-deoxocathasterone (6-deoxoCT), which then proceed through the late C-6 oxidation pathway. In crop plants such as tomato and tobacco, the late C-6 oxidation pathway predominates, resulting

in endogenous brassinosteroids primarily from this pathway. Notably, in *Arabidopsis*, BR6ox connects the late and early C-6 oxidation pathways, and DWF4 can act independently of campestanol. The pathways can branch at campesterol, establishing an early C-22 hydroxylation pathway (Fujioka and Yokota 2003).

4. Signal transduction of brassinosteroids

RLKs (Receptor-like serine/ threonine kinases, the predominant group of plant receptor kinases, are membrane-bound proteins with extracellular ligand-binding domains and cytoplasmic kinase domains. They transmit signals by phosphorylating serine, threonine, or, in some cases, tyrosine residues of target proteins. RLKs respond to various ligands, including signals from biotic sources and plant hormones like brassinosteroids, auxin, and peptides. In the brassinosteroids signaling pathway, RLKs employ signal amplification and repressor inactivation strategies, translating extracellular hormone signals into transcriptional responses within the cell. When brassinolide binds to the receptor kinase BRI1(BRASSINOSTEROID-INSENSITIVE1), on the plasma membrane, it initiates a phosphorylation cascade. This cascade leads to the inactivation of the repressor protein BIN2 (BRASSINOSTEROID-INSENSITIVE2). As a result, transcription factors BES1 (BRI1-EMS SUPPRESSOR1) and BZR1 (BRASSINAZOLE-RESISTANT1) become activated, leading to the expression of specific genes and the propagation of the BR-mediated signal for cellular responses. The BRI1 receptor, part of the plasma membrane LRR subfamily of RLKs, consists of an extracellular domain binding brassinolide, a transmembrane domain, and a cytoplasmic kinase domain with tyrosine, serine, or threonine specificity. When brassinolide binds, BRI1 homodimers

activate and form heterooligomers with BAK1. Both RLKs undergo auto- and transphosphorylation upon activation. Before brassinolide binding, BRI1 interacts with BKI1 (BRI1-KINASE INHIBITOR1), preventing association with BAK1. This interaction acts as a regulatory mechanism in the absence of the BRs hormone. Up on BRI1 activation, BKI1 is released from the plasma membrane, BRI1 and BAK1 dimerize, and BRI1 phosphorylates and activates two plasma membrane–anchored receptor like cytoplasmic kinases (RLCKs), the BR-SIGNALING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1). Activated BSK1 and CDG1 then phosphorylate and activate the Serine/threonine phosphatase BRI1 SUPPRESSOR1 (BSU1). This, in turn, inactivates the repressor protein BIN2. BIN2 is a serine/threonine protein kinase that, in Absence of brassinolide, negatively regulates the closely Related transcription factors BES1 and BZR1 by phosphorylation. Phosphorylation of BES1/BZR1 by active BIN2 has at least two regulatory roles. First, BIN2-mediated phosphorylation of the transcription factors prevents them from shuttling to the nucleus and causes their retention in the cytosol. Second, phosphorylation prevents BES1/BZR1 from binding to target promoters, thus blocking Their activity as transcriptional regulators. In the presence of brassinolide, the activated phosphatase BSU1 dephosphorylates BIN2 and promotes its degradation by the 26S proteasome system, thus blocking its Activity.BES1 and BZR1 Are then dephosphorylated by PROTEIN PHOSPHATASE2A (PP2A), and the active forms of BES1 and BZR1 Move into the nucleus where they regulate the expression of brassinolide response genes (Fig. 2) (Taiz et al. 2015).

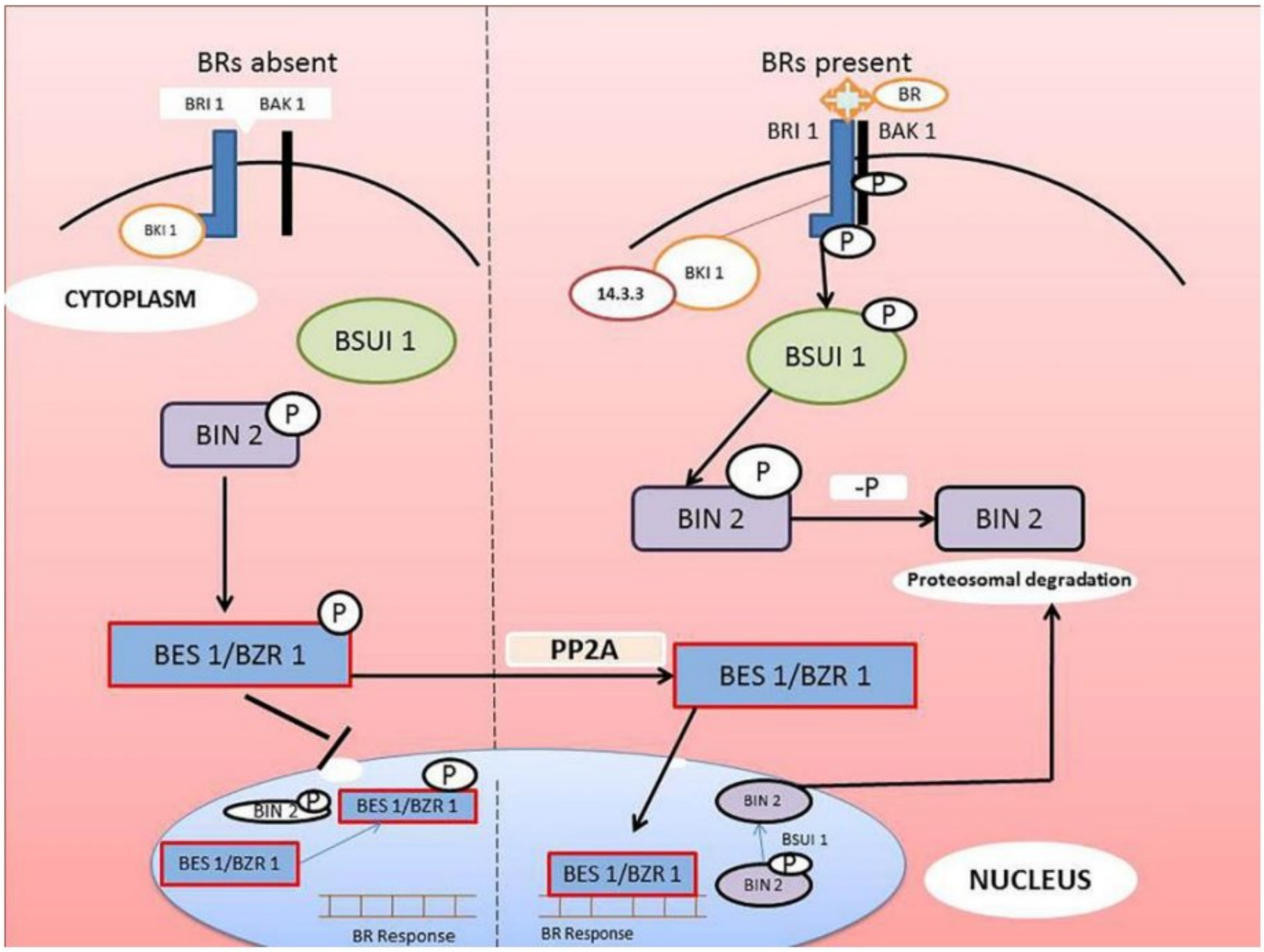

Figure 2. BR signal transduction pathway in plants. The receptor BRI1 is localized on the plasma membrane (PM). The extracellular region consists of a coiled stretch of leucine rich repeat (LRR) sequences containing an Island domain (ID) that functions as part of the Brassinolide (BL) binding site. The intracellular Portion contains a kinase domain (KD) and the C-terminal tail (CT). Signal perception occurs at the cell surface and results in changes in BR-regulated gene expression. BRRE, BRs Response element (Chung and Choe 2013).

5. Functions of brassinosteroids

Brassinosteroids exhibit diverse effects, influencing various cellular responses such as stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton-pump activation, xylem differentiation, and the regulation of gene expression.

5.1 Cell expansion and cell division

Brassinosteroids are naturally occurring compounds that stimulate growth and are present in small quantities in pollen, seeds, and young plant tissues across various plant species. Brassinosteroids play a crucial role in plant growth by influencing both cell division and cell expansion. They induce elongation in various plant structures such as hypocotyls, epicotyls, peduncles, coleoptiles, and mesocotyls. This cell expansion, triggered by brassinosteroids, involves proton extrusion and hyperpolarization of the cell membrane, thereby stimulating and accelerating the cell cycle. The plasticity of the cell wall increases as proton extrusion acidifies the apoplast, activating cell wall loosening enzymes. Brassinosteroids contribute to cell enlargement through their impact on gene expression and enzyme activity. Molecular genetic studies on mutants have provided insights into the necessity of brassinosteroids in cell elongation. In root meristem cells, a cellular analysis combined with the assessment of molecular cell cycle markers and stem cell genes reveals that brassinosteroids positively regulate the size of the root meristem. Brassinosteroids achieve this by increasing cell division, notably by elevating transcript levels of genes encoding cyclin-D3, a regulatory protein in the cell cycle. Interestingly, brassinosteroids can efficiently substitute for cytokinin in Arabidopsis callus and suspension cultures, where they promote growth (Clouse et al. 1998).

5.2 Photosynthesis

Photosynthesis is vital for both plants and life on Earth. Phytohormones are essential in guiding various stages of plant development, from organ formation to aging. They act as key regulators in growth, development, and play a central role in controlling photosynthesis.

Additionally, phytohormones are crucial for safeguarding the photosynthetic machinery during stressful conditions, providing photoprotection (Müller et al. 2021). Brassinosteroids are recognized for their ability to increase the chlorophyll content (Alyemeni et al. 2016). The role of brassinosteroids in enhancing photosynthetic rates and related attributes is widely acknowledged in various plant species. The mechanism is believed to involve the improvement of the photosynthetic carbon reduction cycle efficiency by increasing the content of related enzymes. Exogenous application of 28-homobrassinolide to wheat and mustard, or epibrassinolide to mung bean along with GA3, has demonstrated increased photosynthetic rates. Foliar spray of brassinosteroid solutions to wheat, mustard, and cucumber, as well as the application of epibrassinolide to cucumber seedlings and brassinolide to rice, have shown positive effects on CO_2 assimilation. Furthermore, 24-epibrassinolide application enhanced the light-saturated net CO_2 assimilation rate and carboxylation rate of rubisco in the Calvin cycle. Interestingly, while the epicotyl of cucumber did not respond to epibrassinolide, labeled glucose transport towards the epicotyl was favored. Additionally, foliar application of 28-homobrassinolide positively influenced Hill activity in *Vignaradiata* foliage. Epibrassinolide foliar spray increased rubisco carboxylation rate, RuBP regeneration, and quantum yield of PSII, and it's noted to recover the loss of photosynthetic apparatus from cold stress. Following HBL treatment, initial Hill reaction activity declined, but at subsequent developmental stages, there was an observed enhancement. The efficiency of Photosystem II (ϕPSII), representing the fraction of absorbed photons involved in photochemistry, serves as an indicator of overall photosynthesis. Notably,