Brassinosteroids

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The discovery of brassinosteroids can be traced back to 1941 from Mitchell and Whitehead of the United States Department of Agriculture (USDA, Beltsville, MD) who reported that pollen extracts often have growth-promoting properties on other plant tissues. In 1970, Mitchell et al. reported that the crude extract of pollen from *Brassica napus* (rape) contained "**brassins**" which promoted rapid elongation of internodes in *Phaseolus vulgaris*. This elongation response was distinct from GA-mediated stem elongation. Subsequently, their work leads to the isolation and identification of brassinolide (BL) as the first steroidal plant growth regulator. Another steroid hormone called castasterone (SC) was isolated from insect galls of chestnut (*Castanea* sp.). Since then, a number of related steroidal compounds have been isolated which are collectively called as **brassinosteroids (BRs)**. Structurally, BRs are C-27, C-28, and C-29 steroids with different functional groups on A and B rings and on the side chain. BRs occur in algae, ferns, gymnosperms, and angiosperms. They have not been detected in microorganisms. Brassinolide is a C-28 brassinosteroid and exhibits highest activity among all BRs so far known.

20.1 Biosynthesis and Homeostasis

BRs are synthesized from campesterol (a plant sterol) through formation of many intermediates. Castasterone is the immediate precursor for the formation of brassinolide (BL), which is the most active BR (Fig. 20.1). Bioactive BR levels (homeostasis) are regulated by a variety of catabolic reactions, including hydroxylation, oxidation, sulfonation, epimerization, and conjugation to lipids or glucose. Only a few enzymes responsible for BR catabolism are so far reported. Level of active BR in plant tissue is also regulated by negative feedback mechanism, whereby BR concentration in the tissue above a certain level results in reduction in BR biosynthesis by the downregulation of BR biosynthesis genes and upregulation of genes for BR catabolism.

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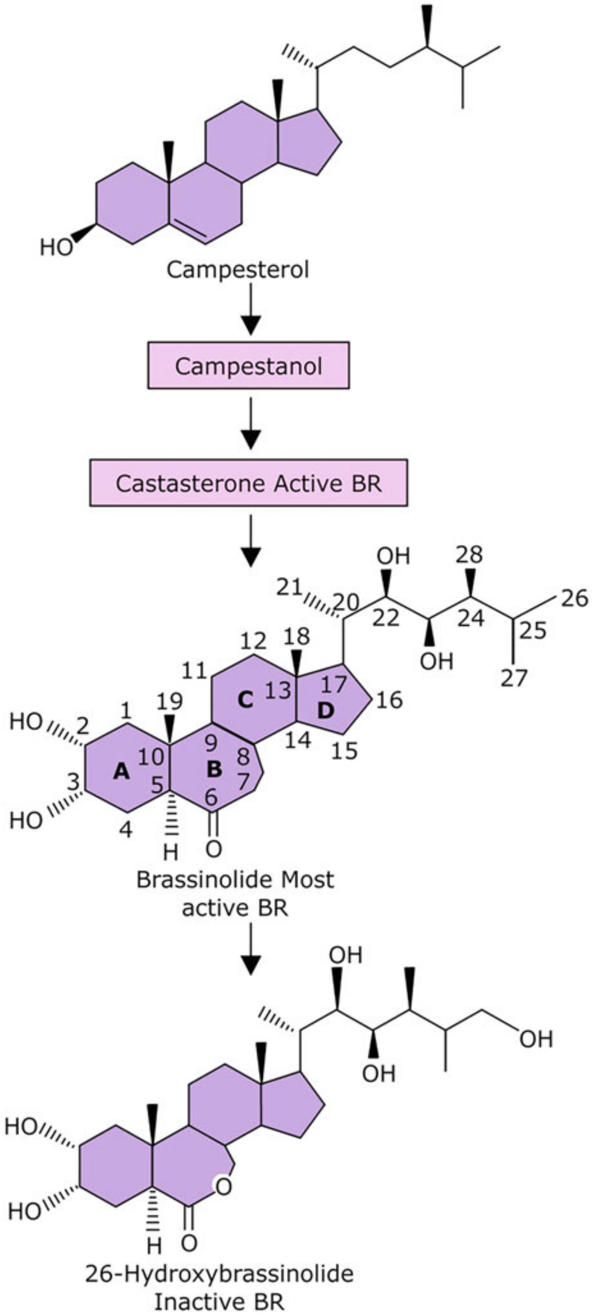
Fig. 20.1 Biosynthetic pathway of brassinosteroids (BRs). Campesterol and campestanol are the inactive precursors of the biologically active compounds castasterone and brassinolide. Brassinolide is the most active naturally occurring BR and is metabolized to inactive 26-hydroxybrassinolide by a cytochrome P450 enzyme encoded by the gene *BAS1*. The biological activity of BRs is dependent on the presence of hydroxyl groups at carbons 2 and 3 of ring A and positions 22 and 23 of the side chain and a seven-membered lactone B-ring



20.2 Functions of Brassinosteroids

20.2.1 Regulation of Photomorphogenesis

BRs prevent photomorphogenesis in etiolated seedlings. This has been extensively worked out using BR mutants with impaired BR synthesis. The role of BRs in repressing photomorphogenesis has further been observed to be operative through different mechanisms as compared to other regulatory mechanisms (e.g., those mediated by COP). Like GA, BRs also suppress photomorphogenesis in dark.

20.2.2 Unrolling and Bending of Grass Leaves

Brassinolide (BL) and other BRs induce a dose-dependent swelling of the adaxial cells of the joint between the rice seedling's leaf blade and sheathing base. This bioassay is very sensitive and is used to determine structure-activity relationship of BR by measuring the angle of lamina bending in response to a defined concentration of BR applied over a period of time (Fig. 20.2).

20.2.3 Other Effects

BRs also induce stem elongation, pollen tube growth, proton pump activation, reorientation of cellulose microfibrils, and xylogenesis. They are required for promotion of GA-mediated cell elongation. GA-induced degradation of DELLA repressor protein also enhances BR response. The signal transduction pathways of BRs and GA also interact with phytochrome pathway by regulating the activity of PIFs (phytochrome-interacting factors).

20.3 Brassinosteroid Mutants

20.3.1 Mutants with Impaired Brassinolide Synthesis (Brassinolide-Sensitive Mutants)

Genetic analysis of de-etiolated mutants grown in dark has led to identification of DE-ETIOLATED2 (DET2) gene which encodes a brassinosteroid biosynthetic gene. *det2* are "loss of function" mutants with low levels of BR and de-etiolated appearance of seedlings even in dark growth conditions. DET2 encodes an enzyme associated with the biosynthesis of brassinolide. The endogenous brassinolide levels in *det2* mutants are less than 10% of that found in wild plants of *Arabidopsis*. The phenotype of dark-grown det2 mutants can be corrected by exogenous application of brassinolide for subsequent photomorphogenic response. Similar to *det2* mutant, some more mutants have also been isolated whose phenotype can be corrected for normal growth by exogenous brassinolide application. The corresponding genes in

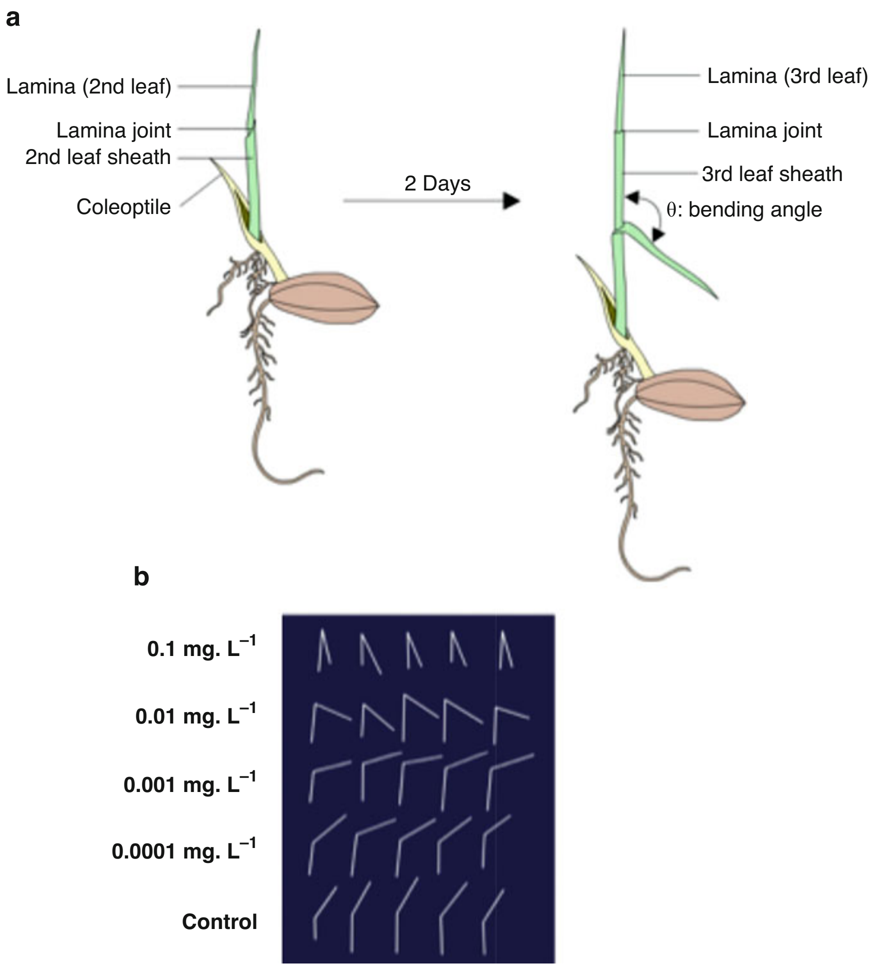


Fig. 20.2 Brassinosteroid bioassay; (**a**) Rice seedling showing the lamina and sheathing leaf sheath. Brassinosteroids to be assayed are applied at the leaf sheath-lamina joint, and the angle of lamina is measured after 2 days of growth. (**b**) Effect of increasing BR concentration on the angle of lamina bending

all these mutants are involved in brassinosteroid biosynthesis, and these mutations have noteworthy effects on the phenotype of light-grown plants and de-etiolation of dark-grown seedlings. The light-grown mutant plants are dwarfed, have short internodes, possess dark green curved leaves, and exhibit reduced apical dominance and fertility. All these observations indicate the role of BR in the inhibition of photomorphogenesis in dark and plant development in light. Dwarf mutants with altered brassinolide synthesis have also been reported from tomato and pea. Like in *Arabidopsis*, the mutants reported from tomato and pea also have aberrations in brassinolide biosynthesis.

20.3.2 Brassinosteroid-Insensitive Mutants (bri)

Another class of mutants were identified in *Arabidopsis* on the basis of their insensitivity to exogenously applied brassinolide. These mutants called as *bri* mutants have a phenotype similar to *det2* mutants, but their growth cannot be normalized by exogenous brassinolide application. The analysis of these mutants enabled identification of proteins associated with BR signal transduction. In these mutants (*bri1*), brassinolide accumulation is higher than in wild-type plants, and the expression of CPD (an enzyme involved in brassinolide biosynthesis) is also increased. This indicates that brassinolide represses its own biosynthesis by feedback mechanism, thereby modulating BR homeostasis. Another class of *Arabidopsis* mutants—*bin2* (brassinosteroid insensitive 2)—are impaired in the expression of a serine/threonine kinase that is a negative regulator of BR signaling.

20.4 Brassinosteroid Signaling Mechanism

BR receptor (BRASSINOSTEROID INSENSITIVE 1; BRI1) on the plasma membrane binds with BR at its extracellular domain. The receptor contains leucine-rich repeats (LRRs) consisting of a series of 20–29-amino acid residues rich in leucines in the extracellular domain. Regions of LRRs are thought to function in protein-protein interactions. It is, however, a 70-amino acid "island domain" interrupting the LRRs on BRI1 receptor, which most likely acts as the site for BR binding on the plasma membrane-associated receptor. BR signaling follows the following steps (Fig. 20.3):

1. Inactive state (homodimer): Inactive BR receptor BRI1 exists as a homodimer on the PM, and in this situation, it coexists with BRI1 KINASE INHIBITOR (BKI1).
2. Phosphorylation: Binding of BR to the receptor (BRI1) induces phosphorylation of the inhibitor (BAK1) at its cytosolic loop and its own phosphorylation (BRI1-P).
3. Loss of inhibitor (BAK1) activity: Phosphorylation of inhibitor makes it inactive, and it is lost from its normal association with the receptor on the PM.
4. Phosphorylation of other cytosolic kinases (BSKs): In the absence of inhibitor activity (BKI1), the phosphorylated receptor (BRI1-P) phosphorylates a small family of cytosolic kinases (BSKs 1, 2, and 3).
5. Cytosolic kinases (BSKs) modulate the activity of phosphatase (BSU1) and another kinase (BIN 2).
6. Phosphatase (BSU1) and kinase (BIN2) regulate the phosphorylation status of transcription factors—BZR1 and BES1 in the cytosol and nucleus.
7. Phosphorylation inhibits the activity of transcription factors BZR1 and BES1 and modulates gene expression by enhancing the rate of their degradation and reduction in their DNA-binding ability.

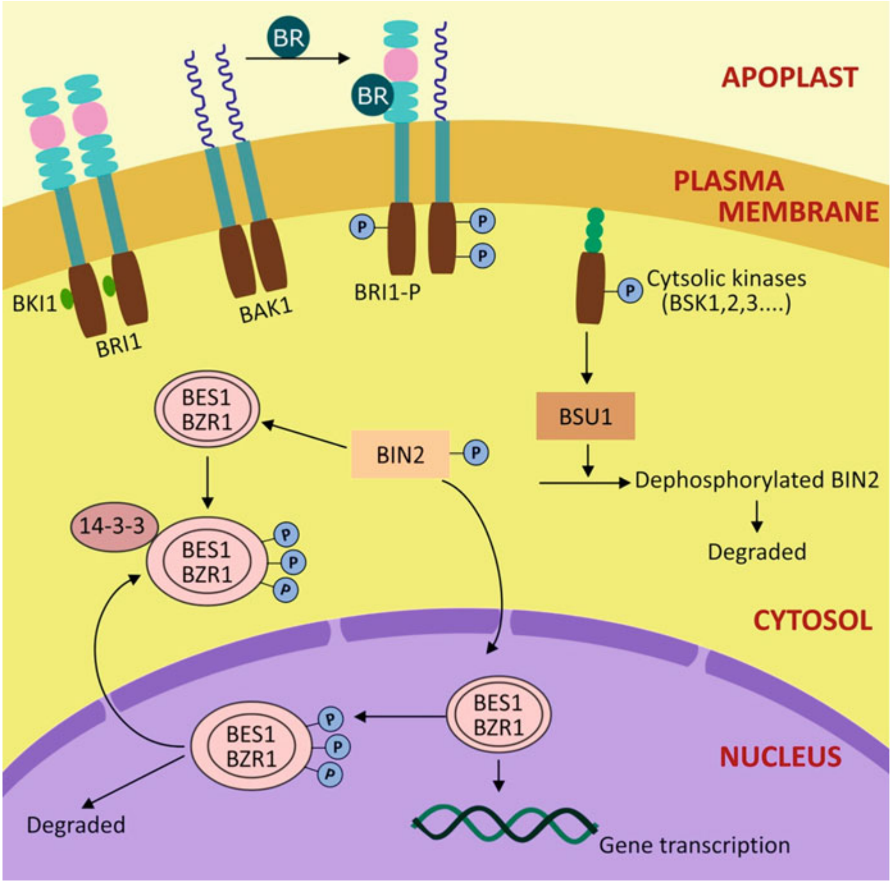


Fig. 20.3 Signal transduction accompanying the action of brassinosteroids

8. BR perception leads to inhibition of BIN2 kinase and stimulation of BES1 (transcription factors), leading to accumulation of unphosphorylated BZR1 and BES1 in the nucleus and causing the regulation of BR-responsive genes.

Noteworthy Features of Brassinosteroid Receptor BRI1 Are

1. In tomato, BR receptor has the ability to bind two different signals—brassinosteroids and **systemin**. Systemin is an 18-amino acid peptide produced by tomato plants and other Solanaceae members in response to insect attack, which is capable of inducing defense-related genes.
2. BRI1 can probably signal from both plasma membrane and **endosomes**. Endosomes are intracellular vesicles created by endocytosis. The significance of using endosomes in addition to PM is that it increases the membrane area

available for signaling interaction. Migration of endosomes by cytoplasmic streaming also enables their proximity to the nucleus to evoke a response.

3. Brassinosteroid receptor inhibitors (BAK1) is a member of kinases called SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASEs; SERKs). Four SERKs have so far been reported which play significant roles in microspore development (SERK1, SERK2), defense signaling (SERK3), brassinosteroid signaling (SERK1, SERK3), and regulation of cell death (SERK3, SERK4). Functions of another kinase (SERK5) are not yet known.

Summary

- Brassinosteroids (BRs) are C-27, C-28, and C-29 steroids with different functional groups on A and B rings and on the side chain. BRs occur in algae, ferns, gymnosperms, and angiosperms. Brassinolide is a C-28 brassinosteroid and exhibits highest activity among all BRs so far known.
- Bioactive BR levels (homeostasis) are regulated by a variety of catabolic reactions, including hydroxylation, oxidation, sulphonation, epimerization, and conjugation to lipids or glucose. Level of active BR in plant tissue is also regulated by negative feedback mechanism, whereby BR concentration in the tissue above a certain level results in reduction in BR biosynthesis by the downregulation of BR biosynthesis genes and upregulation of genes for BR catabolism.
- BRs prevent photomorphogenesis in etiolated seedlings. Brassinolide (BL) and other BRs induce a dose-dependent swelling of the adaxial cells of the joint between the rice seedling's leaf blade and sheathing base.
- BR receptor (BRASSINOSTEROID INSENSITIVE 1; BRI1) on the plasma membrane binds with BR at its extracellular domain. The receptor contains leucine-rich repeats (LRRs) consisting of a series of 20–29-amino acid residues rich in leucines in the extracellular domain. Regions of LRRs are thought to function in protein-protein interactions.
- BR perception leads to inhibition of BIN2 kinase and stimulation of BES1 (transcription factors), leading to accumulation of unphosphorylated BZR1 and BES1 in the nucleus and causing the regulation of BR-responsive genes.
- In tomato, BR receptor has the ability to bind two different signals—brassinosteroids and systemin. BRI1 can probably signal from both plasma membrane and endosomes.

Multiple-Choice Questions

1. Brassinosteroids were first reported in:
   (a) *Arabidopsis thaliana*
   (b) *Brassica napus*
   (c) *Brassica juncea*
   (d) *Phaseolus vulgaris*

2. Which of the following is true for brassinosteroids?
   (a) Stimulate photomorphogenesis in dark
   (b) Stimulate skotomorphogenesis in light
   (c) Prevent photomorphogenesis in dark
   (d) Both a and b
3. Brassinosteroid mutants—bri1 and det2—have similar phenotypes, but exogenous application of brassinolide:
   (a) Can normalize their growth
   (b) Can normalize the growth of only det2
   (c) Cannot normalize the growth
   (d) Can normalize the growth of only bri1
4. CPD enzyme is involved in:
   (a) Brassinosteroid degradation
   (b) Brassinosteroid phosphorylation
   (c) Brassinosteroid biosynthesis
   (d) None of the above
5. bin2 (brassinosteroid insensitive 2) mutants exhibit:
   (a) Overexpression of BR signaling
   (b) Impaired expression of phosphatase
   (c) Impaired expression of ser/thr kinase
   (d) Both b and c

Answers

1. b 2. c 3. b 4. c 5. c

Suggested Further Readings

Clouse SD (2015) A history of brassinosteroid research from 1970 through 2005: thirty-five years of phytochemistry, physiology, genes and mutants. J Plant Growth Regul 34:828–844

Fariduddin Q, Yusuf M, Ahmad I, Ahmad A (2014) Brassinosteroids and their role in response of plants to abiotic stress. Biol Plant 58:9–17