Brassinosteroids Implicated in Growth and Stress Responses

Andrzej Bajguz and Alicja Piotrowska-Niczyporuk

Abstract Brassinosteroids (BRs) are steroidal hormones essential for plant growth and development. They are implicated in plant responses to abiotic environmental stresses such as low and high temperature, drought, salt, infection, pesticides, and heavy metals. BR-regulated stress response is a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of protein synthesis, and the production of various chemical defence compounds. However, the molecular mechanism of BR-induced plant abiotic stress tolerance remains poorly understood. The BR signalling is initiated by a ligand-induced kinase activation followed by receptor oligomerisation. The signal transduction in the cell is mediated through phosphorylation and transcription factors which directly bind to promoters of BR-responsive genes to regulate their expression. BRs that are biosynthesised using sterols as precursors are structurally similar to the cholesterol-derived, human steroid hormones and insect moulting hormones. The biosynthetic pathway of BRs is divided into multiple subunits. Depending on C-22 hydroxylation at campesterol, the BR pathway is further divided into the early and late C-22 oxidation pathways. Similarly, the C-6 position can be oxidised at campestanol or later at 6-deoxocathasterone stage, and thus these are called the early and late C-6 oxidation pathways, respectively. The pathways of BR biosynthesis in plants are well studied. Nevertheless, in order to understand properly the role of BRs during plant development under stress conditions, it seems essential to summarise the experimental data, focusing on the biosynthesis and signal transduction.

Keywords Biosynthesis • Brassinosteroid • Plant stress tolerance • Signal transduction

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Introduction

Brassinosteroids (BRs), a group of plant hormones, have been found in a wide range of organisms from lower to higher plants. BRs have been detected at low concentrations in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, and grain as well as unicellular green algae, pteridophytes, and bryophytes. Thus, it is conceivable that BRs are ubiquitous in the plant kingdom. They also occur in the insect and crown galls of *Castanea crenata*, *Distylium racemosum*, or *Catharanthus roseus*. These plants have higher levels of BRs than the normal tissues. Furthermore, young growing tissues contain higher levels of BRs than mature tissues. Pollen and immature seeds are the richest sources of BRs, while shoots and leaves usually have lower amounts. However, precise spatial and subcellular distribution of BRs still remains unknown (Bajguz and Tretyn 2003).

BRs are characterised by their polyhydroxylated sterol structure. They were first isolated and purified from *Brassica napus* pollen in 1979. The chemical structure of brassinolide (BL), the first BR, and that of the second compound, castasterone (CS), discovered in 1982, was found to be similar to that of ecdysone, the insect moulting steroid hormone (ecdysteroids), and mammalian steroids (e.g. estrogens, androgens, mineralocorticoids, and glucocorticoids). So far, more than 70 BL-related compounds have been identified from plants. Natural BRs have 5α-cholestane skeleton, and their structural variations come from the kind and orientation of oxygenated functions in A ring and B ring. They are divided into free (64) and conjugated (5) compounds. Among the 70 different BRs, BL was shown to possess the greatest growth-promoting activity. CS only exhibits about 10 % of the activity of BL. Other BRs are mainly intermediates of the BL biosynthetic pathway or inactivated products that resulted from various BR catabolic reactions. As inferred from the chemical structure of BL, it was hypothesised that active BRs should possess the following structural requirements. First, the A and B rings must be in the *trans* configuration, which is determined by an α hydrogen at C-5. Second, the B ring should contain a 6-oxo or a 6-oxo-7-oxa group. Third, the hydroxyl groups at C-2 and C-3 in ring A should be *cis* α-oriented. Fourth, the *cis* α-oriented hydroxyl groups at the C-22, C-23, and the C-24 positions should be occupied by either α-oriented methyl or ethyl groups (Fig. 1) (Bajguz and Tretyn 2003).

BRs function in multiple developmental stages, including regulation of gene expression, cell division and expansion, differentiation, programmed cell death, and homeostasis. BRs are implicated in physiological and biochemical response in plants, like vascular differential, stem elongation, leaf bending, epinasty, pollen tube growth, root inhibition, induction of ethylene biosynthesis, activation of proton pumps, photosynthesis, regulation of gene expression, and nucleic acid and protein synthesis (Hayat et al. 2010b). BRs also play a significant role in amelioration of various environmental stresses. More recently, interactions of BRs with other plant hormones, such as abscisic acid (ABA), auxins, cytokinins, gibberellins, and ethylene, have also been found to play a major role in plant stress alleviation. Furthermore, ability of BRs to boost antioxidant system of plants is extensively used to confer

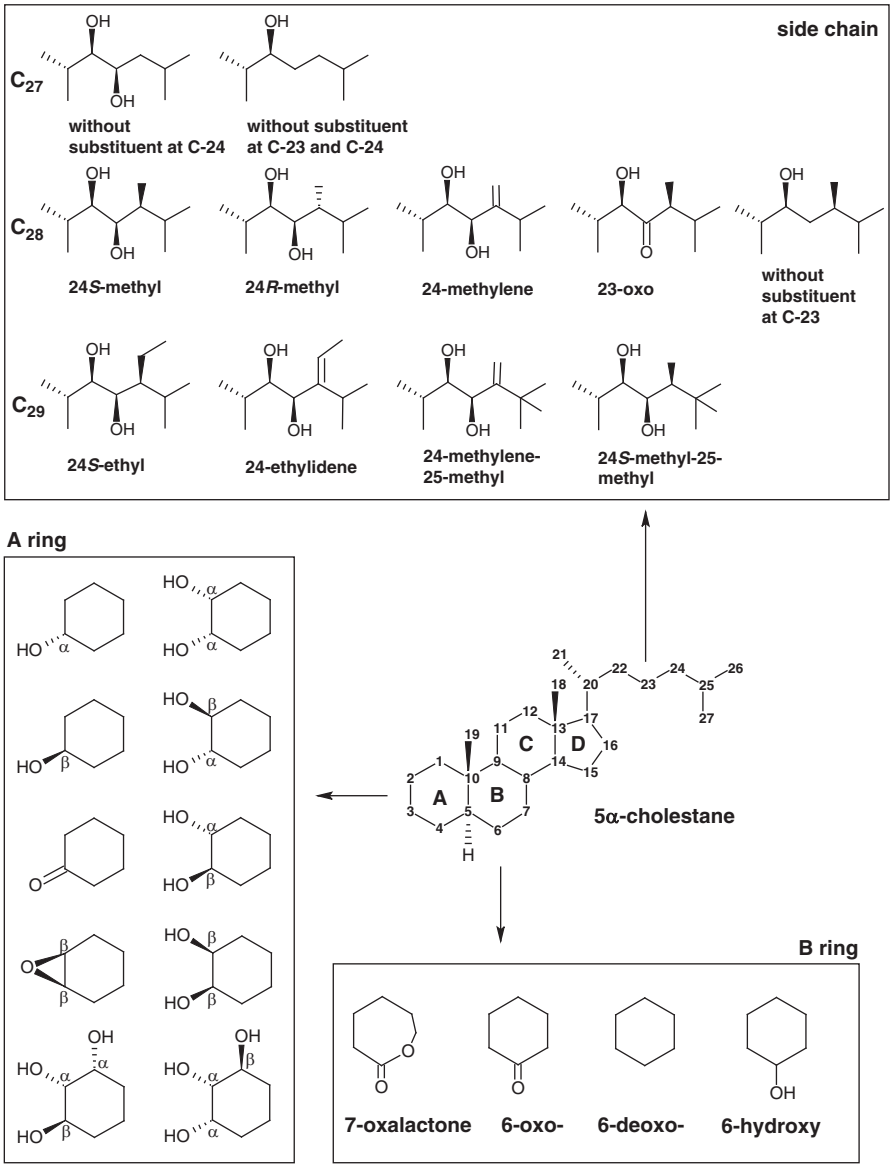

Fig. 1 Structural variations in brassinosteroids (adopted from Bajguz and Tretyn (2003))

resistance in plants against a variety of abiotic stresses, such as drought, heavy metal, pesticides, salinity, and thermal. Although much has been learned about their roles in plant development, the mechanisms by which BRs control stress responses and regulate stress responsive gene expression in plants are not fully acknowledged. Since BRs crosstalk with other plant hormones, it is likely that the stress tolerance

conferring ability of BRs lies in part in their interactions and stimulation of other stress hormones. BRs are not only implicated in plant response to abiotic and biotic stresses but also have medicinal applications (Bajguz and Hayat 2009). At present, our knowledge of the effects of BRs in animals or human is still rather fragmentary. However, it is known that BRs have an anabolic action, anticancer, and antiproliferative properties. BRs have also antiviral activities against herpes simplex viruses type I and II, arenaviruses, measles viruses, and vesicular stomatitis virus. BRs may prove to be promising leads for the development of new generation of drugs, especially against cancer or viral infection (Bajguz et al. 2013).

Brassinosteroid Biosynthesis

Campesterol, one of the major plant sterols, is the precursor of BRs, which is primarily derived from isopentenyl diphosphate (IPP). Sterols are synthesised via the non-mevalonate pathway in lower plants or the mevalonate pathway of isoprenoid metabolism in higher plants. In plants, IPP, the precursor of isoprenoids, is synthesised from acetyl-CoA via mevalonic acid (mevalonate pathway) or by pyruvate and glyceraldehyde 3-phosphate (non-mevalonate pathway). Isoprenoids are synthesised in all living organisms in at least one of two pathways. Plants synthesise isoprenoids by both the mevalonate pathway and the non-mevalonate pathway, segregating these pathways into different compartments: the non-mevalonate pathway synthesises IPP and dimethylallyl diphosphate in plastids, whereas the mevalonate pathway synthesises cytosolic IPP. The non-mevalonate pathway exists in eubacteria, algae (*Chlorella*, *Chlamydomonas*, and *Scenedesmus*) and higher plants (*Lemna* and *Wolffia*) (Bajguz and Asami 2004, 2005; Bajguz 2005; Choe 2006; Zhao and Li 2012).

The major pathway for BR biosynthesis has been established in *Catharanthus roseus* and *Arabidopsis thaliana* by conversion experiments using applied isotope-labelled BR intermediates. In this pathway, campesterol (the precursor of C_{28} BRs) is converted to campestanol (Fig. 2), which is then converted into two biologically active BRs (castasterone and brassinolide) via two parallel pathways, the early and late C-6 oxidation pathways. These oxidative steps are performed by cytochrome P450-type monooxygenases belonging to the closely related CYP85 and CYP90 families. While most of these enzymes were originally identified in *Arabidopsis*, several of their orthologs were soon recognised in other species, e.g. maize, rice, and tomato. BR biosynthesis mutants have defects in cytochrome P450 monooxygenases (P450s or CYPs) (Choe 2006). Enzymes of the BR biosynthetic pathway are summarised in Table 1.

Although metabolic experiments with labelled C_{27} BRs have not yet been performed, the natural occurrence of C_{27} BRs in plant tissues, e.g. tomato and *Arabidopsis* (6-deoxo-28-norcathasterone, 6-deoxo-28-norteasterone, 6-deoxo-28-nortyphasterol, 6-deoxo-28-norcastasterone, and 28-norcastasterone) suggests an in

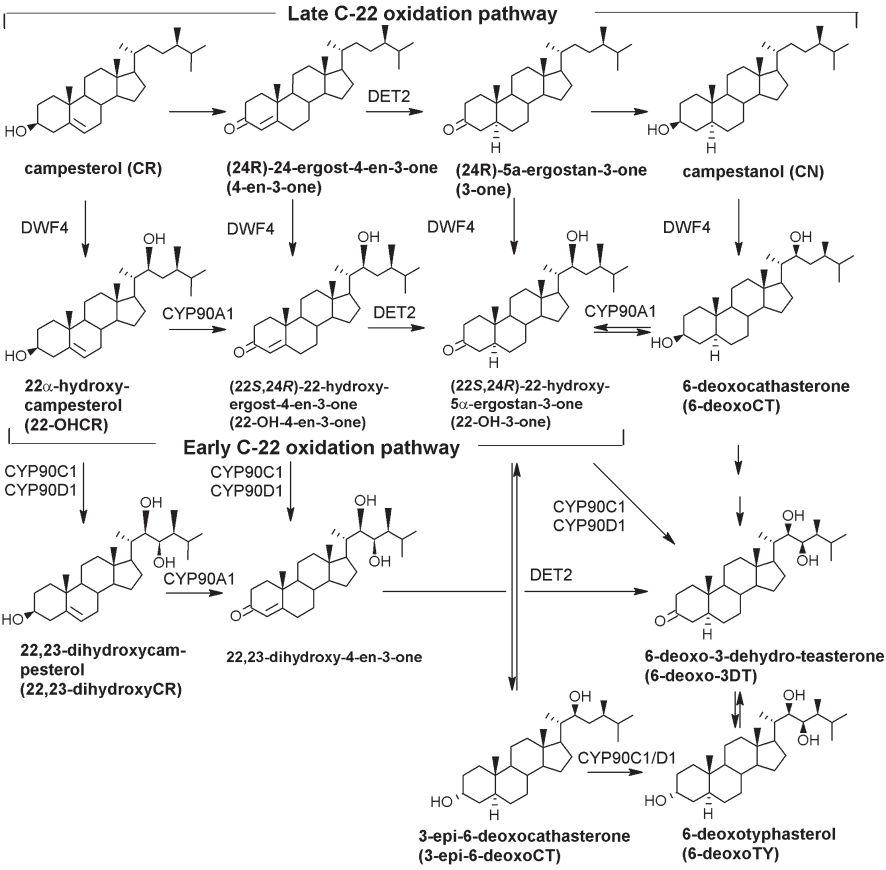

Fig. 2 C-22 oxidation pathways of sterols and their connection established in brassinosteroid biosynthesis in *Arabidopsis thaliana* (adopted from Bajguz (2005), Ye et al. (2011), Zhao and Li (2012))

vivo biosynthetic sequence of 28-nor-22-OH-campesterol → 28-nor-22-OH-4-en-3-one → 28-nor-22-OH-3-one → 6-deoxo-28-norcathasterone. Based on these findings, a biosynthetic pathway of C_{27} BRs has been suggested: cholestanol → 6-deoxo-28-norcathasterone (6-deoxo-28-norCT) → 6-deoxo-28-norteasterone (6-deoxo-28-norTE) → 6-deoxo-28-nor-3-dehydroteasterone (6-deoxo-28-nor-3DT) → 6-deoxo-28-nortyphasterol (6-deoxo-28-norTY) → 6-deoxo-28-norcastasterone (6-deoxo-28-norCS) → 28-norcastasterone (28-norCS) in tomato seedlings. In addition, the cell-free enzyme extract of tomato seedlings catalysed the conversion of cholesterol to cholestanol and 6-deoxo-28-norTE to 28-norCS via 6-deoxo-28-nor-3DT, 6-deoxo-28-norTY, and 6-deoxo-28-norCS. The reactions, named the late C-6 oxidation pathway for C_{27} BRs, have been demonstrated in Figs. 2 and 3 (Fujioka and Yokota 2003; Kim et al. 2004, 2005, 2008; Choe 2006; Choudhary et al. 2012; Joo et al. 2012).

Table 1 Enzymes of the brassinosteroid biosynthetic pathway in *Arabidopsis thaliana* (Schneider 2002; Choe 2006; Ye et al. 2011; Zhao and Li 2012)

Enzyme name	Description	Site of action
CYP85A1	BR C-6 oxidase	22-dihydroxyCR to 22,23-dihydroxy-4-en-3-one
CYP85A1, A2	BR C-6 oxidase	6-deoxoTE to TE, 6-deoxo-3DT to 3DT, 6-deoxoTY to TY, 6-deoxoCS to CS
CYP85A2	BR C-6 oxidase	6-deoxo-28-norTE to 28-norTE, 6-deoxo-28-nor-3DT to 28-nor-3DT, 6-deoxo-28-norTY to 28-norTY, 6-deoxo-28-norCS to 28-norCS, CS to BL
CYP90A1/CPD	Putative BR hydroxylase	22-OHCR to 22-OH-4-en-3-one, 22-OH-3-one to 6-deoxoCT
CYP90B1/DWF4	Steroid C-22 hydroxylase	CR to 22-OHCR, (24*R*)-24-ergost-4-en-3-one to 22-OH-4-en-3-one, (24*R*)-5α-ergostan-3-one to 22-OH-3-one, CN to 6-deoxoCT, 6-oxoCN to CT
CYP90C1/ROT3	BR C-23 hydroxylase	22-OHCR to 22-dihydroxyCR, 22-OH-4-en-3-one to 22-23-dihydroxy-4-en-3-one, 22-OH-3-one to 6-deoxo-3DT, 6-deoxoCT to 6-deoxoTE, CT to TE, 3-epi-6-deoxoCT to 6-deoxoTY
CYP90D1	BR C-23 hydroxylase	
DET2	Steroid-5α-hydroxylase	(24*R*)-24-ergost-4-en-3-one to (24*R*)-5α-ergostan-3-one, 22-OH-4-en-3-one to 22-OH-3-one, 22,23-dihydroxy-4-en-3-one to 6-deoxo-3DT

The *Arabidopsis dwarf4* (*dwf4*), *constitutive photomorphogenesis and dwarfism* (*cpd*) mutants are, through phenotypic rescue experiments using BR intermediates, thought to be blocked in the hydroxylation of C-22 and C-23, respectively. The *dwarf* (*d*) tomato mutant represents a new locus with the *Dwarf* gene (*D*) encoding a P450. It has been classified as CYP85 with high homology to CPD and DWF4. The tomato mutant *dumpy* (*dpy*) has been suggested to be the equivalent of *cpd*. DWARF acts as a C-6 oxidase, catalysing multiple C-6 oxidation reactions including 6-deoxoteasterone (6-deoxoTE) to teasterone (TE), 6-deoxo-3-dehydroteasterone (6-deoxo-3DT) to 3-dehydroteasterone (3-DT), 6-deoxotyphasterol (6-deoxoTY) to typhasterol (TY), and 6-deoxocastasterone (CS) to CS. Most of these reactions were confirmed in yeast using DWARF or its ortholog CYP85A1 (BR6ox1) from *Arabidopsis*. It is the key step linking the late C-6 oxidation pathway to the early C-6 oxidation pathway. The double mutant of *CYP85A1* and *CYP85A2 (BR6ox2)* displays a severe BR-defective phenotype, while the *CYP85A1* null mutant does not show any altered phenotypes and *CYP85A2* only exhibits subtle defective phenotypes (Kim et al. 2005). CYP85A2 catalyses those steps of C-6 oxidation overlapping with CYP85A1, but it is worth noting that only CYP85A2 (and not CYP85A1) is responsible for the Baeyer–Villiger oxidation step converting CS to BL (Clouse and Feldmann 1999; Bishop and Yokota 2001; Shimada et al. 2001; Bishop 2003, 2007; Fujioka and Yokota 2003; Müssig and Altmann 2003; Kim et al. 2005; Choudhary et al. 2012).

Arabidopsis de-etiolated2 (*det2*) was first identified as a mutant with a de-etiolated seedling phenotype when grown in the dark. Recessive mutation of *DET2*

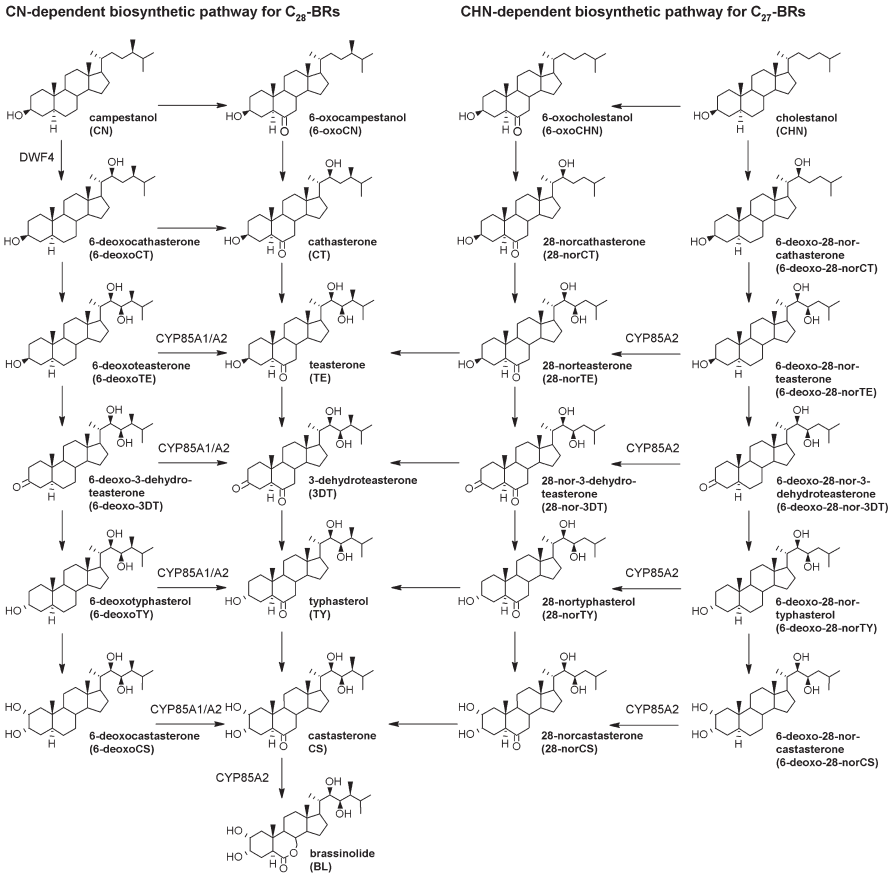

Fig. 3 Biosynthetic pathways for C_{27}- and C_{28}-brassinosteroids in *Arabidopsis thaliana* (adopted from Choe (2006), Ye et al. (2011), Joo et al. (2012), Zhao and Li (2012))

exhibits a typical BR-deficient phenotype including severe dwarfism, dark green colour, delayed flowering, reduced male fertility, and constitutive photomorphogenesis in the dark (Li et al. 1996). Biochemical analyses indicated that DET2 is involved in converting (24*R*)-ergost-4-en-3-one (4-en-3-one) to (24*R*)-5α-ergost-3-one (3-one), which is the second step in the BR-specific biosynthesis pathway (Fujioka et al. 1997; Noguchi et al. 1999a, b). Subsequently, a new subpathway via early C-22 oxidation was found, and in the *det2* mutant, the step converting 22-OH-4-en-3-one to (22*S*, 24*R*)-22-hydroxy-5α-ergost-3-one (22-OH-3-one) is blocked (Fujioka et al. 2002). *det2* mutants have also been identified in other plant species such as pea (*lk*), tomato, and *Pharbitis nil* (Suzuki et al. 2003; Nomura et al. 2004, 2005). DET2 is probably the only known non-P450 catalytic enzyme of the BR-specific biosynthesis pathway.

A T-DNA-tagged dwarfed mutant *dwf4* can only be rescued by BRs but not by other phytohormones (Azpiroz et al. 1998). Feeding experiments have suggested

that DWF4 may contribute to multiple C-22 hydroxylation steps in the BR biosynthetic pathway because only 22α-hydroxylated BRs can rescue the *dwf4* defective phenotypes (Choe et al. 1998). In the early C-22 oxidation pathway, DWF4 was found to catalyse steps like campesterol (CR) to 22-OHCR, 4-en-3-one to 22-OH-4-en-3-one, and 3-one to 22-OH-3-one (Fujioka et al. 2002). In tomato, these steps are catalysed by CYP724B2 and CYP90B3, both of which share a high sequence identity with DWF4 from *Arabidopsis* and rice (Ohnishi et al. 2006b).

In *Arabidopsis* seedlings, *CPD*/*CYP90A1* and *CYP85A2* transcripts were detected mainly in shoots, *ROTUNDIFOLIA3* (*ROT3*)/*CYP90C1* and *CYP90D1* transcripts preferentially in roots, while *DET2* and *DWF4*/*CYP90B1* mRNAs were found in comparable amounts in both the seedling parts (Bancoş et al. 2002). Similar partitioning of the orthologous *CYP90A9*, *CYP90A10*, *CYP85A1*, *CYP85A6*, *CYP90D7*, *LK*, and *CYP90B8* transcripts was observed in pea seedlings (Nomura et al. 2007). The enzyme encoded by the *CPD* (At5g05690) gene was shown to be required for the synthesis of C-23-hydroxylated BRs (Szekeres et al. 1996); gene construct was highly active in expanding rosette leaves, particularly in the adaxial parenchimatic tissues, axillary leaves, and sepals.

Of the *ROT3* (*At4g36380*) and *CYP90D1* (*At3g13730*) genes, which encode functionally redundant C-23 hydroxylases (Ohnishi et al. 2006a), only the expression of the *ROT3* was studied with a *GUS* fusion construct. Early analyses have suggested that ROT3 and its homologue CYP90D1 catalyse different steps in the BR biosynthetic pathway. In young plants, it was found ubiquitous and almost equal in all vegetative organs. *CYP85A1* (*At5g38970*) encodes the C-6 oxidase, and *CYP85A2* (At3g30180) the C-6 oxidase and BL synthase that produce the bioactive BR forms CS, or CS and also BL, respectively (Shimada et al. 2001, 2003; Kim et al. 2005; Nomura et al. 2005). A very similar expression pattern was observed with *Dwarf*, the *CYP85A1* gene of tomato, which was also most active in meristematic regions and developing organs (Montoya et al. 2005). A quantitative comparison of mRNA levels in organs of mature *Arabidopsis* indicated that each of the BR biosynthetic P450 genes has a unique organ-specific expression pattern (Shimada et al. 2003).

Inhibitors of the biosynthesis and metabolism of BRs have complementary roles in the analysis of the functions of BRs in plants to BR-deficient mutants. The P450 inhibitors, clotrimazole and ketoconazole, have been found to suppress the 25-hydroxylation of 24-epiBL (24-epibrassinolide) and BL in tomato cell suspension cultures, indicating that the 25-hydroxylation is catalysed by a P450 enzyme. Recently, the first specific BR biosynthesis inhibitor, brassinazole (Brz), has been synthesised. The application of Brz, a triazole derivative, to plants resulted in growth inhibition or dwarfism but exogenous brassinolide reversed the negative effect. *Arabidopsis* seedlings treated by Brz show a typical BR-deficient mutant phenotype similar to those of *det2* and *cpd*. Brz blocks the conversion of campestanol to 6-deoxoCT, 6-deoxoCT to 6-deoxoTE, 6-oxocampestanol to cathasterone (CT), and CT to TE in BR biosynthetic pathways (Asami and Yoshida 1999; Asami et al. 2003).

The cell cultures produced representatives of C_{28} BRs, such as CT, TE, 3-DT, TY, CS, and BL. The levels of BRs in cell cultures of *C. roseus* have been found to

be comparable to those of BR-rich plant tissues such as pollen and immature seeds. The occurrence of 6-deoxoBRs such as 6-deoxoCS, 6-deoxoTE, and 6-deoxoTY in several plants suggested that the parallel or/and alternative BR biosynthetic route exists. This late C-6 oxidation pathway for C_{28} BRs in *A. thaliana*, *C. roseus*, *L. esculentum*, *Chlorella vulgaris*, and *Marchantia polymorpha* has been investigated. The conversion of 6-deoxoCS to CS via 6α-hydroxyCS has been found in *A. thaliana*. In addition to the early and late C-6 oxidation pathways of C_{28} BRs, cross-links between both branches also exist. The two pathways converge at CS, which ultimately leads to the biosynthesis of BL. Conversion of CS to BL is the final biosynthetic step of BRs. Unfortunately, the biosynthesis of C_{29} BRs is still unclear. An early C-22 oxidation branch, also called the CN-independent pathway, was demonstrated to occur alongside the previously reported CR to CN pathway, and it could be the dominant upstream BR biosynthesis pathway (Fig. 2). Campestanol plays an important intermediate in the BRs biosynthetic pathway. The biosynthetic sequence between campesterol and campestanol leads to completion of the carbon skeleton including trans stereochemistry of the A/B ring junction. The following conversions, campesterol → (24*R*)-ergost-4-en-3β-ol (4-en-3β-ol) → (24*R*)-ergost-4-en-3-one (4-en-3-one) → (24*R*)-5α-ergostan-3-one (3-one) → campestanol, named the late C-22 oxidation pathway, led to 6-deoxoCT. On the other hand, the conversion of campesterol to 6-deoxoCT via intermediates such as (22*S*)-22-hydroxycamesterol, (22*S*,24*R*)-22-hydroxyergost-4-en-3-one (22-OH-4-en-3-one), and (22*S*,24*R*)-22-hydroxy-5α-ergostan-3-one (22-OH-3-one) is now generally accepted as the early C-22 oxidation pathway. Furthermore, the conversion of (22*S*,24*R*)-22-hydroxy-5α-ergostan-3-one to 3-epi-6-deoxocathasterone also exists. Recently, Ohnishi et al. (2006a) reported C-23 hydroxylation shortcuts, leading (22*S*, 24*R*)-22-hydroxy-5-ergost-3-one (22-OH-3-one) and 3-*epi*-6-deoxocathasterone (3-epi-6-deoxoCT) to be directly converted to 3-dehydro-6-deoxoteasterone (6-deoxo-3DT) and 6-deoxotyphasterol (6-deoxoTY), respectively. In addition, the existence of high levels of 6-deoxoCT and 6-deoxoCS in different species analysed suggests that the late C-6 oxidation pathway probably is the predominant BR biosynthesis branch (Fig. 3) (Nomura et al. 2001; Bishop and Yokota 2001; Schneider 2002; Fujioka and Yokota 2003; Choe 2006; Bajguz 2009b; Choudhary et al. 2012).

Brassinosteroids and Abiotic Stress

Brassinosteroids are steroidal plant hormones implicated in the promotion of plant growth and development. One of the most interesting influences of BRs is their ability to confer resistance to plants against various abiotic stress (Fig. 4) (Bajguz and Hayat 2009; Hayat et al. 2010b). Plant responses to different types of stresses are associated with generation of reactive oxygen species (ROS), suggesting that ROS may function as a common signal in signalling pathways of plant stress responses. It was shown that exogenous application of BRs is involved in plant response to oxidative stress (Bajguz 2011). For example, when maize (*Zea mays*) seedlings

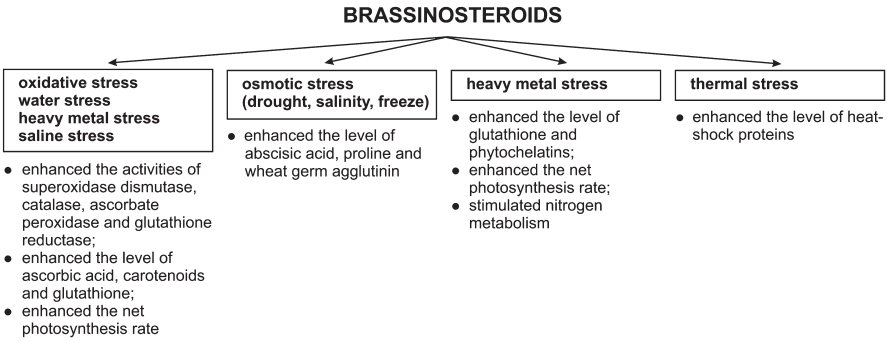

Fig. 4 Effects of brassinosteroids on plants exposed or subjected to abiotic stresses (adopted from Bajguz and Hayat (2009))

treated with BL were subjected to water stress, the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) as well as ascorbic acid and carotenoid contents increased (Li et al. 1998). Rice seedlings exposed to saline stress and treated with BR showed a significant increase in the activities of CAT, SOD, glutathione reductase (GR), and a slight increase in APX (Núñez et al. 2003). *C. vulgaris* responds to heavy metals (cadmium, copper, and lead) by inducing several antioxidants, including several enzymatic systems and the synthesis of low-molecular-weight compounds, such as phytochelatins (PCs). Treatment with BL was effective in increasing the activity of antioxidant enzymes (CAT, GR, and APX) and the content of ascorbic acid, carotenoids, and glutathione (Bajguz 2011). The influence of 24-epiBL on some enzymatic antioxidants in tomato leaf disc under high (40 °C) temperature was reported (Mazorra et al. 2002). Studies on cucumber (*Cucumis sativus*) indicate that BR levels are positively correlated with the tolerance to photooxidative and cold stresses and resistance to *cucumber mosaic virus*. The BR treatment enhanced NADPH oxidase activity and elevated H_2O_2 levels in apoplast. BR-induced H_2O_2 accumulation was accompanied by increased tolerance to oxidative stress (Xia et al. 2009). However, it is still unclear whether BRs directly or indirectly modulate the responses of plants to oxidative stress.

Drought-, salinity-, and freeze-induced dehydration constitute direct osmotic stresses, whereas chilling and hypoxia can indirectly cause osmotic stress via effects on water uptake and loss. Water-stress-induced decline in root nodulation is associated with increase in ABA and decline in cytokinin contents in the nodulated roots (Kang et al. 2009). BRs have the potential to improve root nodulation and pod yield in the irrigated and water-stressed plants, an effect that could be mediated through an influence on cytokinin content in the nodulated roots of *Phaseolus vulgaris*. BR application also resulted in the enhancement of seedling growth, which was evident in terms of seedling length, seedling fresh, and dry weights of sorghum (*Sorghum vulgare*) under osmotic stress (Vardhini and Rao 2003; Upreti and Murti 2004). Similar results have been shown in sugar-beet plants under drought stress, in which a reduction of taproot weight was correlated to stress severity. Treatment with BR

fully compensated for the reduction in biomass caused by mild drought stress. On the other hand, increases in biomass was correlated with increases in acid invertase activity in young leaves, which could have likely provided more assimilates to the plant due to their larger sizes (Schilling et al. 1991). Furthermore, osmotic stress resulted in a considerable reduction in the protein contents in all the three varieties of sorghum. However, BRs not only restored but also stimulated the level of protein and free proline (Vardhini and Rao 2003). 28-homobrassinolide (28-homoBL) also had a stimulatory effect on the growth of drought-tolerant and drought-susceptible wheat (*Triticum aestivum*) varieties under stress conditions. Application of 28-homoBL resulted in increased relative water content, nitrate reductase activity, chlorophyll content, and photosynthesis under both conditions. It also improved membrane stabilisation. These beneficial effects resulted in higher leaf area, biomass production, grain yield, and yield-related parameters in the stress-treated plants. Results obtained by Fariduddin et al. (2009a) indicate that BRs may alleviate drought stress through activation of enzymatic antioxidant system such as CAT, APX, and SOD as well as stimulation of photosynthesis process in *Brassica juncea* plants. In drought-stressed *Chorispora bungeana* plants, BRs inhibited lipid peroxidation, measured in terms of malondialdehyde content, and stimulated antioxidant enzyme activity, chlorophyll content, and photosynthesis. These results suggested that BRs could improve plant growth under drought stress (Li et al. 2012).

Water stress led to oxidative damage. BR treatment of *Zea mays* leaves increased the content of ABA and upregulated the expression of the ABA biosynthetic gene in maize leaves. Moreover, BR treatment induced increases in the generation of nitric oxide (NO) in mesophyll cells of maize leaves, and treatment with the NO donor sodium nitroprusside up-regulated the content of ABA and the expression of ABA biosynthetic gene in maize leaves. These results suggest that BR-induced NO production and NO-activated ABA biosynthesis are important mechanisms for BR-enhanced water stress tolerance in leaves of maize plants (Zhang et al. 2011).

High concentrations of all metals in environment, including those essential for growth and metabolism, exert toxic effects on the metabolic pathways of plants. Plant responds to heavy metal toxicity in different ways, such as by enhancement of the content of PCs, upregulation of antioxidants, accumulation of compatible solutes, accumulation of low-molecular-weight metabolites, and changes in the ABA, auxin, cytokinin, and gibberellin levels. However, BRs are not involved by synthesising de novo in response of algal growth under heavy metal stress but might interact via increasing the contents of other plant hormones (e.g. auxin, cytokinin, and ABA) (Atici et al. 2005; Hsu and Kao 2003; Sharma and Kumar 2002; Bajguz 2011). A recent study indicated that in *C. vulgaris* cultures treated with heavy metals, the endogenous level of BL was very similar to that of control. This finding suggests that the activation of BR biosynthesis is not essential for the growth and development of *C. vulgaris* cultures in response to heavy metal stress (Bajguz 2011). BRs stimulate the synthesis of PCs that are directly involved in detoxification of heavy metals in *C. vulgaris* cells treated with lead. The stimulatory activity of BRs on PC synthesis was arranged in the following order: brassinolide (BL) > 24-epiBL > 28-homoBL > castasterone (CS) > 24-epiCS > 28-homoCS (Bajguz 2002).

The cultures of *C. vulgaris* treated with BRs and heavy metals show a lower bioaccumulation of heavy metals than the cultures treated with metals alone. Application of BRs to *C. vulgaris* cultures reduced the impact of heavy metal stress on growth; prevented chlorophyll, sugar, and protein loss; as well as stimulated the activity of enzymatic and nonenzymatic antioxidant system (Bajguz 2000, 2002, 2010). BRs also reduced the content of cadmium in the seedlings of winter rape (Janeczko et al. 2005) and copper in Indian mustard (Sharma and Bhardwaj 2007). BRs eliminate the toxic effect of cadmium on photochemical pathways in rape cotyledons, mainly by diminishing the damage in reaction centres and O_2 evolving complexes as well as maintaining efficient photosynthetic electron transport (Janeczko et al. 2005). Moreover, Bilkisu et al. (2003) reported that BL during aluminium-related stress stimulated growth of *Phaseolus aureus*. It was shown that changes in the metal content were influenced by 24-epiBL and were dependent on the stage of plant development when the seeds were treated. The application of BRs also improved the performance of mustard (Hayat et al. 2007a), chickpea (Hasan et al. 2008), and tomato (Hayat et al. 2010a) subjected to cadmium stress and also of mung bean (Ali et al. 2008) and mustard (Alam et al. 2007) to aluminium and nickel, respectively. Hasan et al. (2008) reported that BRs enhanced activity of the antioxidant enzymes (CAT, SOD, peroxidase) and proline content in chickpea, which resulted in the improvement of nodulation, nitrogen fixation, and pigment composition, as well as carbonic anhydrase and nitrate reductase activities. A similar pattern of response together with an elevation in the photosynthesis was observed in the plants of mustard and tomato exposed to cadmium through nutrient medium (Hayat et al. 2007a, 2010a, b). The plants treated with 24-epiBL or 28-homoBL showed significantly enhanced growth, photosynthesis, antioxidant enzyme activities, and proline content in aluminium-stressed mung bean plants (Ali et al. 2008) and in *Brassica juncea* that was exposed to different levels of copper (Fariduddin et al. 2009b). In another independent study, the activities of the CAT, peroxidase, carbonic anhydrase, and nitrate reductase enzymes were found to exhibit a significant enhancement by BL treatment in mustard plants grown under nickel stress (Alam et al. 2007). Additionally, these BL-treated and nickel-stressed plants exhibited an elevation in the relative water content and photosynthetic performance. *Raphanus sativus* treated with 24-epiBL in combination with copper enhanced level of phytohormones such as indole-3-acetic acid (IAA) and ABA as well as polyamine contents which may be involved in plant adaptation to the stress factors (Choudhary et al. 2010).

BRs have been reported to alleviate salinity stress on seed germination and seedling growth in many plants. The application of 24-epiBL resulted in substantial improvement in the seed germination and seedling growth of *Eucalyptus camaldulensis* under saline stress (Sasse et al. 1995). BRs removed the salinity-induced inhibition of seed germination and seedling growth in case of rice (*Oryza sativa*). BRs also restored the level of chlorophylls and increased nitrate reductase activity under salt stress. The activity of this enzyme plays a pivotal role in the supply of nitrogen and the growth and productivity of plants, especially in cereals (Anuradha and Rao 2003). The 28-homoBL-treated plants also possessed higher seed yield in

comparison to the plants subjected to NaCl stress, at harvest. Similarly, the spray of 28-homoBL to the foliage or supply through roots of *B. juncea* plants generated from the seeds soaked in NaCl enhanced the growth, nucleic acid content, ethylene, and seed yield (Hayat et al. 2007b).

BRs may also induce tolerance to temperature stress in many plants. For example, leaf spraying of BRs on the rice seedlings at the 4th leaf stage increased plant height and the fresh weights of tops and roots under chilling stress (Fujii and Saka 2001). Extreme temperatures (7 and 34 °C) increased stress symptoms, i.e. necrotic areas on the leaves of bananas. However, in plants treated with a trihydroxylated spirotane, an analogue of BR, the effects of thermal stress were significantly reduced (González-Olmedo et al. 2005). Cool temperature affected leaf emergence with a significant reduction in their number, but application of BR analogue had marked positive effect. Plant height was also significantly reduced by both temperature extremes, whereas the application of BR analogue was effective only in plants exposed to the warmer temperature (González-Olmedo et al. 2005). Application of 24-epiBL minimally increased freezing tolerance of brome grass (*Bromus inermis*) cells by 3–5 °C but markedly enhanced cell viability following exposure to high (40–45 °C)-temperature stress (Wilen et al. 1995).

Treatment of *B. napus* and tomato seedlings with 24-epiBL led to an increase in the basic thermotolerance associated with the higher accumulation of four major classes of heat-shock proteins (hsps): hsp100, hsp90, hsp70, and low-molecular-weight hsps. The higher level of hsps in 24-epiBL-treated seedlings did not correlate with hsp mRNA levels during the recovery period. This finding suggests that 24-epiBL treatment limits the loss of some of the components of the translational apparatus during a prolonged heat stress and increases the level of expression of some of the components of the translational machinery during recovery. The higher hsp synthesis during heat stress resulted in a more rapid resumption of cellular protein synthesis following heat stress and a higher survival rate (Dhaubhadel et al. 1999, 2002). 24-epiBL also induced the expression of mitochondrial small hsps in tomato leaves. BR-treated tomato plants had better photosynthetic efficiency. Significantly higher in vitro pollen germination, enhanced pollen tube growth, and low pollen bursting have been observed in the presence of 24-epiBL at 35 °C, a temperature high enough to induce heat-stress symptoms in tomato, indicating a possible role of BRs during plant growth and reproduction. The beneficial effect of BR application was also observed in fruit yield, which was increased during heat-stressed conditions. This increase in fruit yield was mainly due to increase in fruit number by 24-epiBL application (Singh and Shono 2005).

The exogenously applied BL can also stimulate ABA content in *C. vulgaris* cultures subjected to short-term heat stress (30–40 °C). In parallel, under these conditions treatment with BL resulted in growth levels very similar to those of control cell cultures (nontreated). BL had no significant effect on the content of chlorophyll or sugar in *C. vulgaris* cells. Only a slight effect of BL on the protein content was observed. Under normal growth conditions (25 °C), BL showed a minor increase in the ABA content in *C. vulgaris* cells (Bajguz 2009a).

Signal Transduction of Brassinosteroids

Brassinosteroid Receptor

Recently by developing genetics, genomics, proteomics, and many other approaches performed mainly in *A. thaliana*, a model of BRs signal transduction pathway has been established. The process is commenced by the perception of the hormone ligand by the cell membrane-associated receptor complex, which initiates a relay mediated by phosphorylation/dephosphorylation cascade leading to changes in target gene expression (Gruszka 2013).

BRs are perceived by a plasma-membrane-localised leucine-rich-repeat (LRR) receptor-like kinase (RLK), standing for brassinosteroid-insensitive 1 (BRI1) (Li 2011). BRI1 was isolated and cloned following the identification of a large number of recessive mutant alleles on a single locus (Clouse et al. 1996; Li and Chory 1997). Recent structural studies have confirmed the role of BRI1 as a plasma membrane receptor for BRs (She et al. 2013). BRI1 protein possesses three major domains with unique function in BR perception and receptor activation: a large extracellular domain, a small transmembrane domain, and an intracellular kinase domain (Fig. 5). The extracellular domain of BRI1 contains an amino *N*-terminal signal peptide, a leucine-zipper motif, 24 LRRs, and an island domain located between the 20th and 21st LRRs (Vert et al. 2005; Yang et al. 2011). Further dissection of the extracellular domain of BRI1 revealed a minimal BR-binding region consisting of a 70-amino-acid island domain and its carboxyl *C*-terminal flanking LRR21, which together define a novel steroid-protein-binding element (Kinoshita et al. 2005). The intracellular domain can be further divided into a small intracellular juxtamembrane region (JM), a kinase catalytic domain, and a *C*-terminal tail. The JM domain is required for transducing signal from the outside to the inside of a cell (Wang et al. 2005). Experiments performed on *A. thaliana* plants indicated several Ser/Thr phosphorylation sites within the catalytic domain critical for BR signalling, which include Thr-1049, Ser-1044, and Thr-1045. BRI1 kinase with mutation of Ser-1049A or Ser-1044A/Thr-1045A completely lost its activity in vitro, and transgenic plants carrying these mutated BRI1 also failed to rescue the dwarf phenotype of *bri1-5* (Wang et al. 2005; Yang et al. 2011; Hao et al. 2013).

Given that BRI1 forms homodimer in the absence and presence of BRs, it was proposed that an auto-regulatory mechanism is involved in the activation of BRI1. Without BRs, BRI1 homodimer is kept at quiescent state by its *C*-terminal tail. BR binding induces the conformational change of its kinase domain, and subsequent auto-phosphorylation at a number of sites, including several Ser/Thr residues in the *C*-terminal tail to release its auto-inhibition (Wang et al. 2005). In addition, a specific negative regulator, called BKI1 (BRI1 kinase inhibitor 1), is also required to keep BRI1 at low and basal activity by preventing the interaction of BRI1 with other positive regulators (Wang and Chory 2006; Gruszka 2013).

In the BR receptor complex, besides BRI1, another receptor kinase BAK1 (Fig. 5) (LRR-RK BRI1-associated receptor-like kinase) was also reported to be

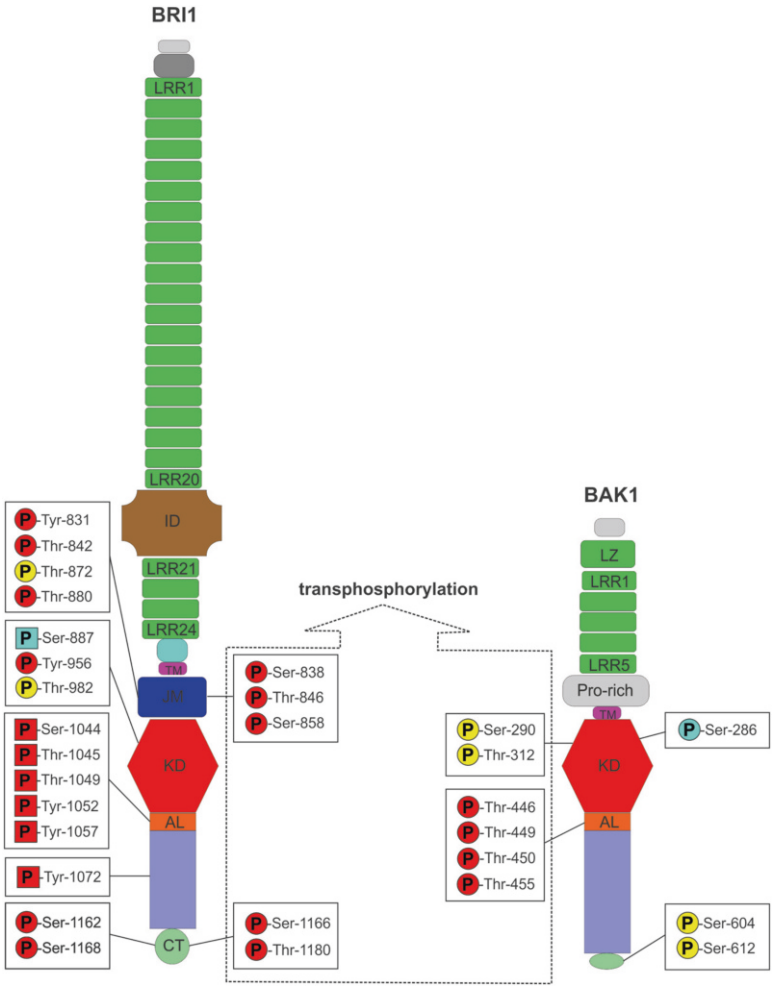

Fig. 5 The structure of BRI1 and BAK1. *LRR* leucine-rich repeats, *ID* island domain, *TM* single-pass transmembrane region, *JM* juxtamembrane region, *KD* kinase domain, *CT* C-terminal region, *LZ* leucine zippers, *pro-rich* proline-rich region, *AL* activation loop of kinases. The putative signal peptide region has been shown as a black box and unassigned regions have been shown as *grey boxes*. The confirmed phosphorylation sites have been marked with *circles*, and putative phosphorylation sites have been marked with *squares* containing the *letter P*. The activation phosphorylation sites have been shown in red, inhibitory sites in *blue*, and residues without significant effect on the kinase activity or not examined experimentally in *yellow* (adopted from Kim and Wang (2010))

required in the activation of BRI1. BRI1 and BAK1 can interact with each other through their kinase domains (Wang et al. 2005). After BR perception by the extracellular domain of BRI1, the kinase domain of BRI1 first phosphorylates and partially activates BAK1, then BAK1 in turn transphosphorylates BRI1 to further enhance the kinase activity of each other (Wang et al. 2008). Before BR binding,

BRI1 is kept inactive by auto-inhibition of its *C*-terminal region and by a negative regulator BKI1. Upon BR perception, BRs induce a conformational change of the intracellular domain of BRI1 playing role as Ser/Thr kinases to autophosphorylate its *C*-terminal tail and phosphorylate BKI1 to release their inhibition on BRI1 activity (Wang and Chory 2006). The pre-activated BRI1 will recruit BAK1 to its proximity to enhance each other's kinase activity via transphosphorylation and to form a fully activated receptor complex (Oh et al. 2009; Hao et al. 2013).

In addition to its critical role in BR signalling for plant growth, BAK1 has been discovered to impact plant MICROBIAL ASSOCIATED MOLECULAR PATTERN (MAMP)-/PATHOGEN-ASSOCIATED MOLECULAR PATTERN (PAMP)-triggered immunity (PTI) through the formation of heterodimers with other pattern-recognition receptors (PRRs) such as flagellin-sensing 2 (FLS2) in a BR-independent manner. Therefore, BAK1 plays key roles in multiple independent pathways by enhancing the signalling output of distinct LRR-RLKs that bind different ligands (Chinchilla et al. 2007).

Substrates of BRI1 Kinase

One of BRI1's substrates is BKI1. BKI1 acts as a negative regulator of BR signalling as indicated by overexpression of BKI1 causing a *bri1*-like dwarf phenotype and inhibiting BR-signalling outputs (Fig. 6) (Wang and Chory 2006). In vitro pull-down assays revealed that the interaction between BRI1 and BAK1 was severely reduced by additional BKI1 protein, suggesting that BKI1 inhibit BR signalling by preventing positive regulators, such as BAK1, from accessing BRI1. Interestingly, BR treatment can rapidly induce the dissociation of BKI1 from plasma membrane, and this process is dependent on a kinase-active BRI1. BKI1 can be phosphorylated by BRI1 kinase, which may lead to the dissociation of BKI1 from BRI1 and plasma membrane through unknown mechanisms (Wang and Chory 2006).

Another BRI1 substrate is polypeptide transthyretin-like protein (TTL) (Nam and Li 2002). TTL is a tetrameric, bifunctional protein with decarboxylase and hydrolase activity, which is phosphorylated by BRI1 and functions as a negative regulator of BR signalling. The exact role of TTL in regulation of this process is not known; however, it has been recently reported that TTL binds kinase-active BRI1 with higher affinity than kinase-inactive BRI1, indicating that TTL may inhibit BRI1 signalling after its activation (Gruszka 2013).

A proteomic analysis led to the identification of other components of the BR receptor complex—BR-signalling kinases (BSKs) belonging to the subfamily of the receptor-like cytoplasmic kinases (RLCK-XII) and functioning as positive regulators of BR signalling. The members of BSK family transmit the signal between membrane-bound receptor complex and cytoplasmic regulators of BR signalling. Two paralogous proteins, BSK1 and BSK3, interact directly with BRI1 in the absence of BR, whereas upon the ligand binding to BRI1, this kinase phosphorylates BSK1 on Ser-230, inducing its activation and release from the

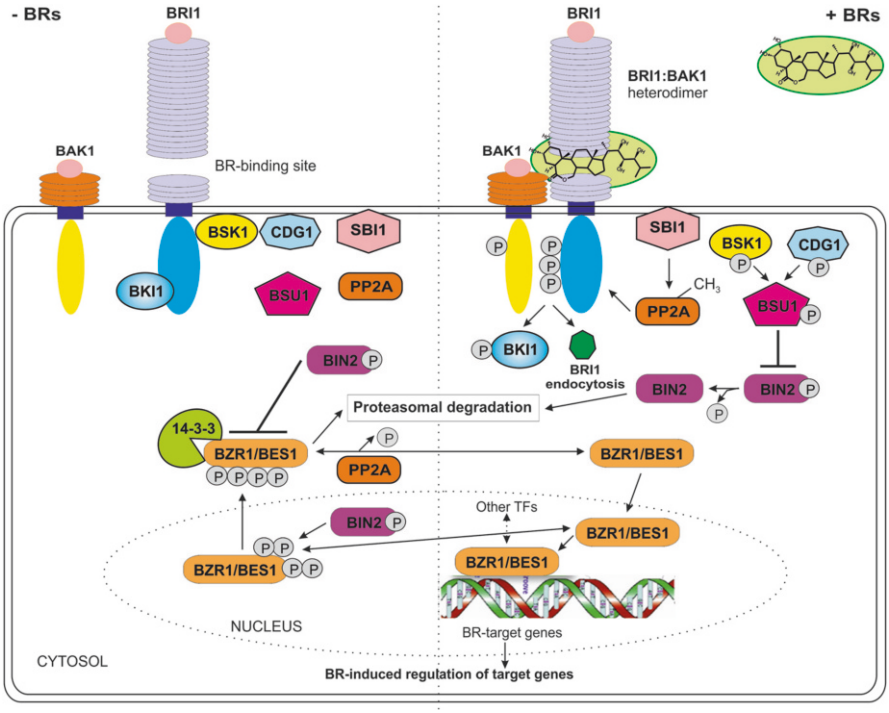

Fig. 6 The model of brassinosteroid signalling in plants (adopted from Bajguz et al. (2013)). Brassinosteroid (BR) signal is perceived by BR-insensitive1 (BRI1) which is a plasma membrane localised leucine-rich repeat (LRR) receptor-like kinase. In the absence of BRs, BRI1 is inactive as a homodimer, due to its binding with the negative regulator BRI1 KINASE INHIBITOR 1 (BKI1) through its cytoplasmic domain. In the presence of BRs, BR binding activates BRI1 kinase activity, through association with its co-receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1)/SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (SERK3) and phosphorylation of BKI1, leading to the disassociation of BKI1 from the plasma membrane. Activated BRI1 phosphorylates the receptor-like cytoplasmic kinases (RLCKs), BR SIGNALLING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH 1 (CDG1), which then activate a phosphatase, BRI1-SUPPRESSOR 1 (BSU1). BSU1 positively regulates BR signalling by dephosphorylating the negative regulator brassinosteroid-insensitive 2 (BIN2). This process facilitates accumulation of unphosphorylated brassinazole-resistant 1 (BZR1) and bri1-EMS-Suppressor 1 (BES1) in the nucleus. BES1 binds to E-box by interacting with BIM1 or MYB30 (TFs) to promote target gene expression. BZR1 could also bind to E-box and BES1 to BRRE, so the functions of the family members may overlap. These are key TFs activating the BR-signalling pathway in plants. Protein phosphatase 2A dephosphorylates BZR1 and also BRI1 in mediating BR signalling. BRI1 degradation depends on PP2A-mediated dephosphorylation that is specified by methylation of the phosphatase, thus leading to the termination of BR signalling

receptor complex. The activated BSK1 interacts with BRI1-supressor1 (BSU1) phosphatase promoting its interaction with the main negative regulator of BR signalling pathway—brassinosteroid-insensitive 2 (BIN2) (Fig. 6) (Tang et al. 2008; Gruszka 2013).

Downstream Events of Brassinosteroid Signalling

A crucial role in BR signalling is played by the serine-threonine kinase BIN2, which is another negative regulator of BR signalling, phosphorylating and thus inhibiting transcription factors regulating expression of target genes (Fig. 6) (Vert and Chory 2006; Yan et al. 2009). The *Arabidopsis BIN2* belongs to a multigene family encoding glycogen synthase kinase 3 (GSK3). GSK3-encoding genes are present in all land plants and in algae, and protists, raising questions about possible ancestral functions in eukaryotes. Studies have revealed that plant GSK3 proteins are actively implicated in hormonal signalling networks during development (e.g. development of generative organs) and as well as in biotic and abiotic stress responses (salinity stress and wounding). BIN2 is encoded by a member of the subfamily of ten related genes—*Arabidopsis* shaggy-like kinases (ASKs) (Vert and Chory 2006). The level of BIN2 protein can be regulated by BR signal likely through a proteasome-mediated protein degradation system, because the exogenously applied BRs can lead to a reduction of BIN2 proteins, and treatment with a proteasome inhibitor, MG132, can promote the accumulation of BIN2 (Peng et al. 2010). In the absence of BR, BIN2 autophosphorylates on Tyr-200 residue, which is required for its kinase activity. BIN2 kinase activity is suppressed by dephosphorylation of the Tyr-200 residue after perception of BR molecule by the BRI1-BAK1/SERK3 receptor complex and initiation of the signalling cascade. BIN2 activity is directly inhibited by BSU1 phosphatase, which dephosphorylates the Tyr-200 residue of BIN2 kinase (Ye et al. 2011; Hao et al. 2013).

A protein phosphatase, BSU1 (BRI1 suppressor protein 1), is a constitutively nuclear-localised Ser/Thr phosphatase (Mora-Garcia et al. 2004; Ryu et al. 2010). BSU1 plays a crucial role in positive regulation of BR signalling by repressing the activity of BIN2 kinase (Fig. 6). BSU1 contains *N*-terminal Kelch-repeat domain and *C*-terminal phosphatase domain and shows basal level of BIN2-binding and dephosphorylation. Activated BSU1 interacts with BIN2 kinase and inactivates it through dephosphorylation of Tyr-200, which is crucial residue for BIN2 activity. BSU1 phosphatase is localised in both the cytoplasm and nucleus; however, it was reported that BR response is mediated mainly by the cytoplasmic fraction of this enzyme. On the contrary, BIN2, which is the direct target of BSU1 phosphatase, operates mainly in the nucleus (Ryu et al. 2010). Therefore, BR perception can activate BRI1, BSKs, and BSU1 to inactive BIN2, resulting in the activation of downstream transcription factors (Kim and Wang 2010).

A Class of Brassinosteroid-Activated Transcription Factors and Their Regulation

The expression of many BR-responsive genes is directly regulated by a class of plant-specific transcription factors including BES1 (BRI1-EMS-suppressor 1), BZR1 (brassinazole-resistant 1) (Fig. 7), and BES1/BZR1 homologues 1–4 (BEH1–4)

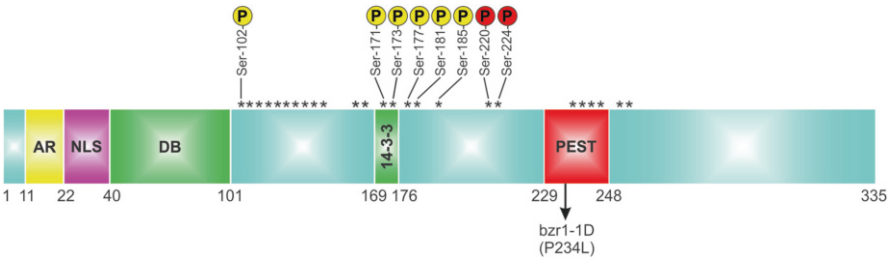

Fig. 7 The structure of the transcription factor BZR1. *AR* an alanine-rich domain, *NLS* nuclear localization signal, *DB* DNA binding domain, *PEST* proline, glutamic acid, serine, threonine rich domain, 14-3-3, binding motif. Putative BIN2 phosphorylation sites (as *blue box*) have been indicated by *asterisks*. *Yellow circles* containing the *letter P* indicate sites phosphorylated by BIN2 in vitro, and *red circles* indicate in vivo phosphorylation sites (adopted from Kim and Wang (2010))

that bind to the promoters of BR-regulated genes, and they are dephosphorylated in response to BR (Gruszka 2013).

BES1 and BZR1 are two major transcription factors that are regulated by BIN2 and mediate BR-regulated gene expression (Fig. 6) (Wang et al. 2002). BES1 and BZR1 are 88 % identical and are composed of DNA-binding domain (DBD), BIN2 phosphorylation domain with more than 20 putative BIN2 phosphorylation sites (Ser/ThrxxxSer/Thr, where x is any amino acid), and a *C*-terminal domain (CTD). The CTD is required for BES1 function as deletion of this domain leads to accumulation of inactive BES1 that acts as a dominant-negative form (Yin et al. 2005). The *C*-terminal domain of BES1 most likely acts as a transcription activation domain as it activates reporter gene expression in yeast. In addition, the *C*-terminal domain also contains a 12-amino-acid docking motif (DM) that binds BIN2, allowing BIN2 to phosphorylate BZR1. Since the same domain is conserved in BES1, it is likely that BIN2 interacts with DM to phosphorylate BES1 as well (Peng et al. 2010).

BIN2 phosphosphorylates BES1 and BZR1 at their central phosphorylation domain and inhibits their function likely through several different but non-exclusive mechanisms, including targeted protein degradation, nuclear export, and cytoplasmic retention by the phosphoprotein-interacting 14-3-3 proteins (Fig. 6). Polypeptides belonging to the group 14-3-3 function as another components of the BR signalling with dual role in regulation of this process. Recently, it has been reported that the 14-3-3 proteins may play a positive role in BR signalling by promoting BKI1 dissociation from the plasma membrane, what in consequence results in repressing of the BKI1 inhibitory effect on the BRI1 receptor (Lillo et al. 2006; Wang et al. 2011; Hao et al. 2013).

BZR1 can bind to a CGTG(T/C)G element, called BR-response element (BRRE) with its *N*-terminal domain to negatively feedback regulating the expression of genes involved in BR biosynthesis, such as *CPD*, *DWF4*, *ROT3*, and *BR6ox* (He et al. 2005). Apparently, BES1 may have a similar function in the feedback regulation of genes encoding BR biosynthetic enzymes (Yin et al. 2005; Vert and Chory 2006). Using transcript profiling and chromatin-immunoprecipitation

microarray experiments, Sun et al. (2010) reported 953 BR-regulated BZR1 target genes, which function in BR promotion of cell elongation and crosstalk between BR and other hormonal and light-signalling pathways at multiple levels.

Nuclear accumulation of dephosphorylated BES1/BZR1 plays important roles in directly regulating the expression of BR-responsive genes. Studies on the subcellular localisation of BES1 and BZR1 using green fluorescent protein (GFP) in *Arabidopsis* showed that, without BRs, BES1 and BZR1 are distributed in both the nucleus and cytoplasm, while BR treatment can rapidly promote the accumulation of BES1/BZR1 in nucleus in *Arabidopsis* hypocotyl cells (Wang et al. 2002; Yin et al. 2002). Later, another study showed that proteins BES1 and BZR1 labelled with GFP (BES1-GFP, BZR1-GFP) can be localised in both the cytoplasm and nucleus, and BR treatment can significantly induce the accumulation of dephosphorylated BES1-GFP and BZR1-GFP in the nucleus (Gampala et al. 2007; Ryu et al. 2010).

When BR levels are low, the GSK3-like kinase BIN2 phosphorylates and inactivates the BZR1 transcription factor to inhibit growth in plants. Brassinosteroid promotes growth by inducing dephosphorylation of BZR1 by protein phosphatase 2A (PP2A). PP2A is a heterotrimeric Ser/Thr phosphatase, which contains as scaffolding subunit A, catalytic subunit C, and a regulatory B subunit that interacts with substrates. Members of the B′ regulatory subunits of PP2A directly interact with BZR1's putative PEST domain containing the site of the *bzr1-1D* mutation. Interaction with and dephosphorylation by PP2A are enhanced by the *bzr1-1D* mutation, reduced by two intragenic *bzr1-1D* suppressor mutations, and abolished by deletion of the PEST domain. Therefore, PP2A plays a crucial function in dephosphorylating and activating BZR1 and completes the set of core components of the brassinosteroid-signalling cascade from cell surface receptor kinase to gene regulation in the nucleus (Tang et al. 2011).

In addition, BZR1 modulates the expression levels of many light-signalling components. Genome-wide protein-DNA interaction analysis revealed BZR1 binding to the promoters of a significant portion of light-regulated genes, suggesting that BR and light signals converge at the promoters of common target genes through direct interaction between BZR1 and some light-signalling transcription factors. BZR1 may also directly interact with phytochrome-interacting factors 4 (PIF4), which is accumulated in the dark to promote morphogenesis. BZR1 and PIF4 interact with each other in vitro and in vivo, bind to nearly 2,000 common target genes, and synergistically regulate many of these target genes, including the PRE family helix-loop-helix factors required for promoting cell elongation. Genetic analysis indicates that BZR1 and PIFs are interdependent in promoting cell elongation in response to BR, darkness, or heat. These results show that the BZR1-PIF4 interaction controls a core transcription network, enabling plant growth co-regulation by the steroid and environmental signals (Lillo et al. 2006; Oh et al. 2012).

Brassinazole (Brz), a specific inhibitor of BR biosynthesis, was used in experiments performed by Bekh-Ochir et al. (2013) to identify Brz-insensitive-long hypocotyls 2-1D (*bil2-1D*) mutant of *Arabidopsis*. The *BIL2* gene encodes a mitochondrial-localised DnaJ/heat-shock protein 40 (DnaJ/Hsp40) family, which is

involved in protein folding. *BIL2*-overexpression plants (*BIL2-OX*) showed cell elongation under Brz treatment, increasing the growth of plant inflorescence and roots, the regulation of BR-responsive gene expression, and the suppression against the dwarfed *BRI1*-deficient mutant. *BIL2-OX* also showed resistance against the mitochondrial ATPase inhibitor oligomycin and higher levels of exogenous ATP compared with wild-type plants. BIL2 participates in resistance against salinity stress and strong light stress. The results indicate that *BIL2* induces cell elongation during BR signalling through the promotion of ATP synthesis in mitochondria (Bekh-Ochir et al. 2013).

In addition, AtMYB30, another transcription factor, is also positively involved in BR signalling by promoting a subset of BR-responsive gene expression (Li et al. 2010). BES1 can interact with AtMYB30 both in vitro and in vivo to promote the expression of downstream target genes. It was discovered that BES1 can also physically interact with interacts-with-Spt6 (IWS1), which participates in RNA polymerase II (RNAPII) post-recruitment and transcriptional elongation processes (Li et al. 2010).

Apart from these transcription factors, BIN2 phosphorylates CESTA transcription factor belonging to the basic helix-loop-helix (bHLH) family. CESTA positively regulates expression of the BR-biosynthesis *CPD* gene by heterodimerisation with the close homologue of CESTA, BRI1-enhanced expression 1 (BEE1). BIN2-mediated phosphorylation of CESTA is assumed to regulate the nuclear localisation of this transcription factor. Based on the results derived from several different approaches, it has been suggested that BIN2 operates both in the nucleus and cytoplasm, and the exact mechanism may depend on developmental stage, tissue type, and BIN2 gene expression level (Clouse 2011; Poppenberger et al. 2011; Hao et al. 2013).

Brassinosteroid Signalling and Stress Tolerance

The molecular mechanisms of BR-induced plant stress tolerance remain poorly understood. Cui et al. (2012) reported that an endoplasmic reticulum (ER)-localised *Arabidopsis* ubiquitin-conjugating enzyme UBC32 is an essential factor involved in both BR-mediated growth promotion and salt stress tolerance. In vivo data in *Arabidopsis* showed that UBC32 is a functional component of the ER-associated protein degradation (ERAD) pathway, which is an important ubiquitin-proteasome system regulating plant growth and development, known to contribute to plant salt tolerance (Liu et al. 2011). UBC32 affects the accumulation of BRI1 and connects the ERAD pathway to BR-mediated growth promotion and salt stress tolerance. A recent study in tomato revealed one possible mechanism of BR-induced abiotic stress tolerance, especially for oxidative and heat stress (Nie et al. 2012). BRs trigger apoplastic H_2O_2 accumulation generated by NADPH oxidase, which is encoded by the RESPIRATORY BURST OXIDASE HOMOLOG 1 (*RBOH1*) gene. The *RBOH*s are involved in plant ROS production and plant response to various

abiotic stresses (Marino et al. 2012). NADPH oxidase in turn activates MAPKs, which play critical roles in plant signal transduction during stress responses (Mittler et al. 2004), giving rise to increased stress tolerance (Hao et al. 2013).

Conclusion Remarks

Brassinosteroids (BRs) are plant hormones implicated in a wide array of fundamental processes in plants ranging from triggering the cell cycle, genome expression, signalling, and plant growth and development to plant adaptation toward abiotic stresses. However, molecular mechanisms underlying BR participation in plant adaptation to stress are not completely understood. Understanding the signal transduction of BRs during abiotic stress is vital in developing plants for stress tolerance. There is an urgent need to identify the signalling components related to the biosynthesis and degradation and their coordination in gene expression events under stress conditions. The characterisation of the molecular mechanisms regulating hormone synthesis, signalling, and action is facilitating the modification of BR biosynthetic pathways for the generation of transgenic crop plants with enhanced abiotic stress tolerance.

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References

Alam MM, Hayat S, Ali B, Ahmad A (2007) Effect of 28-homobrassinolide on nickel induced changes in *Brassica juncea*. Photosynthetica 45:139–142

Ali B, Hasan SA, Hayat S, Hayat Q, Yadav S, Fariduddin Q, Ahmad A (2008) A role for brassinosteroids in the amelioration of aluminum stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot 62:153–159

Anuradha S, Rao SSR (2003) Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigments loss and increased nitrate reductase activity. Plant Growth Regul 40:29–32

Asami T, Yoshida S (1999) Brassinosteroid biosynthesis inhibitors. Trends Plant Sci 4:348–353

Asami T, Mizutani M, Shimada Y, Goda H, Kitahata N, Sekimata K, Han S-Y, Fujioka S, Takatsuto S, Sakata K, Yoshida S (2003) Triadimefon, a fungicidal triazole-type P450 inhibitor, induces brassinosteroid deficiency-like phenotypes in plants and binds to DWF4 protein in the brassinosteroid biosynthesis pathway. Biochem J 369:71–76

Atici Ö, Ağar G, Battal P (2005) Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. Biol Plant 49:215–222

Azpiroz R, Wu Y, LoCascio JC, Feldmann KA (1998) An *Arabidopsis* brassinosteroid-dependent mutant is blocked in cell elongation. Plant Cell 10:219–230

Bajguz A (2000) Blockade of heavy metals accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. Plant Physiol Biochem 38:797–801

Bajguz A (2002) Brassinosteroids and lead as stimulators of phytochelatins synthesis in *Chlorella vulgaris*. J Plant Physiol 159:321–324
Bajguz A (2005) Brassinosteroids: from distribution to metabolism in plants. In: Sharma SK, Govil JN, Singh VK (eds) Recent progress in medicinal plants, vol 10, Phytotherapeutics. Studium, Houston
Bajguz A (2009a) Brassinosteroid enhanced the level of abscisic acid in *Chlorella vulgaris* subjected to short-term heat stress. J Plant Physiol 166:882–886
Bajguz A (2009b) Isolation and characterization of brassinosteroids from algal cultures of *Chlorella vulgaris* Beijerinck (Trebouxiophycaea). J Plant Physiol 166:1946–1949
Bajguz A (2010) An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in *Chlorella vulgaris* cultures under heavy metals stress. Environ Exp Bot 68:175–179
Bajguz A (2011) Suppresion of *Chlorella vulgaris* growth by cadmium, lead, and copper stress and its restoration by endogenous brassinolide. Arch Environ Contam Toxicol 60:406–416
Bajguz A, Asami T (2004) Effects of brassinazole, an inhibitor of brassinosteroid biosynthesis, on light- and dark-grown *Chlorella vulgaris*. Planta 218:869–877
Bajguz A, Asami T (2005) Suppression of *Wolffia arrhiza* growth by brassinazole, an inhibitor of brassinosteroid biosynthesis and its restoration by endogenous 24-epibrassinolide. Phytochemistry 66:1787–1796
Bajguz A, Hayat S (2009) Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem 47:1–8
Bajguz A, Tretyn A (2003) The chemical characteristic and distribution of brassinosteroids in plants. Phytochemistry 62:1027–1046
Bajguz A, Bajguz AJ, Tryniszewska EA (2013) Recent advanced in medicinal applications of brassinosteroids, a group of plant hormones. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 40. Elsevier Science, Amsterdam
Bancoş S, Nomura T, Sato T, Molnár G, Bishop GJ, Koncz C, Yokota T, Nagy F, Szekeres M (2002) Regulation of transcript levels of the *Arabidopsis* cytochrome P450 genes involved in brassinosteroid biosynthesis. Plant Physiol 130:504–513
Bekh-Ochir D, Shimada S, Yamagami A, Kanda S, Ogawa K, Nakazawa M, Matsui M, Sakuta M, Osada H, Asami T, Nakano T (2013) A novel mitochondrial DnaJ/Hsp40 family protein BIL2 promotes plant growth and resistance against environmental stress in brassinosteroid signaling. Planta 237:1509–1525
Bilkisu AA, Xiao-Gang G, Qing-Lei G, Yong-Hua Y (2003) Brassinolide amelioration of aluminium toxicity in mungbean seedling growth. J Plant Nutr 26:1725–1734
Bishop GJ (2003) Brassinosteroid mutants of crops. J Plant Growth Regul 22:325–335
Bishop GJ (2007) Refining the plant steroid hormone biosynthesis pathway. Trends Plant Sci 12:377–380
Bishop GJ, Yokota T (2001) Plant steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant Cell Physiol 42:114–120
Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500
Choe S (2006) Brassinosteroid biosynthesis and inactivation. Physiol Plant 126:539–548
Choe S, Dilkes BP, Fujioka S, Takatsuto S, Sakurai A, Feldmann KA (1998) The *DWF*4 gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in brassinosteroid biosynthesis. Plant Cell 10:231–243
Choudhary SP, Bhardwaj R, Gupta BD, Dutt P, Gupta RK, Biondi S, Kanwar M (2010) Epibrassinolide induces changes in indole-3-acetic acid, abscisic acid and polyamine concentrations and enhances antioxidant potential of radish seedlings under copper stress. Physiol Plant 140:280–296
Choudhary SP, Yu J-Q, Yamaguchi-Shinozaki K, Shinozaki K, Tran PLS (2012) Benefits of brassinosteroid crosstalk. Trends Plant Sci 17:594–605

Clouse SD (2011) Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. Plant Cell 23:1219–1230

Clouse SD, Feldmann KA (1999) Molecular genetics of brassinosteroid action. In: Yokota T, Clouse SD, Sakurai A (eds) Brassinosteroids: steroidal plant hormones. Springer, Tokyo

Clouse SD, Langford M, McMorris TC (1996) A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol 111:671–678

Cui F, Liu L, Zhao Q, Zhang Z, Li Q, Lin B, Wu Y, Tang S, Xie Q (2012) *Arabidopsis* ubiquitin conjugase UBC32 is an ERAD component that functions in brassinosteroid-mediated salt stress tolerance. Plant Cell 24:233–244

Dhaubhadel S, Chaudhary S, Dobinson KF, Krishna P (1999) Treatment of 24-epibrassinolide, a brassinosteroid, increases the basic thermotolerance of *Brassica napus* and tomato seedlings. Plant Mol Biol 40:333–342

Dhaubhadel S, Browning KS, Gallie DR, Krishna P (2002) Brassinosteroid functions to protect the translational machinery and heat-shock protein synthesis following thermal stress. Plant J 29:681–691

Fariduddin Q, Khanam S, Hasan SA, Ali B, Hayat S, Ahmad A (2009a) Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. Acta Physiol Plant 31:889–897

Fariduddin Q, Yusuf M, Hayat S, Ahmad A (2009b) Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. Environ Exp Bot 66:418–424

Fujii S, Saka H (2001) The promotive effect of brassinolide on lamina joint-cell elongation, germination and seedling growth under low-temperature stress in rice (*Oryza sativa* L.). Plant Prod Sci 4:210–214

Fujioka S, Yokota T (2003) Biosynthesis and metabolism of brassinosteroids. Annu Rev Plant Biol 54:137–164

Fujioka S, Li J, Choi Y-H, Seto H, Takatsuto S, Noguchi T, Watanabe T, Kuriyama H, Yokota T, Chory J, Sakurai A (1997) The *Arabidopsis deetiolated2* mutant is blocked early in brassinosteroid biosynthesis. Plant Cell 9:1951–1962

Fujioka S, Takatsuto S, Yoshida S (2002) An early C-22 oxidation branch in the brassinosteroid biosynthetic pathway. Plant Physiol 130:930–939

Gampala SS, Kim TW, He JX, Tang W, Deng Z, Bai MY, Guan S, Lalonde S, Sun Y, Gendron JM (2007) An essential role for 14-3-3 proteins in brassinosteroid signal transduction in *Arabidopsis*. Dev Cell 13:177–189

González-Olmedo JL, Córdova A, Aragón CE, Pina D, Rivas M, Rodríguez R (2005) Effect of an analogue of brassinosteroid on FHIA-18 plantlets exposed to thermal stress. InfoMusa 14:18–20

Gruszka D (2013) The brassinosteroid signaling pathway—new key players and interconnections with other signaling networks crucial for plant development and stress tolerance. Int J Mol Sci 14:8740–8774

Hao J, Yin Y, Fei S-Z (2013) Brassinosteroid signaling network: implications on yield and stress tolerance. Plant Cell Rep 32:1017–1030

Hasan SA, Hayat S, Ali B, Ahmad A (2008) 28-homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. Environ Pollut 151:60–66

Hayat S, Ali B, Hasan SA, Ahmad A (2007a) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot 60:33–41

Hayat S, Ali B, Hasan SA, Ahmad A (2007b) Effect of 28-homobrassinolide on salinity-induced changes in *Brassica juncea*. Turk J Biol 31:141–146

Hayat S, Hasan SA, Hayat Q, Ahmad A (2010a) Brassinosteroids protect *Lycopersicon esculentum* from cadmium toxicity applied as shotgun approach. Protoplasma 239:3–14

Hayat S, Mori M, Fariduddin Q, Bajguz A, Ahmad A (2010b) Physiological role of brassinosteroids: an update. Indian J Plant Physiol 15:99–109

He JX, Gendron JM, Sun Y, Gampala SS, Gendron N, Sun CQ, Wang ZY (2005) BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science 307:1634–1638

Hsu YT, Kao CH (2003) Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Cell Environ 26:867–874

Janeczko A, Kościelniak J, Pilipowicz M, Szarek-Łukaszewska G, Skoczowski A (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43:293–298

Joo S-H, Kim T-W, Son S-H, Lee WS, Yokota T, Kim S-K (2012) Biosynthesis of a cholesterol-derived brassinosteroid, 28-norcastasterone, in *Arabidopsis thaliana*. J Exp Bot 63:1823–1833

Kang Y-Y, Guo S-R, Li J, Duan J-J (2009) Effect of root applied 24-epibrassinolide on carbohydrate status and fermentative enzyme activities in cucumber (*Cucumis sativus* L.) seedlings under hypoxia. Plant Growth Regul 57:259–269

Kim TW, Wang ZY (2010) Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol 61:681–704

Kim T-W, Chang SC, Lee JS, Takatsuto S, Yokota T, Kim S-K (2004) Novel biosynthetic pathway of castasterone from cholesterol in tomato. Plant Physiol 135:1231–1242

Kim G-T, Fujioka S, Kozuka T, Tax FE, Takatsuto S, Yoshida S, Tsukaya H (2005) CYP90C1 and CYP90D1 are involved in different steps in the brassinosteroid biosynthesis pathway in *Arabidopsis thaliana*. Plant J 41:710–721

Kim BK, Fujioka S, Takatsuto S, Tsujimoto M, Choe S (2008) Castasterone is a likely end product of brassinosteroid biosynthetic pathway in rice. Biochem Biophys Res Commun 374:614–619

Kinoshita T, Caño-Delgado AI, Seto H, Hiranuma S, Fujioka S, Yoshida S, Chory J (2005) Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature 433:167–171

Li J (2011) Direct involvement of leucine-rich repeats in assembling ligand-triggered receptor-coreceptor complexes. Proc Natl Acad Sci U S A 108:8073–8074

Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 90:929–938

Li J, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science 272:398–401

Li L, van Staden J, Jäger AK (1998) Effects of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjected to water stress. Plant Growth Regul 25:81–87

Li L, Ye H, Guo H, Yin Y (2010) *Arabidopsis* IWS1 interacts with transcription factor BES1 and is involved in plant steroid hormone brassinosteroid regulated gene expression. Proc Natl Acad Sci U S A 107:3918–3923

Li YH, Liu YJ, Xu XL, Jin M, An LZ, Zhang H (2012) Effect of 24-epibrassinolide on drought stress-induced changes in *Chorispora bungeana*. Biol Plant 56:192–196

Lillo C, DeLong A, Burlingame AL, Sun Y, Wang Z-Y, Vert G, Chory J (2006) Downstream nuclear events in brassinosteroid signalling. Nature 441:96–100

Liu L, Cui F, Li Q, Yin B, Zhang H, Lin B, Wu Y, Xia R, Tang S, Xie Q (2011) The endoplasmic reticulum-associated degradation is necessary for plant salt tolerance. Cell Res 21:957–969

Marino D, Dunand C, Puppo A, Pauly N (2012) A burst of plant NADPH oxidases. Trends Plant Sci 17:9–15

Mazorra LM, Núñez M, Hechavarria M, Coll F, Sánchez-Blanco MJ (2002) Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biol Plant 45:593–596

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Montoya T, Nomura T, Yokota T, Farrar K, Harrison K, Jones JGD, Kaneta T, Kamiya Y, Szekeres M, Bishop GJ (2005) Patterns of *Dwarf* expression and brassinosteroid accumulation in tomato reveal the importance of brassinosteroid synthesis during fruit development. Plant J 42:262–269

Mora-Garcia S, Vert G, Yin Y, Cańo-Delgado A, Cheong H, Chory J (2004) Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in *Arabidopsis*. Genes Dev 18:448–460

Müssig C, Altmann T (2003) Genomic brassinosteroid effects. J Plant Growth Regul 22:313–324
Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110:203–212
Nie WF, Wang MM, Xia XJ, Zhou YH, Shi K, Chen Z, Yu JQ (2012) Silencing of tomato RBOH1 and MPK2 abolishes brassinosteroid-induced H_2O_2 generation and stress tolerance. Plant Cell Environ 36:789–803
Noguchi T, Fujioka S, Choe S, Takatsuto S, Yoshida S, Yuan H, Feldmann KA, Tax FE (1999a) Brassinosteroid-insensitive dwarf mutant of *Arabidopsis* accumulate brassinosteroids. Plant Physiol 121:743–752
Noguchi T, Fujioka S, Takatsuto S, Sakurai A, Yoshida S, Li J, Chory J (1999b) *Arabidopsis det*2 is defective in the conversion of (24*R*)-24-methylcholest-4-en-3-one to (24*R*)-24-methyl-5α-cholestan-3-one in brassinosteroid biosynthesis. Plant Physiol 120:833–839
Nomura T, Sato T, Bishop GJ, Kamiya Y, Takatsuto S, Yokota T (2001) Accumulation of 6-deoxocathasterone and 6-deoxocastasterone in *Arabidopsis*, pea and tomato is suggestive of common rate-limiting steps in brassinosteroid biosynthesis. Phytochemistry 57:171–178
Nomura T, Jager CE, Kitasaka Y, Takeuchi K, Fukami M, Yoneyama K, Matsushita Y, Nyunoya H, Takatsuto S, Fujioka S, Smith JJ, Kerckhoffs LHJ, Reid JB, Yokota T (2004) Brassinosteroid deficiency due to truncated steroid 5α-reductase causes dwarfism in the *lk* mutant of pea. Plant Physiol 135:2220–2229
Nomura T, Kushiro T, Yokota T, Kamiya Y, Bishop GJ, Yamaguchi S (2005) The last reaction producing brassinolide is catalyzed by cytochrome P450s, CYP85A3 in tomato and CYP85A2 in *Arabidopsis*. J Biol Chem 280:17873–17879
Nomura T, Ueno M, Yamada Y, Takatsuto S, Takeuchi Y, Yokota T (2007) Roles of brassinosteroids and related mRNAs in pea seed growth and germination. Plant Physiol 143:1680–1688
Núñez M, Mazzafera P, Mazorra LM, Siqueira WJ, Zullo MAT (2003) Influence of a brassinsteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. Biol Plant 47:67–70
Oh MH, Wang X, Kota U, Goshe MB, Clouse SD, Huber SC (2009) Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in *Arabidopsis*. Proc Natl Acad Sci U S A 106:658–663
Oh E, Zhu J-Y, Wang Z-Y (2012) Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol 14:802–810
Ohnishi T, Sazatmari A-M, Watanabe B, Fujita S, Bancos S, Koncz C, Lafos M, Shibata K, Yokota T, Sakata K, Szekeres M, Mizutani M (2006a) C-23 hydroxylation by *Arabidopsis* CYP90C1 and CYP90D1 reveals a novel shortcut in brassinosteroid biosynthesis. Plant Cell 18:3275–3288
Ohnishi T, Watanabe B, Sakata K, Mizutani M (2006b) CYP724B2 and CYTP90B3 function in the early C-22 hydroxylation steps of brassinosteroid biosynthetic pathway in tomato. Biosci Biotechnol Biochem 70:2071–2080
Peng J, Zhao J, Zhu Y, Asami T, Li J (2010) A direct docking mechanism for a plant GSK3-like kinase to phosphorylate its substrates. J Biol Chem 285:24646–24653
Poppenberger B, Rozhon W, Khan M, Husar S, Adam G, Luschnig C, Fujioka S, Sieberer T (2011) CESTA, a positive regulator of brassinosteroid biosynthesis. EMBO J 30:1149–1161
Ryu H, Kim K, Cho H, Hwang I (2010) Predominant actions of cytosolic BSU1 and nuclear BIN2 regulate subcellular localization of BES1 in brassinosteroid signaling. Mol Cells 29:291–296
Sasse JM, Smith R, Hudson I (1995) Effect of 24-epibrassinolide on germination of seed of *Eucalyptus camaldulensis* in saline conditions. Proc Plant Growth Regul Soc Am 22:136–141
Schilling G, Schiller C, Otto S (1991) Influence of brassinosteroids on organ relations and enzyme activities of sugar-beet plants. In: Cutler HG, Yokota T, Adam G (eds) Brassinosteroids: chemistry, bioactivity and applications. American Chemical Society, Washington, DC, pp 208–219
Schneider B (2002) Pathways and enzymes of brassinosteroid biosynthesis. Progress Bot 63:286–306

Sharma P, Bhardwaj R (2007) Effects of 24-epibrassinolide on growth and metal uptake *Brassica juncea* L. under copper metal stress. Acta Physiol Plant 29:259–263

Sharma SS, Kumar V (2002) Responses of wild type and abscisic acid mutants of *Arabidopsis thaliana* to cadmium. J Plant Physiol 159:1323–1327

She J, Han Z, Zhou B, Chai J (2013) Structural basis for differential recognition of brassinolide by its receptors. Protein Cell 4:475–482

Shimada Y, Fujioka S, Miyauchi N, Kushiro M, Takatsuto S, Nomura T, Yokota T, Kamiya Y, Bishop GJ, Yoshida S (2001) Brassinosteroid-6-oxidases from *Arabidopsis* and tomato catalyze multiple C-6 oxidations in brassinosteroid biosynthesis. Plant Physiol 126:770–779

Shimada Y, Goda H, Nakamura A, Takatsuto S, Fujioka S, Yoshida S (2003) Organ-specific expression of brassinosteroid-biosynthetic genes and distribution of endogenous brassinosteroids in *Arabidopsis*. Plant Physiol 131:287–297

Singh I, Shono M (2005) Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. Plant Growth Regul 47:111–119

Sun Y, Fan XY, Cao DM, He K, Tang W, Zhu JY, He JX, Bai MY, Zhu S, Oh E (2010) Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in *Arabidopsis*. Dev Cell 19:765–777

Suzuki Y, Saso K, Fujioka S, Yoshida S, Nitasaka E, Nagata S, Nagasawa H, Takatsuto S, Yamaguchi I (2003) A dwarf mutant strain of *Pharbitis nil*, Uzukobito (*kobito*), has defective brassinosteroid biosynthesis. Plant J 36:401–410

Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A, Altmann T, Redei GP, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. Cell 85:171–182

Tang W, Kim TW, Oses-Prieto JA, Sun Y, Deng Z, Zhu S, Wang R, Burlingame AL, Wang ZY (2008) BSKs mediate signal transduction from the receptor kinase BRI1 in *Arabidopsis*. Science 321:557–560

Tang W, Yuan M, Wang R, Yang Y, Wang C, Oses-Prieto JA, Kim T-W, Zhou H-W, Deng Z, Gampala SS, Gendron JM, Jonassen EM (2011) PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nature Cell Biol 13:124–132

Upreti KK, Murti GSR (2004) Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. Biol Plant 48:407–411

Vardhini BV, Rao SSR (2003) Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regul 41:25–31

Vert G, Chory J (2006) Downstream nuclear events in brassinosteroid signalling. Nature 441:96–100

Vert G, Nemhauser JL, Geldner N, Hong F, Chory J (2005) Molecular mechanisms of steroid hormone signaling in plants. Annu Rev Cell Dev Biol 21:177–201

Wang X, Chory J (2006) Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science 313:1118–1122

Wang ZY, Nakano T, Gendron JM, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T (2002) Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell 2:505–513

Wang X, Li X, Meisenhelder J, Hunter T, Yoshisa S, Asami T, Chory J (2005) Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. Dev Cell 8:855–865

Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD (2008) Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell 15:220–235

Wang H, Yang C, Zhang C, Wang N, Lu D, Wang J, Zhang S, Wang ZX, Ma H, Wang H (2011) Dual role of BKI1 and 14-3-3s in brassinosteroid signaling to link receptor with transcription factors. Dev Cell 21:825–834

Wilen RW, Sacco M, Gusta LV, Krishna P (1995) Effects of 24-epibrassinolide on freezing and thermotolerance of bromegrass (*Bromus inermis*) cell cultures. Physiol Plant 95:195–202

Xia X-J, Wang Y-J, Zhou Y-H, Tao Y, Mao W-H, Shi K, Asami T, Chen Z, Yu J-Q (2009) Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. Plant Physiol 150:801–814

Yan Z, Zhao J, Peng P, Chihara RK, Li J (2009) BIN2 functions redundantly with other *Arabidopsis* GSK3-like kinases to regulate brassinosteroid signaling. Plant Physiol 150:710–721

Yang C-J, Zhang C, Lu Y-N, Jia-Qi Jin J-Q, Wang X-L (2011) The mechanisms of brassinosteroids' action: from signal transduction to plant development. Mol Plant 4:588–600

Ye H, Li L, Yin Y (2011) Recent advances in the regulation of brassinosteroid signaling and biosynthesis pathways. J Integr Plant Biol 53:455–468

Yin Y, Wang ZY, Mora-Garcia S, Li JM, Yoshida S, Asami T, Chory J (2002) BES1 accumulates in the nucleus in response to brassionsteroids to regulate gene expression and promote stem elongation. Cell 109:181–191

Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J (2005) A new class of transcription factors mediates brassinosteroid-regulated gene expression in *Arabidopsis*. Cell 120:249–259

Zhang A, Zhang J, Zhang J, Ye N, Zhang H, Tan M, Jiang M (2011) Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves. Plant Cell Physiol 52:181–192

Zhao B, Li J (2012) Regulation of brassinosteroid biosynthesis and inactivation. J Integr Plant Biol 54:746–759