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Genes Involved in Brassinosteroids's Metabolism and Signal Transduction Pathways

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ABSTRACT

Brassinosteroids (BRs) are plant steroids essential for the normal growth and development, which carry an oxygen moiety at C-3 and additional ones at one or more of the C-2, C-6, C-22 and C-23 carbon atoms. In the past few years, application of molecular genetics allowed significant progress on the understanding of the BRs biosynthetic pathway regulation and on the identification of several components of their signal transduction pathway, as well. Search in eletronic databases show dozens of records for brassinosteroid-related genes for the last twelve months, demonstrating the big efforts being carried out in this field. This review highlights the recent advances on the characterization of genes and mutations that are helping to unravel the molecular mechanisms involved in the BRs synthesis/metabolism, perception and response, with especial emphasis on their role in plant cell elongation. Aspects of the involvement of BRs on the regulation of cell cycle-controlling proteins are discussed as well.

Key words: Brassinolide, cell elongation, dwarf, signal perception

INTRODUCTION

The most recently discovered class of the plant growth regulators, the brassinosteroids (BRs), is a family of about 60 phytosteroids (Bajguz and Tretyn, 2003). Application of molecular genetics in the past few years has led to rapid progress toward understanding these steroids's biosynthetic pathway regulation and the identification of several components of their signal transduction pathway as well. Indeed, BRs signaling is now considered to be one of the best-understood signal transduction pathways in plants (Russinova et al., 2004).

The genetic approach used to identify the components of the BR biosynthetic and signal transduction pathways have relied on the isolation and characterization of mutants deficient in BRs biosynthesis or response. The availability of these mutants has played a major role in the increase in the knowledge concerning BRs (Clouse and Sasse, 1998; Li and Chory, 1999; Bishop and Yokota, 2001; Clouse, 2002). BR-deficient mutants usually result from lesions in genes encoding for BR biosynthetic enzymes and are rescued to the wild type phenotype by exogenously supplied BRs (Szekeres et al., 1996; Choe et al., 1998, 2000). Whereas, BR-insensitive mutants usually result from lesions in genes encoding for the receptor or other elements in the BRs signal transduction pathway, and although these mutants may present the same phenotype as BRs-deficient mutants, they are not rescued by exogenously supplied BRs (Clouse et al., 1996; Li et al., 2001b; Yin et al.,

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2002). Some characteristics are typical for both, BR-deficient and BR-insensitive mutants. In *Arabidopsis thaliana*, for example, all BR mutants show male sterelity or reduced female fertility, drastic dwarfism and reduced apical dominance. When grown in darkness, these mutants present reduced hypocotyl elongation, unregulated opening of cotyledons, and emergence of primary leaves (Creelman and Mullet, 1997). The BR-deficient and BR-insensitive mutants and their correspondent cloned genes are powerful tools intensively used to investigate not only the physiological functions but also the biosynthetic and signal transduction pathways of BRs.

Microarray analysis of BR-regulated genes in *Arabidopsis* showed that 59 up-regulated genes and 36 down-regulated genes were reproducibly regulated by brassinolide (BL) (Goda et al., 2002), the most active BR. From this list, P450 genes and transcription factor genes predominated among down-regulated genes, while auxin-related genes and genes that were implicated in cell elongation or cell wall organization predominated among up-regulated genes. Yang *and* colleagues (2003), using microarray analysis in plants of rice, identified 12 genes with strongly enhanced expression after BL treatment, 14 genes with weakly enhanced expression after BL treatment and 12 genes with repressed expression, indicating that the broader use of this technique would very likely significantly increase the knowledge about BR-regulated genes in a wide variety of plant species in a near future.

This review highlights the advances on the characterization of genes and mutations that are helping to unravel the molecular mechanisms involved in the BRs synthesis/metabolism, perception and response, with especial emphasis on their role in plant cell elongation. Aspects of the involvement of BRs on the regulation of activity of D-type cyclins, proteins thought to be critical for the stimulation of cell division, and also to play key roles in linking the plant cell cycle to extracellular and developmental signals, are discussed as well. Having insight the large number and complexity of cross-talk networks between BRs and other plant growth regulators, along with the limited length for the reviews, these interactions will not be discussed in this review.

Genes Involved in the Biosynthesis of BRs

The BR biosynthetic pathways consist of two major parts, sterol biosynthesis and a BR-specific pathway. Analysis of BR metabolic gene expressions indicate that BR homeostasis is maintained through feedback expressions of multiple genes, each of which is involved not only in BR-specific biosynthesis and inactivation, but also in sterol biosynthesis (Tanaka et al., 2005).

The *dwf* (dwarf) group

Nine BR *dwarf* loci in *Arabidopsis*, *dwf1* to *dwf8*, and *dwf12*, have been reported. The *dwf1*, *dwf5*, and *dwf7* mutants are defective in sterol biosynthesis, and a second group including *dwf3, dwf4*, *dwf6*, and *dwf8* belong to the BR specific pathway. Only *dwf2* and *dwf12* mutants are insensitive to bioactive BRs. *dwf1*, *dwf2*, *dwf3*, and *dwf6* were found to be allelic to *diminuto1* (*dim1*), *cabbage1* (*cbb1*) from *Arabidopsis* and possibly *lkb* (from pea); *bri1*, *cbb2* and 18 *bin* (from *Arabidopsis*); *Arabidopsis cpd*, tomato *dpy* and *Arabidopsis cbb3*; and *Arabidopsis det2*, respectively (see Table 1). As expected, except for *dwf2* and *dwf12* alleles, all of the dwf mutants are highly responsive to exogenously supplied BRs.

***dwf1*.** The *dwf1* was the first BR-related gene identified to be cloned (B.P. Dilkes *and* K.A. Feldmann, personal communication) (Choe et al., 1998). The three alleles *dwarf1* (*dwf1*), *dim* (Takahashi et al., 1995; Klahre et al., 1998) and *cbb1* (Kauschmann et al., 1996), were defective in the synthesis of campesterol from 24-methylenecholesterol and *lkb* is the equivalent mutant in pea (*Pisum sativum*) (Nomura et al., 1997; 1999). Analysis of the endogenous levels of BR intermediates showed that 24-methylenecholesterol in *dwf1* accumulated to 12 times the level found for the wild type (Choe et al., 1999a). In the *lkb* mutant the levels of BL, castastherone (CS), another very active BR, and 6-deoxoCS, campestanol, and campesterol were severely reduced in young shoots, however, levels of 24-methylenecholesterol were elevated, compared to those of wild-type plants (Nomura et al., 1997, 1999). Furthermore, the deduced amino acid sequence of *dwf1* showed significant similarity to a flavin adenine dinucleotide-binding domain conserved in various oxidoreductases, suggesting an enzymatic role for *dwf1* (Choe et al., 1999a).

dwf 3. *dwf3* mutants have only been rescued by 23-alpha-hydroxylated BRs. These mutants have also been found to be allelic to *cpd* (Choe et al., 1998), a mutant described below.

dwf4. The *dwf4* mutant was also shown to be defective in the BR biosynthetic pathway, more specifically in a steroid 22α– hydroxylation (CYP90B1), presenting 513 aminoacids and 43% identity and 66% similarity with the *cpd* gene (Choe et al., 1998), described below, which catalized a key regulatory step in BRs biosynthesis (Choe et al., 2001). Choe *and* co-workers (2001) have shown that transgenic *Arabidopsis* plants overexpressing *dwf4* (*aod4*) presented dramatical increase in hypocotyl length in both light- and dark-grown as compared to wild type. Analysis of endogenous levels of BRs in *dwf4* and *aod4* revealed that *dwf4* accumulated the precursors of the 22 α-hydroxylation steps, while overexpression of *dwf4* resulted in enhanced levels of downstream compounds relative to controls.

dwf5, dwf6, dwf7, dwf8, dwf11 and ***dwf13.*** *dwarf5* (*dwf5*) has been shown to be defective in the reduction of 5-dehydroepisterol to 24-methylenecholesterol (Bishop *and* Yokota, 2001). In addition to that metabolite tracing with C-13-labeled precursors in *dwf5* demonstrated that the mutant phenotype was caused by loss-of-function mutation in a sterol Δ^7 reductase gene (Choe et al., 2000). *dwarf6* (*dwf6*) and its allele deetiolated2 (*det2*), described below, were mutants in a 5-α-steroid reductase (Li et al., 1996; Li et al., 1997) that linked campesterol to campestanol by catalyzing the conversion of (24*R*)-24-methylcholest-4-en-3-one to (24*R*)-24-methyl-5α-cholestan-3-one (Fujioka et al., 1997; Noguchi et al., 1999; Bishop and Yokota, 2001).
Feeding studies with intermediates of the BR biosynthetic pathway and analysis of endogenous BRs and intermediates of the sterol biosynthetic pathway have shown that the defective step in *dwf7* resided before the production of 24-methylenecholesterol in the sterol biosynthetic pathway, more specifically the dehydrogenation of episterol to 5-dehydroepisterol, indicating that *dwf7* was an allele of the previously cloned *Arabidopsis sterol1* (*ste1*) gene (Choe et al., 1999b; Bishop *and* Yokota, 2001). Similar studies in the dwf8 mutant indicated that the biosynthetic defect in this mutant was located in the most downstream step yet to be genetically identified (Choe et al., 1998, personal communication).
dwarf11, a rice (*Oryza sativa*) dwarf mutant, was defective for a novel cytochrome P450 (CYP724B1), which showed homology to enzymes involved in BR biosynthesis. The *dwarf 11* gen is feedback-regulated by BL. Several lines of evidence suggest that the *dwarf11*/*cyp724b1* gene plays a role in BR synthesis and may be involved in the supply of 6-deoxotyphasterol and typhasterol in the BR biosynthesis network in rice (Tanabe et al., 2005).
An *Arabidopsis* BR-insensitive mutant, named *dwf12* was recently reported (Choe et al., 2002) as follows. The *dwarf* (*d*) mutant of tomato represented a new locus encoding a P450 that was classified as CYP85 (Bishop et al., 1999). The CYP85A family members (e.g. CYP85A1; CYP85A3, CYP85A2) catalyze the formation of CS from 6-deoxoCS (Nomura et al., 2005) via the hydroxylated form of 6-deoxoCS which is the key step linking the late C-6 oxidation pathway to the early C-6 oxidation pathway (Bishop et al., 1999). The CYP85A family members have high homology to CPD and DWF4 (Bishop et al., 1999).

cpd. Rescue of the *Arabidopsis cpd* (constitutive photomorphogenesis and dwarfism) mutant showed that CPD/CYP90A1, a member of the CYP90 family, functions as C-23 steroid hydroxylase (Szekeres et al., 1996). Rescue of the dwarf phenotypes of cpd mutants by BR intermediates indicates that CYP90A1 and CYP90B1 are responsible for the C-23 and C-22 side chain hydroxylation reactions in both the early and late C-6 oxidation pathways of BR biosynthesis (Choe et al., 1998; Li and Chory, 1997). The *Rotundifolia3* (rot*3*) gene, encoding the enzyme CYP90C1, has been recently shown to be required for the late steps in the BR biosynthesis pathway. *rot3* seems to be required for the conversion of typhasterol to CS, an activation step in the BR pathway (Kim et al., 2005).

cpy. The *dumpy* (*dpy*) mutant of tomato (*Lycopersicon esculentum* Mill.) exhibited short stature, reduced axillary branching, and altered leaf morphology (Koka et al., 2000). Application of BL and CS to dpy plants resulted in a nearly complete rescue of the mutant phenotype, while the late C-6 oxidation pathway intermediates 6-

deoxoteasterone, 6-deoxo-3-dehydroteasterone, 6-deoxotyphasterol, and 6-deoxoCS also presented significant effect on dpy leaf development, although the overall plant height and branching were not as pronounced as with CS and BL (Koka et al., 2000). However, intermediates upstream of 6-deoxoteasterone, including campesterol, campestanol, and 6-deoxocathasterone, had no noticeble effect on leaf phenotype or height. The considerable difference in leaf morphology between 6-deoxoteasterone-treated dpy plants and later pathway intermediates, compared with 6-deoxocathasterone-treated plants and earlier pathway intermediates-treated plants, suggested that conversion of 6-deoxocathasterone to 6-deoxoteasterone might be blocked in the dpy mutant. Measurement of the endogenous BR levels showed that both dpy and wild-type plants presented similar levels of the BR precursors 24-methylenecholesterol, campesterol, and campestanol. However, dpy plants had two times the level of 6-deoxocathasterone compared to the wild type and less than 50% the amount of 6-deoxoteasterone. Koka *and* collegues (2000) found that dpy plants had a 25-fold reduction in 6-deoxoCS levels, and CS was below the detection limit in the mutant. Those results showed clearly that dpy was in fact BR deficient and the biochemical data were consistent with the feeding experiments, which suggested that dpy mutants had reduced ability to convert 6-deoxocathasterone into 6-deoxoteasterone (Koka et al., 2000). Finally, since the *cpd* gene of *Arabidopsis* likely encode a C-23 steroid hydroxylase responsible for the conversion of cathasterone to teasterone (Szekeres et al., 1996), and also possibly hydroxylate 6-deoxocathasterone to 6-deoxoteasterone, *dpy* was the putative tomato homolog of *cpd* (Koka et al., 2000).

***det2* (*"deetiolated"2*).** The *Arabidopsis det2* gene (Chory et al., 1989) was shown to encode a protein that shares significant sequence identity with steroid 5α-reductases of mammals, enzymes involved with steroids metabolism in animals (Li et al., 1996). Mutants *det2* presented only 8-15% of the campestanol levels found in the wild type and less than 10% of the wild type levels of other BRs. Mutants det2 were also unable to convert H^2-campesterol into H^2-campestanol, demonstrating that det2 mutantes were deficient in brassinoesteroids (reviewed in Li and Chory, 1999). Additional support to this finding came from rescue experiments in which all intermediates of the BRs biosynthetic pathway after the reaction catalyzed by *det2* were able to rescue det2 mutant phenotypes (Li et al., 1996; Li and Chory, 1999). The analysis of the endogenous BR contents indicated that the *lk* mutant might be the equivalent of *Arabidopsis det2* in pea. Indeed, the pea gene homologous to the *det2* gene, *Psdet2*, had been cloned, and the *lk* mutation was found to result in a putative truncated PsDET2 protein. Thus, it was concluded that the *lk* mutation was due to a defect in the steroidal 5 α-reductase [3-oxo-5-α-steroid 4-dehydrogenase] gene (Nomura et al., 2004).

***bas1-d*.** The mechanisms connecting light and hormone signaling are still not well understood. Neff *and* co-workers (1999) identified a new *Arabidopsis* mutant, *bas1-D* (phy*B a*ctivation-tagged suppressor1-dominant), caused by the enhanced expression of the cytochrome P450 monooxigenase: CYP734A1, formerly CYP72B1. The *Arabidopsis* bas1-D mutation suppressed the long hypocotyl phenotype caused by mutations in the photoreceptor phytochrome B (phyB). Seedlings with reduced *bas1* expression are hyperresponsive to BRs in a light-dependent manner and display reduced sensitivity to light under a variety of conditions. Biochemical evidences suggest that *bas1* and *sob7* (Suppressor of Phyb-4 7) gene, a homolog of *bas1*, act redundantly reducing the level of active BRs through unique mechanisms. Overexpression of *sob7* results in a dramatic reduction in endogenous CS levels, and although single null-mutants of *bas1* and *sob7* have the same level of CS as the wild type, the double null-mutant has twice the amount. Application of BL to overexpression lines of *bas1* or *sob7* results in enhanced metabolism of BL, although only *bas1* overexpression lines confer enhanced conversion to 26-OHBL, suggesting that *sob7* and *bas1* convert BL and CS into unique products (Turk et al., 2005).

An *Arabidopsis* mutant, *shrink1-d* (*shk1-d*), has been shown to be caused by activation of the *cyp72c1* gene, a member of the cytochrome P450 monooxygenase gene family similar to *bas1*/cyp*734a1* that regulates BR inactivation. However, unlike *bas1*/*cyp734a*1 the expression of *cyp*72*c*1 is not changed by application of exogenous BL (Takahashi et al., 2005).

Table 1 - Summary of the available/proposed informations for genes involved in the biosynthesis or signal transduction pathway of brassinosteroids

Gene	Encoded protein	Metabolic step affected/physiological defect	Allele/ Equivalents	References
dwf1	Oxido-reductase	24-methylenecholesterol → 24-methyldesmosterol 24-methylenecholesterol → campesterol	*dim1*, *cbb1*, *lkb*	Feldmann et al., 1989; Takahashi et al., 1995 Klahre et al., 1998; Nomura et al., 1999 Kauschmann et al., 1996; Choe et al., 1999a.
dwf2	Receptor-kinase	Insensitivity	*bri1*, *cbb2*, *bin* (n) *lka*, *cu3*, *abs1*	Clouse et al., 1996; Li & Chory, 1997, 1999b Bishop & Yokota, 2001; Montoya et al., 2002
dwf3	23α- hydroxylase (CYP90A1)	6-deoxocathasterone → 6-deoxoteasterone	*cpd*, *cbb3*	Szekeres et al., 1996Choe et al., 1998
		cathasterone → teasterone	*dpy*	Clouse & Sasse, 1998
dwf4	22α- hydroxylase (CYP90B1)	Campestanol → 6-deoxocathasterone 6-oxocampestanol → cathasterone		Azpiroz et al., 1998 Choe et al., 1998
dwf5	Δ^7 sterol reductase	Mevalonic acid → 24-methylenecholesterol		Choe et al., 2000
dwf6	5α-steroid reductase	(24R)-24-methylcholest-4-ene-3-one → (24R)-24-methyl-5α-cholestane-3-one	*det2*, *lk* (?)	Choe et al., 1998
dwf8	?	Sterol biosynthetic pathway		Choe et al., 1998, personal communication
dwf7	Δ^7 sterol C-5 desaturase	Mevalonic acid → 24-methylenecholesterol	*sterol 1*	Choe et al., 1999b
dwf8	Unknown			Reviwed in Li & Chory, 1999
dwf11	Cytochrome P-450 (CYP724b1)	BR biosynthesis		Tanabe et al., 2005
dwf12	Glycogen synthase kinase	Insensitivity		Choe et al., 2002
dwf(d)	Cytochrome P-450 (CYP85)	6-deoxoCS→CS		Bishop et al., 1999
bas	C-26 hydroxylase (CYP734A1)	Brassinolide → 26-OH brassinolide		Neff et al., 1999; Takahashi et al., 2005; Turk et al., 2005
brd1	BR-6-oxidase	Multiple C-6 oxidations		Mori et al., 2002
sax	?	3β,22-dihydroxyl-$\Delta^{5,6}$ →3-oxo-22-hydroxy-$\Delta^{4,5}$		Ephritikhine et al., 1999
bak1-1D	Protein kinase	Insensitivity		Li et al., 2002
bim1	(bHLH) proteins	Transcription factor		Yin et al., 2005
bee1,2, 3	(bHLH) proteins	Transcription factors		Friedrichsen et al., 2002
bes1	Nuclear protein (bri1 supression)	Insensitivity		Yin et al., 2002
bin2	Glycogen synthase quinase	Insensitivity		Li et al., 2001a
brs1	Carboxypeptidase	Modulation of signal transduction		Li et al., 2001b
bru1	XET	Inibition of signal transduction		Oh et al., 1998
bsu1	Nuclear phosphatase	Modulation of signal transduction		Mora-Garcia et al., 2004
bzr1	Nuclear protein	Insensitivity		Wang et al., 2002
tch4	XET	Inibition of signal transduction		Xu et al., 1995

***sax*.** Phenotypic rescue experiments of the *sax1* mutation of *Arabidopsis* using synthetic BRs suggest that *sax1* is involved in the conversion of 3β,22-dihydroxyl-$\Delta^{5,6}$ precursors to 3-oxo-22-hydroxy-$\Delta^{4,5}$ steroids, the first step of the hypotetical C22α-hydroxy pathway. Furthermore, recovery of wild-type phenotypes of root elongation in the light and of hypocotyl elongation in the dark suggests that *sax1* is mutated in a step of the pathway that is active in light and dark as well (Ephritikhine et al., 1999).

***brd1*.** *brd1*(BR-dependent 1), a recessive rice (*Oryza sativa* L. Cv. Nipponbare) mutant showing reduction in cell length also displays constitutive photomorphogenesis under darkness (Mori et al., 2002). Exogenously supplied BL has been shown to considerably restore the normal phenotypes. In addition to that, the analyses of endogenous sterols and BRs indicated that BR-6-oxidase, a BR biosynthesis enzyme, would be defective, and, indeed, a 0.2-kb deletion was found in the genomic region of o*sbr6*ox (a rice BR-6-oxidase gene) in the *brd1* mutant, indicating that the defect of BR-6-oxidase caused the *brd1* phenotype.

***bls1*.** Recently, an *Arabidopsis* mutant impaired in light- and BR induced responses as well as in sugar signaling has been described (Laxmi et al., 2004). For its photomorphogenic responses, *bls1* (BR, light and sugar1) resembles BR biosynthetic mutants cpd and *det2*, however, *bls1* is not allelic to either of these mutants. In addition to that, the mutant retains a normal perception/sensing mechanism for BRs.

Genes Involved in the Signal Transduction Pathway of BRs

bri1. From *Arabidopsis*, Clouse *and* colleagues (1996) identified the first BL insensitive mutant, BL insensitive1 (*bri1*) (Figure 1). BRI1 has been cloned and shown to encode a receptor kinase with an extracellular domain (Li and Chory, 1997), which appear to contain 24 rather than 25 leucine-rich repeats (LRRs), with LRR21 (formerly LRR22) being an unusual methionine-rich repeat (Vert et al., 2005). The intracellular region could be subdivided into a juxtamembrane, followed by a canonical serine/threonine kinase and a short C-terminal extension (Vert et al., 2005). It has been recently demonstrated that brassinolide binds directly to both, native and recombinant BRI1 proteins (Kinoshita et al., 2005). Friedrichsen *and* colleagues (2000) demonstrated that a BRI1::GFP (GFP, green fluorescent protein) fusion protein was located at the plasma membrane, which, along with the protein acting Ser/Thr phosphorylation suggested that BRs were perceived at the cell surface. A dramatic increase of BL binding activity in the membrane fractions of the BRI1::GFP transgenic plants was also found, which was due to an increase of binding sites with similar binding afinities (Wang et al., 2001).

Since all of the over 20 BR-insensitive mutants reported to date, such as *cbb2*, 18 *bin* and 3 alleles of *dwf2*, were all allelic to *bri1* (Clouse and Sasse 1998; Li and Chory, 1999), one might conclude that: 1. BRI1 was the only unique and specific component of the BRs signal transduction pathway; 2. The other components of the pathway are either redundant or shared with other signalling cascade (Li and Chory, 1999; Li et al., 2001b); 3. Mutations in other genes involved in BR signal transduction are lethal (Koka et al., 2000). Recently, Nam and Li (2004) identified an *A. thaliana* transthyretin-like protein (TTL) as a potential *bri1* substrate. TTL interacts with BRI1 in a kinase-dependent way in yeast and is phosphorylated by BRI1 *in vitro*. In addition, TTL presents a similar expression pattern with BRI1 and is associated with the plasma membrane.

The *curl-3* (*cu-3*) mutant (*Lycopersicon pimpinellifolium* [Jusl.] Mill.) shows severe dwarfism, modified leaf morphology, de-etiolation, and reduced fertility, and all of these features are strikingly similar to the *bri1* mutant of *Arabidopsis*. Elongation in primary roots of wild-type *L. pimpinellifolium* seedlings was strongly inhibited by BR application, while *cu-3* mutant roots were capable to elongate at the same BR concentration (Koka et al., 2000). The tomato partially BR-insensitive mutant *altered brassinolide sensitivity1* (*abs1*) was a weak allele of *cu3* (Montoya et al., 2002). Using sequence analysis of tBRI1 in the mutants *cu3* and *abs1*, Montoya and co-workers (2002) demonstrated that *cu3* was a nonsense mutant and that *abs1* is a missense mutant. The *lka* pea dwarf has increased levels of endogenous BRs and is insensitive to BL treatment (Nomura et al., 1997). Indeed, the *lka* mutant has been shown to be defective in the BR receptor kinase PsBRI1 (Nomura et al., 2003).

bak1-1d. Li *and* co-workers (2002) identified in *Arabidopsis* a dominant genetic suppressor of *bri1*, *bak1-1d* (bri1-associated receptor kinase 1-1Dominant). This gene encodes an LRR-RLK serine/threonine protein kinase, which interact with *bri1*. *bri1* and *bak1* can phosphorylate each other, being the autophosphorylation activity of *bak1* enhanced by *bri1*. Expression of a *bak1*dominant-negative mutant allele results in severe dwarf phenotype, mimicking the phenotype of null *bri1* alleles. The BRI1-BAK1 receptor complex is now thought to initiates BR signaling (Russinova et al., 2004). Studies using a*tserk3* (*Arabidopsis* Somatic Embryogenesis Receptor-like Kinase3) a gene identical to bak*1-1d*, showed that *bri1*, but not a*tserk3*, homodimerizes in the plasma membrane, while *bri1* and a*tserk3* preferentially heterodimerize in the endosomes (Russinova et al., 2004). Today, *bak1* is believed to act as a coreceptor and/or downstream target of *bri1* (Vert et al., 2005).

***bzr1* and *bes1*.** A dominant mutation in *Arabidopsis*, *bzr1-1d*, suppresses BR-deficient and BR-insensitive (*bri1*) phenotypes (Wang et al., 2002). BZR1 is a transcriptional repressor that binds directly to the promoters of feedback-regulated BR biosynthetic genes (He et al., 2005). The BZR1 protein accumulates in the nucleus of elongating cells of dark-grown hypocotyls and has been shown to be a positive regulator of the BR signaling pathway (Wang et al., 2002). Thus, BZR1 coordinates BR homeostasis and signaling by playing dual roles in regulating BR biosynthesis and downstream BR responses.

Yin *and* co-workers (2002) have identified in *Arabidopsis bes1*, a novel plant-specific transcription factor (Li and Deng, 2005). *bes1* is a semidominant suppressor of *bri1* which exhibits constitutive BR response phenotypes such as constitutive expression of BR-response genes. *bes1*, which accumulates in the nucleus in response to BRs, is phosphorylated and apparently is destabilized by the glycogen synthase kinase-3 (GSK-3) *bin2*, a negative regulator of the BR pathway (see more details below).

BZR1-BES1 family of proteins directly binds to and regulates BR-responsive genes, which establish a link between hormonal signal transmission in the cytoplasm and transcriptional status change in the nucleus (Li and Deng, 2005). Through the use of a yeast two-hybrid approach using BES1 as a bait, Yin and co-workers (2005) identified the bHLH transcription factor BIM1 in *Arabidopsis*. BIM1 and BES1 have been shown to be able to bind CANNTG E-box motifs in the promoter of a *saur-ac1* BR responsive gene and likely to form a heterodimer. Many bHLH transcription factors are known to bind to those E-box motifs (reviewed in Vert et al., 2005).

***brs1*.** Li *and* collegues (2001b) identified in *Arabidopsis brs1-1d*, for bri1 suppressor-Dominant, which encoded a secreted serine carboxypeptidase (Zhou and Li, 2005). *brs1-1d* suppressed multiple *bri1-5* defects. This suppression was selective for *bri1*, and dependent on BR, and a functional BRI1 protein kinase domain. In addition, overexpressed *brs1* missense mutants, predicted to abolish BRS1 protease activity, failed to suppress *bri1-5*.

***bin2-1* and *bin2-2*.** BR insensitive 2 (*bin2-1* and *bin2-2*) mutants, isolated from *Arabidopsis* (Li et al., 2001b) exhibit insensitivity to BL, but hypersensitivity to ABA. *bin2* has been found to encode an *Arabidopsis* ortholog of human glycogen synthase kinase β and *Drosophila* SHAGGY protein kinase (Li *and* Nam, 2002). Interestingly, a mutation in the same kinase gene has also been found to be carried by the ultracurvata1 (*ucu1*) *Arabidopsis* mutant (Perez-Perez et al., 2002). Phenotype and map position of *bin2* mutations indicate that these mutants are likely to be alleles of the *ucu1* gene (Berná et al., 1999). Overexpression of *bin2* in a weak bri1 mutant background generated a more-pronounced dwarf phenotype. In contrast, co-suppression studies of *bin2* transcription indicate that reduced *bin2* levels have only a weak effect on plant growth. When seen together, these data indicate that *bin2* is a negative regulator of BL signaling. It has also been thought that BRI1 may interact with and phosphorylate BIN2 to inactivate it (Li *and* Nam, 2002), but BIN2 do not present close homology to the consensus sequence surrounding the preferred serines that BRI1 is likely to phosphorylate (Oh et al., 2000), consequently, BRI1 is thus not likely to interact directly with the BIN2 kinase. Li *and* Nam (2002) have also suggested that BIN2 is constitutively active in the absence of BRs and phosphorylates positive BR signaling proteins to inactivate them.

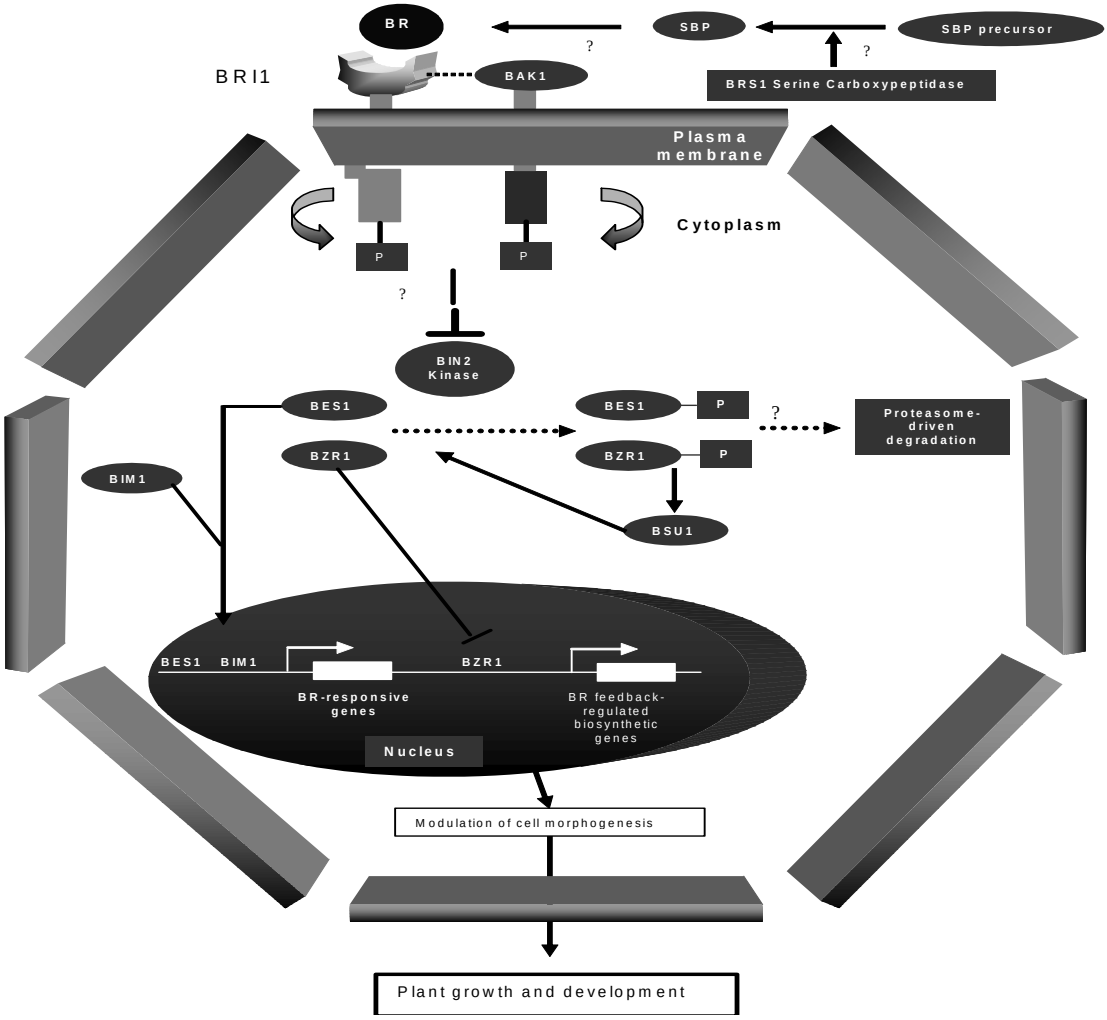

Figure 1 - Working model for the main genes involved in brassinosteroid signal transduction pathway in *Arabidopsis*.

Brassinosteroids (BRs) are perceived at the plasma membrane by direct binding to the extracellular domain of the BRI1 receptor S/T kinase or before that, it complexes with a still unidentified steroid binding protein (SBP), which may require processing by a serine carboxypeptidase (BRS1). BAK1, which encodes for an LRR-RLK serine/threonine protein kinase, interact *in vitro* and *in vivo* with BRI1. BRs are thought to bind to three points in BRI1, its receptor: the 2α, 3α -hydroxyls (Wada et al. 1981), the B ring lactone and the 22α, 23α -hydroxyls (Kishi et al. 1986). BR perception initiates a signaling cascade through BIN2, and the BSU1 phosphatase, which in turn modulates the phosphorylation state and stability of the nuclear transcription factors BES1 and BZR1, two positive regulators of the BR signaling cascade. Interrogation marks indicate proposed but not demonstrated steps.

More recently, Sun and Allen (2005) have shown that transgenic *Arabidopsis* plants expressing cotton *bin2* cDNAs show reduced growth and similar phenotypes to the semi-dominant *bin2* mutant plants, indicating that the cotton *bin2* genes encode functional *bin2* isoforms (Sun and Allen, 2005).

***bsu1*.** The *bsu1-1d Arabidopsis* mutant was identified through a *bri1* supressor screen by activation tagging (Mora-Garcia et al. 2004). *bsu1* encodes a plant-specific nuclear phosphatase with a long, Kelch-repeat-containing N-terminal region hooked up to a C-terminal S/T phosphatase domain. *bsu1* partially suppresses the dwarf phenotype of the *bin2* mutant. Furthermore, in *bsu1* mutants BES1 accumulates in its hypophosphorylated form and *in vitro* BIN2-phosphorylated BES1 is dephosphorylated in the presence of BSU1 protein. Finally, RNAi knock-down plants display a compact phenotype resembling weak *bri1* alleles, which provide further support for a model where BSU1 directly counters the effects of BIN2 on BES1, and likely BZR1 (reviewed in Vert et al., 2005).

***dwf12*.** Choe *and* colleagues (2002) identified two new BR-insensitive mutants (*dwarf12-1d* and *dwf12-2d*). The semidominant dwf1*2* mutants displayed the typical morphology of previously reported BR dwarfs but they also exhibited several unique phenotypes such as severe downward curling of the leaves. Similar to bri1 mutants, *dwf12* plants accumulated CS and BL, providing further evidence that DWF12 was a component of the BR signaling pathway that included BRI1. Cloning of the *dwf12* gene revealed that DWF12 belonged to a member of the glycogen synthase kinase 3β family, but differently from the human glycogen synthase kinase 3β, DWF12 lacked the conserved serine-9 residue in the auto-inhibitory N terminus. In addition, *dwf12-1d* and *dwf12-2d* encode changes in consecutive glutamate residues in a highly conserved TREE domain. While comparing their findings with previously published data on two other *Arabidopsis* mutants, *bin2* and *ucu1*, which contain mutations in this TREE domain, Choe *and* colleagues suggested that the TREE domain was of critical importance for adequate functioning of DWF12/BIN2/UCU1 in the BR signalling cascade. *dwarf 9*, 10 and 11 mutations have not been reported yet.

***bee1*, *bee2*, and *bee3*.** Friedrichsen *and* co-workers (2002) identified in *Arabidopsis* three genes, which shared high sequence identity, encoding putative basic helix-loop-helix (bHLH) proteins called BR Enhanced Expression (BEE1, BEE2, and BEE3). These functionally redundant, transcription factors, are induced within 30 minutes of treatment with BL being this induction dependent of functional BRI1 and independent of *de novo* protein synthesis, making these genes the first early response genes characterized in the BR signal transduction pathway. BEE1, BEE2, and BEE3 were active throughout the plant, and mutants lacking all three proteins were less responsive to BRs (Friedrichsen et al., 2002 and Vert et al., 2005).

bru1. Zurek *and* Clouse (1994) identified *bru1* in soybean, a gene specifically regulated by BRs during the early stages of elongation. BR application resulted in increased plastic extensibility of the elongating soybean epicotyls walls within 2 h, with a concomitant increase in BRU1 mRNA levels. A direct relationship between the level of BRU1 transcripts and both, magnitude of BR-induced stem elongation and plastic extensibility of the cell wall was also demonstated. BRU1 encodes a protein that shows significant homology to various xyloglucan endotransglycosylases (XETs) (Zurek *and* Clouse, 1994), enzymes that specifically cleave xyloglucan chains and transfer a fragment of the cleaved chain to an acceptor xyloglucan (Fry, 1995), being the mechanism of BR regulation of BRU1 posttranscriptional. Enzyme assays with the recombinant protein showed that BRU1 was indeed a functional XET. Moreover, a linear relationship has been observed between BR concentrations and extractable XET activities in BR-treated soybean epicotyls (Oh et al., 1998). In addition to that structural changes that reduced or impaired stem elongation in soybean resulted in proportional reduction in *bru1* expression (Clouse, 1997). When seen together, these data strongly suggested an involvement of BRU1 in BR-stimulated stem elongation.

***tch4*.** The *Arabidopsis* TCH(for touch)4 gene was identified as a XET by sequence similarity and

enzyme activity (Xu et al., 1995). The recombinant TCH4 protein has been shown to be capable of catalyzing the transfer of xyloglucan polymer segments to radioactively labeled xyloglucan-derived oligosaccharides to form labeled products (Fry et al., 1992), demonstrating that TCH4 was able to modify a major component of the plant cell wall (Xu et al., 1995). It has also been demonstrated that plants of *Arabidopsis* exposed to 24-epiBL, a highly active BR (Mandava, 1988), presented higher levels of TCH4 mRNA, compared to untreated plants. In addition to that, TCH4 expression has been shown to be restricted to expanding tissues and organs that underwent cell wall modification (Xu et al., 1995).

cycd3. Progression through the eukaryotic cell cycle is regulated at the G1/S and G2/M check points by distinct families of cyclin-dependent kinases (CDKs), whose activities are determined by co-ordinated binding of different types of cyclins (Pines, 1995). Among all cyclins, the D-types are thought to be critical for the stimulation of cell division (Hu et al., 2000). D-type cyclins (CycD) play key roles in linking the *Arabidopsis* cell cycle to extracellular and developmental signals, but their regulation at the post-transcriptional level or of their cyclin-dependent kinase (CDK) partners is poorly understood (Healy et al., 2001). In *Arabidopsis*, overexpression of *cycd3* enable initiation and maintanence of cell division in the absence of cytokinin, which suggests that cytokinins activate cell division through induction of *cycd3* (Riou-Khamlichi et al., 1999). Hu *and* co-workers (2000) identified genes that responded to BR in *det2* suspension culture, and found that epi-BL upregulated transcription of *cycd3*. Epi-BL was also able to substitute cytokinin in culturing of *Arabidopsis* callus and suspension cells. In addittion to that the epi-BL-driven induction of *cycd3* was demonstrated to involve *de novo* protein synthesis, but no protein phosphorylation or dephosphorylation. *cycd3* induction was also found to occur in cells of bri1, a BR-insensitive mutant, which suggested that BR induced *cycd3* transcription through a previously unknown signal pathway in plants. These findings led Hu *and* co-workers to conclude that epi-BL stimulated cell division through *cycd3* induction.

bru2. A novel cDNA from BL-treated rice seedlings, tentatively named BR-upregulated gene 2 (*bru2*), was isolated (Sasuga et al., 2000). The *bru2* could encode an actin effector protein that control polymerization of actin molecules, which provided evidence for the involvement of BRs on the orientation of microtubules in plant cells (Bishop *and* Yokota, 2001).

exo. The *exordium* (*exo*) protein has been identified as a regulator of BR-responsive genes in *A. thaliana* (Coll-Gracia et al., 2004). The *exo* gene was characterized as a BR-up-regulated gene. *exo* overexpression resulted in increased transcript levels of the BR-up-regulated *kcs1*, e*xp5*, *delta - tip*, and *agp4* genes, thought to be involved in the mediation of BR-promoted growth. In addition to that *exo* overexpressing lines showed enhanced vegetative growth, resembling the features of BR-treated plants.

Conclusions and Future Perspectives

BRs are perceived at the plasma membrane through direct binding to the BRI1 receptor. BR-driven changes in gene expression are achieved mainly through the modulation of the phosphorylation state of the transcription factors, BES1 and BZR1, involved, respectively, in the promotion of BR responses and repression of BR-feedback regulated genes. Microarrays analysis has helped to identify a new whole group of BR-related genes (Goda et al., 2002; Yang et al., 2003), which is predicted to make significantly contribution to the elucidation of the full BR signal transduction pathway.

It is expected, as a practical goal, that detailed insight into the regulation of BRs biosynthesis, signal transduction pathway and action mechanism, will provide new tools for the improvement of crops. For example, ripening control in orchards (Simmons et al., 2006) is expected to be improved, while improved management of shoot architecture in horticulture and forestry has already been achived (Patent BR 000057, Pereira-Netto et al., pending). Furthermore, improved knowledge on BR-related genes might also help to unravel unknown action mechanisms of steroids in animals, especially in mammals.

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RESUMO

Genes envolvidos nas vias de biossíntese e de transdução de sinal de brassinoesteróides.
Brassinoesteróides são esteróides vegetais, essenciais para o crescimento e o desenvolvimento, que apresentam um oxigênio no carbono C-3 e oxigênios adicionais em um ou mais dos átomos de carbono C-2, C-6, C-22 e C-23. Nos últimos anos, a aplicação de técnicas de genética molecular possibilitou progresso significativo no entendimento da regulação da via biossintética e na identificação de vários componentes da via de transdução de sinal de brassinoesteróides. Buscas em bases de dados eletrônicas mostram dúzias de registros para genes relacionados a brassinoesteróides nos últimos doze meses, demonstrando os grandes esforços desenvolvidos neste campo. Esta revisão destaca os recentes avanços na caracterização de genes e mutações que estão auxiliando na elucidação dos mecanismos moleculares envolvidos na síntese/metabolismo, e percepção e resposta de brassinoesteróides, com ênfase especial no seu papel no alongamento de células vegetais. Aspectos do envolvimento de BRs na regulação de proteínas que controlam o ciclo cellular também são discutidos.

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