A Brassinosteroid-Insensitive Mutant in Arabidopsis thaliana Exhibits Multiple Defects in Growth and Development

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Brassinosteroids are widely distributed plant compounds that modulate cell elongation and division, but little is known about the mechanism of action of these plant-growth regulators. To investigate brassinosteroids as signals influencing plant growth and development, we identified a brassinosteroid-insensitive mutant in Arabidopsis thaliana (L.) Heynh. ecotype Columbia. The mutant, termed bril, did not respond to brassinosteroids in hypocotyl elongation and primary root inhibition assays, but it did retain sensitivity to auxins, cytokinins, ethylene, abscisic acid, and gibberellins. The bril mutant showed multiple deficiencies in developmental pathways that could not be rescued by brassinosteroid treatment, including a severely dwarfed stature; dark green, thickened leaves; male sterility; reduced apical dominance; and de-etiolation of dark-grown seedlings. Genetic analysis suggests that the Bril phenotype is caused by a recessive mutation in a single gene with pleiotropic effects that maps 1.6 centimorgans from the cleaved, amplified, polymorphic sequence marker DHS1 on the bottom of chromosome IV. The multiple and dramatic effects of mutation of the BR1 locus on development suggest that the BR1 gene may play a critical role in brassinosteroid perception or signal transduction.

Brassinosteroids are plant-growth-promoting natural products with structural similarities to animal steroid hormones (Mandava, 1988). When BRs are applied exogenously at nanomolar to micromolar levels in a number of test systems, they have a marked effect on cell elongation and/or proliferation that is distinct from that of auxins, cytokinins, and GAs, although they interact with these plant hormones and also with environmental signals, such as light and temperature, in complex ways (Adam and Marquardt, 1986). BRs are found throughout the plant kingdom and have been shown by microchemical techniques to occur endogenously at levels sufficient to promote, in planta, the physiological effects observed in bioassay systems (Sasse et al., 1992). Taken together, these facts suggest that BRs are endogenous plant-growth regulators and may serve as one of the signals controlling plant growth and development (Sasse, 1991).

Despite the large body of physiological data gathered since the first publication of a BR structure (Grove et al., 1979), unequivocal proof that BRs are essential for plant growth and development has so far been unavailable. Furthermore, the molecular mechanism of BR action is currently unclear. One might argue from structural considerations that they act by a mechanism similar to that of animal steroid hormones. In general, such hormones act via a soluble receptor/ligand complex that binds to nuclear sites to regulate the expression of specific genes (Evans, 1988; Mangelsdorf et al., 1995). We and others have shown that BR regulates gene expression in Glycine max (Clouse and Zurek, 1991; Clouse et al., 1992; Zurek and Clouse, 1994; Zurek et al., 1994) and Arabidopsis thaliana (Clouse et al., 1993; Xu et al., 1995) and that this modulation of specific gene expression is correlated with cell elongation, but information on a BR receptor is lacking.

The use of mutants of A. thaliana that are deficient in or insensitive to plant hormones has been invaluable in dissecting the molecular mechanisms of ABA, auxin, ethylene, and GA action (Estelle and Klee, 1994; Finkelstein and Zeevart, 1994; Ecker, 1995). Hormone-insensitive mutants usually result from lesions in genes encoding hormone biosynthetic enzymes and are rescued to wild-type phenotype by treatment with the hormone. For example, the ga1, ga2, and ga3 mutants of A. thaliana are dwarfs that are restored to wild-type height by spraying with GA (Finkelstein and Zeevart, 1994). The GA1 locus has been cloned by genomic subtraction (Sun and Kamiya, 1994) and shown to encode ent-kaurene synthetase A, a critical enzyme in GA biosynthesis (Sun and Kamiya, 1994). Hormone-insensitive mutants may show the same phenotype as hormone-deficient mutants but are not rescued by hormone treatment. Hormone-insensitive mutants usually result from lesions in genes encoding the hormone receptor or elements of the signal transduction pathway. The ETR1 locus of A. thaliana was identified by a screen for ethylene resistance (Bleecker et al., 1988), and positional cloning of the ETR1 gene with subsequent characterization showed that the ETR1 locus encoded an ethylene receptor (Chang et al., 1993).

Our goal is to identify the BR receptor and other components of the BR signal transduction pathway using

**Abbreviations**: BR, brassinosteroid; CAPS, cleaved, amplified, polymorphic sequences; Col-0, Columbia ecotype; EBR, 24-epibrassinolide; EMS, ethylmethane sulfonate; Ler, Landsberg erecta ecotype; SSLP, simple sequence length polymorphism; XET, xyloglucan endotransglycosylase.

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a mutational analysis in *A. thaliana*. BRs have a range of physiological effects, but promotion of stem elongation is perhaps the best characterized (Clouse et al., 1992). Employing the inability to elongate stems in the presence of BR as a screen for BR insensitivity was found to be impractical for technical reasons (S.D. Clouse, M. Langford, T.C. McMorris, unpublished data). However, like auxin, BR often inhibits primary root elongation even though shoot growth is stimulated by hormone application (Roddick and Juan, 1991). The *axr1* (Lincoln et al., 1990), *axr2* (Wilson et al., 1990), and *aux1* (Pickett et al., 1990) auxin-resistant mutants and the *ckr1/ein2* (Su and Howell, 1992; Ecker, 1995) cytokinin-resistant mutant were selected because of the ability of the mutant plants to elongate roots in the presence of hormone concentrations that inhibited root growth in wild-type *A. thaliana*. We have previously shown that BRs exhibit a similar inhibitory effect on Arabidopsis root growth and that EMS-mutagenized plants can be selected that are insensitive to this response (Clouse et al., 1993). We recently presented a preliminary report on the first BR-insensitive mutant in *A. thaliana*, *bril*, that has a severely dwarfed phenotype and reduced apical dominance and is male-sterile (Clouse and Langford, 1995). In this paper we provide additional genetic and physiological analysis of *bril*.

**MATERIALS AND METHODS**

**Screening of Mutagenized Plants**

*M*₂ seeds of *A. thaliana* (Col-0 background) mutagenized with EMS were obtained from Lehle Seeds (San Antonio, TX). The *M*₂ seeds were supplied in bulked parental groups of 8,000 seeds representing 1,000 M₁, parents. *M*₂ seeds (approximately 71,000 total) were sterilized by treatment with absolute ethanol (10 min) followed by a 30% commercial bleach solution containing 0.1% Triton X-100 (20 min). Seeds were then rinsed five times in a large volume of sterile water and suspended in 0.2% agarose, and between 100 and 200 seeds were pipetted along straight lines in 150 × 100 mm Petri plates containing 1% agar, 2% Suc, and standard mineral salts (Estelle and Somerville, 1987). EBR was synthesized as previously described (McMorris and Patil, 1993) and added to the medium after autoclaving at either 10⁻⁶ or 10⁻⁷ M final concentration. The plates were placed vertically in a growth chamber at 23°C with a 16-h light/8-h dark cycle at 50 μE m⁻² s⁻¹ intensity. After 6 d of incubation, *M*₂ plants, whose roots elongated substantially (>9 mm), were picked and grown to maturity to produce *M*₃ progeny from individual *M*₂ picks were then compared to wild type for their ability to elongate roots in the presence of 10⁻⁷ M EBR. *M*₃ plants that were male-sterile, such as *bril*, were backcrossed to wild-type Col-0 to generate the *M*₄₀, which was selfed to create a segregating *M*₄₀ population. EBR insensitivity of the mutant phenotype was confirmed in *M*₄₀ individuals followed by a second round of backcrossing and selfing to generate *M*₅₀ mutant individuals for additional analysis.

**Hormone Sensitivity Assays**

Because *bril* is male-sterile, pure stocks of *bril* seed were not available. Therefore, segregating *F₂* progeny from a backcross of *bril* × Col-0 were used for all experiments. Excess *F₂* seeds were sterilized and plated on agar medium as described above with the exception that plates were incubated for 3 d at 4°C in the dark to increase uniform germination before transfer to the growth chamber. All hormones were added after autoclaving and cooling of the media to <55°C. Root length of all seedlings was measured to the nearest 0.1 mm after 7 or 8 d using a dissecting scope with a stage micrometer, and plants were tagged individually and grown in soil for 2 months to determine phenotype. Data points represent 20 measurements ± se for those plants determined to be *bril*, compared with 20 measurements ± se for Col-0 seedlings treated in the same manner.

**Ethylene Experiments**

Seeds were sterilized as described above and plated on 1% agar containing mineral salts (Estelle and Somerville, 1987) and 2% Suc, pH 5.8, poured into the bottom of 1-quart Mason jars whose lids were fitted with rubber septums. Jars were wrapped in aluminum foil and placed at 4°C for 3 d. Ethylene was injected to a final concentration of 1 μL/L and the jars were placed at 23°C for 4 d. Ethylene, CO₂, and O₂ were monitored daily by GC. Final measurements of root and hypocotyl length were made using a dissecting microscope with a stage micrometer.

**Plant Growth**

Plants for crossing and phenotypic analysis were grown in growth chambers or shaking liquid culture as previously described (Clouse et al., 1993).

**Chromosome Mapping**

A single plant showing the *Bril* phenotype (Col-0 background) was pollinated with Ler pollen yielding a fertile *F₁* hybrid, which was selfed to generate a segregating *F₂* population of 70 mutant and 212 wild-type individuals. DNA was isolated from individual mutant plants by placing the entire plant in a microfuge tube with a small amount of liquid nitrogen, followed by grinding with a plastic pestle. After sublimation of the nitrogen, 500 μL of buffer (100 mM Tris, 100 mM Na₂EDTA, 250 mM NaCl, pH 8.0) were added and grinding was continued. Fifty microliters of 10% sarkosyl were then added, followed by 5 μL of proteinase K (10 mg/mL), and the tubes were incubated at 55°C for approximately 1 h. After centrifugation (14,000 rpm, 10 min), the supernatant was extracted twice with phenol:chloroform:isoamyl alcohol (24:24:1, v/v), and 0.35 volumes of 5 mM NaCl and 2 volumes of 100% ethanol were added to the final aqueous phase to precipitate DNA free from polysaccharides (Mettler, 1987). The pellets were washed twice in 75% ethanol and resuspended in 100 μL of sterile water, and 1.5 μL were used per 20-μL PCR reaction. DNA from individual plants showing the wild-type phenotype was isolated in a similar manner except that a small
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mortar and pestle was used for grinding whole plants and the buffer was increased to 5 mL/g tissue. Genotypes of the F2 wild-type plants were determined by F3 progeny analysis. Linkage to known molecular markers was determined by SSLP (Bell and Ecker, 1994) and CAPS (Konieczny and Ausubel, 1993) analysis using primers from Research Genetics (Huntsville, AL).

RESULTS

Phenotype and Genetics of the bri1 Mutant

From a screen of approximately 70,000 EMS-mutagenized M2 seedlings of A. thaliana, an individual was selected that showed root elongation in the presence of $10^{-6}$ or $10^{-7}$ M EBR, a concentration that inhibited the elongation of wild-type Col-0 roots by up to 75%. This mutant consistently exhibited long roots and very short hypocotyls when placed on various EBR concentrations and showed a striking phenotype when grown to maturity, which suggested that a fundamental pathway controlling growth and development had been compromised by the mutation. Figure 1a shows that the bri1 mutant is an extreme dwarf that reaches less than 10% of the height of wild-type Col-0 plants grown under the same conditions. Two-month-old bri1 plants did not exceed 1.5 cm, whereas Col-0 plants of the same age were greater than 15 cm in height. Figure 1, b and c, shows that the leaf morphology of the bri1 mutant was distinctly different from that of the wild type. Rosette leaves were shorter in the mutant than in the wild type, had a thickened, curled appearance, and were darker green, and their petioles failed to elongate. Preliminary microscopic evaluation of the bri1 mutant suggests that reduced cell size rather than cell number is responsible for the dwarf phenotype (data not shown). Figure 1d shows that 4-month-old plants had a bushy appearance, indicating reduced apical dominance when compared to mature wild-type plants.

bri1 plants failed to yield viable seeds even when self-pollinated by hand and grown at either 23 or 15 to 18°C. However, when pollen from Col-0 or Ler wild-type plants were placed on bri1 stigmas, siliques containing fertile F1 seeds developed, indicating that the bri1 mutant is most likely male-sterile but female-fertile. We have not yet examined the mechanism of male sterility. The F1 resembled wild type in appearance, and selfed F1 plants gave an F2 population that segregated in a 3:1 wild-type-to-mutant phenotypic ratio, suggesting that the Bri phenotype is caused by a single recessive Mendelian allele. Table I summarizes the F2 phenotypic segregation of a number of bri1 x Col-0 and bri1 x Ler crosses. In all cases examined, those plants showing the dwarf phenotype also were BR insensitive, as determined by the root-elongation assay. Moreover, after two generations of backcrossing interspersed with two generations of selfing, there was no segregation of the dwarf phenotype and BR insensitivity.

To determine the map position of the bri1 locus, DNA was isolated from individual F2 progeny of a bri1 x Ler cross and analyzed for linkage to SSLP (Bell and Ecker,
Hormone Sensitivity of the bril Mutant

The bril mutant was identified by the presence of long roots when grown on a concentration of EBR that inhibited wild-type root elongation. Figure 2 shows that 0.5 \( \mu \text{M} \) EBR caused, on average, 82\% inhibition of root elongation in wild-type Col-0 when compared to a buffer control. In a segregating \( F_2 \) population from a cross of the bril mutant with wild-type Col-0, those individuals showing Col-0 phenotype gave a similar response to the wild-type parent with respect to inhibition of root elongation by 0.5 \( \mu \text{M} \) EBR. However, those individuals with the Bril phenotype were completely insensitive to the effect of 0.5 \( \mu \text{M} \) EBR on root elongation. This insensitivity was confirmed over a wide range of EBR concentrations, as shown in Figure 3. Concentrations of EBR greater than 5.0 nM resulted in a rapid decrease in root length of wild-type Col-0 seedlings, reaching a maximum inhibition at 0.5 \( \mu \text{M} \). In contrast, bril mutants maintained the same root length on EBR concentrations ranging from 10.0 nM to 0.5 \( \mu \text{M} \) as they did when they were grown on control medium. An EBR concentration of 1.0 \( \mu \text{M} \) did result in a slight inhibition of bril root elongation, but the mutant roots were still over five times longer than roots of Col-0 seedlings grown on 1.0 \( \mu \text{M} \) EBR. EBR concentrations greater than 1.0 \( \mu \text{M} \) were not attempted so as to preserve the limited amounts of the compound available.

The inhibition of root elongation in wild-type Arabidopsis seedlings seems to be a general effect of most plant hormones tested (Bleecker et al., 1988; Lincoln et al., 1990; Pickett et al., 1990; Wilson et al., 1990; Su and Howell, 1992; Clouse et al., 1993). To determine if the bril mutation confers insensitivity specifically to BRs or results in insensitivity to multiple hormones, as has been observed for aax1, aax2, aux1, and ckr1, we compared root elongation of Col-0 and bril seedlings on a number of plant hormones. Figure 4 shows that concentrations of 2,4-D from 5 to 100 nM resulted in greater inhibition of bril root elongation than in wild-type, and that at 0.5 and 1.0 \( \mu \text{M} \) the inhibition was equally severe in both genotypes. Therefore, the bril mutant retains complete sensitivity to 2,4-D. Figure 5 shows that bril and Col-0 are equally sensitive to 0.5 \( \mu \text{M} \) IAA, 6-benzylaminopurine, and kinetin, and that bril is hypersensitive to 0.5 \( \mu \text{M} \) ABA. bril appears to be slightly less sensitive to 0.5 \( \mu \text{M} \) GA3 than Col-0, but nowhere does it approach the insensitivity observed with 0.5 \( \mu \text{M} \) EBR (cf. Fig. 2).

To determine the sensitivity of bril to ethylene, dark-grown seedlings were used in an experiment similar to that described by Bleecker et al. (1988) for the analysis of the etr1 ethylene-sensitive mutant. It is interesting that the bril mutant grown in the dark in air had short, thickened hypocotyls and fully opened cotyledons characteristic of the det2 and cop class of photomorphogenic mutants (Chory and Susek, 1994). Figure 6 shows that even though...
The inhibitory effect of BRs on primary root elongation in wild-type Arabidopsis thaliana proved to be a facile and reproducible screen for BR insensitivity (Clouse et al., 1993). While the root assay allowed the initial identification of bril, mutant plants exhibited a phenotype that extended far beyond the simple root response to BR, which suggested that the entire developmental program had been altered. Analysis of several thousand F2 progeny showed no segregation between root elongation in the presence of BR and the altered developmental morphology. Therefore, it is most likely that the short stature, altered leaf structure, reduced apical dominance, and male sterility are caused by a mutation in a single gene with pleiotropic effects that also confers insensitivity to BRs. However, after two backcrosses and two rounds of selfing, it cannot be completely ruled out that the dwarf phenotype and the BR insensitivity are caused by independent mutations in genes that are very tightly linked.

Another Arabidopsis dwarf that has a very similar phenotype to bril is det2, a de-etiolated mutant that shows characteristics of a light-grown plant even when it is grown in the dark (Chory and Susek, 1994). Very recently, the DET2 locus has been cloned by chromosome walking and has been found to have significant homology to a mammalian 5α steroid reductase (Li et al., 1996), an enzyme involved in steroid metabolism. Moreover, the Det2 mutant phenotype is rescued by BR treatment, indicating that det2 is a BR biosynthetic mutant (Li et al., 1996). The fact that det2 (a single locus that is nonallelic to bril and that can be rescued to wild type by BR treatment) has a closely related phenotype to bril strongly suggests that the single locus conferring insensitivity to BR is the same locus controlling the developmental aspects of the Bril phenotype. In the cases of both det2 and bril, it is likely that the dwarf phenotype is caused by a lack of BR action, either through reduced synthesis or reduced response, respectively.

It is not surprising that both a BR-insensitive and a BR-deficient mutant show a dwarfed growth habit, since BRs have long been known to promote stem elongation. BR-promoted elongation of young vegetative tissue has been observed in mung bean, azuki bean, and pea epicotyls (Yopp et al., 1981; Gregory and Mandava, 1982; Mandava, 1983).

**DISCUSSION**

Reduced apical dominance, and male sterility are caused by a mutation in a single gene with pleiotropic effects that also confers insensitivity to BRs. However, after two backcrosses and two rounds of selfing, it cannot be completely ruled out that the dwarf phenotype and the BR insensitivity are caused by independent mutations in genes that are very tightly linked.

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known that auxin also promotes stem elongation (Taiz, 1988); bean, sunflower, and cucumber hypocotyls (Mandava et al., 1981; Katsumi, 1985); Arabidopsis peduncles (Clouse et al., 1993); and maize mesocotyls and wheat coleoptiles (Yopp et al., 1981; Sasse, 1985). We have previously shown that BR is a potent enhancer of epicotyl elongation in soybeans and we have found that BRs modulated cell-wall mechanical properties and gene expression in this system (Clouse et al., 1992; Zurek et al., 1994). It is well known that auxin also promotes stem elongation (Taiz, 1984), and comparisons of auxin- versus BR-stimulated growth showed that the kinetics of elongation and effects on gene expression were quite different for BR and auxin (Clouse et al., 1992; Zurek and Clouse, 1994).

Also of interest is whether BR-induced growth, like auxin-induced cell elongation, is ultimately mediated by cell-wall loosening. The biochemical basis of wall relaxation was predicted nearly 20 years ago to involve enzymes that cleave and rejoin cell-wall polymers, particularly xyloglucans (Albersheim, 1976). XET is an enzyme that specifically cleaves a xyloglucan chain and transfers a fragment of that chain to an acceptor xyloglucan (Fry, 1995). Several groups (Smith and Fry, 1991; Nishitani and Tominaga, 1992; Fry et al., 1992; Fanutti et al., 1993) have proposed a wall-loosening function for this enzyme during cell elongation, or it may have other important roles in elongation such as xyloglucan biosynthesis or integration of new xyloglucan polymers into the wall (McQueen-Mason et al., 1993).

We have cloned a BR-regulated gene from soybean called BRUL that shows high sequence homology to XETs from other species (Zurek and Clouse, 1994) and whose transcript levels are correlated with the extent of BR-promoted stem elongation and increases in plastic extensibility of the cell wall (Zurek et al., 1994). We recently found that the recombinant BRU1 protein has XET activity and that extractable XET activity in soybean epicotyls is increased by BR treatment (W. Romanow, R. Smith, S.D. Clouse, unpublished data). Therefore, XETs may play a role in BR-modulated stem elongation in soybean.

A similar story is unfolding in A. thaliana. TCH4, which is 70% identical to BRU1 at the amino acid level, shows increased transcript levels in BR-treated Arabidopsis tissue and has also been found to encode a XET (Xu et al., 1995). Moreover, transgenic A. thaliana plants harboring TCH4 promoter:GUS fusions show highest reporter gene expression in elongating tissue (Xu et al., 1995). Therefore, a likely explanation for the dwarf phenotype of br1 is that BR insensitivity results in reduced XET activity (along with reduced expression of other unidentified molecular components of the cell-elongation machinery), which results in shortened cells. We are currently examining TCH4 expression and XET activity in the br1 mutant.

The specificity of the hormone insensitivity of br1 lends additional support to the importance of BRs in growth and development. Based on the root-elongation assay, br1 is as sensitive to auxins, GAs, cytokinins, ABA, and ethylene as wild-type Col-0. Therefore, if insensitivity to BR in the root assay and the developmental phenotype of br1 are caused by a single gene, it is compelling evidence that BR activity is essential for growth and development. Furthermore, besides cell elongation, BRs must play a role in male fertility and apical dominance. Based on early physiological studies, it was suggested that the effects of BR are mediated through auxin or that BR increases tissue sensitivity to endogenous auxins (Mandava, 1988). We previously showed that BR can act independently of auxin in elongating soybean epicotyls (Clouse et al., 1992) and that auxin-
insensitive mutants such as axr1 (Lincoln et al., 1990) and 
dgf (Daniels et al., 1989) retained sensitivity to BR (Clouse
et al., 1993; Zurek et al., 1994). The complete sensitivity of
br1 to auxin demonstrated here provides additional evi-
dence of the independence of BR and auxin action.

Our primary motivation in studying BR-insensitive
mutants is to identify the BR receptor and clarify the BR
signal transduction pathway. Hormone insensitivity
generally results from one of three mechanisms: (a) a
mutation in the receptor prevents the hormone from
binding, or the receptor fails to become activated even
when bound to the ligand; (b) the receptor is functional
but a mutation in a critical step in the signal transduction
pathway has occurred; or (c) the applied hormone is not
actually the active endogenous compound and an en-
zyme that converts the applied hormone to the active
form has been mutated. In view of the proven biological
activity of numerous forms of BRs, including brassinol-
ide, EBR, 28-homobrassinolide, and castasterone (Mc-
Morris et al., 1994), and the fact that we have shown
insensitivity of the br1 mutant to more than one BR (S.D.
Clouse, M. Langford, T.C. McMorris, unpublished data),
option c is unlikely and br1 probably represents a mu-
tation in class a or b. A collaborative effort between the
Chory and Sakurai groups to determine endogenous BRs
in Arabidopsis will help to clarify this issue (J. Chory,
personal communication).

Original views of the mechanism of animal steroid hor-
mone action assumed a single protein receptor/signal
transduction model in which the steroid ligand diffused
through the cell membrane, bound to the steroid receptor
in the cytosol or nucleus, and the receptor/ligand complex
interacted directly with hormone-responsive elements in
the promoters of steroid-regulated genes (Evans, 1988). It is
now known that steroid receptors interact with heat-shock
proteins in the cytosol, are regulated by phosphorylation,
and can complex with nonsteroidal transcription factors in
the nucleus (Beato et al., 1995). Therefore, although the
steroid signal transduction pathway in animals is more
complex than previously thought, it is still quite short. If
BRs have a similar mechanism of action in plants, a small
set of BR-insensitive mutants will define the entire signal
transduction pathway. Even if the BR pathway differs from
that of animals and is more complex, isolation of hormone-
insensitive mutants in Arabidopsis is still a very fruitful
approach, as has been amply demonstrated in ethylene
(Ecker, 1995) and ABA (Finkelstein and Zeevart, 1994) sig-
nal transduction studies.

We are engaged currently in the positional cloning of the
BRI1 locus, which is greatly facilitated by the recent pub-
lication of a complete physical map of chromosome IV
(Schmidt et al., 1995). The dramatic and pleiotropic effects
of the br1 mutation suggests that the BRI1 gene lies very
early in the signal perception/transduction pathway.
Therefore, cloning and characterization of the BRI1 locus
should lead to a major advance in our understanding of BR
action. The BR-insensitive (br1) and BR-deficient (det2)
mutants will be valuable resources for further understand-
ing the molecular mode of BR action. Furthermore, the

phenotype of these mutants provides strong genetic evi-
dence that BRs are essential for normal plant growth and
development.

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