J Plant Growth Regul (2003) 22:325–335
DOI: 10.1007/s00344-003-0064-1

J P G R
Journal of Plant Growth Regulation

Brassinosteroid Mutants of Crops

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Abstract

Plant steroid hormones, brassinosteroids (BRs), were originally isolated from extracts of pollen because of their growth-promoting properties and their potential use for enhancing crop production. Mutants in the biosynthesis, metabolism, and signaling of brassinolide (BL), the most bioactive BR, are important resources in helping to establish BRs' essential role in plant growth and development. The dark green and distinctive dwarf phenotype of BR-related mutants identified in pea, tomato, and rice highlights the importance of BRs in crops. These mutants are helping to elucidate both the conserved and the unique features of BR biosynthesis and signaling. Such insights are providing the key knowledge and understanding that will enable the development of strategies towards the production of crops with enhanced qualities.

Introduction

One of the key questions concerning world food production is, ''How can crops be produced that have the yield and quality characteristics that the current population desires?'' Better understanding of the processes that contribute to increased yield and/or quality of the crop is of fundamental importance and has played and will continue to play an important role in determining the funding of plant science research. The discovery, in the 1940s (Mitchell and Whitehead 1941) of pollen extracts enhancing plant growth was therefore of great significance and led to the funding of the isolation and characterization of the bioactive component in pollen (Mitchell and others 1970). Further analysis of the growth-promoting activity in pollen led to the identification of the plant steroid hormone brassinolide (BL) (Grove and others 1979), which is the most bioactive member of a group of steroids generically called brassinosteroids (BRs). Since this important discovery the biosynthetic pathway leading to the production of BL (Figure 1) has been elucidated utilizing skills from diverse disciplines including chemistry, biochemistry, physiology, and genetics.

Recently, the most significant and rapid breakthroughs relating to brassinosteroid research have been made by adopting a genetic approach and identifying mutants involved in brassinosteroid synthesis, metabolism, signaling, and response, using the model plant *Arabidopsis thaliana*. These advances in cloning the associated genes and placing them into appropriate pathways of action have been highlighted in the accompanying reviews of this special issue. The most salient features of BR biosynthesis and signaling mutants in *Arabidopsis* is that they are dark green dwarfs that often exhibit a deetiolated phenotype when grown in the dark (that is, they undergo photomorphogenic development in the absence of light). BR biosynthesis mutants respond to exogenous BR application whereas the signaling mutants generally do not (Clouse and others 1996; Li and others 1996; Szekeres and others 1996). Analysis of the BR content in such

Received: 1 May 2003; accepted: 15 August 2003; Online publication: 18 December 2003
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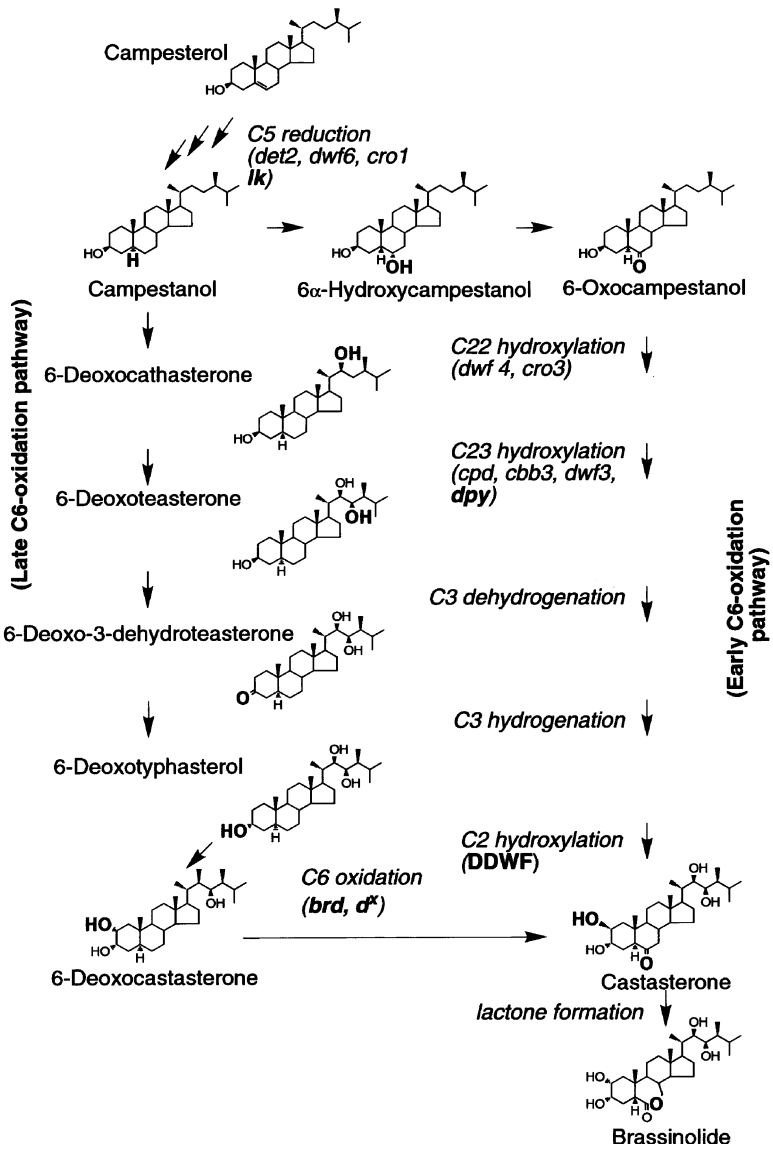

Figure 1. Simplified BL biosynthetic pathway. BL biosynthesis occurs via two distinct routes according to the C-6 oxidation status of the BR, namely, early and late C-6 oxidation pathways. Intermediates of the late C-6 oxidation pathway are shown and larger **bold font** moieties in the steroid structure highlight the common changes in the molecule for both pathways. Text in italics indicates the conversion taking place. In parentheses mutants that have been identified in *Arabidopsis* are listed in regular font and crop mutants/enzymes discussed in this review are in bold font. Information for this figure has been based on data in a recent review by Fujioka and Yokota (2003).

mutants highlights the deficiency of BRs in the biosynthesis mutants whereas increased levels are present in the signaling mutants (Noguchi and others 1999). The increased level of BRs in the signaling mutants is associated with increased transcription of BR biosynthesis genes and the homeostatic level of BRs appears to be regulated by a feedback mechanism (Bancos and others 2002).

Concurrent to the rapid advances made in *Arabidopsis*, crucial discoveries in BR research have been made in crops, including the identification of BR biosynthesis and BR signaling mutants. These mutants not only confirm the conserved role of BRs in plant growth and development but also highlight novel features that would not have been revealed if only a limited number of plant species were studied. The focus of this review is to highlight the similarities and differences in the role of BRs in plant development between model and crop systems. This is achieved by describing the characterization of the biosynthesis and the signaling mutants in selected crops, namely, pea, tomata, and rice. Novel discoveries relating to BRs in crops are also presented. At the end of the review some of the future prospects in crop development utilizing the knowledge gained from studies in crops is presented.

BR Mutants of Pea

When adopting a genetic approach to unravel the complexity of plant growth and development, it is worthwhile to remember Mendel and his selection of the garden pea to provide an account of the laws of inheritance. In his studies Mendel utilized a dwarf mutant and, although this mutant was found

Table 1. BR Biosynthesis Mutants in Crops

Crop	Mutation	*Arabidopsis* equivalent	Function	References
Pea	*lk*	*det2*	5α-reduction	T. Yokota and T. Nomura, personal communication
Pea	*lkb*	*dwf1, dim*	24-methylenecholesterol to campesterol conversion	Nomura and others 1999; Schultz and others 2001
Tomato	*d, d*^{x}	none	C-6 oxidation	Bishop and others 1996, 1999
Tomato	*dpy*	*cpd?*	C-23 hydroxylation?	Koka and others 2000
Rice	*brd1*	none	C-6 oxidation	Mori and others 2002; Hong and others 2002

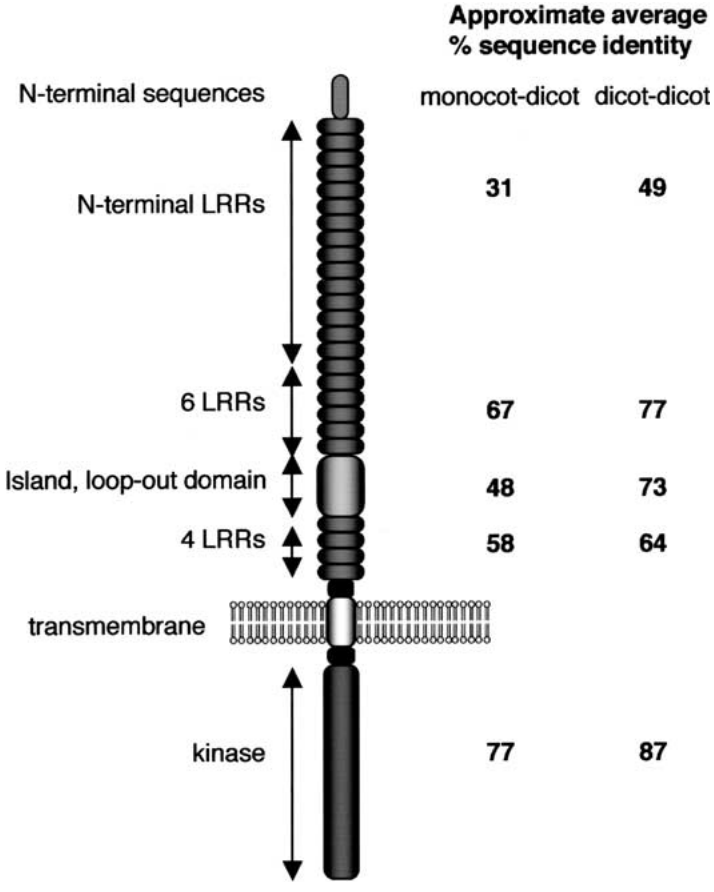

Figure 2. Schematic of domain structure of BRI1 and homology of known BRI1 genes. Simplified predicted domain structure of BRI1 from *Arabidopsis* (Li and Chory 1997). Averages of sequence identity of regions were generated using sequence from known BRI1 homologues for which mutations have been identified, that is, monocot, rice (Yamamuro and others 2000) and dicots, pea (Nomura and others 2003), tomato (Montoya and others 2002), and *Arabidopsis* (Li and Chory 1997).

to be defective in the synthesis of the plant hormone gibberellin (GA) (Martin and others 1997), it is conceivable that he could have utilized the distinctive dwarf phenotypes of the recently characterized BR dwarfs, if they had been available to him. These BR mutants have been characterized by a dedicated group of researchers that have maintained pea as their major research material for studying plant hormones and have taken advantage of the relative ease with which enough (approximately 100 g) tissue can be obtained for BR analysis as compared to the more favored model system, *Arabidopsis*, where this can be more difficult.

The BR-related dwarf mutants of pea were first characterized as a group of mutants that appeared to be insensitive to GA (Reid and Potts 1986; Reid and Ross 1989). As described below, these pea mutants have been shown to be defective in either BR biosynthesis or BR signaling.

Pea BR Biosynthesis Mutants

Several lines of evidence have confirmed that the dwarf pea mutants *lk* and *lkb* are BR biosynthesis mutants. *lk* is the smallest BR-related dwarf in pea and this dwarfism can be rescued by BR application.

Table 2. BR Signaling Mutants in Crops

Crop	Mutation	*Arabidopsis* equivalent	Function	Reference
Pea	*lka*	*bri1*	LRR–RLK	Nomura and others 2003
Tomato	*cu3, abs*	*bri1*	LRR–RLK	Koka and others 2000; Montoya and others 2002
Rice	*d61-1,-2*	*bri1*	LRR–RLK	Yamamuro and others 2000

Analysis of BR content suggests that the *lk* mutant is defective in the orthologous gene to the *Arabidopsis DEETIOLATED2* homologue (T. Nomura and T. Yokota, personal communication; Table 1). The *lkb* dwarfism is also rescued by exogenous BR application (Nomura and others 1997), and analysis of BR content indicates that the *lkb* mutant is defective in the early steps of BR synthesis (Nomura and others 1999). More recently, the *lkb* mutant has been shown to be defective in the pea equivalent of the *Arabidopsis* DWF1/DIM gene (Schultz and others 2001) that is involved in the conversion of 24-methylenecholesterol (Table 1).

Pea BR Signaling Mutants

At the same time *lkb* was assessed for its response to BL, the *lka* mutant of pea was found to be defective in BR response (Nomura and others 1997). Initial BR quantification experiments showed that this mutant had increased levels of endogenous BRs (Nomura and others 1999), which is similar to that seen in the *Arabidopsis* BR response mutant *brassinolide insensitive 1* (*bri1*) (Noguchi and others 1999). BRI1 encodes a putative leucine-rich repeat receptor-like kinase (Li and Chory 1997) (Figure 2), and recently the *lka* mutation has been shown to co-segregate with a sequence mutation in a pea homologue of BRI1 (PsBRI1) (Nomura and others 2003; Table 2). Nomura and others (2003) also highlight that PsBRI1 is phylogenetically more closely related to the BRI1 homologues from other species in which dwarfing mutations have been recovered than to other closely related sequences; this is discussed in more detail later in this review. Interestingly, in comparing the dwarf phenotypes of *lka* and *lk* mutants, the *lk* mutant is more dwarfed, suggesting that the *lka* mutation is probably not a null allele of a PsBRI1 homologue.

Novel Discoveries Utilizing Pea

The identification of the pea *lk*, *lka*, and *lkb* mutants as being defective in BR biosynthesis and response has provided insights into BRs' role in pea development, and a novel finding is that these pea mutants do not exhibit de-etiolation. When grown in the dark the pea BR mutants are shorter than wt, however, they do not express light-regulated genes or show leaf development similar to the de-etiolated *lip* mutant of pea (Symons and others 2002). This suggests that in pea BRs do not play a direct role in the etiolation or de-etiolation response. Further evidence of this was observed when endogenous levels of BRs were analyzed in light- and dark-grown tissue. If BRs help promote etiolation, increased concentrations may be expected in dark-grown tissue. However, this was not observed and light-grown tissue was found to have increased concentrations of BRs (Symons and others 2002). In addition to this, no rapid decrease in BRs was observed when dark-grown seedlings were transferred to light (Symons and others 2002). Conceivably, it may be the case that the sensitivity to BRs of light- and dark-grown tissue are different, with dark-grown tissues being more sensitive, and the relative concentrations of BRs may not represent their signaling potential. In addition to this is the novel finding that when wt *Arabidopsis* seedlings are grown under the appropriate culture conditions (vertically oriented plates and close contact to the medium), the seedlings exhibit cotyledon expansion and leaf development (Azpiroz and others 1998). Furthermore, the *Arabidopsis* BR mutant *dwf4* does not show induction of a light-regulated CAB::GUS fusion, which suggests that the de-etiolated phenotype of *dwf4* may be merely a consequence of the dwarfism (Azpiroz and others 1998; Choe and others 1998). These results indicate that further careful examination of BRs' role in light-regulated growth and development in both *Arabidopsis* and crops is needed to help clarify the part played by BRs in the de-etiolation/etiolation of plants.

As mentioned above, pea has been used to study light-regulated development, and the expression of the pea small G protein Pra2 has been identified as being light regulated. Recently, it has been suggested that Pra2 plays a pivotal role in integrating light and BR signaling through interacting with and regulating the activity of a cytochrome P450 enzyme, DDWF, that catalyzes the C-2 hydroxylation of BRs (Kang and others 2001). These authors also

indicated that DDWF is transcriptionally upregulated in the dark which would suggest that increased concentrations of BRs would be observed in dark-grown etiolated material. As discussed previously, these findings seem to conflict with the levels observed in light- and dark-grown pea seedlings (Symons and others 2002). In addition to this is the apparent observation that DDWF does not appear to have a homologous sequence in the *Arabidopsis* genome, which may suggest that this Pra2–DDWF interaction is not a universal system of regulating BR levels. It will be highly informative therefore to utilize the pea BR mutants to further clarify Pra2's role in the etiolation of pea seedlings, for example, testing whether the increased levels of BRs in the *lka* mutant reflect increased levels of Pra2 expression/activity.

BR Mutants of Tomato

Tomato is a highly valuable crop and is used as a model system for studying certain aspects of plant growth and development. Some of the first genetic linkage experiments in tomato were carried out in the early 1900s, which included the use of the tomato dwarf mutation (*d*). More recently this mutation has been identified as being defective in BR synthesis (Bishop and others 1996, 1999). Numerous tomato mutants have been isolated and a carefully maintained collection of these mutants, including those that are dwarf, exists at the Rick Stock Centre (http:://tgrc.ucd.edu). In addition to this genetic resource, many laboratories have developed tomato as a model system for studying fruit production and disease resistance. This interest has led to the development of many molecular resources including a high-density genetic map, introgression lines, transposon tagging lines, an extensive collection of ESTs, and numerous genomic and cDNA libraries. Furthermore, tomato can be transformed relatively easily to generate lines over- or underexpressing genes involved in BR signaling or response. These features have made tomato an attractive crop in which to study BRs, and the recent exploitation of these resources has been beneficial in identifying mutants in BR biosynthesis and response.

Tomato BR Synthesis Mutants

The easily scored seedling phenotype of the *dwarf* mutant enabled easy screening for a targeted transposon tagging experiment. *D* was transposon tagged and the corresponding gene was cloned. It was found to encode a cytochrome P450 enzyme that showed significant homology to the *Arabidopsis CONSTITUTIVE PHOTOMORPHOGENIC AND DWARFISM* (*CPD*) gene (Bishop and others 1996; Szekeres and others 1996). More recent work has shown that *Arabidopsis* has two highly homologous genes to the tomato *Dwarf* gene (Shimada and others 2001, 2003) and that *CPD* is a member of a different family of P450 enzymes (Szekeres and others 1996).

Phenotypic rescue experiments of the *d* allele using exogenous BR application proved inconclusive. However, use of the strong *extreme dwarf* (d^x) allele was more informative and showed that BL enabled partial phenotypic recovery (Bishop and others 1999). BR quantification of wt and d^x plants highlighted that in tomato vegetative tissue the late C-6 oxidation pathway of BRs is the predominant pathway leading to the synthesis of castasterone (CS), the immediate precursor of BL. Interestingly, BL was not detected in either wt or d^x vegetative tissue, and the major difference between the mutant and wt BR content was that the d^x mutant lacks CS but has increased concentrations of the precursor 6-deoxocastasterone. These data and the conversion assays carried out using yeast that express the DWARF enzyme have shown that it functions in the C-6 oxidation of BRs (Bishop and others 1999) (Table 1) and *Arabidopsis* homologues also show this activity (Shimada and others 2001, 2003).

Further screens for mutations involved in BR synthesis in tomato have been carried out, and the *dumpy* (*dpy*) mutant has been identified as a candidate for being defective in BR synthesis (Koka and others 2000). *dpy* has a similar phenotype to the tomato d^x mutant and can be rescued to a wild-type phenotype by exogenous application of certain BR intermediates. These rescue experiments suggest that *dpy* is defective in the C-23 hydroxylation of BRs, that is, the tomato equivalent of *cpd* (Koka and others 2000) (Table 1). The quantification of endogenous BRs in this mutant, however, indicates only a relatively small twofold reduction in the level of 6-deoxoteasterone, the intermediate predicted to be generated by the tomato *cpd* homologue. The cloning of *dpy* is therefore needed to help clarify the genetic lesion in this mutant.

Tomato BR Signaling Mutants

At the same time as identifying *dpy*, Koka and others (2000) screened the tomato dwarf collection maintained at the Rick Stock Centre for BR signaling mutants. In this screen they identified the *curl3* (*cu3*) mutant as being BR insensitive and having

characteristics of being defective in a BRI1 homologue. Degenerate primers based on conserved sequences in the BRI1 kinase domain were used to isolate the BRI1 homologue of tomato (tBRI1), and sequence analysis has shown that *cu3* has a mutation in tBRI1 (Montoya and others 2002). The *cu3* allele contained a nonsense mutation in one of the LRRs of tBRI1 and a novel weak allele (*abs*) of tBRI was identified as harboring a mutation in the kinase domain (Montoya and others 2002) (Table 2). Similar to other BRI mutants, both *cu3* and *abs* mutants have increased levels of BR intermediates and increased expression of a BR biosynthesis gene, *Dwarf* (Montoya and others 2002). Interestingly, in both *cu3* and *abs* mutants BL was not detected and only intermediates of the late C-6 oxidation pathway were observed, suggesting that in the tissues from which BRs were analyzed BL may not be the major bioactive BR but CS may be. In addition, the *abs* mutant is defective in the feedback regulation of the tomato *Dwarf* gene. These data provide further evidence for the essential role of BRI1 homologues in the transcriptional regulation of BR-regulated genes.

Novel Discoveries Utilizing Tomato

Similar to the pea mutants, tomato BR biosynthesis mutants do not appear to exhibit a dramatic de-etiolated phenotype, although when grown in the dark the d^x, *dpy*, and *cu3* mutants are short and exhibit cotyledon expansion (Bishop and others 1999; Koka and others 2000). In tomato, further molecular investigation is required to clarify whether BR mutants show expression of light-regulated genes in the dark and have a de-etiolated phenotype similar to the *det2* mutation of *Arabidopsis* (Chory and others 1991).

The cloning of *Dwarf*, the identification of two dwarf homologues in *Arabidopsis*, and the lack of identification of an *Arabidopsis* mutant equivalent to *Dwarf* highlight the fact that it is possible to identify novel mutations that may not be revealed through studying only *Arabidopsis*. Tomato may, in fact, be a good system to recover further novel mutations, as it may be the case that tomato, even with a larger genome size, may have less genetic redundancy than *Arabidopsis* (Van der Hoeven and others 2002).

Another highly novel finding has been the recent observation that the putative systemin receptor, SR160, has sequence homology to BRI1 (Scheer and Ryan 2002). Sequence analysis between tBRI1 and SR160 indicates that these sequences are the same and highlights the possible dual role of tBRI1/SR160 LRR–RLK in systemin and BR signaling (Montoya and others 2002). This novel finding has been reviewed in two recent commentaries (Yin and others 2002a; Szekeres 2003).

Systemin is a peptide product from the preprotein prosystemin and when it is applied to tomato it initiates responses, for example, induction of proteinase inhibitor (PIN) gene expression, that also occur in wounding (Ryan and others 2002). SR160 is a 160-kD protein that was isolated via its ability to bind photoaffinity-labeled systemin (Scheer and Ryan 1999, 2002). However, the lack of a mutant prevented these authors from confirming the role of tBRI1/SR160 in systemin signaling. The tomato dwarf mutants *cu3* and $cu3^{-abs}$ have mutations in tBRI1/SR160 and will therefore offer opportunities to dissect the putative dual role of this receptor. It is worthwhile to note that systemin is a Solanaceae-specific peptide hormone and that this raises interesting questions as to whether in *Arabidopsis* and/or other crops there is/are equivalent peptide(s) to systemin that can bind BRI1 homologues. These possible differences in function are worthy of further investigation.

BR Mutants of Rice

Rice is an important monocot crop and, because of its relatively small genome size in comparison to other monocots, it has been adopted as a model system. The rice genome has been recently sequenced (Goff and others 2002; Yu and others 2002) and more complete annotation is expected sometime in 2005. In addition, T-DNA insertional mutants are being generated so that both forward and reverse genetics can be adopted. Numerous dwarf mutants are available in rice and have been classified into distinct morphological groups. Although these mutants have been in existence for some time, it is only relatively recently that there has been a concerted effort to characterize this germplasm at the molecular level to assess whether any of these dwarf mutants are defective in BR biosynthesis and signaling. Rice is therefore an excellent system for revealing insights in BRs' role in the growth and development of monocots.

Rice BR Synthesis Mutants

Two research groups have isolated rice BR-deficient mutants in the independently named *brd1* gene, namely, *brassinosteroid-dependent-1* (Mori and others 2002), and *brassinosteroid-deficient dwarf-1* (Hong and others 2002). Mori and others (2002) identified a strong *brd1* allele in a T-DNA transformation ex-

periment, although this mutant was not T-DNA tagged but was- in fact a deletion mutant. Hong and others (2002) identified three alleles of *brd1: brd1-1* and *brd1-2* being strong null alleles and *brd1-3* being a weak allele.

Phenotypically plants with the strong alleles are very dwarfed with twisted/crinkled leaves and have poor fertility. This twisted morphology is most likely the consequence of altered vascular development, which is highly consistent with BRs' role in promoting tracheary element formation. BR analysis of wt and mutant tissue showed that the mutants had increased concentrations of intermediates in the late C-6 oxidation pathway compared with the early C-6 oxidation pathway. This suggested that the *brd* mutants were likely to be defective in the rice equivalent of the tomato *Dwarf* gene and sequence analysis of the rice *Dwarf* (*OsDwarf*) homologue from these mutants confirmed this. The strong alleles were found to be deletion (nonsense) derivatives and the weak alleles to be missense mutants (Mori and others 2002; Hong and others 2002) (Table 1). Currently, only multiple mutant alleles of this gene have been reported in rice as being defective in BR synthesis; however, in the extensive dwarf collection more BR-related mutants are likely to be identified. It is worthwhile to note that, in common with *Arabidopsis*, BR intermediates of both late and early C-6 oxidation pathway were detected in the mutants and wt.

Rice BR Signaling Mutants

BR-insensitive mutants have been identified in rice based on their dwarf habit and lack of BR-induced leaf bending in the lamina joint test (Yamamuro and others 2000). The two chemically induced mutants initially identified were found to be allelic and subsequently referred to as *d61-1* and *d61-2*. BR profiles of light-grown mutant material showed increased concentrations of BR intermediates, and, when grown in the dark, these mutants lacked normal etiolation. These data suggested that the *d61* mutants were defective in the rice homologue of BRI1, and in a suitable mapping population the dwarf phenotype was shown to cosegregate with a BRI1 homologue from rice (OsBRI1). Subsequently, sequence analysis of OsBRI1 in *d61-1* indicated that this was a missense mutant in the kinase domain of OsBRI1, whereas the stronger allele was a missense mutation in a leucine-rich repeat just prior to the island region in the extracellular domain of OsBRI1 (Yamamuro and others 2000) (Table 2). Interestingly, phenotypes of certain lines antisense OsBRI1 are more severe than those of the *d61* mutant alleles and are similar to the strong alleles of the *Brd* gene, suggesting that the *d61* mutants are not null alleles. The lack of identification of a null allele of OsBRI1 may highlight the essential nature of this gene in the growth and development of rice or functional redundancy, which is discussed later in this review.

Novel Discoveries Utilizing Rice

Similar to the isolation of the tomato d/d^x mutants, the isolation of the rice *brd* mutants indicates that there is a lack of genetic redundancy in rice for C-6 oxidation of BRs compared with that observed in *Arabidopsis*. It is also quite surprising that in rice further mutations that are similar to the strong *brd* alleles have not yet been reported, as these would be candidates to be defective in other steps of BR synthesis. It is also interesting to note that, similar to tomato, BL was not detected in the rice tissue analyzed for BR quantification, indicating the need for further clarification as to whether CS and other BRs are bioactive.

The isolation of OsBRI1 has highlighted some unique features of the evolution of the BRI1 receptor. The most striking feature is the lack of three leucine-rich repeats in the extracellular domain compared with that isolated in the dicots (*Arabidopsis*, tomato, and pea). In addition to this, the island domain that was previously thought to be important for BL binding is not as conserved as other regions of the receptor (Figure 2). Evolutionary distances between monocots and dicots would suggest that it is not a surprising observation that the rice sequence BRI1 has less sequence identity to the dicot sequences than the dicot sequences have between themselves. However, the apparent lack of high homology in the extracellular domain, even though recognizing the same ligand, is worthy of further investigation.

Homologues or Orthologues?

A common theme emerging from the comparison of BR synthesis and signaling mutants in the above-selected species is that similar dwarfs have been recovered with mutations in similar genes. It seems highly likely, therefore, that the isolated homologous sequences represent orthologous genes. In *Arabidopsis*, however, there is increased genetic redundancy as there are three closely related sequences to BRI1 (BRI1Like—BRL), two of which have been identified as binding BR (Yin and others 2002a). Another example of genetic redundancy can be observed in the *Arabidopsis* C-6 oxidation of

BRs, as there are apparently two redundant homologous P450 sequences. Although this large number of closely related homologues in one species may be the consequence of a relatively recent gene duplication event(s), it is perhaps surprising that in some cases a phenotype is observed, for example, *bri1* dwarf phenotype, that is not masked by such genetic redundancy, and in other cases a phenotype is not recovered. The relatively weak dwarfing phenotypes of the identified BRI1 mutations in pea and rice may be a consequence of genetic redundancy masking the fact that these are null mutants. However, if pea and rice have less genetic redundancy, a null mutation in BRI1 may result in a lethal phenotype and hence only weak alleles are observed. More sequence information of the existing germplasm is needed to provide better understanding in these areas.

As mentioned previously, the phylogenetic grouping of the BRI1 sequences in which mutations have resulted in dwarfism indicates that these sequences are orthologues (Yin and others 2002a; Nomura and others 2003). The recent observation that a mutation in the BRI1-like gene causes altered vascular development (Clay and Nelson 2002) indicates that the additional homologous sequences may reveal novel BR-related, tissue-specific functions. Therefore, it will be exciting to determine whether crops will have genes equivalent to these functions.

As mentioned, the identification of orthologous sequences offers exceptional opportunities to reveal key sequence motifs that are associated with BR signaling/biosynthesis. What is surprising is that in the analysis of four closely related BRI1 homologues of pea, rice, tomato, and *Arabidopsis*, no obvious BR-binding motif has been identified as a highly conserved region (Montoya and others 2002). In fact, in the LRR regions and island domain where the ligand interaction is likely to take place the homology is less than in the kinase domain (Figure 2). It will be highly informative when the 3D structure of the BRI1 extracellular domain is resolved, as this will enable the identification of the key residues involved in ligand interaction and resolve whether these residues are conserved between species.

Future Prospects

It is becoming evident that with the results obtained from studying BRs and the associated mutants in crops it will be possible to develop strategies that utilize BR-related traits so that crops are manipulated for improved productivity/quality. Of crucial significance will be the necessity to enhance our knowledge of BR action in important crops such that the rapid advances in our understanding of BR biosynthesis and signaling that take place in the model plant species *Arabidopsis* can be exploited quickly.

The two major strategies possible for increasing crop performance are either via chemical application (BR application and/or BR biosynthesis inhibitor application) or via genetic approaches (altering the level of expression of BR biosynthesis or signaling genes). It is also feasible that a combination of these strategies could be used on wt or BR mutants. Previous work has shown the potential benefit of treating crops with BRs to improve stress tolerance and crop yield. Paradoxically BR mutants are also more stress tolerant although they usually have reduced yield. It will be important to understand the basis of this apparent paradox to maximize the potential of a BR-related strategy in crop improvement.

Application Strategies

After the discovery of the growth-promoting pollen extract and BL much effort was focused on testing applications of different BRs on crops to see if any improvement in crop performance could be obtained (Steffens 1991). Although improvements in crop performance were noted, the results from this work were not conclusive. In fact, because BRs are not widely used today in crop production one can assume the costs of synthesis and application outweigh any gain in productivity. However, with more recent advances in the synthesis of synthetic analogues (see accompanying review by Back and Pharis 2003), it seems more likely that new formulations will be possible, enabling better crop performance. Some examples of the physiological responses that indicate BR-treated crops may show enhanced performance are thermotolerance (Dhaubhadel 1999) and disease resistance (Nakashita and others 2003). It is also conceivable that better crop performance may be achieved by inhibiting BR synthesis and partially phenocopying the mutant phenotypes. Inhibitors of BR biosynthesis, for example, brassinazoles, are highlighted in the accompanying review by Asami and others (2003).

Genetic Approaches

There is a rapidly increasing array of genes involved in BR biosynthesis and signaling that has been identified in *Arabidopsis* which can potentially be used to manipulate plant architecture. It is already

known that overexpression of *Dwarf* in tomato (Bishop and others 1999) increases plant height and that overexpression of the *DWARF4* gene, encoding the *Arabidopsis* BR C-22 hydroxylase, in both *Arabidopsis* and tobacco increases plant height and seed yield (Choe and others 2001). In addition to the increased expression of biosynthesis genes towards increasing plant growth, it is possible to utilize the overexpression of some of the genes involved in BR signaling to enhance plant growth, for example, BRI1 (Wang and others 2001), BAK1 (Li and others 2002; Nam and Li 2002), BZR1 (Wang and others 2002), and BES1 (Yin and others 2002b) [for a summary of the function of these components see Clouse (2002) and the accompanying review by Peng and Li (2003)]. It is likely that careful use of the overexpression of such genes in selected tissues will enable better crop performance.

Conversely, it may be possible to generate better crops from dwarfing plants in specific tissues or at different stages of development. Antisense or RNAi approaches to reduce the expression of BR biosynthesis or signaling components will enable this. An alternative approach is to overexpress genes that inactivate BRs. Overexpression of the BAS1 gene can reduce BR levels and generate dwarf plants in both *Arabidopsis* and tobacco (Neff and others 1999). Such dwarfing technologies may be useful in the prevention of lodging or the need to frequently cut turf grass.

Conclusions

The importance of BRs in crops has been shown through the identification of mutants in BR biosynthesis and signaling. It is an exciting time with the need of not only identifying further mutants in crops to determine the extent of conservation of BR biosynthesis and signaling, but also careful analysis of the existing biomaterials to discern the possible benefits for crop improvement. In addition to this, it will also be important to see if any of the current novel findings observed in the crops may in fact be conserved in other species but have not yet been observed. The next few years will prove to yield important information in these areas.

Acknowledgements

I thank Takao Yokota and Takahito Nomura for their personal communications and helpful comments on the manuscript. I also thank Miklos Szekeres for his invaluable help and advice on the manuscript. Grateful thanks to both the reviewer and Steve Clouse for their stimulating comments in helping in the revision process of preparing this manuscript. The Bishop Lab acknowledges support from the BBSRC, the Royal Society, the HFSPO, and the British Council.

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