J Plant Growth Regul (2006) 25:245–251
DOI: 10.1007/s00344-006-0041-6

J P G R
Journal of Plant Growth Regulation

Characterization of *CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM* Homologs in Rice (*Oryza sativa* L.)

Tomoaki Sakamoto[1] and Makoto Matsuoka[2]*

[1]*Field Production Science Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Nishi-Tokyo, 188-0002, Japan;* [2]*Bioscience and Biotechnology Center, Nagoya University, Nagoya 464-8601, Japan*

Abstract

To enhance our understanding of brassinosteroid (BR) biosynthesis in rice, we attempted to identify putative rice homologs of *Arabidopsis CYP90A1/CPD* and related mutants. Two candidate genes, designated *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2*, are located on chromosomes 11 (2.0 cM) and 12 (1.9 cM), respectively. Based on sequence similarity with the *Arabidopsis CYP90A1/CPD* gene, we predict that the *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* gene products function as C-23α hydroxylases in the BR biosynthesis pathway. Both are broadly expressed in wild-type rice, and their expression is regulated by a feedback mechanism. A retrotransposon insertion mutant of *CYP90A3/OsCPD1*, *oscpd1*-1, did not produce any BR-deficient phenotype or feedback upregulation of genes for BR biosynthesis enzymes. These results indicate that if, as predicted, the *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* genes do function in the BR biosynthesis pathway, they may each have enough capacity to catalyze BR biosynthesis on their own. As a consequence, the *oscpd1-1* mutant may not be deficient in endogenous BRs. Interestingly, BR biosynthesis enzymes except C-6 oxidase are encoded by plural genes in rice but by single genes in *Arabidopsis* (again, except C-6 oxidase). On the basis of these findings, we discuss the differences in BR biosynthesis between rice and *Arabidopsis*.

Key words: Brassinosteroid; *CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM* (*CPD*); Cytochrome P450 monooxygenase; Retrotransposon *Tos17*; Rice

Introduction

Brassinosteroids (BRs) are endogenous phytohormones that are involved in cell and stem elongation, dark-adapted morphogenesis (skotomorphogenesis), responses to environmental stress, and tracheary element differentiation (Clouse and Sasse 1998; Sasse 2003). The major pathway for BR biosynthesis was recently established in *Arabidopsis*, and a number of dwarf mutants have been identified as BR-deficient (Bishop and Yokota 2001; Fujioka and Yokota 2003). Brassinosteroid biosynthesis starts with the

Received: 22 April 2006; accepted: 28 June 2006; Online publication: 26 September 2006
*Corresponding author; e-mail: makoto@nuagr1.agr.nagoya-u.ac.jp

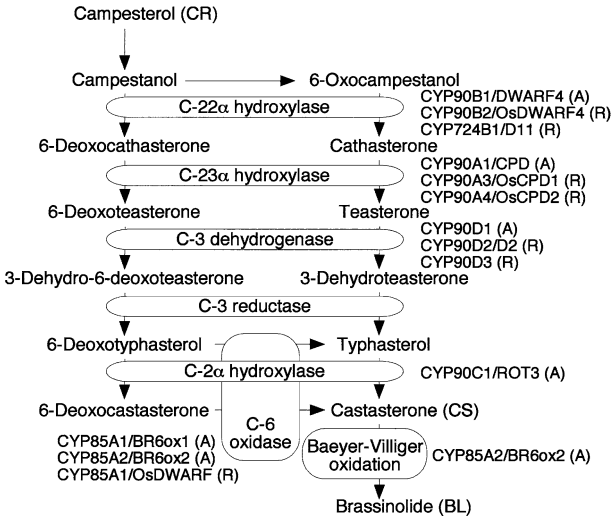

Figure 1. BR biosynthesis pathway and related enzymes in rice and *Arabidopsis*. A, *Arabidopsis thaliana* L.; R, rice (*Oryza sativa* L.).

plant sterol campesterol (CR), and the bioactive BRs castasterone (CS) and brassinolide (BL) are synthesized by sequential reactions catalyzed mainly by 6 enzymes (Figure 1). Brassinolide has not been detected in rice, so CS is considered the bioactive BR in rice. Among the 6 enzymes involved in BR biosynthesis, 5 are cytochrome P450 monooxygenases (CYPs): in *Arabidopsis*, C-22α hydroxylase is encoded by *CYP90B1/DWARF4* (Azpiroz and others 1998; Choe and others 1998); C-23α hydroxylase, by *CYP90A1/CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM* (*CPD*) (Szekeres and others 1996; Mathur and others 1998); C-3 dehydrogenase, by *CYP90D1* (Kim and others 2005); C-2α hydroxylase, by *CYP90C1/ROTUNDIFOLIA3* (*ROT3*) (Kim and others 2005); and C-6 oxidase, by *CYP85A1/BR6ox1* and *CYP85A2/BR6ox2* (Shimada and others 2001, 2003). Because *CYP85A1/BR6ox1* and *CYP85A2/BR6ox2* function redundantly in BR biosynthesis, the severe BR-deficient phenotype is apparent only in *cyp85a1/cyp85a2* double mutants, whereas *cyp85a1* or *cyp85a2* single mutants show no or very weak phenotype (Nomura and others 2005). In contrast, *CYP90B1/DWARF4* (*dwarf4*) and *CYP90A1/CPD* (*cpd*) each show a severe BR-deficient phenotype (Szekeres and others 1996; Azpiroz and others 1998; Choe and others 1998; Mathur and others 1998), indicating that hydroxylation of C-22 or C-23 in *Arabidopsis* is catalyzed by a single enzyme, *CYP90B1/DWARF4* or *CYP90A1/CPD*, respectively.

To elucidate the functions of BR in rice, we have extensively screened and analyzed rice BR-related mutants (Yamamuro and others 2000; Hong and others 2002, 2003, 2005; Sakamoto and others 2006). In contrast to *Arabidopsis cyp85a1* and *cyp85a2*, rice *BR-deficient dwarf1* (*brd1*), a loss-of-function mutant of *CYP85A1/OsDWARF*, showed a severe BR-deficient phenotype (Hong and others 2002). So far, we have screened more than 100 BR-related mutants and identified 13 rice mutants showing a severe BR-deficient phenotype; interestingly, all of them have alleles of *brd1* (T. Sakamoto and M. Matsuoka, unpublished results). However, *osdwarf4-1*, a loss-of-function mutant of a rice homolog of *Arabidopsis CYP90B1/DWARF4* (*OsDWARF4*), and *ebisu dwarf* (*d2*), a loss-of-function mutant of a rice homolog of *Arabidopsis CYP90D1* (*CYP90D2/D2*), showed a weak BR-deficient phenotype (Hong and others 2003; Sakamoto and others 2006). In addition, no mutant corresponding to the rice homolog of *Arabidopsis CYP90A1/CPD* has been identified.

Among the *Arabidopsis* BR-deficient mutants, *cpd* showed the most severe dwarf phenotype (plant height was less than 5% of wild-type plants), which is similar to that of the *brassinosteroid insensitive1* (*bri1*) mutant, a loss-of-function mutant of *Arabidopsis* BR-receptor gene *BRI1* (Clouse and others 1996; Li and Chory 1997). Therefore, CYP90A1/CPD is considered to be a rate-limiting enzyme in BR biosynthesis in *Arabidopsis*. In this study, we attempted to identify rice homologs of *Arabidopsis CYP90A1/CPD* and related mutants to enhance our understanding of BR biosynthesis in rice. On the basis of the results, we discuss the redundancy of genes for BR biosynthesis enzymes in rice.

Materials and Methods

Plant Materials and Treatments

Seeds of *oscpd1-1* and *oscpd1-2*, *Tos17* insertion lines of *CYP90A3/OsCPD1* (NF4874 and NE1525), were kindly provided by the Rice Genome Resource Center at the National Institute of Agrobiological Sciences. Seedlings of wild-type rice (*Oryza sativa* L. cv. 'Nipponbare') and mutants were grown in a greenhouse at 28°C under ambient light conditions. For expression analysis, wild-type and mutant seeds were sown on agar medium and grown for 1 week. To test the effect of exogenously applied BR on the expression of *OsCPDs*, we added 10^{-6} M BL to the growth medium.

Sequence Analysis

A BLAST search was performed against the rice DNA databases of the Rice Genome Research Program (http://rgp.dna.affrc.go.jp/), the Knowledge-based

Oryza Molecular Biological Encyclopedia (KOME; http://cdna01.dna.affrc.go.jp/cDNA/), Gramene (http://www.gramene.org/), the Torrey Mesa Research Institute (http://www.tmri.org/index.html), and the Beijing Genomics Institute (http://btn.genomics.org.cn/rice). The deduced amino acid sequences were aligned using the CLUSTALW program (http://www.ddbj.nig.ac.jp/E-mail/homology.html) with standard parameters, and the phylogenetic tree was obtained with the neighbor-joining method.

Expression Analysis

To determine the accumulation level of *OsCPD* transcripts in wild-type plants, we performed reverse-transcription polymerase chain reaction (RT-PCR) with DNase-treated total RNAs prepared separately from various organs of rice using the Advantage RT-for-PCR Kit (Clontech Laboratories, Palo Alto, CA, USA). For feedback analysis, total RNAs were prepared from whole plants of wild-type and mutants. Quantitative RT-PCR (qRT-PCR) was performed with an iCycler iQ real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The expression level was normalized against the values obtained for histone H3, which was used as an internal reference gene. The primer sequences were 5′-TTCTTCTCCATCCCCTTTCCTCTCGCCA-3′ and 5′-CACCCTCCGCCTCAAGAAGCTCCTCAA-3′ for *OsCPD1*, and 5′-TTCTTCTCCATCCCCTTTCCTCTCG CCT-3′ and 5′-CACCCTCCGCCTGAAGAAGCTCC TCCAC-3′ for *OsCPD2*. These primers specifically amplified the target gene sequences (data not shown).

Results and Discussion

Isolation of CYP90A1/CPD-like Genes in Rice

We screened for rice *CYP90A1/CPD*-like genes by computer using the predicted amino acid sequences encoded by *Arabidopsis CYP90A1/CPD* (Szekeres and others 1996) as probes in all available rice DNA databases. Rice candidate sequences detected during this process were also used reiteratively as probes for searches. We found two candidates, designated *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2*; in contrast, *CYP90A1/CPD* is encoded by a single gene in *Arabidopsis*. Mapping analysis revealed that *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* are located on the short arm of chromosomes 11 (2.0 cM) and 12 (1.9 cM), respectively. The presence of duplicated segments between chromosomes 11 and 12 has been reported (Wu and others 1998; Guyot and Keller 2004). Because the gene order and the sequence of 10 genes around *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* (within 50 kb) are highly conserved (Figure 2a), we consider the two *CYP90A1/CPD*-like genes to be a result of recent segmental duplication in the rice genome.

The predicted open reading frames (ORFs) of both *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* consist of 7 exons and encode proteins of 501 amino acids. The similarity of the deduced amino acid sequences of CYP90A3/OsCPD1 and CYP90A4/OsCPD2 is 98%, and the sequences are most closely related to *Arabidopsis* CYP90A1/CPD (62% amino acid identity; Figure 2b). The structures of CYP90A3/OsCPD1 and CYP90A4/OsCPD2 are similar to that of CYP90A1/CPD throughout their lengths: 5 domains found in cytochrome P450s—proline-rich, A (also referred to as dioxygen-binding), B (steroid-binding), C, and heme-binding—are highly conserved (Figure 2c). In addition, functionally important amino acid residues—threonine in domain A, which is thought to bind molecular oxygen, and cysteine in the heme-binding domain, which links to a heme prosthetic group by a thiolate bond—are all conserved (Figure 2c). Although the activity of CYP90A1/CPD has not been determined *in vitro*, conservation of domains and important amino acid residues suggests that both CYP90A3/OsCPD1 and CYP90A4/OsCPD2 may have the same function as *Arabidopsis* CYP90A1/CPD.

Expression of *OsCPD* Genes in Rice Plants

In wild-type rice, *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* were broadly expressed in a similar manner in all the organs we tested, namely vegetative shoot apices, leaf sheaths, leaf blades, elongating stems, roots, immature panicles, and flowering panicles (Figure 3a). We compared the *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* expression level in wild-type and BR-related mutant seedlings by qRT-PCR analysis (Figure 3b). The levels of *CYP90A3/OsCPD1* transcripts in BR-deficient *brd1-1* and BR-insensitive *d61-4* were each 2.8 times that in the wild-type. Similarly, the levels of *CYP90A4/OsCPD2* transcripts in *brd1-1* and *d61-4* were 2.1 and 2.4 times, respectively, that in the wild-type. In contrast, when the wild-type seedlings were treated with BL, levels of *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* transcripts decreased to 45% and 30%, respectively, of that of untreated wild-type seedlings (Figure 3b). A similar decrease was observed in the *brd1-1* background. These results indicate that expression of both *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* is regulated by a feedback mechanism.

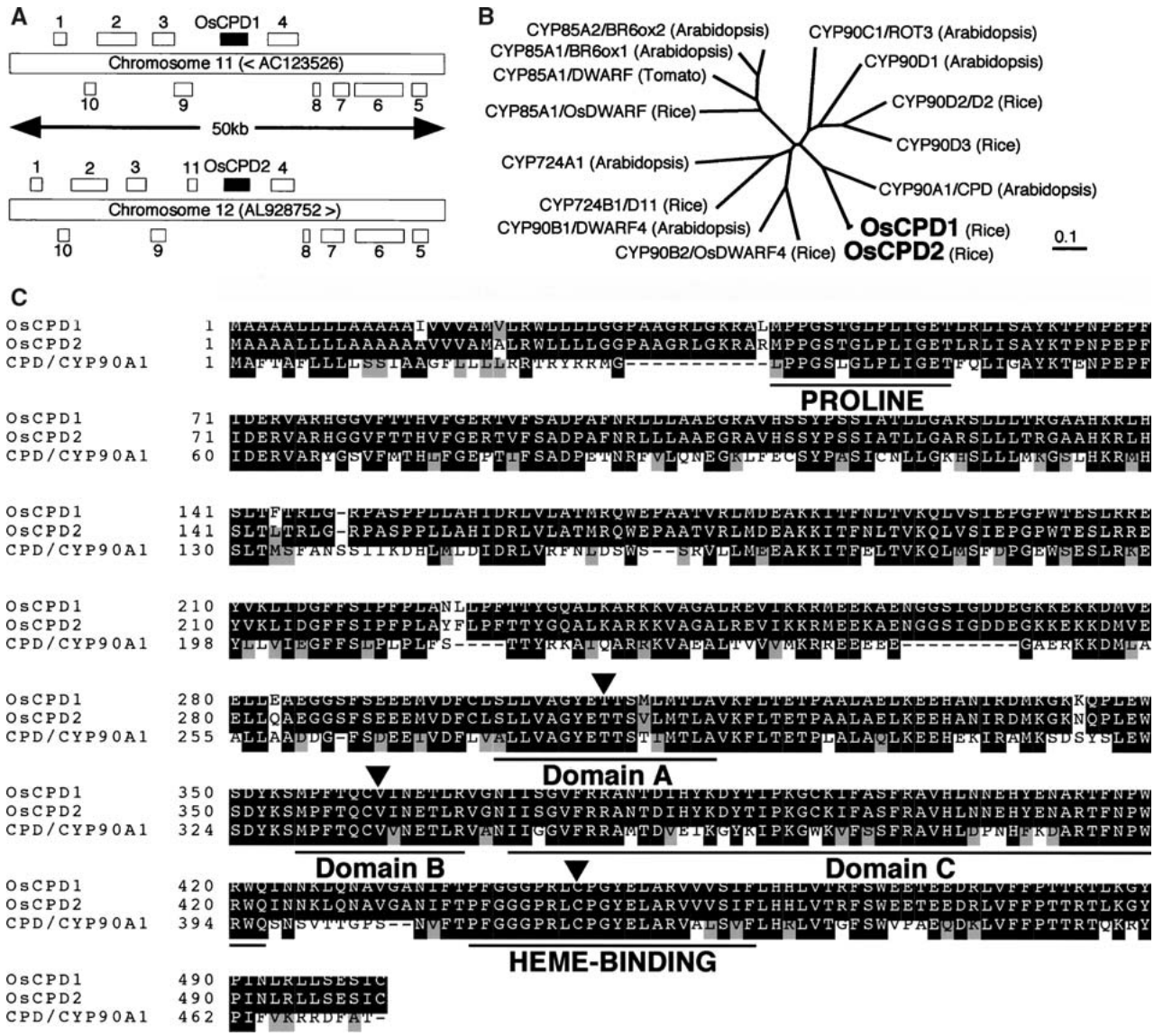

Figure 2. Molecular characterization of *OsCPD* genes. **a.** Chromosomal location of *OsCPD* genes. Annotations of predicted ORFs are as follows: 1, hypothetical protein; 2, galactonolactone dehydrogenase; 3, RNA polymerase; 4, hypothetical protein; 5, strictosidine synthase; 6, 2-isopropylmalate synthase; 7, Zn-finger protein; 8, ring finger protein; 9, response regulator; 10, hypothetical protein; 11 hypothetical protein. **b.** Phylogenetic relationships among BR-biosynthetic cytochrome P450s. Bar, 0.1 amino-acid substitutions per site. **c.** Amino-acid sequence alignment comparing OsCPD1, OsCPD2, and CPD. Exact matches are boxed in black; shaded boxes indicate conservative substitutions. Five domains found in cytochrome P450s—proline-rich, A (dioxygen-binding), B (steroid-binding), C, and heme-binding—are underlined. Arrowheads, functionally important amino acid residues.

Characterization of *OsCPD*-related mutants

Through large-scale screening of rice mutant collections to obtain BR-related mutants, we selected more than 100 dwarf mutants as candidates. We have already characterized about 40 mutants and identified 7 genes encoding BR biosynthesis enzymes or BR signaling components. However, neither *CYP90A3/OsCPD1* nor *CYP90A4/OsCPD2* has been identified yet. Therefore, we screened mutant lines caused by the retrotransposon *Tos17* (http://tos.nias.affrc.go.jp/~miyao/pub/tos17/). Transposons of *Tos17* are activated during tissue culture, and several copies of *Tos17* are inserted randomly into the rice genome (Hirochika 2001; Kumar and Hirochika 2001). In this system, it is possible to screen for mutants by inserting *Tos17* into genes of interest from a large pool of regenerated plants.

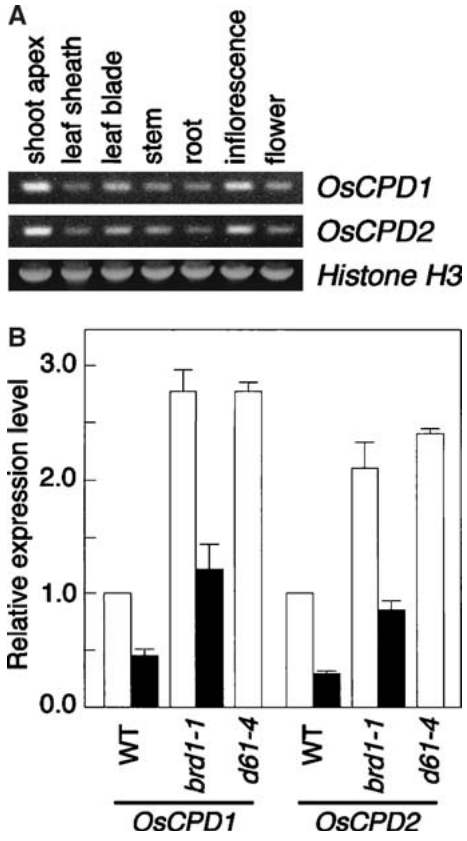

Figure 3. Expression of *OsCPD* genes. **a.** Expression of *OsCPD* genes in various organs of wild-type rice. Histone H3 was used as a control. **b.** Feedback regulation of *OsCPD* gene expression in BR-deficient (*brd1-1*) and BR-insensitive (*d61-4*) mutants. White and black bars indicate control and BL treatment plants, respectively. The ratio between each gene level and the histone H3 level obtained from wild-type was arbitrarily set at 1.0. qRT-PCR was performed in triplicate, and mean values with SD are shown.

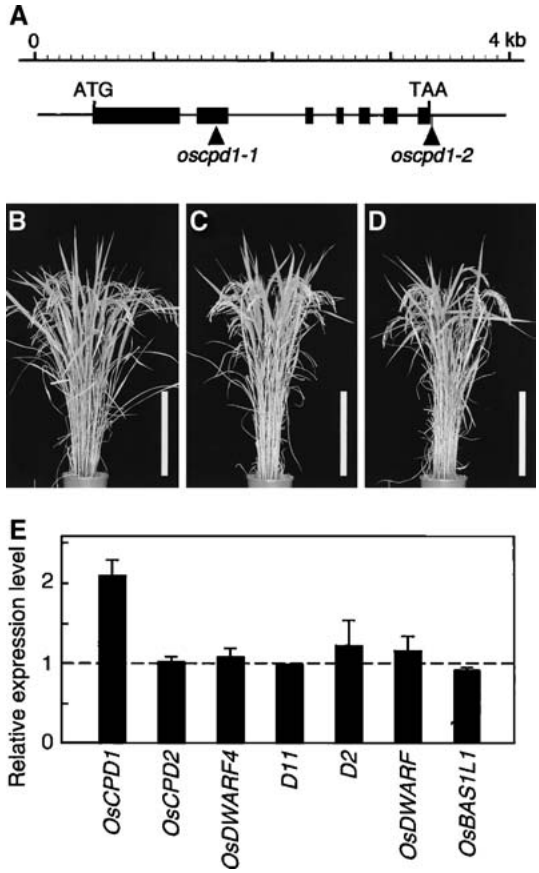

Figure 4. Loss-of-function mutants of *CYP90A3/OsCPD1*. **a.** Genomic structure of *CYP90A3/OsCPD1* and the insertion sites of a rice retrotransposon, *Tos17*. (**b–d**) Comparison of gross morphology between the original cultivar 'Nipponbare' (**b**), *oscpd1-1* (**c**), and *oscpd1-2* (**d**) mutants. Bar represents 30 cm. **e.** Expression of genes for endogenous BR biosynthesis enzymes (*OsCPD2*, *OsDWARF4*, *D11*, *D2*, and *OsDWARF*) and for a catabolic enzyme (*OsBAS1L1*) in *oscpd1-1* seedlings. The ratio between each gene level and the histone H3 level obtained from the wild-type was arbitrarily set at 1.0. qRT-PCR was performed in triplicate, and mean values with SD are shown.

Sequences flanking the *Tos17* insertions in two mutant lines, NF4874 and NE1525, were completely matched to the partial genomic sequence of *CYP90A3/OsCPD1*. We designated the two lines *oscpd1-1* (NF4874) and *oscpd1-2* (NE1525). In *oscpd1-1*, *Tos17* insertion into exon 2 causes a premature termination in domain A (Figure 4a). In *oscpd1-2* in contrast, *Tos17* is inserted 21 bp after the stop codon. Because mutated CYP90A3/OsCPD1 protein in *oscpd1-1* lacks most functional domains of CYPs, we consider *oscpd1-1* to be a null allele. However, neither *oscpd1-1* nor *oscpd1-2* showed any abnormal phenotypes and both resembled the wild-type (Figures 4b–d).

Next, we compared the expression levels of genes for BR biosynthesis enzymes in wild-type and *oscpd1-1* mutant seedlings by qRT-PCR analysis (Figure 4e). Interestingly, only the level of *CYP90A3/OsCPD1* transcripts in *oscpd1-1* was increased (it was doubled); the feedback upregulation of genes for other biosynthesis enzymes (*CYP90A4/OsCPD2*, *OsDWARF4*, *D11*, *D2*, and *OsDWARF*) and the downregulation of a gene for a catabolic enzyme (*OsBAS1L1*) were not observed. Therefore, loss of function of *CYP90A3/OsCPD1* stimulated its own expression but did not affect the expression of the other BR-related genes. These results indicate that if, as predicted, the *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* genes do function in the BR biosynthesis pathway, they may each have enough capacity

Table 1. Genes for BR Biosynthesis CYP Enzymes in Rice and *Arabidopsis*

CYP group	Function	Method	Gene name	Plant	Reference
CYP90B	C-22α hydroxylation	Recombinant protein assay	*CYP90B1/DWARF4*	*Arabidopsis*	Azpiroz and others 1998; Choe and others 1998; Fujita and others 2006
			CYP90B2/OsDWARF4	Rice	Sakamoto and others 2006
CYP724B			*CYP724B1/D11*		
CYP90A	C-23α hydroxylation	Mutant feeding assay	*CYP90A1/CPD*	*Arabidopsis*	Szekeres and others 1996; Mathur and others 1998
	Not determined		*CYP90A3/OsCPD1*	Rice	This report
			CYP90A4/OsCPD2		
CYP90D	C-3 dehydration	Mutant feeding assay	*CYP90D1*	*Arabidopsis*	Kim and others 2005a
			CYP90D2/D2	Rice	Hong and others 2003
			CYP90D3		
CYP90C	C-2α hydroxylation	Mutant feeding assay	*CYP90C1/ROT3*	*Arabidopsis*	Kim and others 2005a
CYP85A	C-6 oxidation	Recombinant protein assay	*CYP85A1/BR6ox1*	*Arabidopsis*	Shimada and others 2001
			CYP85A2/BR6ox2		Shimada and others 2003
			CYP85A1/OsDWARF	Rice	Hong and others 2002
	Baeyer-Villiger oxidation	Recombinant protein assay	*CYP85A2/BR6ox2*	*Arabidopsis*	Kim and others 2005b Nomura and others 2005

to catalyze BR biosynthesis on their own. As a consequence, the *oscpd1-1* mutant may not be deficient in endogenous BRs.

Redundancy of Genes for BR Biosynthesis Enzymes in Rice

For C-3 dehydroxylation, *Arabidopsis* has one gene, *CYP90D1* (Figure 1). In contrast, two *CYP90D* genes, *CYP90D2/D2* and *CYP90D3*, were identified in the rice genome (Hong and others 2003). Although the expression level of *CYP90D3* was much less than that of *CYP90D2/D2*, the very weak phenotype of the loss-of-function mutant of *CYP90D2/D2*, *d2*, indicates that *CYP90D3* is also a functional gene controlling bioactive BR synthesis for normal rice development. For C-22α hydroxylation, like *Arabidopsis*, rice has one *CYP90B* gene, *CYP90B2/OsDWARF4*. However, biochemical and genetic analyses revealed that another enzyme, CYP724B1/D11, also catalyzes C-22α hydroxylation redundantly with CYP90B2/OsDWARF4 in rice (Sakamoto and others 2006).

As shown in this study, CYP90A1 is encoded by two genes, *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2*, in rice, but by a single gene, *CYP90A1/CPD*, in *Arabidopsis*. These observations imply that BR biosynthesis enzymes (except CYP85A) are encoded by plural genes in rice, but by single genes in *Arabidopsis* (again except CYP85A) (Table 1). Genes for rice gibberellin (GA) biosynthesis enzymes also have increased copy numbers (Sakamoto and others 2004). Early steps in bioactive GA synthesis are sequentially catalyzed by two diterpene cyclases (CDP synthase and *ent*-kaurene synthase) and two CYPs (*ent*-kaurene oxidase and *ent*-kaurenoic acid oxidase, KAO). Interestingly, these enzymes (except *ent*-kaurenoic acid oxidase) are encoded by small gene families in the rice genome, but by a single gene in *Arabidopsis* (again except KAO). Genetic analysis revealed that GA biosynthesis in rice is catalyzed mainly by one gene product, and some of the remaining genes in these families are involved in the biosynthesis of diterpene phytoalexins rather than GA (Sakamoto and others 2004). In contrast, enzymes for later steps in GA biosynthesis such as GA 20-oxidase and GA 3-oxidase are encoded by plural genes in both *Arabidopsis* and rice. These results suggest that the copy numbers of genes for GA biosynthesis enzymes have increased in the rice genome, but the duplicated genes for early step enzymes rapidly lost their original GA biosynthesis activities, even though the original genes retained them. One possible explanation for this phenomenon is that multiple copies of enzyme genes for the early steps of GA biosynthesis might be deleterious to growth and development, as discussed by Aubourg and others (2002). Because the duplicated genes for BR biosynthesis enzymes retain their original BR biosynthesis activities, as in the case of later enzyme genes for GA biosynthesis in both *Arabidopsis* and rice, multiple copies of BR

biosynthesis enzyme genes are not deleterious but necessary for normal growth and development in rice.

ACKNOWLEDGMENTS

We thank Drs. Akio Miyao and Hirohiko Hirochika (National Institute of Agrobiological Sciences) for providing mutant seed materials. T.S. was supported by the Rice Genome Project of the Ministry of Agriculture, Forestry and Fisheries of Japan (IP-1010) and a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT). M.M. was supported by a Grant-in-Aid for Centers of Excellence from MEXT.

REFERENCES

Aubourg S, Lecharny A, Bohlmann J. 2002. Genomic analysis of the terpenoid synthase (AtTPS) gene family of *Arabidopsis thaliana*. Mol Genet Genomics 267:730–745.

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

Bishop GJ, Yokota T. 2001. Plant steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant Cell Physiol 42:114–120.

Choe S, Dilkes BP, Fujioka S, Takatsuto S, Sakurai A and others. 1998. The *DWF4* gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in brassinosteroid biosynthesis. Plant Cell 10:231–243.

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

Clouse SD, Sasse JM. 1998. Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451.

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

Fujita S, Ohnishi T, Watanabe B, Yokota T, Takatsuto S and others. 2006. *Arabidopsis* CYP90B1 catalyses the early C-22 hydroxylation of C27, C28 and C29 sterols. Plant J 45:765–774.

Guyot R, Keller B. 2004. Ancestral genome duplication in rice. Genome 47:610–614.

Hirochika H. 2001. Contribution of the Tos17 retrotransposon to rice functional genomics. Curr Opin Plant Biol 4:118–122.

Hong Z, Ueguchi-Tanaka M, Shimizu-Sato S, Inukai Y, Fujioka S and others. 2002. Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase, prevents the organized arrangement and polar elongation of cells in the leaves and stem. Plant J 32:495–508.

Hong Z, Ueguchi-Tanaka M, Umemura K, Uozu S, Fujioka S and others. 2003. A rice brassinosteroid-deficient mutant, *ebisu dwarf* (*d2*), is caused by a loss of function of a new member of cytochrome P450. Plant Cell 15:2900–2910.

Hong Z, Ueguchi-Tanaka M, Fujioka S, Takatsuto S, Yoshida S and others. 2005. The rice *brassinosteroid-deficient dwarf2* mutant, defective in the rice homolog of *Arabidopsis DIMINUTO/DWARF1*, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone. Plant Cell 17:2243–2254.

Kim GT, Fujioka S, Kozuka T, Tax FE, Takatsuto S and others. 2005a. CYP90C1 and CYP90D1 are involved in different steps in the brassinosteroid biosynthesis pathway in *Arabidopsis thaliana*. Plant J 41:710–721.

Kim TW, Hwang JY, Kim YS, Joo SH, Chang SC and others. 2005b. *Arabidopsis* CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. Plant Cell 17:2397–2412.

Kumar A, Hirochika H. 2001. Applications of retrotransposons as genetic tools in plant biology. Trends Plant Sci 6:127–134.

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

Mathur J, Molnar G, Fujioka S, Takatsuto S, Sakurai A and others. 1998. Transcription of the *Arabidopsis CPD* gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. Plant J 14:593–602.

Nomura T, Kushiro T, Yokota T, Kamiya Y, Bishop GJ and others. 2005. The last reaction producing brassinolide is catalyzed by cytochrome P-450s, CYP85A3 in tomato and CYP85A2 in *Arabidopsis*. J Biol Chem 280:17873–17879.

Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M and others. 2004. An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol 134:1642–1653.

Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S and others. 2006. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nature Biotechnol 24:105–109.

Sasse JM. 2003. Physiological action of brassinosteroids: an update. J Plant Growth Regul 22:276–288.

Shimada Y, Fujioka S, Miyauchi N, Kushiro M, Takatsuto S and others. 2001. Brassinosteroid-6-oxidases from Arabidopsis and tomato catalyze multiple C-6 oxidations in brassinosteroid biosynthesis. Plant Physiol 126:770–779.

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

Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A and others. 1996. Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell 85:171–182.

Wu J, Kurata N, Tanoue H, Shimokawa T, Umehara Y and others. 1998. Physical mapping of duplicated genomic regions of two chromosome ends in rice. Genetics 150:1595–1603.

Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S and others. 2000. Loss of function of a rice *brassinosteroid insensitive1* homolog prevents internode elongation and bending of the lamina joint. Plant Cell 12:1591–1606.