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Differential expression of three 1-deoxy-D-xylulose-5-phosphate synthase genes in rice

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Abstract

1-Deoxy-D-xylulose-5-phosphate synthase (DXS) encoded by a multigene family in plants, catalyzes the first step in the methylerythritol 4-phosphate (MEP) pathway. Three rice DXS-related sequences (OsDXS) were identified from available rice databases. The open reading frame of three OsDXS genes (*dxs1*, *dxs2*, and *dxs3*) were amplified against cDNA template. Ratio of their transcript levels in etiolated rice leaf was 9:181:1. While the expression levels were not changed along the growth stages of etiolated culture, UV-irradiation of the etiolated rice induced the expression of *dxs3* up to nine-fold compared with that of unirradiated control. In the case of light-illumination, the relative expression of *dxs1* based on unilluminated control increased two-fold. The differential expression of three OsDXS genes suggested their distinct and complementary roles in the control of the first step of the MEP pathway in response to environmental stimuli.

Introduction

1-Deoxy-D-xylulose-5-phosphate synthase (DXS), the first enzyme in the methylerythritol 4-phosphate (MEP) pathway, catalyzes the transketolase reaction converting pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose 5-phosphate (Lichtenthaler 1999). DXS is encoded by a multigene family in plants. Three DXS cDNAs were identified from *Medicago truncatula*, but two DXS proteins were characterized by heterologous functional expression (Walter *et al*. 2002). In addition, three *Arabidopsis* DXS proteins were predicted from genomic and EST sequences, but only one DXS protein was proved to have a biochemical function (Estevez *et al*. 2000, Rodriguez-Concepcion & Boronat 2002). However, the presence of extra functional DXS enzymes in *Arabidopsis thaliana* was suggested by *cla1* mutants, defective in a DXS gene, still accumulated low levels of plastid isoprenoids (Estevez *et al*. 2000).

The presence of several DXS isoforms suggests that biosynthesis of photosynthetic isoprenoids and of terpenoid photoalexins are dependent on particular DXS activities (Lichtenthaler 1999, Walter *et al*. 2002). As previously reported for terpene synthases (Trapp & Croteau 2001), the differential expression of several DXS isoforms could explain why the albino phenotype of *cla1* mutants was not rescued by the other two putative DXS isoforms (Rodriguez-Concepcion & Boronat 2002).

Reverse transcription quantitative-PCR (RT Q-PCR) is a sensitive, reproducible, and high-throughput measurement of gene expression. Previously, we reported that RT Q-PCR can discriminate the expression level of each gene in a multigene family and measure the relative expression among the isoforms (Kim *et al*. 2004).

In this work, we reported the differential expression patterns of three rice DXS (OsDXS) genes suggested their distinct and complementary roles in the control of the first step of the MEP pathway during plant development and in response to environmental stimuli.

Materials and methods

Biological materials

Following incubation at 25 °C for 2 days, the day when rice seeds began sprouting was recounted as day 0. At day 6, etiolated rice (*Oryza sativa* L. ssp. *Japonica*) plants, Hwasung cultiva, were irradiated at 254 nm with a 84 W UV lamp at 10 cm for 5 min or illuminated by white light at intensity of 4,200 Lux. Rice leaves were frozen by snap freezing in liquid N_2 along the growth stages of the etiolated seedlings and at various times after UV-irradiation or light-illumination.

Sequence analysis

The DXS-related sequences were searched from databases of Rice PIPELINE in National Institute of Agrobiological Sciences (NIAS) (http://cdna01.dna.affrc.go.jp/cDNA/ANNOTATE/Annotate_clone_search2.html) and of TIGR Rice Gene Index (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species = rice). Chloroplast transit peptide was predicted by the ChloroP algorithm version 1.1 (http://www.cbs.dtu.dk/services/ChloroP). A Phylogenetic tree of DXS proteins from plants and *E. coli* was constructed using the program TreeTop from GeneBee (http://www.genebee.msu.su/services/phtree_reduced.html).

RNA extraction and RT Q-PCR

Total RNA was extracted by the guanidinium thiocyanate/phenol/chloroform method and incubated with RNase-free DNase I (Sigma). RT Q-PCR for the measurement of transcript levels of isoforms in a sample was described previously (Kim *et al*. 2004). Primers used for the RT Q-PCR listed in Table 1 were designed by Primer3 (http://www.broad.mit.edu/cgi-bin/primer/primer3.cgi) and synthesized by Bioneer (Daejon, Korea). The products of RT Q-PCR were run on 2% (w/v) agarose gel electrophoresis and showed an equal-sized single band as predicted in template sequence. The data of RT Q-PCR were normalized to the 18S ribosomal RNA levels (Kim *et al*. 2003). Each data point represents the average of more than three experiments and the error bars indicate the standard deviation of individual experiments unless mentioned otherwise.

Results and discussion

Three OsDXS-related sequences have been identified from available rice databases (Table 1). The genomic sequences of OsDXS are widely distributed across the chromosomes and their predicted sizes of open reading frame were similar. The open reading frames of three OsDXS genes (*dxs1*, *dxs2*, and *dxs3*) were amplified against rice cDNA template to show the predicted sizes of

Table 1. List of primers used for the RT Q-PCR.

Gene	Chromosome	Locus (cM)	Source	ORF[a] (a.a.)	cTP[b]	ESTs[c]	PCR (bp)	Primer Sequence (forward/reverse)
dxs1	5	75–77.4	TC263109	720	–	14	139	CTCAAGGGAGGGAAGAACAA
								ACACCTGCTTGTTGTCGTTG
dxs2	6	10.4	TC262788	722	–	25	263	TGTTGTGGAGCTCGCTATTG
								TCCTCCCACCTAGATCCCTT
dxs3	7	38.4	TC276717	713	Y	9	146	ACCTCCTCGGGAAGAAGAAC
								GAGGGACACCTGCTTGTTGT

[a]Number of amino acid residues in predicted open reading frame.
[b]Prediction of N-terminal chloroplast transit peptide by ChloroP algorithm.
[c]Total number of ESTs in TIGR rice collections (274,018 ESTs).

PCR products (data not shown). The least abundant ESTs were those from the OsDXS3 gene, only which was predicted to contain a putative plastid targeting sequence. In the case of eight rice diterpene cyclases, recently identified and characterized for the biosynthesis of gibberellins and diterpenoid phytoalexins, only half of them were predicted to contain chloroplast transit peptide even though they all are very likely targeted to plastids (data not shown). It is necessary to find out the localization of OsDXS proteins for the functional analysis.

Phylogenetic analysis of OsDXS proteins showed that OsDXS1 was grouped into class 1 for housekeeping function and OsDXS3 into class 2 for secondary isoprenoids biosynthesis (Walter *et al*. 2002). However, OsDXS2 formed a separate branch in the tree, including DXS of *E. coli* and two biochemically unidentified DXS proteins of *Arabidopsis* (Figure 1). Homology of *Capsicum annuum* transketolase (Y15781) to these DXS amino acid sequences ranged between 9 and 16% and homologies among plant DXS proteins were at least 46% (Bouvier *et al*. 1998).

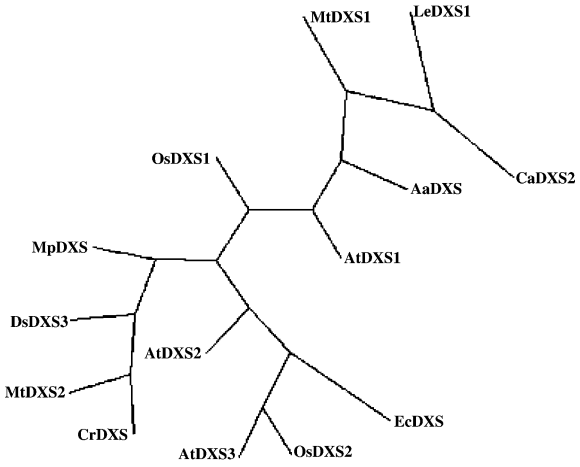

Fig. 1. Phylogenetic tree of DXS proteins from plants and *E. coli*. The tree was constructed using the program TreeTop from GeneBee. The plastid targeting sequences were not excluded from the analysis of DXS proteins; AaDXS (*Artemisia annua*, AF182286), AtDXS1 (*Arabidopsis thaliana*, U27099), AtDXS2 (At3g21500), AtDXS3 (At5g11380), CaDXS2 (*Capsicum annuum*, Y15782), CrDXS (*Catharanthus roseus*, AJ011840), EcDXS (*Escherichia coli*, AF035440), LeDXS1 (*Lycopersicon esculentum*, AF143812), MpDXS (*Mentha* × *piperita*, AF019383), MtDXS1 (*Medicago truncatula*, AJ430047), MtDXS2 (AJ430048), OsDXS1 (*Oryza sativa*, this work, TC263109), OsDXS2 (this work, TC262788), and OsDXS3 (this work, TC276717).

Three OsDXS genes in a leaf sample at day 6 of etiolated culture were expressed at the ratio of 9:181:1 (Figure 2), which is in good order of total number of ESTs in Table 1. Interestingly, from the same sample, we have reported the presence of three rice HMGR genes at the similar ratio of 7:185:1 (Kim *et al*. 2004).

The expression levels of OsDXS genes were not changed along the growth stages of etiolated culture condition (Figure 3a). In contrast, UV-irradiation of the etiolated rice leaves induced the expression level of *dxs3* up to nine-fold compared with that of unirradiated control (Figure 3b), whereas levels of *dxs1* and *dxs2* decreased up to two-fold. In the case of light-illumination (Figure 3c), the expression of *dxs1* was induced, but *dxs3* repressed.

The differential expression patterns of each DXS gene suggested their distinct roles in the control of the first step of the MEP pathway. The appearance of green pigment in the light-illuminated cleoptiles of etiolated rice was coincident with the time for positive regulation of *dxs1* to light, suggesting that the expression of OsDXS1 might be involved in chlorophyll biosynthesis. As *Arabidopsis* DXS gene was detected at low level in etiolated culture and positively regulated by light (Mandel *et al*. 1996, Souret *et al*. 2002), OsDXS1 might be closely related to *Arabidopsis* DXS. Since phytoalexins such as

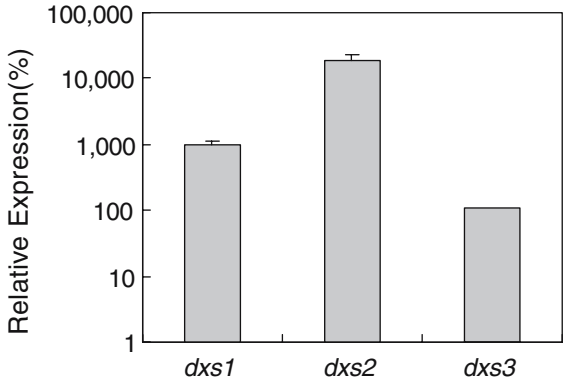

Fig. 2. Transcript levels of three OsDXS genes in leaves at day 6 of etiolated culture. Relative expression levels of three OsDXS genes in a sample based on that of *dxs3* were determined by diluting the cDNA concentration and displayed in a log scale.

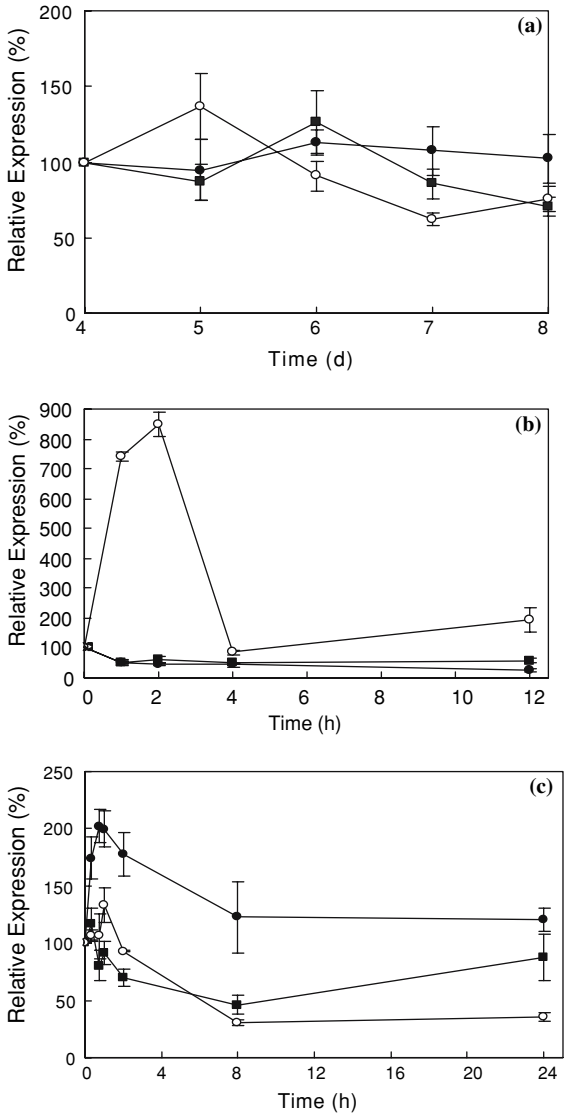

Fig. 3. Relative expressions of *dxs1* (●), *dxs2* (■), and *dxs3* (○) along the growth stages (a) and at various times after UV-irradiation (b) and light-illumination (c) based on day 6 of etiolated control. The data of RT Q-PCR were normalized to the 18S ribosomal RNA levels. Each data point represents the average of three or four experiments and the error bar indicates the standard deviation of individual experiments.

momilactones A and B were detected in abundance from UV-irradiated, dark-grown rice cleoptiles (Wickham & West 1992), the induction of *dxs3* by UV-irradiation indicated that OsDXS3 might be involved in the biosynthesis pathway of rice phytoalexins. Because of the highest transcript level and the constitutive expression, OsDXS2 might be involved in primary metabolism except the chlorophyll biosynthesis.

Wide distribution of ESTs in the available rice database, amplification of the open reading frame from cDNA template, and differential expression patterns of three genes according to the environmental conditions strongly suggested that three OsDXSs are all expressed and functional. Even though functional analysis of each proposed DXS-homologs with biochemical or genetic approach is needed to confirm the predictions generated by the sequence-based analysis. We are now cloning the open reading frame of OsDXS for heterologous expression and biochemical characterization. To our knowledge, this is the first report that all three OsDXS genes are expressed for the complementary roles in the control of the first step of the MEP pathway.

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References

Bouvier F, d'Harlingue A, Suire C, Backhaus RA, Camara B (1998) Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. *Plant Physiol.* **117**: 1423–1431.

Estevez JM, Cantero A, Romero C, Kawaide H, Jimenez LF, Kuzuyama T, Seto H, Kamiya Y, Leon P (2000) Analysis of the expression of *CLA1*, a gene that encodes the 1-deoxy-xylulose 5-phosphate synthase of the 2-C-methyl-D-erythritol-4-phosphate pathway in *Arabidopsis*. *Plant Physiol.* **124**: 95–103.

Kim B-R, Nam H-Y, Kim S-U, Chang Y-J (2003) Normalization of reverse transcription quantitative-PCR with housekeeping genes in rice. *Biotechnol. Lett.* **25**: 1869–1872.

Kim B-R, Nam H-Y, Kim S-U, Chang Y-J (2004) Reverse transcription quantitative-PCR of three genes with high homology encoding 3-hydroxy-methylglutaryl-CoA reductase in rice. *Biotechnol. Lett.* **26**: 985–988.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **50**: 47–65.

Mandel MA, Feldmann K, Herrera-Estrella L, Rocha-Sosa M, Leon P (1996) CLA1, a novel gene required for chloroplast

development, is highly conserved in evolution. *Plant J*. **9**: 649–658.

Rodriguez-Concepcion M, Boronat A (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiol*. **130**: 1079–1089.

Souret FF, Weathers PJ, Wobbe KK (2002) The mevalonate-independent pathway is expressed in transformed roots of *Artemisia annua* and regulated by light and culture age. *In Vitro Cell. Dev. Biol*. **38**: 581–588.

Trapp S, Croteau R (2001) Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genetics* **158**: 811–832.

Walter MH, Hans J, Strack D (2002) Two distantly related genes encoding 1-deoxy-D-xylulose 5-phosphate synthases: differential regulation in shoots and apocarotenoid-accumulating mycorrhizal roots. *Plant J*. **31**: 243–254.

Wickham KA, West CA (1992) Biosynthesis of rice phytoalexins: Identification of putative diterpene hydrocarbon precursors. *Arch. Biochem. Biophys*. **293**: 320–332.