Evolution and expression analysis of starch synthase III and IV in rice

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Abstract
Plants contain at least five subfamilies of starch synthases, granule bound starch synthase (GBSS) and starch synthases I, II, III, and IV (SSI, SSII, SSIII, SSIV). In this work, two members of SSIII and SSIV, respectively, were cloned and designated OsSSIII-1/-2 and OsSSIV-1/-2 in rice. Together with six other previously reported genes, the SS gene family in rice therefore is known to be duplicated and to comprise ten SS genes distributed among the five subfamilies. The starch synthase activity of each SS was confirmed by expression and enzyme activity assay in E. coli. Expression profile analysis with reverse transcription-PCR, western blotting and zymogram, indicates that OsSSIII-2 and OsSSIV-1 are mainly expressed in endosperm, while OsSSIII-1 and OsSSIV-2 are mainly expressed in the leaves. With a similar pattern of genes encoding other enzymes for starch synthesis, (such as GBSS, SSII, ADP-glucose pyrophosphorylases, and branching enzymes), it is suggested that two divergent groups of these genes should be classified in rice. Group I genes are preferentially expressed in the endosperm and function on storage starch synthesis. Group II genes are mainly expressed in leaves and some of them in the early developing endosperm, and function on transient starch synthesis in rice.

Key words: Gene duplication, gene expression, Oryza sativa L, starch biosynthesis, starch synthase.

Introduction
Plant starches are comprised of two classes of glucose homopolymers. Firstly, there is amyllose, which is a lightly branched linear molecule with a degree of polymerization of 1000 to 5000 Glc units. Secondly, amylopectin, which has a much larger polymer unit (with a degree of polymerization of $10^5$–$10^6$ Glc units) and contains frequent $\alpha$-1,6 branch linkages. In higher plants, biosynthesis of starch occurs in plastids with the involvement of a series of biosynthetic enzymes, including ADP-Glc pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE), and debranching enzyme (DBE) (Smith et al., 1997; Myers et al., 2000; James et al., 2003).

The $\alpha$-glucan chains of both types of polymers are elongated by starch synthase (ADP-Glc: $\alpha$-1,4 glucan $\alpha$-4-glucosyl-transferase; EC 2. 4. 1. 21). Starch synthase catalyses the transfer of $\alpha$-D-glucose from ADP-Glc to the non-reducing end of the chain by an $\alpha$-1,4-linkage. Five subfamilies of starch synthases have been identified in higher plants, including granule-bound starch synthase (GBSS), starch synthase I (SSI), starch synthase II (SSII), starch synthase III (SSIII), and starch synthase IV (SSIV). GBSS is essential for amyllose synthesis and is exclusively bound to the starch granule. SSI, SSII, SSIII, and SSIV (named as SSV in dicots) are responsible for amylopectin chain elongation with their distribution between the granular and soluble fractions (Ball and Morell, 2003; Li et al., 2003). Each class of SS genes plays a distinct role in the synthesis of amylopectin. Analysis of a rice mutant with a retrotransposon inserted into a gene that encodes for SSI indicates that amylopectin is depleted in chains of DP8-12 and enriched in chains of DP6 and 7 in the mutant. This suggests a distinct capacity of SSI for the synthesis of chains with DP8-12 from DP6-7 (Nakamura, 2002). SSII has a specific role in the synthesis of intermediate-length chains (B2 and B3 chains) (Fontaine et al., 1993; Craig et al., 1998; Edwards et al., 1999; Morell et al., 2003). Mutations eliminating SSIII activity are known in maize as...
**Materials and methods**

**Plant material**

The indica rice (*Oryza sativa L.*) variety Zhe733 and japonica rice variety Zhongdang104 (for zymogram analysis) were grown in a greenhouse at 22-28 °C (night/day) and 80% relative humidity. The grains were harvested several times during maturation while the leaves and roots were taken from 4-leaf-stage seedlings.

For the sugar effect test, the 4-leaf-stage seedlings were cultured for 2 d under dark condition to deplete endogenous sugars before treatments with sugars. After 2 d, the second and third leaves were excised from the plants (now about 1 cm in length) and then transferred and incubated in rice nutrition solution (Yoshida et al., 1976) with different sugar or sugar analogues in the dark at 28 °C. Excised leaves were also incubated in a rice solution without sugar as a control. The leaf samples were harvested after 12 h of sugar treatments.

cDNA cloning of OsSSIII and OsSSIV

The TBLASTN searching of the GenBank (http://www.ncbi.nlm.nih.gov) databases with the conserved C-terminal amino acids of rice starch synthase genes yielded four highly homologous rice genomic clones: AL606645 (OSIN00079), AP005441, AP003292, and AC121365. The predicted polypeptides encoding by AL606645 and AP005441 bear an analogy to maize SSIII/DU1, named as OsSSIII-1 and OsSSIII-2, while those of AP003292 and AC121365 bear an analogy to wheat SSIV, named as OsSSIV-1 and OsSSIV-2, respectively. The OsSSIII-1 cDNA clone containing the complete coding domain sequence (CDS) and an OsSSIII-1 cDNA fragment (lack 560 bp at the 5’ region of the putative ORF) were isolated by screening the immature seed cDNA library (Jiang et al., 2004). For sequencing, the positive clones were converted to pTriplEx2. The plasmid was prepared using GFX™ Micro Plasmid Prep Kit (Amerham Pharmaccia Biotech), and then the cDNA fragments were subcloned into the pBSK vector and sequenced (MegaBACE™1000, Amerham Pharmaccia Biotech). To clone the two putative SSIV cDNA, two primer pairs were designed for each gene. S411 [5’-AGT GCC CCT CCT CGC CCT GTT G (–24 to –3 bp, A –ATG is No. 1)] and S412 [5’-GTC GTA TTT GAG AAC AAT CTC (+1540 to +1563 bp)]; S413 [5’-AAA AGA GGT ACC GGA AAC AGT CCT GCC CCT CGC CCT CCT GCT G-3’ (+2893 to +2917 bp)]; S421 [5’-TTCC CCC AGC CCT CGC ATC GGA TTA (-36 to –3 bp)] and S422 [5’-TCA CTT CGG CCA AAC CAC CAA CC (+1308 to +1330 bp)]; S423 [5’-AAA AAA GGT ACC ACT AGT CCT GGC TCG CAC ATC ATC C (+1252 to +1276 bp)] and S424 [5’-AAA AAA GAG CTC TGC TGC TGC TGC TGC TGC CAC CAC CAC CAC CAC CAC C (+2720 to +2745 bp)]. Respectively, the amplified fragments were cloned in the pUCm-T vector and sequenced (MegaBACE™1000, Amerham Pharmaccia Biotech). GenBank accession numbers of these four cDNA clones were AF432915 (OsSSIII-1), AY100469 (OsSSIII-2), AY373257 (OsSSIV-1), and AY373258 (OsSSIV-2), respectively.

**Expression of OsSSIII and OsSSIV in E. coli**

The following primers were used for PCR modification of the N-termini of OsSSIIIa, OsSSIIIb, OsSSIVa, and OsSSIVb: pS311 [5’-AAA TGG CAT ATG ATG GAC TTT GTT TTC TCT-3’] and pS312 [5’-AAA TGG CAT ATG ATG GAC TTT GTT TTC TCT-3’]. These primers were used to introduce A –ATG is No. 1) and pS311 [5’-AAA TGG CAT ATG ATG GAC TTT GTT TTC TCT-3’] and pS312 [5’-AAA TGG CAT ATG ATG GAC TTT GTT TTC TCT-3’]. These primers were used to introduce

**Semiquantitative RT-PCR analysis**

Total RNA from leaves, roots, and grains of plants were isolated with Trizol (GIBICOL). PCR amplifications were performed on first DNA strand corresponding to 2 μg of total RNA, using eight specific primer sets (Table 1). All specific primers were designed to span intron sequences, thus allowing the signals resulting from genomic DNA contamination to be accounted for. Primers (Table 1) that amplify Actin (Genbank accession number X16280) from rice were used as a control. The amplified fragments were cloned in the pUCm-T vector and sequenced (MegaBACE™1000, Amerham Pharmaccia Biotech). Thermocycling time and temperature were as follows: 95 °C for 5 min, followed by 30 cycles (or as indicated) of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final extension period 72 °C for 7 min. PCR products were separated on 1% agarose gels, and stained with ethidium bromide.

**Gel electrophoresis and immunoblot analysis**

Native PAGE was carried out using 7.5% (resolving gel) and 2.5% (stacking gel) polyacrylamide and activity staining was performed as
previously described (Jiang et al., 2003). Each lane was loaded with 50 μg of the crude extract from leaves (L) or endosperms. Starch synthase activity was detected after incubation of the gel in 50 mM Tricine-NaOH (pH 8.5), 0.5 M sodium citrate, 25 mM potassium acetate, 2 mM EDTA, 2 mM DTT, 0.1% (w/v) potato amylopectin, and the indicated content of ADP-glucose at the indicated temperature for 10 h.

In immunoblot analysis, the following antisera were used: antiseria (Anti-ZmSSIII), raised against the N-terminal region of the maize SSIII protein, were the generous gift of Dr Alan M Myers (Iowa State University, Ames) and are described by Cao et al. (1999). The rabbit polyclonal antiseria (designated Anti-OsSSI and Anti-OsSSIII) were raised against the deduced mature OsSSI or the C-terminus of OsSSIIIa protein, were the generous gift of Dr Alan M Myers (Iowa State University, Ames) and are described by Cao et al. (1999). The rabbit polyclonal antiseria (designated Anti-OsSSI and Anti-OsSSIII) were raised against the deduced mature OsSSI or the C-terminus of OsSSIII-1 expressed in E. coli (Jiang et al., 2004). The Anti-OsSSI-2 and Anti-OsSSIII-3 are described by Jiang et al. (2004). Protein extracts were separated by 7.5% Native-PAGE or 7.5% SDS–PAGE, transferred to polyvinylidenedifluoride (PVDF) membranes (AMRESCO), and visualized using rabbit anti-SS antiserum followed by alkaline phosphatase–conjugated goat anti-rabbit serum (Santa Cruz, USA).

### Results

**Cloning and characterization of OsSSIII and OsSSIV genes**

BLAST analysis of the rice genome (http://www.ncbi.nlm.nih.gov/BLAST/) using the conserved C-terminus of starch synthase amino acid sequences, revealed four new homologous genes. According to the putative amino acid sequences, two genes were homologous to maize SSIII/DUI, designated OsSSIII-1 and OsSSIII-2, and the other two genes homologous to wheat SSIV, designated OsSSIV-1 and OsSSIV-2. The cDNA of these four genes were cloned by screening a cDNA library or RT-PCR. According to the cDNA sequences, rice SSIII genes contain 16 exons separated by 15 introns as in wheat. The sequences of exon3 are the variable repeat region (Li et al., 2000), and vary between OsSSIIIa and OsSSIIIb in length. The structures of the SSIV genes contain 16 exons separated by 15 introns, the first three exons in the 5' region of the genes are variable in length.

**Duplication and distribution of SS gene families in rice**

The four genes of SSIII-1/-2 and SSIV-1/-2 cloned in this case together with the other six genes cloned previously (Wang et al., 1990; Baba et al., 1993; Dian et al., 2003; Jiang et al., 2004), compose 10 members of the SS gene family in rice with five different subfamilies distributed over eight rice chromosomes. SSIII-1, SSIII-2, SSIV-3, and SSIV-4 were located on chromosomes 4, 8, 1, and 5, respectively. However, many genes clustered around the loci of SSIII-1 and SSIII-2 and the loci of the SSIV-1 and SSIV-2 were identical (Fig. 1). This observation indicated that the duplications of SSIII and SSIV were associated with the duplication of a large chromosome segment during evolution. In addition to the 10 genes, a truncated SSI gene was identified on chromosome 10 and a truncated SSIII-3 gene (Accession number BAC16084) on chromosome 7. The putative sequence of amino acids of the truncated SSI shared high identity with C-terminus of OsSSI (Fig. 2).

**Domain organization of OsSSIII and OsSSIV and expression of OsSSIII and OsSSIV in E. coli**

As is the case in the SSIII protein in maize and wheat (Gao et al., 1998; Li et al., 2000), rice SSIII proteins also contain four distinct regions. These are a putative transit peptide region (47/49 amino acids for OsSSIII1-1/2) (identified using the ChloroP neural network analysis of the 100 amino acids at the N-terminus of each sequence), a variable repeat region, a SSIII specific region, and a C-terminal region that contains the catalytic domain (Fig. 3A). The SSIII specific region in OsSSIII-1/2 was composed by a set of three repeated units of amino acid similarity in each gene. Thirteen amino acids were conserved in these repeat units (Fig. 3B). Rice SSIV proteins contain three distinct regions: a putative transit peptide region (78/33 amino acids for SSIV-1-2), a region homologous to Smc (COG1196)/myosin tail 1 (pfam01576) and a C-terminal catalytic domain region (Fig. 3A).

The deduced amino acid sequences of the C-terminal catalytic domain region from the ten OsSS proteins were aligned with one prokaryotic glucogen synthase (E. coli glucogen synthase, EcGS) using the Clustal W program. Nine conserved regions were identified. The invariant residues were listed (Fig. 3C). The first homologous region contains the consensus motif KXGGL, which is believed to be the ADP-Glc binding site of starch synthase (Furukawa et al. 1990).

### Table 1. Primer pairs for semiquantitative PCR amplification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer pairsa</th>
<th>Fragment sizeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsSSI</td>
<td>F, 5'-GGGAGTTGCAATTGCACATACAG;</td>
<td>R, 5'-ATAAGAACACAGAGGCGACCGAGAAGATGG</td>
</tr>
<tr>
<td>OsSSII-2</td>
<td>F, 5'-GGGAGTTGCAATTGCACATACAG;</td>
<td>R, 5'-TGTTTCTTGTCCGGTGTCGCGATGC</td>
</tr>
<tr>
<td>OsSSIII-1</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSIV-2</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSIV-1</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSII-1</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSIV-2</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSIII-1</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
<tr>
<td>OsSSIV-1</td>
<td>F, 5'-TTTATGCTGGTGCCGACTTG; R, 5'-GAACTCAACCCGGCGAGATAC</td>
<td>577</td>
</tr>
</tbody>
</table>

*a* F. forward primer; R, reverse primer. 
*b* Numbers indicate the size (in bp) of amplified fragments.
To determine whether the OsSSIII and OsSSIV genes encode authentic starch synthases, expression (Fig. 4) of OsSSIII-1, OsSSIII-2, OsSSIV-1, and OsSSIV-2 genes in E. coli (BL21) were tested. The activities of starch synthase were increased by 4.1-fold, 4.8-fold, 2.7-fold, and 2.4-fold above baseline activity level of E. coli glycogen synthase, for each of the four genes, respectively. This indicated that the OsSSIII and OsSSIV genes encoded functional starch synthase enzymes in rice.

**Fig. 1.** Gene arrangement of the two SSIII gene loci (A) and two SSIV gene loci (B) in rice. Location of the four genes, anchor RFLP markers and Kosambi values (cM) are indicated on the chromosomes (the High-Density Rice Genetic Map on http://rgp.dna.afrc.go.jp/publicdata). The genomic sequences were analysed with GeneMark (http://www.ebi.ac.uk/genemark) and Genscan (http://genes.mit.edu/GENSCAN.html). The orthologous genes are listed.

Fig. 2. The amino acid sequence alignment of OsSSI and the other destroyed OsSSI orthologous gene in rice. The highly homologous rice genomic sequences were yielded by tBLASTn searching of the GenBank databases queried with OsSSI protein. The middle lane shows the conserved amino acids.

**Fig. 3.** (A) Domain structure of the rice SSIII and SSIV amino acid sequences showing the conserved motifs. Putative transit peptide cleavage sites were identified using the ChloroP neural network analysis of the 100 amino acids at the N terminus of each sequence. (B) SSIII-specific region. Alignment of the deduced amino acid sequence of the three repeated units of OsSSIII using Clustal W software. Asterisks indicate 100% conserved, double dots similar, single dots related amino acids; dashes depict gaps. Numbers refer to amino acids. (C) Identification of residues invariant to E. coli glycogen synthase (EcGS) and rice starch synthases. Invariant residues that are specific to rice starch synthases are marked with an asterisk. All amino acid numbers correspond to the sequence of EcGS (Accession number NC_000913).
Organ expression profile of OsSSIII and OsSSI genes

The spatial expression of OsSSIII-1, OsSSIII-2, OsSSIIV-1, and OsSSIV-2 was examined in storage and non-storage organs of rice by RT-PCR (Fig. 5A). The result shows that OsSSIII-1 and OsSSIV-2 were expressed mainly in leaves and weakly in endosperms, while OsSSIII-2 and OsSSIIV-1 mainly in endosperms and weakly in leaves. Total proteins extracted from rice leaves and endosperms were electrophoresed in SDS–PAGE gels using antibodies to maize SSIII polypeptide (N-terminus) (anti-ZmSSIII) and anti OsSSIII-1 polypeptide (C-terminus) (anti-OsSSIII-1). Both antibodies were bound to 230 kDa proteins from rice endosperms and 170 kDa proteins from rice leaves and endosperms (Fig. 5B). The calculated molecular mass of OsSSIII-1 and OsSSIII-2 was 138 kDa and 201 kDa, respectively. The apparent molecular mass of SS as estimated on SDS-PAGE, was often noted to be larger than the calculated molecular mass (Knight et al., 1998), suggesting that the 230 kDa protein should be OsSSIII-1 and the 170 kDa protein should be OsSSIII-1. This result is consistent with the expression patterns of OsSSIII-1 and OsSSIII-2b transcripts. OsSSIII-1 was expressed at the early developing stage of endosperm, while OsSSIII-2 reached maximum levels at the middle age. The transcription of...
OsSSIII-1 reached maximum levels in endosperm at the late developmental stage (Fig. 5C). Western blotting also indicated that OsSSIII-2 protein maintains higher levels at the middle developmental stage of rice endosperms (Fig. 5D).

Zymogram analysis for SS activities in rice endosperm and leaf extracts

The zymogram experiment was employed to identify further the expression of different SS genes in rice endosperms and leaves. Figure 6A shows that at least four SS activity bands were detected in rice leaves and five SS activity bands in rice endosperms. Immunoblotting showed that the lowest mobility activity band in endosperms should be that of OsSSIII-2, and in leaves should be that of OsSSIII-1 (Fig. 6B). Rice SSIII-2 showed temperature-sensitive activity, with notably lower starch synthase activity at 37 °C than in 25 °C. Figure 6B also shows that the expression of OsSSI was not tissue-specific, whilst OsSSII-2 was leaf-specific and OsSSII-3 was endosperm-specific. The mobility of OsSSII-2 in leaves and OsSSII-3 in endosperms is seen as equal. SSI and SSII showed an identified band in many rice cultivars such as Zhenongda104, Nipponbare and Kasalath (the data of last two cultivars is not shown).

Influence of sugar level on SS gene expression in rice leaves

It has been demonstrated that the gene expression of OsGBSSII is regulated by sugar level in excised leaves (Dian et al., 2003). The effects of sugars on the other SS genes that expressed in rice leaves were tested in this case. Excised leaves were treated with 175 mM of mannitol (Mal), sucrose (Suc), 3-O-methyl-glucose (3-OMG), and 2-deoxyglucose (2-DOG), respectively. RT-PCR analysis indicated that accumulation of OsSSI, OsSSII-2, and OsSSIII-1 transcripts were up-regulated by sucrose, but not for OsSSIII-2 transcripts. Glucosamine eliminated the accumulation of these transcripts induced by sucrose (Fig. 7). Sequence analysis has revealed the sugar response elements in the promoter regions (up to 2000 bp from the initiation codon of ATG) of the three SS genes: OsSSIIIa, SP8BFIBSP8BIB (TACTATT) (Ishiguro and Nakamura, 1992), −1431, SURE2STPAT21 (AATACTAAT) (Grierson et al., 1994), −1758; OsSSI, SURE1STPAT21 (AATA-GAAAA) (Grierson et al., 1994), −830; OsSSII-2, SURE1STPAT21, −362. This suggested that the sugar regulation of these SS genes in rice leaves is consistent and the sugar response elements are active in rice.
After separation of SS isoforms by native-polyacrylamide gel electrophoresis, the gels were incubated at 25 °C or 37 °C for 20 min, and then the enzyme reaction was performed with the addition of 8 mM ADPGlc at 25 °C or 37 °C for 10 h. Each lane was loaded with 50 μg of the crude extract from leaves (L) or endosperms (E). 104, rice cultivar zhenongda 104 (japonica); 733 rice cultivar zhe733 (indica).

The expression patterns of the five SS gene subfamilies were investigated as a first step toward understanding their respective functions. Previous observations indicated that the transcripts and proteins of the OsSSIII-2 and OsGBSSII genes were detected mainly in leaves, while OsSSIII-3 and GBSSII (Wx) primarily in endosperms (Harn et al., 1998; Vrinten and Nakamura, 2000; Dian et al., 2003; Jiang et al., 2004). The present observation with reverse transcription-PCR, western blotting or zymogram, indicate that OsSSIII-2 and OsSSIV-I are mainly expressed in endosperm, while OsSSIII-1 and OsSSIV-2 are mainly expressed in leaves (Figs 5, 7). In rice endosperms, OsSSIII-I was expressed at an early developing stage, while OsSSIII-2 reached maximum levels at the middle stage. In rice leaves, as for the OsGBSSII, the expression of OsSSI, OsSSII-2, and OsSSIIIa is regulated by sugars, suggesting that sugar regulation of these SS genes converged during rice evolution. The consistent regulation of these OS genes allows starch synthesis to be modulated in response to the accumulation of sugars in rice leaves under conditions such as high light and nitrogen or phosphate starvation.

In cereals, evidence is increasing that the different subfamilies of genes for starch synthesis may be partitioned into two gene groups, with one group expressed in the endosperm and a second mainly expressed in other tissues. For instance, BEIIb (RBE3) is specifically expressed in endosperm, while BEIIa (RBE4) is mainly expressed in vegetative tissues and in the early developing endosperms in cereals (Yamanouchi and Nakamura, 1992; Gao et al., 1996; Morell et al., 1997; Sun et al., 1998; Jiang et al., 2003; Mutisya et al., 2003). Transcripts of AgpL1 and AgpS1a genes were specifically detected in endosperms, while AgpL2, AgpS1b, and AgpS2 are mainly expressed in leaves of maize, wheat, barley, and rice (AgpS1a and AgpS1b are encoded by a single gene in wheat, barley, and rice). AgpL2, AgpS1b, and AgpS2 are likewise expressed in the early developing endosperm in wheat, barley, and rice (Burton et al., 2002; Johnson et al., 2003). Thus, in cereals, the duplicated starch synthesis genes diverged into two
groups: Group I, preferentially expressed in endosperms, including AgpL1, AgpS1a, GBSSI, SSII-3 (SSIIa), SSII-2 (DU1), SSIV-1 (may be including SSIV-2), and BEIIb (RBE3), and Group II, mainly expressed in other tissues, and some in the early developing endosperms, including AgpL2, AgpS1b, AgpS2, GBSSI, SSII-2 (SSIIb), SSIII-1, SSIV-2 (may be including SSIV-1), and BEIIa (RBE4). The single member subfamily genes of SSI and BEI (RBE1) are expressed constitutively and function in both groups (Fig. 8). Thus the preservation of these duplicate genes is likely to be by subfunctionalization (Lynch and Force, 2000), with the primary function of the Group II genes on transient starch synthesis, and Group I genes on storage starch synthesis in rice.

Expression profiles of the corresponding genes suggest a two-step model for starch biosynthesis in rice

It is already known that angiosperm seeds accumulate storage protein, oil, and carbohydrate during seed filling and degrade them to support early seedling growth upon germination. The relative abundance of storage reserves varies among seeds of different species. Many oilseeds produce 50–70% oil, some legumes contain 40% protein, whereas most cereal seeds contain 70–85% of the seed dry weight as starch (Baud et al., 2002). In developing legume seeds, the model of detailed gene expression regarding the control of starch and protein synthesis has been established through the proteomics research on Medicago truncatula seed development (Gallardo et al., 2003). Contrapuntal networks of gene expression during the Arabidopsis seed-filling period reveal that the genes involved in carbohydrate metabolism peaks are expressed in early seed development, those required for oil synthesis are expressed later, and the genes for oil-body and storage-protein are expressed later still (Ruuska et al., 2002). Although the cytosolic localization of AGPase in cereal endosperms has been suggested to have functional significance for partitioning large amounts of carbon into starch when sucrose is plentiful (Beckles et al., 2001), the accumulation of storage components requires the co-ordination of many genes that encode the enzymes of the corresponding pathways (Ruuska et al., 2002). In rice, the expression of Group II starch synthesis genes peaks early in seed development, similar to that in the developing seeds of Arabidopsis (Fig. 8B), whilst the expressed pattern of Group I starch synthesis genes was consistent with the age of the rapid accumulation of starch in the endosperms. This suggests that this is one of the primary factors in the synthesis and storage of starch in rice endosperms (Fig. 8B).

The expression profiles of these genes are likely to suggest a two-step model for starch biosynthesis in rice endosperms. Group II genes synthesize the early transient starch mainly in leaves and Group I genes synthesize the storage starch in rice endosperms. This result is also in support of the hypothesis of subfunctionalization regarding the preservation of the duplicated genes in the genome (Lynch and Force, 2000). Therefore this can help in understanding the co-evolution of the relevant genes regarding the functional divergences in duplicated genes as revealed in gene expression changes in plants.

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