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Identification and analysis of eight peptide transporter homologs in rice

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ABSTRACT

Peptide transporters (PTRs) have diverse substrates and underlie many biological processes in plants. To better understand their function and regulation, we have analyzed the transport capabilities of eight rice PTRs (OsPTRs) in a yeast ptr2 mutant strain and their expression patterns in plants. A yeast ptr2 complementation assay demonstrated that only OsPTR6 transports Gly–His and Gly–His–Gly, and OsPTR6 shows substrate selectivity for di-/tripeptides; however, the other seven proteins could not transport the five tested di-/tripeptides. The expression patterns for the eight OsPTRs were distinct in tissues under normal growth conditions. In germinating seeds, seven of the eight OsPTRs were expressed at various levels, but OsPTR5 was not expressed. In grain-filling seeds, expression of OsPTR4, OsPTR7 and OsPTR8 increased from early to late stages of seed development and that of OsPTR2, OsPTR3 and OsPTR6 decreased. Expression of OsPTR1, OsPTR2, OsPTR5, OsPTR7 and OsPTR8 were upregulated by drought and salt, and OsPTR4 was downregulated. However, only OsPTR1 displayed a slight upregulation by cold. This study showed that rice OsPTRs have distinctive transport functions for substrates and differing expression patterns during both development and response to stress.

1. Introduction

Nitrogen (N) is the most important nutrient for plant growth and productivity. Plants have evolved diverse and complex transport systems to facilitate uptake and reallocation of nitrogenous compounds [1,2]. Besides uptake of inorganic nitrogen molecules such as nitrate and ammonium by specific transporters, plants also take up organic nitrogen as a nitrogen source through their corresponding transporters in the form of amino acids, small peptides and proteins [3,4].

Organic nitrogen sources (amino acids and peptides) in the soil play important roles in the nitrogen economy of grasslands, particularly in nitrogen-limited terrestrial ecosystems. There is evidence that plants can use di-/tripeptides as a nitrogen source [3,5]. Peptide transporters have important roles in organic nitrogen translocation for plants. Furthermore, nitrogen remobilization from older to younger leaves is also an important process for sustaining growth of developing organs. A significant contribution for nitrogen remobilized from older parts to growing young leaves and for filling seeds has been observed in rice [6], tobacco [7], and soybean [8]. In plants, peptide transporters have been demonstrated to be involved in recycling of organic nitrogen and regulating germination of barley, wheat, rice and maize grains from the endosperm to the embryo by physiological tests [9–11].

Peptide transport in plants is accomplished by two distinct gene families: the OPTs (the oligopeptide transporters) and the PTRs (the peptide transporters) [12]. Higher plants contain a far greater number of genes for these transporters than do other eukaryotes. This may indicate the relative importance of small peptides and their transport to plant growth and metabolism. The OPT family transports tetra- and pentapeptides, while the peptide transporter (PTR) family transports di- and tripeptides [12,13]. Stacey et al. consider that an important distinction between the two families is the much more selective nature of the OPT family for peptides when compared to the PTR family [14]. However, substrates for the vast majority of OPTs have not been discovered, and for those few that have had their substrates identified, these molecules range from diverse peptides to modified tripeptides that are bound to metals [13–16]. Nine members of the OPT family have been found in the model plants *Arabidopsis* and rice [13,16], and some rice OPT members (OsOPTs) have been demonstrated to transport ferrous iron [16].

For the PTR family, the best-characterized plant PTR is the carrier responsible for mobilization of peptides across the scutellum from the endosperm to the embryo during barley grain germination. The barley scutellar peptide transporter HvPTR1 has been functionally characterized by expression in Xenopus oocytes [17] and shown to

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be localized to the plasma membrane of scutellar epithelial cells [18]. Several PTR members have also been identified from *Arabidopsis* and exhibit various functions in the plant [19–21]. AtPTR5 facilitates peptide transport into germinating and possibly maturing pollen, ovules, and seeds. In contrast, AtPTR1 has a role in uptake of peptides as the sole nitrogen source by roots [21].

Although barley, wheat, rice, and maize demonstrate rapid uptake of peptides into the scutellum in previous physiological experiments, some differences exist among these four cereals [11]. A large gene family of small peptide transporters exists in the *Arabidopsis* and rice genomes. More than 50 NRT1/PTR homologs have been found in *Arabidopsis*, and 80 are present in rice [12]. However, physiological functions for these genes are unknown. Here, we report the primary functions and expression patterns for eight rice PTRs (OsPTRs).

2. Materials and methods

2.1. Sequence analysis

Protein alignments obtained using ClustalX1.83 [22] were used as starting points for phylogenetic analysis. Multiple sequence alignments were displayed and manually edited using GeneDoc2.6 [23]. Unrooted phylogenetic and molecular evolutionary analyses were constructed using MEGA4.0 [24] by the neighbor-joining method [25] with a Poisson correction model [26], using 1000 replicates for bootstrap analysis. Sequence logos for two conserved motifs were generated using WebLogo2.8 [27] using default parameters. The isoelectric point (pI), molecular weight and grand average of hydropathy (GRAVY) were estimated using the ProtParam tool from ExPASy (http://us.expasy.org/tools/protparam.html) [28]. HMMTOP 2.0 (http://www.enzim.hu/hmmtop/html/document.html) [29] was used to estimate the number of transmembrane (TM) domains. Hydrophobicity plots were generated based on the Kyte–Doolittle method using protein sequence analysis software (DNASTAR) with default parameters [30].

2.2. Plant materials and growth conditions

Rice (*Oryza sativa* 'Zhonghua 11') was cultivated in an experimental field in the South China Botanical Garden under natural growth conditions to collect grain-filling seeds. For collection of roots, germinating seeds and treated seedlings, seeds were imbibed for 2 days. Germinating seeds were then grown in nutrient solution [31] in a growth chamber under a 14 h light/10 h dark cycle at 28 ± 1 °C. The nutrient solution consisted of NH_4NO_3 (1.43 mM), NaH_2PO_4 (0.37 mM), K_2SO_4 (0.5 mM), $CaCl_2$ (1.00 mM) and $MgSO_4$ (1.6 mM). Nutrient solutions were changed weekly and the pH was maintained between 5.0 and 6.5 by adding sulfuric acid.

For salt treatment, 7-day-old seedlings were transferred to a beaker containing a 200 mM NaCl solution for 3 h. For drought treatment, 7-day-old seedlings were kept in folded tissue paper for 3 h at 28 ± 1 °C. For cold treatment, seedlings were kept at 4 ± 1 °C for 3 h. Seedlings were kept in water for 3 h at 28 ± 1 °C as controls.

To collect grain-filling seeds, rice plants were grown in the field under natural growth conditions. When spikelets bloomed, pollinating spikelets were marked with a marker pen then grain-filling seeds were collected at 2, 4, 10, 20 and 29 days after pollination (dap).

2.3. Semi-quantitative and quantitative RT-PCR analysis

Total RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. To remove contaminating genomic DNA, RNA was treated with DNase I. DNase-treated RNA samples (0.5 μg) were reverse transcribed with an anchored oligo-(dT) primer and 200 U Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) in a volume of 20 μL according to the manufacturer's instructions. Semi-quantitative RT-PCR (sq-RT-PCR) was performed in a 20 μL reaction volume containing 1 μL cDNA solution, 1× PCR buffer, 0.25 μM dNTPs, 1.0 μM gene-specific primers and 0.5 U Taq polymerase (Takara, Otsu, Japan) under the following conditions: 94 °C for 3 min (1 cycle), 94 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min (30–32 cycles) and 72 °C for 10 min (1 cycle). Quantitative RT-PCR (q-RT-PCR) was performed with an ABI real-time thermal cycling system using SYBR-Green to detect gene expression abundance, and the actin 1 gene (Os03g0718100) was used as an internal control. The cDNA reaction mixture was diluted three times and 2 μL was used as a template in a 15 μL PCR reaction. Amplifications were carried out after pre-incubation at 95 °C for 10 s, followed by 35 cycles of denaturation at 95 °C for 5 s, annealing at 58 °C for 10 s and extension at 72 °C for 15 s. All runs used a negative control without target DNA and all reactions were performed in three replicates. Gene-specific primers are listed in Supplementary Tables 1 and 2. The specificity of these primers for each OsPTR was confirmed by sequencing the RT-PCR products before performing the sq-RT-PCR and q-RT-PCR.

2.4. Heterologous complementation in Saccharomyces cerevisiae and growth assays

Clones corresponding to full-length cDNAs of OsPTR1 (AK100112), OsPTR2 (AK068351), OsPTR3 (AK101055), OsPTR4

Table 1
Basic information of eight rice OsPTRs.

Gene name	Locus ID[a]	Protein						FL-cDNA[b]	Identity/similarity (*E*-value) to ScPTR2;AtPTR1;AtNRT1.1
		Length (aa)	MW (kDa)	PI	TM[c]	GRAVY[d]	AI[e]		
OsPTR1	Loc_Os07g01070	582	62.7	8.87	11	0.37	93.8	AK100112	23/41%(6e-29);62/76%(0.0);41/61%(5e-123)
OsPTR2	Loc_Os12g44100	588	62.8	9.06	12	0.33	95.2	AK068351	19/34%(4e-09);36/56%(2e-107);34/51%(9e-88)
OsPTR3	Loc_Os10g33210	610	66.4	9.17	12	0.27	98.6	AK101055	20/39%(4e-19);45/64%(3e-147);39/58%(1e-118)
OsPTR4	Loc_Os07g41250	577	63.5	6.75	12	0.41	103.0	AK101099	22/40%(3e-20);43/63%(3e-147);36/55%(9e-102)
OsPTR5	Loc_Os04g50940	570	61.9	4.96	11	0.57	101.3	AK070216	23/47%(8e-15);42/61%(6e-135);35/53%(1e-92)
OsPTR6	Loc_Os04g50950	593	65.1	5.47	12	0.46	99.9	AK101480	21/38%(1e-18);41/61%(7e-132);36/54%(4e-93)
OsPTR7	Loc_Os01g04950	580	63.1	6.22	11	0.30	96.8	AK070036	25/41%(1e-30);67/81%(0.0);41/61%(2e-125)
OsPTR8	Loc_Os03g51050	593	65.6	5.12	11	0.29	95.4	AK072691	23/42%(6e-35);62-76%(0.0);42/62%(4e-130)

[a] Locus ID was adopted from The MSU Rice Genome Annotation Project Database.
[b] Corresponding full-length cDNA in GeneBank.
[c] Number of transmembrane domains.
[d] Grand average of hydropathicity.
[e] Aliphatic index.