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Full Length Article

Differential Expression Pattern of Splice Variants of Amino Acid Transporter Genes from Rice Grown under Various Nitrogen Conditions and during Development

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Abstract

Amino acid transporters (AATs) play indispensable roles in nutrient uptake and allocation for plants. Alternative splicing of gene's post transcription can increase diversity of protein and allow novel ways to regulate growth and development of plant. In this study, 11 *OsAAT* alternative-splicing genes with 24 kinds of predicted mRNAs were identified from 85 rice *AAT* genes; of these, 23 alternative-splicing open reading frames were actually expressed in rice. Quantitative real time-polymerase chain reaction results showed that the predicted primary splice variant 1 was the main alternative splice variant for 8 genes because of its higher expression levels but splice variant 2 was also an important variant existing individually. The expression of splice variants in a gene was regulated by nitrogen (N) and the expression of 5 genes was up-regulated at higher concentration of all forms of N and that of nine genes was up-regulated at higher nitrate concentration. The mRNA levels of splice variants were also regulated during leaf and panicle development of rice. It indicated that alternative splice variants of *OsAAT* family were regulated with a natural variation in expression ratio grown under various nitrogen conditions in rice. © 2017 Friends Science Publishers

Keywords: Amino acid transporter; Rice; Alternative splicing; Expression profile; Nitrogen; Development

Introduction

Inorganic nitrogen (N) is mainly absorbed in the form of nitrate and ammonium. It is converted to amino acids directly in the roots or after translocation to the leaves. The amino acids are then transported to the roots, leaves, flowers, pollens and embryos. Amino acids require transporters from source to sink organs (Coruzzi and Bush, 2001; Tegeder, 2012) and amino acid transporters (AATs) are cellular membrane proteins for their transport. They play a critical role in different processes of plants such as seed development, abiotic and pathogen stresses (Schulze *et al*., 1999; Paungfoo-Lonhienne *et al*., 2008; Näsholm *et al*., 2009). More than 60 *AtAATs* have been identified in *Arabidopsis* (Andrews *et al*., 2009) and more than 80 in rice (Lu *et al*., 2012; Zhao *et al*., 2012).

Plant AAT family includes amino acid/auxin permease (AAAP) family and the APC family (Marschnert *et al*., 1997). The AAAP family includes amino acid permeases (AAPs), aromatic and neutral amino acid transporters (ANTs), proline transporters (ProTs), auxin transporters (AUXs), lysine and histidine transporters (LHTs) and γ-aminobutyric acid transporters (GATs). APC family includes amino acid/choline transporters, cationic amino acid transporters and polyamine H^+-symporters (Fischer *et al*., 1998; Gillissen *et al*., 2000; Ortiz-Lopez *et al*., 2000).

The *AAT* gene family members have been identified in *Arabidopsis* (Tegeder, 2012), rice (Lu *et al*., 2012), poplar (Wu *et al*., 2015), *Solanum tuberosum* L. (Ma *et al*., 2016) and *Glycine max* L. (Cheng *et al*., 2016). This showed that AAP transporters play an important role in loading of amino acids for nitrogen sink and supply (Tegeder and Ward, 2012). AtAAP1 regulates amino acid transport to the root cells or embryos (Lee *et al*., 2007; Sanders, 2009). Furthermore, this transporter functions in the uptake of glutamate and neutral amino acids when present at soil concentrations in *Arabidopsis* (Perchlik *et al*., 2014). AtAAP2 localizes to the phloem and plays a major role in N transfer from the xylem to phloem (Zhang *et al*., 2010). *AtAAP3* is preferentially expressed in the vascular tissue of root (Okumoto *et al*., 2004). AtAAP5 transports amino acids at low concentrations in the roots (Svennerstam *et al*., 2008) and AtAAP6 regulates amino acid composition of the phloem (Hunt *et al*., 2010). AtAAP8 transports amino acids to the endosperm during early embryogenesis (Schmidt *et al*., 2007). Recently, AtAAP8 is shown to be localized in the

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plasma membrane and functions in phloem loading (Santiago and Tegeder, 2016). AtLHT1 plays a role in cellular amino acid uptake in root epidermis and leaf mesophyll (Hirner *et al*., 2006). AtProTs are responsible for the transport of proline, glutamic acid, glycine betaine and gamma-aminobutyric acid (GABA; Grallath, 2005). *AtGAT1* is highly expressed at higher GABA concentration or in the event of wounding and senescence and the protein it encodes is a high-affinity transporter for GABA (Meyer *et al*., 2006). AtANT1 transports aromatic and neutral amino acids such as arginine (Chen *et al*., 2001). AtAUX1 could facilitate auxin uptake and regulates the root gravitropism (Bennett *et al*., 1996; Marchant *et al*., 1999). Although the functions of many AATs in *Arabidopsis* have been identified, few studies have investigated the AAT family members in rice. Whole genome analysis suggested the presence of 85 or 79 AAT homologous genes in rice (Lu *et al*., 2012; Zhao *et al*., 2012). The biomass and yield of rice is significantly influenced when some of the *OsAAT* genes are knocked out (Lu *et al*., 2012; Peng *et al*., 2014).

Alternative splicing of gene's post transcription can allow novel ways to increase the diversity of mRNAs (Graveley, 2001; Kriventseva *et al*., 2003). Alternative splicing is divided into 7 types: alternative donor, alternative acceptor, alternative terminal exon, skipped exon/retained exon, initiation within an intron, termination within an intron and retained intron/spliced intron (Campbell *et al*., 2006; Wang and Brendel, 2006).

The roles of the *AAT* genes in plants, especially in rice are not known completely and diverse alternative splicing regulation might be important for N uptake and utilization. In this study, we analysis 11 alternative-splicing genes of the rice *OsAAT* family and uncover alternative splicing diversification of these genes by conducting bioinformatics and expression regulation.

Materials and Methods

Identification of *OsAAT* Alternative Splicing Genes

The *OsAAT* family gene sequences were acquired from Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) and the alternative-splicing genes were predicted. These full-length corresponding alternative splicing mRNA sequences were checked from the Rice Annotation Project (http://rapdb.dna.affrc.go.jp/).

Protein Structure and Phylogenetic Analysis of *OsAAT* Alternative Splicing Genes

Chemical properties of the proteins were analyzed using Protparam (http://web.expasy.org/protparam/). Their subcellular localization was predicted using Wolf PSORT (http://psort.hgc.jp/) and transmembrane domains were analyzed using TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Rice Materials and Treatments

For expression analysis of the *OsAAT* alternative-splicing genes under normal conditions, total RNA of Zhonghua 11 (ZH11) was extracted from the different tissues of rice plants grown in a controlled field of Wuhan Institute of Bioengineering. For N treatments, rice seeds were soaked in water and germinated at 28°C for 2 days and then cultured in basic nutrient solution (Yoshida *et al*., 1976) with one of the following as the N source: 0.5 mM KNO_3, 2.0 mM KNO_3, 5.0 mM KNO_3, 0.25 mM $(NH_4)_2SO_4$, 1 mM $(NH_4)_2SO_4$, 2.5 mM $(NH_4)_2SO_4$, 0.25 mM NH_4NO_3, 1 mM NH_4NO_3 or 2.5 mM NH_4NO_3.

RNA Extraction and Quantitative Real-time Polymerase Chain Reaction Analysis

Total RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, China). The first-strand of cDNA was synthesized from 3 μg of total RNA treated with DNase I by using M-MLV reverse transcriptase (Promega, China). The first-strand cDNA was used as a template for full-length cDNA PCR amplification and for real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) after normalizing to rice *Actin1* (AB047313). The full-length cDNA-PCR amplification was performed in a 20 μL reaction volume containing 1× PCR buffer, 1.0 μM dNTPs, 1 μL cDNA solution, 1.0 μM gene-specific primers and 0.5 U Taq polymerase (Takara, China) under the following conditions: 94°C for 3 min (1 cycle); 94°C for 30 s, 55°C for 30 s and 72°C for 90 s (40 cycles) and 72°C for 10 min (1 cycle). The primers for transcription level detection of these splice variants were designed by using different open reading frame (ORF), 5′UTR or 3′UTR sequences between splice variants in a gene. If the shorter-splice variant had fewer exons than the longer-splice variant the primers were designed for the common sequences between the two splice variants. Next, one of the two splice variants accounts for half of the expression level for both the splice variants. The positions of primers for amplification in qRT-PCR are shown as red arrows in Fig. 1. The qRT-PCR analysis was performed in a 20 μL reaction volume containing 1×PCR buffer, 0.25 μM dNTPs, 1 μL cDNA solution, 1.0 μM gene-specific primers and 0.5 U Taq polymerase (Takara, China) under the following conditions: 94°C for 2 min (1 cycle); 94°C for 30 s, 55°C for 30 s and 72°C for 30 s (40 cycles) and 72°C for 1 min (1 cycle).

Results

Identification of Alternative Splice Variants from Rice AAT Gene Family

In all, 85 *AAT* genes (*OsAAT*s) are known to be present in the rice (*Oryza sativa* L.) genome. The alternative splicing genes from this family were identified by assessing all the

members of the *OsAAT* family by searching the Rice Genome Sequence Annotation (http://rice.plantbiology.msu.edu/). Eleven putative alternative splicing *OsAAT* genes were found; they were predicted to produce a total of 24 alternatively spliced mRNAs that have 23 different ORFs (Table 1). The exon number of the 11 *OsAAT* genes ranged from 3 to 12 (Fig. 1). Eleven *OsAAT*s were classified into 5 kinds of alternative splicing types (Campbell *et al*., 2006). Two genes (*OsATL7* and *OsAAP5*) belonged to the kind that showed initiation within an intron; four (*OsAUX1*, *OsProT3*, *OsAAP1* and *OsAAP4*), alternative acceptors; two (*OsProT1* and *OsAUX2*), skipped exon/retained exon; one (*OsATL4*), retained intron/spliced intron; and two (*OsAAP13* and *OsATL4*), termination within an intron. Two kinds of alternative splicing types were identified for *OsAAP4* and *OsATL4*.

The corresponding mRNAs for the putative alternative splicing mRNAs were determined by checking the full-length mRNA sequences from the Rice Annotation Project (http://rapdb.dna.affrc.go.jp/); 17 kinds of mRNAs were found in this database except seven mRNAs of *OsAAP4-2*, *OsAAP4-3*, *OsAAP5-2*, *OsAAP13-2*, *OsAAP14-2*, *OsProT1-2* and *OsProT3-2*. Further, 23 full-length cDNA ORFs of splice variants (*OsATL7-1*, *OsATL7-2*, *OsAUX1-1*, *OsAUX1-2*, *OsAAP5-1*, *OsAAP5-2*, *OsProT1-1*, *OsProT1-2*, *OsAAP13-1*, *OsAAP13-2*, *OsAAP14-1*, *OsAAP14-2*, *OsAUX2-1*, *OsATL4-1*, *OsATL4-2*, *OsATL4-3*, *OsProT3-1*, *OsProT3-2*, *OsAAP1-1*, *OsAAP1-2*, *OsAAP4-1*, *OsAAP4-2* and *OsAAP4-3*) were amplified using PCR by using the RT-PCR cDNA template (Fig. 2). Thus, the splice variants were identified. Twenty four alternative splicing mRNAs of the 11 *OsAAT* genes were predicted to encode 23 kinds of proteins that had 229 to 574 amino acids residues (Table 2). Their predicted proteins belonged to AAT-like, auxin transporter, amino acid permease or proline transporter. These splice variant proteins were predicted to be localized at the membrane and contained 5 to 12 transmembrane helices; different transmembrane helices were present between splice variants in one splicing gene except *OsAAP1* (Fig. 3; Table 3). However, one splice variant (OsAAP4-3) had no transmembrane helices; this could be because of the recombination of exons, causing remarkable changes in amino acids (Table 4).

To further confirm the phylogenetic relationship of the proteins encoded by the *OsAAT* alternative splice variants, we compared their amino acid sequences (Fig. 4). The analysis of phylogenetic relationship revealed that 10 alternative splicing proteins, including OsAAP1 (OsAAP1-1 and OsAAP1-2), OsAAP4 (OsAAP4-1 and OsAAP4-2), OsAAP5 (OsAAP5-1 and OsAAP5-2), OsAAP13 (OsAAP13-1 and OsAAP13-2) and OsAAP14 (OsAAP14-1 and OsAAP14-2) were clustered into the AAP subfamily; four alternative splicing proteins, including OsAUX1 (OsAUX1-1 and OsAUX1-2) and OsAUX2 (OsAUX2-1 and OsAUX2-2) were clustered into the AUX subfamily; four alternative splicing proteins, including OsProT1 (OsProT1-1 and OsProT1-2) and OsProT3 (OsProT3-1 and OsProT3-2) were clustered into the ProT subfamily; and five alternative splicing proteins, including OsATL4 (OsATL4-1, OsATL4-2 and OsATL4-3) and OsATL7 (OsATL7-1 and OsATL7-2) were clustered into the ATL subfamily. However, the protein OsAAP4-3 was not clustered into the AAP subfamily.

Root Expression Pattern of *OsAAT* Alternative Splicing Genes under Various Nitrogen Conditions

Root is the main organ for the uptake of N as well as amino acids. The comprehensive roles of the alternative splicing mRNA variants of the *OsAAT* genes in response to various N levels in rice roots were determined by their expression patterns when using inorganic N (NO_3^-, NH_4^+ or NH_4NO_3) as the sole N source by using qRT-PCR (Fig. 5; all primer sequences used in this study are listed in Table 5). When the difference in the expression of the alternatively spliced mRNAs within a gene was compared, higher level of the first spliced mRNA variants was noted for *OsAAP5*, *OsAAP13*, *OsAUX2*, *OsATL4*, *OsProT3*, *OsAAP1*, *OsAAP4* and higher levels of the second spliced mRNA variants were found for *OsATL7*, *OsProT1* and *OsAAP14*. This indicated that the spliced mRNA variants for a gene differed in response to N availability. Two spliced mRNA variants of only one gene (*OsAUX1*) were expressed at a similar level (Fig. 5B).

Roots subjected to nitrate treatment showed the up-regulation of thirteen alternative splice variants of nine genes (*OsATL7-2*, *OsAUX1-1/2*, *OsAAP5-1/2*, *OsAAP14-2*, *OsAUX2-1*, *OsATL4-1*, *OsProT3-1*, *OsAAP1-1* and *OsAAP4-1/2/3*) and only one gene (*OsAAP13-1*) showed down-regulation of the alternative splice variant (Fig. 5). After treatment with ammonium, 11 alternative splice variants of nine genes (*OsATL7-2*, *OsAUX1-2*, *OsAAP5-1*, *OsAAP14-1/2*, *OsAUX2-1/2*, *OsATL4-1*, *OsProT3-1*, *OsAAP1-1* and *OsAAP4-3*) were up-regulated and three alternative splice variants of 3 genes (*OsAUX1-1*, *OsAAP13-1* and *OsAAP4-1*) were down-regulated. After nitrate ammonium treatment, seven alternative splice variants of six genes (*OsATL7-2*, *OsAUX1-1*, *OsAAP5-1*, *AUX2-1/2*, *OsATL4-1* and *OsAAP4-1*) were up-regulated and two alternative splice variants of two genes (*OsAAP13-1* and *OsAAP4-3*) were down-regulated. Interestingly, the expression of *OsAUX1-1* and *OsAAP4-1/2* was up-regulated after nitrate treatment, but down-regulated after ammonium or nitrate ammonium treatment.

Leaf Expression Pattern of *OsAAT* Alternative Splice Genes under Various Nitrogen Conditions

The leaf expression pattern of the *OsAAT* alternative splice variants in response to various N levels was investigated after inorganic N treatment (NO_3^-, NH_4^+ or NH_4NO_3) by

Table 1: Information of rice alternative splicing amino acid transporter genes (*OsAAT*s)

Gene name		Transcript ID in MSU[c]	Transcript ID in RAP[c]	No. of Introns	No. of Exons	Predicted function
by Lu *et al.* (2012)	by Zhao *et al.* (2012)					
OsAAT7-1	*OsATL7-1*	LOC_Os01g61044.1	AK064301	4	5	Amino acid transporter-like
OsAAT7-2	*OsATL7-2*	LOC_Os01g61044.2	AK102220	4	5	Amino acid transporter-like
OsAAT9-1	*OsAUX1-1*	LOC_Os01g63770.1	AK103239	6	7	Auxin transporter
OsAAT9-2	*OsAUX1-2*	LOC_Os01g63770.2	AK068536	3	4	Auxin transporter
OsAAT12-1	*OsAAP5-1*	LOC_Os01g65660.1	AK073884	5	6	Amino acid permease
OsAAT12-2	*OsAAP5-2*	LOC_Os01g65660.2	Not found	3	4	Amino acid permease
OsAAT16-1	*OsProT1-1*	LOC_Os01g68050.1	AK241733	6	7	Proline transporter
OsAAT16-2	*OsProT1-2*	LOC_Os01g68050.2	Not found	4	5	Proline transporter
OsAAT35-1	*OsAAP13-1*	LOC_Os04g39489.1	AK071044	6	7	Amino acid permease
OsAAT35-2	*OsAAP13-2*	LOC_Os04g39489.2	Not found	3	4	Amino acid permease
OsAAT40-1	*OsAAP14-1*	LOC_Os04g56470.1	XM_0157786001	6	7	Amino acid permease
OsAAT40-2	*OsAAP14-2*	LOC_Os04g56470.2	Not Found	5	6	Amino acid permease
OsAAT45-1	*OsAUX2-1*	LOC_Os05g37470.1	AK111659	5	6	Auxin transporter
OsAAT45-2	*OsAUX2-2*	LOC_Os05g37470.2	AK111849	6	7	Auxin transporter
OsAAT51-1	*OsATL4-1*	LOC_Os06g16420.1	AK120497	4	5	Amino acid transporter-like
OsAAT51-2	*OsATL4-2*	LOC_Os06g16420.2	AK066102	3	4	Amino acid transporter-like
OsAAT51-3	*OsATL4-3*	LOC_Os06g16420.3	AK099920	2	3	Amino acid transporter-like
OsAAT58-1	*OsProT3-1*	LOC_Os07g01090.1	AK066298	6	7	proline transporter
OsAAT58-2	*OsProT3-2*	LOC_Os07g01090.2	Not found	5	6	Proline transporter
OsAAT60-1	*OsAAP1-1*	LOC_Os07g04180.1	AK106110	5	6	Amino acid permease
OsAAT60-2	*OsAAP1-2*	LOC_Os07g04180.2	AK103862	4	5	Amino acid permease
OsAAT72-1	*OsAAP4-1*	LOC_Os12g09300.1	AK069508	4	5	Amino acid permease
OsAAT72-2	*OsAAP4-2*	LOC_Os12g09300.2	Not found	3	4	Amino acid permease
OsAAT72-3	*OsAAP4-3*	LOC_Os12g09300.3	Not found	2	3	Amino acid permease

[a,b] Gene names are from Lu et al. (2012) and Zhao et al. (2012); [c] Full-length cDNA accession numbers of *OsAATs* obtained from MSU Rice Genome Annotation Project Database or The Rice Annotation Project (RAP) identified using RT-PCR

Table 2: Protein information of rice alternative splicing amino acid transporter genes (*OsAAT*s)

Gene name	Amino acid number	Molecular weight (D)	Theoretical pI	Localization
OsATL7-1	459	47870.3	9.43	Membrane
OsATL7-2	456	47688.0	7.73	Membrane
OsAUX1-1	492	54762.1	8.27	Membrane
OsAUX1.2	282	31354.3	9.26	Membrane
OsAAP5-1	465	50002.3	8.87	Membrane
OsAAP5-2	375	40484.6	9.08	Membrane
OsProT1-1	447	49025.0	9.20	Membrane
OsProT1-2	354	38484.6	8.60	Membrane
OsAAP13-1	466	50747.0	8.38	Membrane
OsAAP13-2	229	24339.0	7.07	Membrane
OsAAP14-1	469	51377.9	8.89	Membrane
OsAAP14-2	409	44980.4	9.60	Membrane
OsAUX2-1	503	47688.0	7.73	Membrane
OsAUX2-2	482	53358.3	8.77	Membrane
OsATL4-1	448	48121.6	6.79	Membrane
OsATL4-2	402	43002.5	6.23	Membrane
OsATL4-3	274	29277.6	6.29	Membrane
OsProT3-1	434	47663.9	9.30	Membrane
OsProT3-2	384	42329.0	9.69	Membrane
OsAAP1-1	487	52864.1	8.80	Membrane
OsAAP1-2	460	49716.9	9.15	Membrane
OsAAP4-1	468	50891.3	8.52	Membrane
OsAAP4-2	341	36748.3	6.69	Membrane
OsAAP4-3	371	43411.0	12.26	Nucl: 9.0, Cyto: 3.0, Plas: 1.0

Nucl: Nucleus, Cyto: Cytoplasm, Plas: Plastid

using qRT-PCR (Fig. 6). When the difference in the expression of the alternatively spliced mRNAs within a gene was compared, higher level of the first spliced mRNA variant was found in *OsAAP5*, *OsAAP13*, *OsAUX2*, *OsProT3*, *OsAAP1*, *OsAAP4* and higher levels of the second spliced mRNA variants were found in *OsATL7*, *OsProT1* and *OsAAP14*. The spliced mRNA variants of only two genes (*OsAUX1* and *OsATL4*) were expressed at a similar level (Fig. 6B, H). *OsAUX2-3*, *OsATL4-2* and *OsATL4-3* showed higher expression in the leaves (Fig. 6G, H). Although *OsAUX2-3* has the same ORF as that of *OsAUX2-2*, the expression level of *OsAUX2-3* was significantly different from that of *OsAUX2-2* in the leaves when different 3′-untranslated regions were used as primers for qRT-PCR. Different mRNAs of alternatively spliced *OsAAT* genes were shown to exist either in rice roots or in leaves in response to various N concentrations.

In leaves subjected to nitrate treatment (Fig. 6) the expression of *OsATL7-2*, *OsProT1-1/2*, *OsAUX2-1* and *OsAAP4-1* was up-regulated and that of *OsATL7-2*, *OsAAP13-1*, *OsALT4-1/2* and *OsAAP1-1* was down-regulated. After ammonium treatment the expression of *OsProT1-1* and *OsAAP4-1* was up-regulated, but that of *OsATL7-2*, *OsAAP13-1*, *OsATL4-2/3* and *OsAAP1-1* was down-regulated. After nitrate ammonium treatment, the expression of *OsProT1-1* was up-regulated, but that of *OsAAP1-1* was down-regulated.

Leaf Expression Pattern of *OsAAT* Alternative Splicing Genes at the Reproductive Stage

Systematic characterization of the participation of *OsAAT* splicing genes in leaf amino acid transport or mobilization was performed by conducting qRT-PCR by using leaf

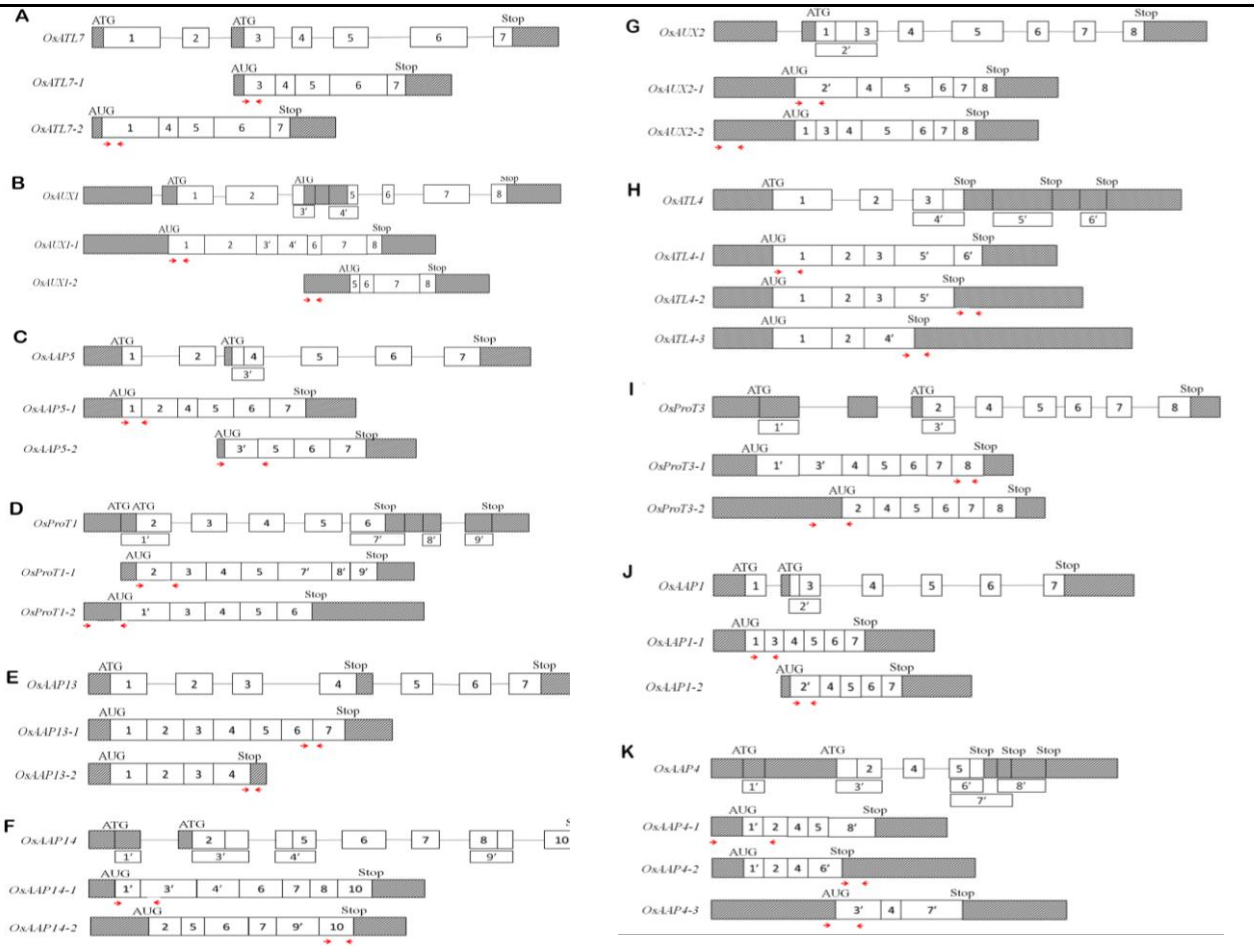

Fig. 1: Alternative splice variant structure of *OsAAT* genes. *OsXX* represents the DNA sequence of splice variants. *OsXX-1*, *OsXX-2*, *OsXX-3* represent mRNA sequences of each splice variant. The OsAAT splice variant sequences were acquired from http://rice.plantbiology.msu.edu/ and selected from the predicted alternative splice variants. Gray box represents UTR. White box represents a potential exon region for splice variants. Black line represents potential intron region for splice variants. Red arrow represents the position site of forward and reverse primers for the detection of the expression level of splice variants in qRT-PCR

tissues during the entire reproductive stage (Fig. 7). The results indicated that the expression levels were different among splice variants within a gene of most *OsAAT* splicing genes (*OsAUX1*, *OsAAP5*, *OsProT1*, *OsAAP13*, *OsProT3*, *OsAAP1*, *OsAAP14* and *OsAAP4*) at four stages. The expression of splice variant 1 was higher than that of splice variant 2 for six splicing genes (*OsAUX1*, *OsAAP5*, *OsProT1*, *OsAAP13*, *OsProT3* and *OsAAP1*); however, the expression of splice variant 1 was lower than that of splice variant 1 for two splicing genes *OsAAP14* and *OsAAP4*. Only three genes *OsATL7*, *OsAUX2* and *OsATL4* showed similar expression among different alternative splice variants. The expression of *OsATL7-1/2*, *OsAUX1-1/2*, *OsAAP5-1*, *OsProT1-1*, *OsAAP14-2*, *OsProT3-1* and *OsAAP-1* was up-regulated in the leaves from the early booting stage to mature stage, but that of *OsAAP13-1* was down-regulated along with development (Fig. 7E).

Panicle Expression Pattern of *OsAAT* Alternative Splicing Genes at the Reproductive Stage

In this study, qRT-PCR was used to monitor the expression changes of the 24 alternative splicing mRNAs in developing panicles (Fig. 8). The first alternatively spliced variants of *OsAAP5*, *OsAAP13*, *OsAUX2* and *OsProT3*, but the second alternatively spliced variants of *OsAAP4* and *OsAAP14* were highly expressed in the panicles. Further, qRT-PCR showed that *OsATL7-1*, *OsAAP5-1*, *OsProT1-1* and *OsAAP1-1* were highly expressed highest in the early developing panicles (P1); *OsAAP13-1*, *OsAAP14-2*, *OsAUX2-1*, *OsATL4-2/3* and *OsAAP1-2* showed the highest expression level in the middle developing panicles (P2–P3); and *OsAAP4-1/2* showed the highest expression level in mature panicles (P4).

Table 3: List of the full-length cDNA PCR amplified primers used in this study

Name	Sequence (5'-3')	Note
OsATL7-1-OF	ATGGTGTCCAAGAAGACCTCCATC	The PCR fragment is 1380 bp
OsATL7-1-OR	CTACCTACCTGCGGCATGGCCTCC	
OsATL7-2-OF	ATGACGCCGCCGGCGAGCACGGGC	The PCR fragment is 1371bp
OsATL7-2-OR	CTACCTACCTGCGGCATGGCCTCC	
OsAUX1-1-OF	ATGGTGCCGCGCGAGCAGGCGGAG	The PCR fragment is 1479 bp
OsAUX1-1-OR	CTAGTGGTGCGGCAATGGCACCGG	
OsAUX1-2-OF	ATGACCACCTATACCGCTTGGTAC	The PCR fragment is 849 bp
OsAUX1-2-OR	CTAGTGGTGCGGCAATGGCACCGG	
OsAAP5-1-OF	ATGAACAAGAACGCCGCACCGGAA	The PCR fragment is 1398 bp
OsAAP5-1-OR	GCTGACAGTTTTGAAAGGGGTTGCAAC	
OsAAP5-2-OF	ATGAACAAGAACGCCGCACCGGAA	The PCR fragment is 1128 bp
OsAAP5-2-OR	ATGCCAAAAATATTTTCAGGTCCT	
OsProT1-1-OF	ATGGCTGCTTCATCGCTCGACGCCGAG	The PCR fragment is 1344 bp
OsProT1-1-OR	TTACATGTCCGCGAAGAAATGGTA	
OsProT1-2-OF	ATGAAGTGTGCGTGTGTGAGAGAGT	The PCR fragment is 1065 bp
OsProT1-2-OR	TCACGCCATCAACGGGGAGGAGGA	
OsAAP13-1-OF	ATGGCGCTCGGCGACGGGGACGAC	The PCR fragment is 1401 bp
OsAAP13-1-OR	TTAGCCTAGCTTCTGGCTGATGAG	
OsAAP13-2-OF	ATGGCGCTCGGCGACGGGGACGAC	The PCR fragment is 690 bp
OsAAP13-2-OR	TCAAAGCCTCAAAGGATGAACAGA	
OsAAP14-1-OF	ATGGCGCCGCAGCTGCCGCTCGAG	The PCR fragment is 1410 bp
OsAAP14-1-OR	CTAGCCAAGCCTCTTTCTGATGAC	
OsAAP14-2-OF	ATGGACGTGCGTAGCGCACATTAT	The PCR fragment is 1230 bp
OsAAP14-2-OR	CTAGCCAAGCCTCTTTCTGATGAC	
OsAUX2-OF	ATGGTGCCGGCCGGCGACCAGGCG	The PCR fragment is 1512 bp
OsAUX2-OR	CTAGTGGCGCGGCGGAGCCGGCAG	
OsATL4-1-OF	ATGGGTGGAGGCGTCACGGAGCGG	The PCR fragment is 1347 bp
OsATL4-1-OR	TCAGGCTATGGAAGGGGAACTTTTCC	
OsATL4-2-OF	ATGGGTGGAGGCGTCACGGAGCGG	The PCR fragment is 1209 bp
OsATL4-2-OR	TTACCTTAGAGTGATCGCGGCTGG	
OsATL4-3-OF	ATGGGTGGAGGCGTCACGGAGCGG	The PCR fragment is 825 bp
OsATL4-3-OR	CTAAAAGAAAATTGAAAGAAAGTCTTC	
OsProT3-1-OF	ATGAACATCGACATGGCCAATTCC	The PCR fragment is 1305 bp
OsProT3-1-OR	TCACAGATCAGCAAACAAATGGTA	
OsProT3-2-OF	ATGGTCCCTTTAGGTTGGATTGGT	The PCR fragment is 1155 bp
OsProT3-2-OR	TCACAGATCAGCAAACAAATGGTA	
OsAAP1-1-OF	CTAGCTAAGCTAGGAATTGAA	The PCR fragment is 1485 bp
OsAAP1-1-OR	TCATGAGGAGACGCTGAATGGCTT	
OsAAP1-2-OF	CAAGCTAATGGGTTGGTAATAACA	The PCR fragment is 1407 bp
OsAAP1-2-OR	TCATGAGGAGACGCTGAATGGCTT	
OsAAP4-1-OF	ATGGACAGGAGAGCAGTAGTGTAT	The PCR fragment is 1407 bp
OsAAP4-1-OR	TCAGTTGACAGTCTTGAAGGGTGC	
OsAAP4-2-OF	ATGGACAGGAGAGCAGTAGTGTAT	The PCR fragment is 1026 bp
OsAAP4-2-OR	TCATCGTCGATCGCCGCCCGCCAT	
OsAAP4-3-OF	ATGATGATGATGATGATGATGGTGATGATCAG	The PCR fragment is 1116 bp
OsAAP4-3-OR	TCACGTAGTAGGTCGCGTTGATGA	

The OsAAT splice variants sequences were acquired from http://rice.plantbiology.msu.edu/, and the full-length cDNAs PCR amplified primers were designed through region of position of start codon and and stop codon. The length of PCR fragment of some primers contains some 5'UTR and 3' region because the GC ratio is too high in start codon and and stop codon in some splice variants

Discussion

We found that 11 genes of the 85 or 79 *OsAAT* genes were alternatively spliced (Lu *et al*., 2012; Zhao *et al*., 2012). They could be classified into five kinds of alternative splicing patterns. The highest proportion (31.25%) of their alternative splicing types was selective receptor sites (AA) and the second (25%) was intron splicing/intron retention. The highest alternative splicing type (probability, 53.5%) in rice was intron splicing and intron retention and the second (15.1%) was selective receptor site (AA) (Wang *et al*., 2006). The amino acid residues of these spliced proteins ranged from 229 to 574 and the molecular weights and isoelectric potentials ranged from 24 to 63 KDa and 5.57 to 12.26, respectively (Table 2). *OsAAP1* has two splice variants and variant OsAAP1-1 transports both positively and neutral charged basic amino acids in rice (Taylor *et al*., 2015). Five *OsAAPs* of rice AAT family have splice variants and the number or length of trans-membrane helices differed between the splice variants of a gene. These findings suggest that some *OsAAP* splice variants play a broad role in rice growth and development. AUX members could support specific regulatory inputs from different signals or different interactions (Reed, 2001). We indicated that both *OsAUX1* and *AtAUX2* have two splice variants and their expression levels differed between splice variants

Table 4: Analysis of transmembrane domain in OsAAT splice variants proteins

Splice variants	Amount	Start and end site of trans-membrane domain										
		TM1	TM2	TM3	TM4	TM5	TM6	TM7	TM8	TM9	TM10	TM11
OsATL7-1	11	39-61	66-88	114-136	163-182	194-216	236-258	271-293	321-343	363-385	390-409	422-444
OsATL7-2	10	55-77	115-137	157-179	191-213	233-255	268-290	318-340	360-382	387-406	419-441	
OsAUX1-1	10	88-110	149-171	181-198	203-225	240-262	275-297	323-345	365-382	387-409	438-460	
OsAUX1-2	6	31-53	66-88	113-135	155-172	177-199	228-250					
OsAAP5-1	11	20-42	52-74	109-130	150-172	177-199	224-246	267-289	304-326	370-389	393-415	427-449
OsAAP5-2	9	21-40	60-82	87-109	134-156	177-199	214-236	280-299	303-325	337-359		
OsProT1-1	10	38-60	67-89	116-138	159-181	185-204	225-247	262-284	351-370	374-396	413-435	
OsProT1-2	7	59-81	85-107	133-155	175-194	201-223	243-262	281-303				
OsAAP13-1	9	35-57	59-81	118-140	160-182	186-208	276-298	313-335	393-415	435-457		
OsAAP13-2	5	35-57	59-81	118-140	160-182	197-219						
OsAAP14-1	9	24-46	53-75	114-136	157-176	180-202	271-293	308-330	396-418	438-460		
OsAAP14-2	8	43-65	97-114	121-143	168-190	211-233	248-270	336-358	378-400			
OsAUX2-1	11	66-85	100-122	161-183	193-210	215-237	252-274	287-309	335-357	377-394	399-421	441-463
OsAUX2-2	10	79-101	140-162	172-189	194-216	231-253	266-288	314-336	356-373	378-400	420-442	
OsATL4-1	11	31-53	58-80	111-133	153-175	182-204	224-243	254-286	313-335	355-377	382-401	414-436
OsATL4-2	10	31-53	58-80	111-133	153-175	182-204	224-243	264-286	313-335	355-377	382-401	
OsATL4-3	6	31-53	58-80	111-133	153-175	182-204	224-246					
OsProT3-1	11	30-52	56-78	104-126	141-163	168-190	210-232	252-274	289-311	339-358	362-384	396-418
OsProT3-2	10	5-27	54-76	91-113	118-140	160-182	202-224	239-261	289-308	312-334	346-368	
OsAAP1-1	9	45-67	71-93	130-152	194-216	287-309	326-348	391-410	414-432	452-474		
OsAAP1-2	9	7-29	44-66	103-125	167-189	260-282	299-321	364-383	387-405	425-447		
OsAAP4-1	9	20-42	49-71	108-130	142-164	174-196	265-287	304-326	384-406	429-451		
OsAAP4-2	7	20-42	49-71	108-130	142-164	174-196	265-287	297-319				

The OsAAT splice variants sequences was acquired from http://rice.plantbiology.msu.edu/) and the analysis of sequence of protein use TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/

under exogenous N. *AtAUX1* was shown to regulate root gravitropism and change the distribution of indole-3-acetic acid to promote the formation of lateral roots (Bennett *et al*., 1996; Marchant *et al*., 1999). AtLAX3, an AtAUX1 homologous protein, could influence the lateral root prominence rate and regulate cell wall remodeling (Lee *et al*., 2011). Our results suggest that some *OsAUX* splice variants might play various roles in rice growth and development.

Amino acid transporters could transport a range of different types of amino acids in the roots directly (Miller *et al*., 2008; Svennerstam *et al*., 2008) or indirectly via other amino acids that are transferred from ammonium to glutamine by glutamine synthetase (Sonoda *et al*., 2003). Substrate specificity and expression profiles of AATs have been identified in *Arabidopsis* (Fischer *et al*., 1995). AAPs are highly regulated by environmental signals (Grallath *et al*., 2005). The *PtAAT* genes might play a critical role in abiotic stress signaling in *Populus*, because their expression was either increased or repressed after the PEG and cold treatments (Wu *et al*., 2015). However, little is known about the role of the *OsAAT* alternative splicing genes in response to environmental signal, especially N in the roots and leaves. We showed some clues about the role of *OsAAT* alternative splicing genes in response to N by determining their expression levels (Fig. 5 and 6). Splice variants for an *OsAAT* gene showed differential expression and responded differently to N in the rice roots and leaves. After nitrate, ammonium or ammonium nitrate treatments the alternative splice variants of *OsAAT* genes showed differential expression in the roots. Wide divergence of transmembrane

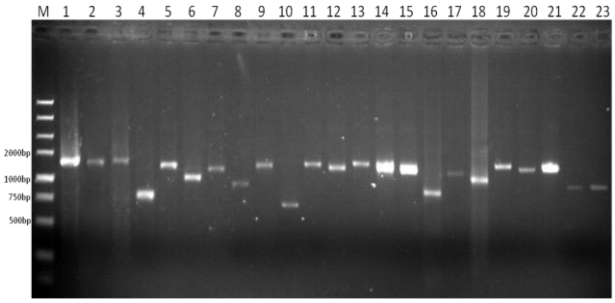

Fig. 2: The full-length cDNA amplification of *AAT* splice variants. Lane 1: *OsATL7-1*, Lane 2: *OsATL7-2*, Lane 3: *OsAUX1-1*, Lane 4: *OsAUX1-2*, Lane 5: *OsAAP5-1*, Lane 6: *OsAAP5-2*, Lane 7: *OsProT1-1*, Lane 8: *OsProT1-2*; Lane 9: *OsAAP13-1*, Lane 10: *OsAAP13-2*, Lane 11: *OsAAP14-1*, Lane 12: *OsAAP14-2*, Lane 13: *OsAUX2-1*, Lane 14: *OsATL4-1*, Lane 15: *OsATL4-2*, Lane 16: *OsATL4-3*, Lane 17: *OsProT3-1*, Lane 18: *OsProT3-2*, Lane 19: *OsAAP1-1*, Lane 20: *OsAAP1-2*, Lane 21: *OsAAP4-1*, Lane 22: *OsAAP4-2*, Lane 23: *OsAAP4-3*

helices are noted in splice variants of *OsAAP5*, *OsProT1*, *OsAAP13*, *OsAAP14*, *OsATL4*, *OsAAP4* and expression levels in response to N are different in the rice root and leaf. These findings suggest that N could regulate alternative splice variants of *OsAAT* genes, because of the divergence of protein structure in splice variants.

Some genes of the *AAT* family have been characterized. It has shown different functions during the uptake of amino acids by the root, transporting amino acids

Table 5: List of the real-time quantitative RT-PCR primers used in this study

Name	Sequence (5'-3')	Note
OsATL7-1F	TGGTGTCCAAGAAGACCTCCAT	Detection of transcription level of *OsATL7-1*
OsATL7-1R	ACGACGCTCGTCGACACGTTG	
OsATL7-2F	GTTCAACGTGTCGACGAGCATCAT	Detection of transcription level of *OsATL7-2*
OsATL7-2R	TACCGCAGCATGAATTCCACGGAG	
OsAUX1-1F	AGGAGGCCATCGTGGCTGACA	Detection of transcription level of *OsAUX1-1*
OsAUX1-1R	CGACCTGGTTAGAGGCGCAGCT	
OsAUX1-2F	GTCACTTCGCAGCGCCAGTGTA	Detection of transcription level of *OsAUX1-2*
OsAUX1-2R	GCTTGTCCAGCCGGTCGTTGA	
OsAAP5-1F	AACGCCGCACCGGAAGACGT	Detection of transcription level of *OsAAP5-1*
OsAAP5-1R	TAGGCGTTGGCGAGCAGAGT	
OsAAP5-2F	CTTGTTGGTTAAACTGAACGC	Detection of transcription level of *OsAAP5-2*
OsAAP5-2R	AACGCCAGCATGAGCACCGT	
OsProT1-AF	TGGCTGCTTCATCGCTCGACG	Detection of both transcription level of *OsProT1-1* and *OsProT1-2*
OsProT1-AR	CAACCCAGCGGCGCCATCAT	
OsProT1-2F	ATGCGACCGAGCTACAATGCCGTC	Detection of transcription level of *OsProT1-2*
OsProT1-2R	TCGGCGTCGAGCGATGAAGC	
OsAAP13-1F	GACTTCGCCAACGCCTGCAT	Detection of transcription level of *OsAAP13-1*
OsAAP13-1R	CGCAGGAGGTTCACCCGACA	
OsAAP13-2F	ATACCGGACTTCCACGACAT	Detection of transcription level of *OsAAP13-2*
OsAAP13-2R	GCACTAGTACTACGACTGAGCA	
OsAAP14-1F	ACCCACAACGCACCGGGAATCT	Detection of transcription level of *OsAAP14-1*
OsAAP14-1R	TCTCCGAGCCAGGAGACCTGTAGC	
OsAAP14-AF	CCCGTCGAGATGTACTGCGTGCAG	Detection of both transcription level of *OsAAP14-1* and *OsAAP14-2*
OsAAP14-AR	TTCCACCGAGCCGACGAACGCGAA	
OsAUX2-1F	ACGACCAACCACGAACGACCTCGT	Detection of transcription level of *OsAUX2-1*
OsAUX2-1R	ATCCCGAGCTGCGAGAAGGA	
OsAUX2-AF	AGTCTCCTCACTCTCGACTACTCT	Detection of both transcription level of *OsAUX2-1* and *AUX2-2*
OsAUX2-AR	TGCCACAGGAGGCTCGTCAT	
OsAUX2-3F	TGCCTTTGTGTAGTAGGACAGC	Detection of transcription level of *OsAUX2-3*
OsAUX2-3R	ACGACGCAAAGTTGCCAACAAC	
OsATL4-1F	GCGCGGTGTTCAACCTGTCG	Detection of transcription level of *OsATL4-1*
OsATL4-1R	AGCTCGATGGAGGCGTCGGT	
OsATL4-2F	CATTCCGAGCATCTGGGATGCCT	Detection of transcription level of *OsATL4-2*
OsATL4-2R	ACGGAATAAATATCCCAGCCA	
OsATL4-3F	TGATGTTCCCGACTTGGCAT	Detection of transcription level of *OsATL4-3*
OsATL4-3R	AAGCAACCCTATTCGGGCTT	
OsProT3-AF	TTGGCGACTTCATGAGCCTGA	Detection of both transcription level of *OsProT3-1* and *OsProT3-2*
OsProT3-AR	TGCCAGGATATCTGCAAGGT	
OsProT3-2F	AGTGAGGGTTCCCGAACACACCTC	Detection of transcription level of *OsProT3-2*
OsProT3-2R	GGATCAAGCCACATGTCCCACCAA	
OsAAP1-1F	GCCTTCAACCTCGCCGAGTC	Detection of transcription level of *OsAAP1-1*
OsAAP1-1R	GTTATGACCGAGAACGCCACCA	
OsAAP1-2F	CGGACGAACACTTGGATGCA	Detection of transcription level of *OsAAP1-2*
OsAAP1-2R	GTTATGACCGAGAACGCCACCA	
OsAAP4-1,2F	TGGCACTCACCCTTGCACAC	Detection of both transcription level of *OsAAP4-1* and *OsAAP4-2*
OsAAP4-1,2R	CCGTCCACACCGTCCCTTGT	
OsAAP4-2,3F	TTGCTGCAGGTGTTCGCGCA	Detection of both transcription level of *OsAAP4-2* and *OsAAP4-3*
OsAAP4-2,3R	ATCGTCCGCAGCACCAGCTTCAG	
OsAAP4-3F	ACTTGAGCTCTCTGCATTGGGT	Detection of transcription level of *OsAAP4-3*
OsAAP4-3R	AGCGGTAGCAATTGGCGAGGA	
OsActin-F	CGGTGTCATGGTCGGAAT	Detection of transcription level of *OsActin*
OsActin-R	GCTCGTTGTAGAAGGTGT	

The OsAAT splice variants sequences were acquired from http://rice.plantbiology.msu.edu/and the RT-PCR primers were designed by Primer 5.0 software

into the phloem or partitioning of amino acids to the seeds (Tegeder, 2014). AtAAP1 transports amino acids at the roots of *Arabidopsis* (Lee *et al*., 2007) and regulates amino acids at developing embryos (Sanders *et al*., 2009). AtAAP6 affects the amino acid content of the *Arabidopsis* (Hunt *et al*., 2010). *PtAAP11* is specifically expressed in xylem cells of poplar (Couturier *et al*., 2010). AtLHT1 could uptake amino acid both at the root epidermis and leaf mesophyll (Chen and Bush, 1997; Hirner *et al*., 2006). LeProT1 could transport proline, glycine betaine and GABA in tomato pollen (Schwacke *et al*., 1999).

During the reproductive stage, flag leaves develop from functional to senesce leaves, which are a source for amino acids and the sink of seeds (Fang *et al*., 2013). Rice *OsAAP5* is highly expressed in the leaves, but *OsProT1*, *OsAUX1* and *OsAUX2* expressed highly in the panicles (Zhao *et al*., 2012). Rice OsAAP6 functions as a positive regulator of grain protein content (Peng *et al*., 2014).

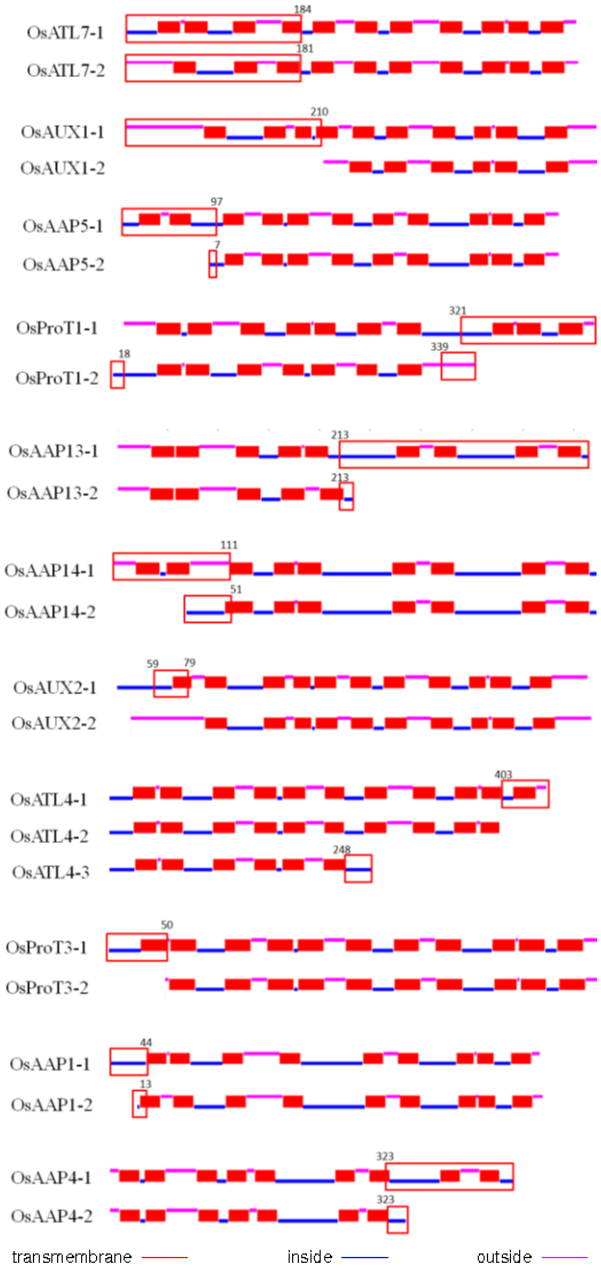

Fig. 3: Prediction of transmembrane domain in OsAAT splice variants proteins. The OsAAT splice variants sequence was acquired from http://rice.plantbiology.msu.edu/) and the analysis of sequence of protein use TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The red box represents specific region of splice variant, and the number represents the position site of amino acids

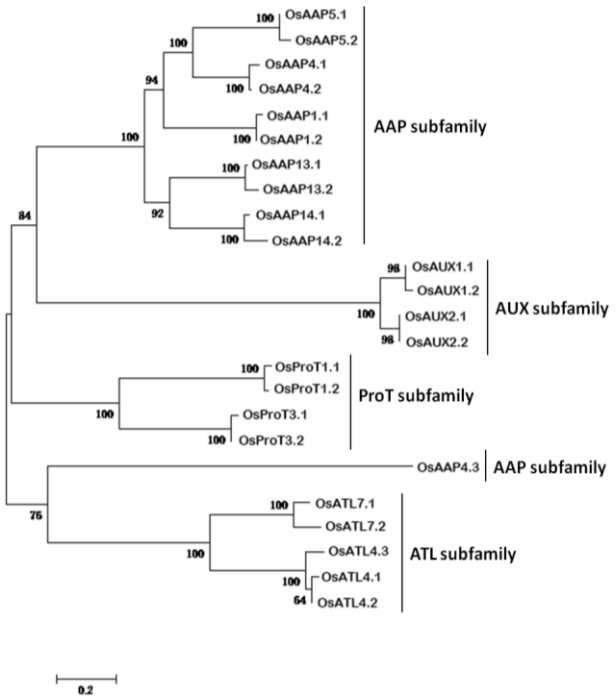

Fig. 4: Phylogenetic tree of OsAAT alternative splice variants based on amino acid sequence. The tree was drawn according to results generated by MEGA4.0 analysis using the neighbor-joining method with an amino acid and Poisson correction model. Bootstrap values calculated for 1,000 replicates are indicated at corresponding nodes. The 11 OsAAT alternative splice variants from rice were clustered into four sections. Locus IDs of OsAAT splice variants from rice are given in Table 1

Alternative splice variants of *OsAAT* genes showed differential expression during leaf development (Fig. 7). Expression of *OsALT7-1/2*, *OsAUX1-1*, *OsAAP14-2* and *OsProT3-1* increased from young to old leaves. However, the expression of *OsAUX1-1*, *OsAAP5-1*, *OsProT1-1*, *OsAUX2-2* and *OsAAP1-1* increased at the early stage and then decreased at the late stage. These changes in gene expression suggest that these genes play differential roles when flag leaves develop from young to old. In the developing panicles, alternative splice variants of *OsAAT* genes also showed differential expression (Fig. 8). These findings suggested that different alternative splice variants of *OsAAT* genes play different roles during panicle development.

Conclusion

Eleven identified *OsAAT* genes showed differential expression via alternative splicing to adapt to environmental nutrition and developmental conditions. This study provides a new perspective on the wide divergence and regulation of splice variants in rice *AAT* gene family. These data might provide an insight into further understanding the functions of *AAT* members and their roles in rice growth and development.

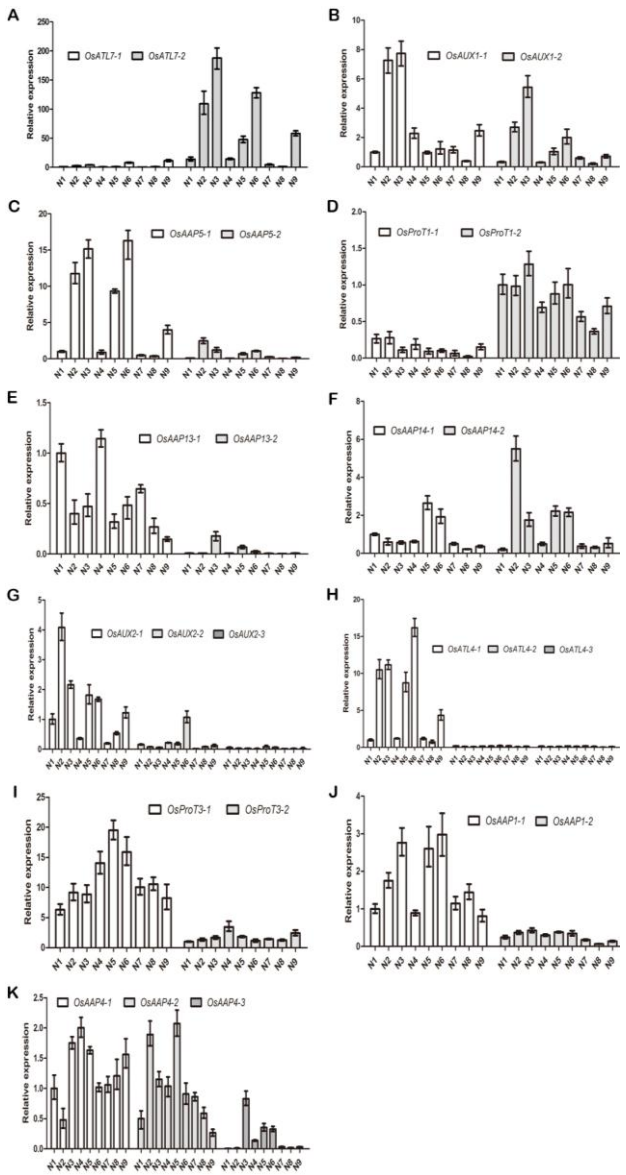

Fig. 5: Root expression patterns of *OsAAT* alternative splice variants under different nitrogen conditions. For N treatments, seeds were soaked in water and germinated at 28°C for 2 days, and then cultured in the basic nutrient solution (Yoshida et al., 1976) with one of the following as the N source: 0.5 mM KNO_3 (N1), 2.0 mM KNO_3 (N2), 5.0 mM KNO_3 (N3), 0.25 mM $(NH_4)_2SO_4$ (N4), 1 mM $(NH_4)_2SO_4$ (N5), 2.5 mM $(NH_4)_2SO_4$ (N6), 0.25 mM NH_4NO_3 (N7), 1 mM NH_4NO_3 (N8), or 2.5 mM NH_4NO_3 (N9). After one week of culture, roots of seedlings were harvested for total RNA isolation. Rice *Actin1* gene (AB047313) was used as an internal control. The ratio of relative expression set to 1 was the expression level of *OsATL7-1*, *OsAUX1-1*, *OsAAP5-1*, *OsProT1-2*, *OsAAP13-1*, *OsAAP14-1*, *OsAUX2-1*, *OsATL4-1*, *OsProT3-2*, *OsAAP1-1*, and *OsAAP4-1* for each splice variant after treatment with 0.5 mM KNO_3 (N1)

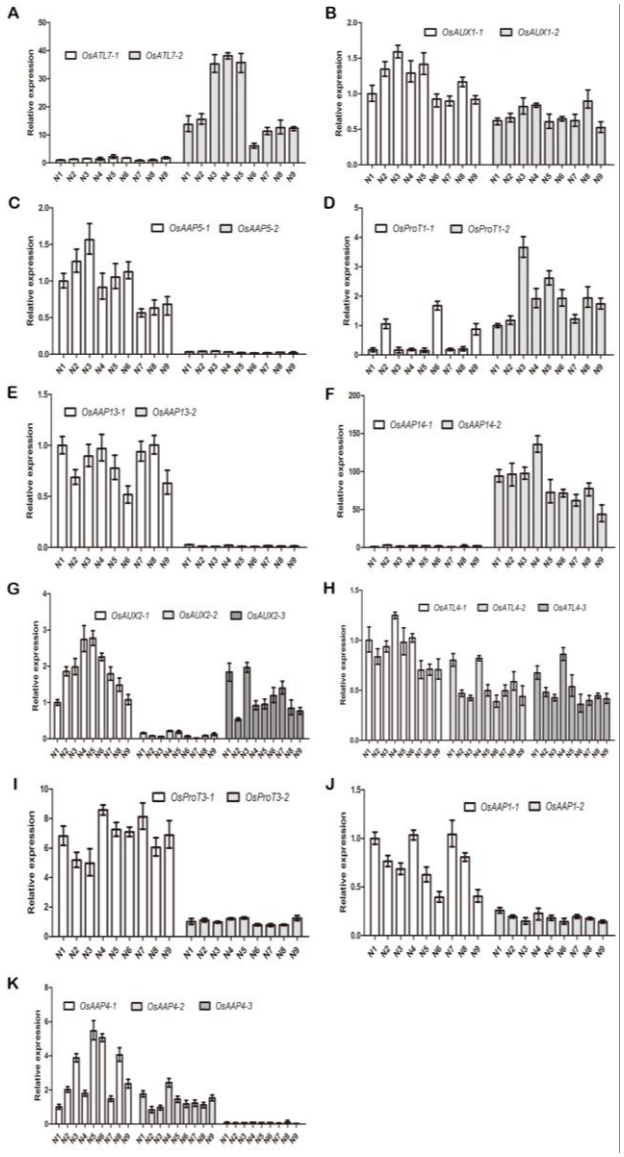

Fig. 6: Leaf expression patterns of *OsAAT* alternative splice variants after different nitrogen treatments. Experimental conditions were the same as those shown Fig. 5. The ratio of relative expression set to 1 was the expression level of *OsATL7-1*, *OsAUX1-1*, *OsAAP5-1*, *OsProT1-2*, *OsAAP13-1*, *OsAAP14-1*, *OsAUX2-1*, *OsATL4-1*, *OsProT3-2*, *OsAAP1-1*, and *OsAAP4-1* for each splice variant after treatment with 0.5 mM KNO_3 (N1)

Acknowledgments

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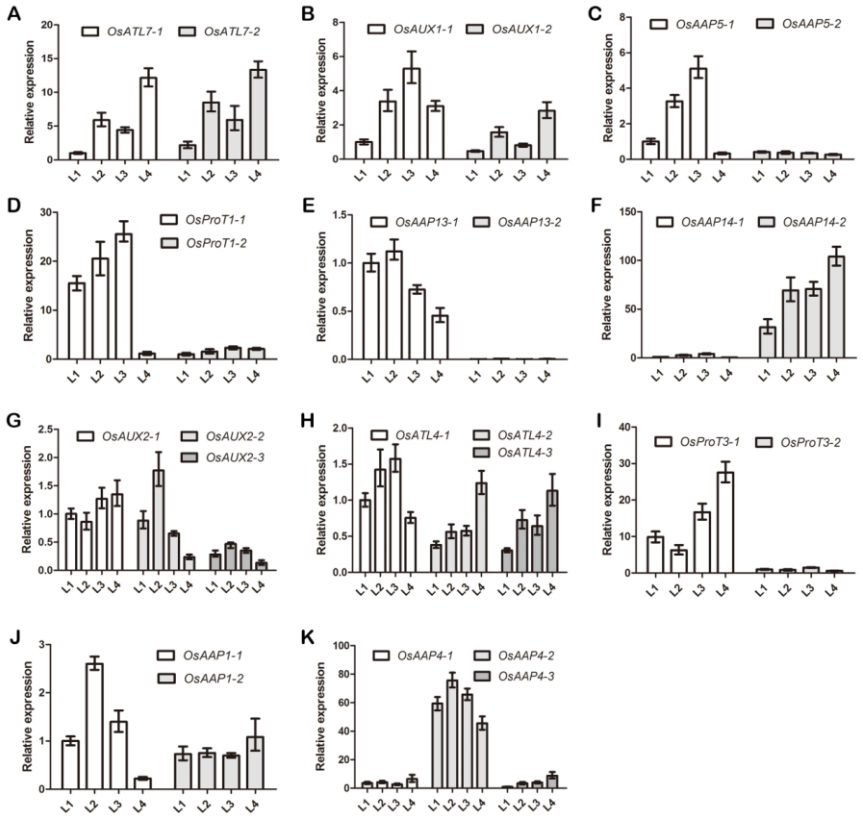

Fig. 7: Leaf expression patterns of *OsAAT* alternative splice variants at different reproductive stages. Flag leaves at booting stage (L1), heading stage (L2), filling stage (L3), and mature stage (L4) of reproductive stage (90 d to 120 d after planting) in a paddy field were harvested for total RNA isolation. Rice *Actin1* gene (AB047313) was used as an internal control. The ratio of relative expression set to 1 was the expression level of *OsATL7-1*, *OsAUX1-1*, *OsAAP5-1*, *OsProT1-2*, *OsAAP13-1*, *OsAAP14-1*, *OsAUX2-1*, *OsATL4-1*, *OsProT3-2*, *OsAAP1-1*, and *OsAAP4-1* for each splice variant at the booting stage (L1)

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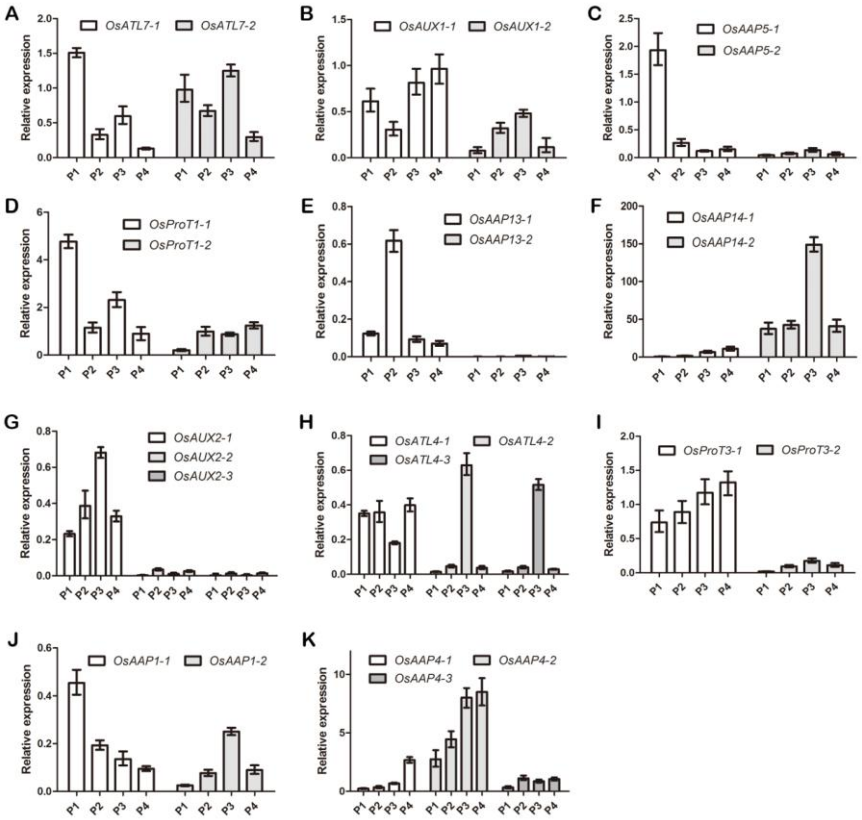

Fig. 8: Panicle expression patterns of *OsAAT* alternative splice variants at different reproductive stages. Panicles at booting stage (P1), heading stage (P2), filling stage (P3), and mature stage (P4) of reproductive stage (90 d to 120 d after planting) in a paddy field were harvested for total RNA isolation. Rice *Actin1* gene (AB047313) was used as an internal control. The ratio of relative expression set to 1 was the expression level of *OsATL7-1*, *OsAUX1-1*, *OsAAP5-1*, *OsProT1-2*, *OsAAP13-1*, *OsAAP14-1*, *OsAUX2-1*, *OsATL4-1*, *OsProT3-2*, *OsAAP1-1*, and *OsAAP4-1* for each splice variant at the booting stage (L1)

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