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Comparative genome analysis of cytokinin biosynthesis genes (IPTS) reveals conserved orthologs cross Poaceae crops

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ABSTRACT

Isopentenyltransferase (*IPT*) catalyzed the synthesis of cytokinin and subsequently played important roles in cytokinin regulatory processes. *IPT* proteins were encoded by small multigene family in *planta*. Poaceae is the most important family for food crops (e. g. rice, maize and wheat). To understand the evolutionary patterns of IPT members in Poaceae crops, we surveyed *IPT* genes from the genomes of four fully sequenced Poaceae (Brachypodium, rice, sorghum and maize) in 2010. The results showed that Poaceae *IPT* genes could be divided into nine ortholog groups. The members from each ortholog group were located in the colinearity chromosome regions cross Poaceae species, and distinct from those of any paralog groups, indicating gene expansions within *IPT* family happened in the common cereal ancestor before the divergence of Poaceae plants. Duplication analysis revealed that the deletion and retention of segmental duplicates were shared by the orthologs cross Poaceae species. Organ-dependent expression patterns and protein properties of members among Poaceae orthologs were also revealed to be similar among the orthologs. These results suggested conserved evolution features of Poaceae *IPT* genes, and further reflected a conserved biological function among the orthologs.

Key words : Cytokinin, evolution, isopentenyltransferase (*IPT*), ortholog, Poaceae

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INTRODUCTION

Cytokinins (CKs), which are chemically N6-substituted purine derivatives, are a class of plant hormone and play a crucial role in many fundamental processes such as shoot and root development, senescence, reproduction and pathogen defense in *planta* (Ashikari *et al.,* 2005; Choi *et al.,* 2011; Qin *et al.,* 2012). Thus, the fine-tuned control of CKs catabolism is required in ensuring the proper regulation of CKs functions. The homeostasis of CKs level in plant cells is mainly regulated by the rates of CKs biosynthesis, which is catalyzed by isopentenyltransferase (*IPT*) enzyme (Mok and Mok, 2001; Frebort *et al.,* 2011; Spichal, 2012).

The CKs biosynthetic protein IPT was firstly identified in plant pathogenic bacterium *Agrobacterium tumefaciens* (Barry *et al.,* 1984), and was further found to present in almost all living organisms including bacteria, yeast, animals and plants (Kakimoto, 2001; Frebort *et al.,* 2011). In green plants, the IPT enzymes were indentified with *de novo* adenylate IPT (EC 2.5.1.27) and tRNA IPT (EC 2.5.1.8) (Takei *et al.,* 2001; Golovko *et al.,* 2002). The adenylate *IPT* adds an isopentenyl group to the N^6 atom of activated adenine (AMP, ADP or ATP), while the tRNA IPT acts to tRNA integrated adenine base.

IPT enzyme was encoded by multigene family with a varying number of members in different plant species. The diverse functions of *IPT* members were reflected by their differences in biochemical characteristics and cellular/subcellular locations (Kasahara *et al.,* 2004). The studies of Arabidopsis *IPT* mutants indicated that adenylate *IPT* was involved in the synthesis of isopentenyladenine and trans-zeatin type CKs, whereas tRNA *IPTs* were required for cis-zeatin type CKs production (Miyawaki *et al.,* 2006). In case of subcellular locations, *AtIPT2* and *AtIPT4* were located in cytosol, while the other IPTs targeted to mitochondria or plastid (Kasahara *et al.,* 2004). Expression profiling revealed that *AtIPT2*

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showed ubiquitously expression manners, while others showed organ-dependant expressions (i. e. *AtIPT1* and *AtIPT3* were abundant in root, while *AtIPT5* was abundant in shoot/leaves) (Takei *et al.,* 2004; Miyawaki *et al.,* 2006; Hirose *et al.,* 2008).

In contrast to the deep knowledge of *IPT* gene functions in Arabidopsis, the molecular basis of cytokinin synthesis in Poaceae plants is rather scarce. Only a few individual *IPT* members have been functionally characterized. For example, 10 *IPT* genes were identified from rice; within which two members (*OsIPT2* and *OsIPT3*) played roles in the formation and maintenance of shoot apical meristem (Sakamoto *et al.,* 2006). In maize, 11 *IPT* genes were identified and the putative functions of *ZmIPT2* in kernel development were reported (Brugiere *et al.,* 2008).

Poaceae is a family in the Class Liliopsida (the monocots) of the flowering plants. Plants of this family are usually called grasses and represent the most important plants of food crops (e. g. wheat, maize and rice). Poaceae plants diverged from a common ancestor ~60 million years ago (Salse *et al.,* 2008), and shared high degrees of colinearity cross their genomes (Bolot *et al.,* 2009). These colinearities provided a framework within which genes function characterized in one species could potentially be transferred to its orthologs in other species. Here, we conducted bioinformatic analyses of the *IPT* gene family in the four fully sequenced grass genomes of rice, Brachypodium, sorghum and maize. The results provided clues about the origin and evolution of *IPTs* in Poaceae plants, and further provided strong evidences to predict their ortholog functions cross Poaceae species.

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MATERIALS AND METHODS

Bioinformatic Identification of *IPT* Genes

The putative proteins or coding sequences of the completely sequenced plants were retrieved from the following database by Blast methods : rice (*Oryza sativa* ssp. *japonica* cv. Nipponbare; release v. 6.1, http://rice.plantbiology.msu.edu/); Brachypodium (*Brachypodium distachyon;* release v. 1.0, Joint Genome Institute, http://modelcop.org/); sorghum (*Sorghum bicolour* cv. Moench; release v. 1.0, http://phytozome.net/) and maize (*Zea may* cv. B 73; release v. 5b.60, http://www.maizesequence.org/). The corresponding sequences of the lower plants moss (*Physcomitrella patens;* release v. 1.2), spikemoss (*Selaginella moellendorfii;* release v. 1.0), single cellular algae (*Chlamydomonas reinhardtii;* release v. 3.1) and multicellular algae (*Volvox carteri;* release v. 1.0) were obtained from the Plaza web site (http://bioinformatics.psb.ugent.be/plaza) or the Joint Genome Institute (www.jgi.doe.gov). The peptide sequences, which were less than 60% of the average peptide length of each family, were considered as pseudogenes or non-functional sequences and neglected from this work.

Phylogenetic Analysis of *IPT* Genes

Multiple sequence alignment was constructed using CLUSTALW method, and the alignment result was then imported into the MEGA 4.0 software to build the phylogenetic tree with the neighbour-joining method (Tamura *et al.,* 2007). To estimate evolutionary distance, the proportions of amino acid differences were computed using Poisson Correction Distance. The pair-wise deletion option was used to circumvent the gaps and missing data. The reliability of different phylogenetic clusters was evaluated by the bootstrap test (1000 bootstrap replications).

Comparative Genome Analysis of *IPT* Genes

Location of the rice whole genome duplications (WGD) (inner-species colinearity) was extracted from http://rice.plantbiology.msu.edu (*Oryza sativa* ssp. *japonica* cv. Nipponbare; release v. 6.1). Macro-colinearity cross cereal genome was extracted from http://chibba.agtec.uga.edu/duplication, which was previously described by Bolot *et al.* (2009). Micro-colinearity for each aimed gene was performed using ±10 genes from either side of the aimed gene as queries for BLAST searches to the genomes of the sequenced grass species.

Prediction of Isoelectric Point and Subcellular Localization of *IPT* Proteins

Isoelectric point (pI) of IPTs was predicted by the Compute pI/Mw solfware provided in http://www.expasy.ch/tools/

pi_tool.html, and the subcellular localization by TargetP solfware in CBS database (http://www.cbs.dtu.dk/services/TargetP).

Expression Analysis of Rice and Maize *IPT* Genes

Maize plants (inbred line B73) were grown in field in summer 2010 and the corresponding tissues were collected for *ZmIPT* gene expression analysis. Roots and leaves of maize seedlings were harvested at five-leaf stage (28-day old plants after germination). Mature tassels (male tissue) and immature ears (female tissue) were collected at silking stage. Embryo and endosperm were separately collected from intact seeds after 3-day germination.

The expression profiles of maize *IPT* genes were examined using quantitative RT-PCR method (qPCR). The PCR reactions were performed using SYBR Green dye, and data were analyzed using 7500 SDS software 1.3 (Applied Biosystems). Expression of the *ZmGAPDH* gene (NM_001111943.1) was served as an internal control for organs specific expression (Zhao *et al.,* 2012). Three biological replicates of qPCR were performed for each sample. The primers for the aimed or control genes were as under :

ZmGAPDH-F : 5'-CTGGTTTCTACCGACTTCCTTG-3',
ZmGAPDH-R : 5'-CGGCATACACAAGCAGCAAC-3';
ZmIPT1-F : 5'-GGTAGATGCTGATCTTCAAGTCCTG-3',
ZmIPT1-R : 5'-CCGCATCATATATGCTGCATACTTC-3';
ZmIPT2-F : 5'-GCTGACTTGGAAGAAGCAGGTGTG-3',
ZmIPT2-R : 5'-GTTCCCTTCTTCAGTTACTGCAACAC-3';
ZmIPT3-F : 5'-CCACGAGCAGCAGGAAAGGTGG-3',
ZmIPT3-R : 5'-CTCGGCTCCCGGGACTCTTGAC-3';
ZmIPT3b-F : 5'-GAGCAGCAGCAGCAGCAAAGGTG-3',
ZmIPT3b-R : 5'-CGGACCTTCGACGCGTCATCAC-3';
ZmIPT4-F : 5'-GTGCGCTCCTTCCTGCGC-3',
ZmIPT4-R : 5'-GTCATTAGTTATCCTGCGACGACAG-3';
ZmIPT5-F : 5'-TGGAGGAGCAAGAGTACAGCAGCAG-3',
ZmIPT5-R : 5'-TCTTCCATTCGCTGAGCTGGTGAG -3';
ZmIPT6-F : 5'-GACGCCACGGAGGTGTTCCTGA-3',
ZmIPT6-R : 5'- CATGCTGCTGTACTCTTGTTGGTC-3';
ZmIPT7-F : 5'-GTGGGAGACGGACGTCGTCAGC-3',
ZmIPT7-R : 5'-CAAGAAACGGTCCTTGTCCTTGTCC-3';
ZmIPT8-F : 5'-GAGCTTCCTTCTAGAGCTGGACG-3',
ZmIPT8-R : 5'-GAAAGTGACAGCAGCTGAGGACC-3';
ZmIPT9-F : 5'-GTAGCAGGATAACGGACGACGAAG-3',
ZmIPT9-R : 5'-CCATCGCACCAAGTAATGGTGTC -3';
ZmIPT10-F : 5'- CCCACAAGAGTTCCTTGACTTCC-3',
ZmIPT10-R : 5'-GAGCCATCGACCCAGTGGTAAATC-3'.

The tissue expression data of *OsIPTs* were extracted from GENEINVESTIGATOR V3 (https://www.genevestigator.ethz.ch). By TMEV software, relative gene expression data were gene-wise normalized by subtraction of the mean level of the selected tissues for each gene, and subsequently displayed with the signal $\log_2$ value.

RESULTS AND DISCUSSION

Phylogeny Analysis of *IPT* Genes in Poaceae

Four sequenced plants (rice, Brachypodium, sorghum and maize) were selected for the comparative genome analysis of *IPT* gene in Poaceae. After searching the most recent rice genome annotation database and maize sequenced database using the published *IPT* proteins as queries (Takei *et al.,* 2001; Sakamoto *et al.,* 2006), none of any novel homologous sequence was found, indicating that all the rice and maize *IPT* members were obtained in previous works and included in this work. We further conducted the similar searches to identify putative *IPTs* from Brachypodium and sorghum, and obtained nine BdIPTs (named from BdIPT1 to BdIPT9) and eight SbIPTs (named from SbIPT1 to SbIPT8) sequences (Table 1). To understand the evolutionary phylogeny of Poaceae *IPT* genes, we also collected the *IPT* members from the dicot plant poplar and Arabidopsis, the basal land plants moss and spikemoss, and the green algae *Chlamydomonas reinhardtii* and *Volvox carteri.* The numbers of *IPT* genes from each selected plant were summarized in Table 2.

Phylogenetic tree of *IPTs* from some sequenced plants had been constructed in previous works (Vyroubalova *et al.,* 2009; Frebort *et al.,* 2011). Here, the sequences of *Selaginella moellendorfii*, Brachypodium and sorghum members were included, and similar phylogenetic tree with several evolutionary groups was found for green plant *IPT* proteins (Fig. 1). Nevertheless, each group from this update tree showed a large evolutionary distance from other groups, indicating an early divergence among the groups. Within these groups, the prokaryotic tRNA *IPT* group were assigned together with those members from algae and lower plants, suggesting the ancient evolutionary origin of the group. Furthermore, the prokaryotic- and eukaryotic tRNA *IPTs* groups were found to uniformly contain one gene member from each higher plant species (Table 2; Fig. 1). By contrast,

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Table 1. *IPT* genes in rice, Brachypodium, sorghum and maize

IPT	Rice			*Brachypoduim distachyon*			Sorghum			Maize		
	Gene name[a]	gene id	Chr (Mbp)	Gene name[b]	Gene id	Chr (Mbp)	Gene name[b]	Gene id	Chr (Mbp)	Gene name[c]	Gene id	Chr (Mbp)
IPTa	*OsIPT1*	OS03G24240	3 (13.80)	*BdIPT1*	BD4G15770	4 (16.41)	*SbIPT1*	SB06G014810	6 (40.98)	*ZmIPT2*	GRMZM2G084462	2 (62.52)
	OsIPT2	OS03G24440	3 (13.92)	*BdIPT2*	BD4G27860	4 (33.17)						
IPTb	*OsIPT3*	OS05G47840	5 (27.36)	*BdIPT3*	BD2G17400	2 (15.46)	*SbIPT2*	SB09G027683	9 (56.74)	*ZmIPT8*	GRMZM2G025429	6 (162.74)
IPTc	*OsIPT4*	OS03G59570	3 (33.90)	*BdIPT4*	BD1G04580	1 (3.07)	*SbIPT3*	SB01G003950	1 (3.22)	*ZmIPT5*	GRMZM2G116878	1(291.56)
										ZmIPT6	AC210013.4_FG005	5 (3.32)
IPTd	*OsIPT5*	OS07G11050	7 (6.09)	*BdIPT5*	BD1G54060	1 (52.43)	*SbIPT4*	SB02G006400	2 (8.02)	*ZmIPT4*	GRMZM2G018046	2 (160.73)
										ZmIPT9	GRMZM2G104559	7 (19.42)
IPTe	*OsIPT6*	OS07G09220	7 (4.83)	*None*			*None*			*None*		
IPTf	*OsIPT7*	OS05G24660	5 (14.20)	*BdIPT6*	BD2G13410	2 (11.88)	*SbIPT5*	SB03G014490	3 (19.74)	*ZmIPT7*	GRMZM2G436770	3 (67.17)
IPTg	*OsIPT8*	OS01G49390	1 (28.40)	*BdIPT7*	BD2G46920	2 (47.32)	*SbIPT6*	SB03G031570	3 (59.96)	*ZmIPT3b*	GRMZM2G415751	3 (207.25)
										ZmIPT3	GRMZM2G393014	8 (151.59)
IPTh	*OsIPT9*	OS01G73760	1 (42.72)	*BdIPT8*	BD1G70200	1 (68.61)	*SbIPT7*	SB03G047160	3 (74.11)	*ZmIPT1*	GRMZM2G097258	3 (145.44)
IPTi	*OsIPT10*	OS06G51350	6 (31.10)	*BdIPT9*	BD1G29490	1 (25.06)	*SbIPT8*	SB10G031125	10 (60.77)	*ZmIPT10*	GRMZM2G102915	6 (81.72)

[a]Gene names of rice *IPT* sequences were based on Sakamoto *et al.* (2006).
[b]Gene names of Brachypodium and sorghum *IPT* sequences were arranged in this work.
[c]Gene names of maize *IPT* sequences were based on Brugiere *et al.* (2008) and Vyroubalova (2009).

Table 2. Number of members of *IPT* identified in the different investigated species

Species	Low-rank	Logogram	*IPT*			
			Prokaryotic tRNA-IPT	Eukaryotic tRNA-IPT	Adenylate *IPT*	Total *IPT*
C. reinhardtii	Chlorophyte	Cr	1	0	0	1
V.carteri	Chlorophyte	Vc	1	0	0	1
P. patens	Bryophyte	Pp	7	0	0	7
S. moellendorffii	Gymnospermae	Sm	2	0	0	2
A. thaliana	Dicot	At	1	1	7	9
P. trichocarpa	Dicot	Pt	1	1	7	9
B. distachyon	Monocot	Bd	1	1	7	9
O. sativa	Monocot	Os	1	1	8	10
S. bicolor	Monocot	Sb	1	1	6	8
Z. mays	Monocot	GRMZM	1	1	9	11

the other group members of *IPTs* from Poaceae plants were grouped into several independent clades distinct from the dicot members, suggesting an early divergence of the monocot and dicot members before the monocot/dicot split.

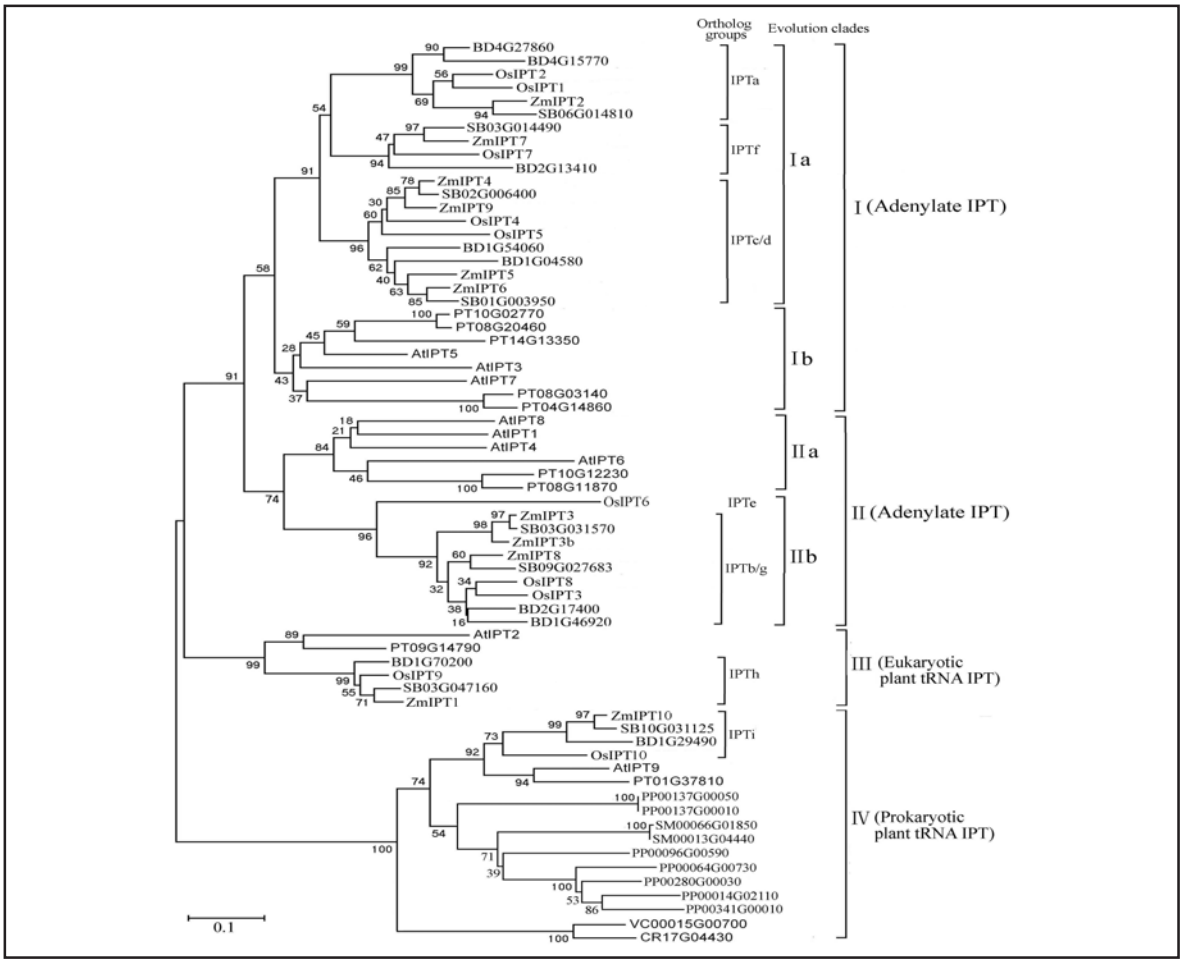

Fig. 1. Phylogenetic tree of *IPT* family genes in plants. Proteins encoded by *IPT* genes in plants were identified by Blastp searches. The phylogenetic tree was generated by protein sequence alignment with ClustalW using the neighbor-joining method with 1000 bootstrap replicates. Plant *IPT* proteins were assigned into four evolutionary groups, and the corresponding clades of each group are indicated at the right. The name of each *Poaceae IPT* ortholog group was also indicated at the right.

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Comparative Genome Analysis of *IPT* Genes Cross Poaceae Species

Because of the early divergence of most Poaceae *IPT* members from the dicot members (Fig. 1), we further focused on the evolution analysis of *IPT* family genes only in Poaceae species. For better expressing the following comparative genome analysis results, we first divided the Poaceae *IPT* genes into several ortholog groups by analyzing the chromosome locations of rice members, and distinguished them by adding a lowercase suffix (Table 1). Our results showed that *OsIPT1* (*Os03G24240*) and *OsIPT2* (*Os03G24440*) were likely to be tandem duplicates, which were located in the adjacent chromosome segments of ~124 kb distance on *Os03*. We then took the tandem duplicates as a gene of two copies, and classed them into the same ortholog group. Thus, we assigned the 10 rice *IPT* genes into nine groups from *IPTa* to *IPTi* according to the published rice gene names (Table 1). In addition, maize genome contained additional whole genome duplication (commonly named allotetraploidization) by comparing to the other three genomes (Wei *et al.,* 2007; Bolot *et al.,* 2009). The allotetraploidization arising *IPT* duplicates were also taken as one gene and assigned into the same group (Table 1).

Cross-species comparison at micro-colinearity level showed that most of the Brachypodium, sorghum and maize *IPT* genes (30 out of the total 38) were mapped to their chromosomes colinearized to the six rice *IPT* gene regions (*IPTb, c, d, e, g* and *i*); and subsequently grouped according to their rice colinearity orthologs (Table 1; Fig. 2). Nevertheless, *IPTh* of rice (*OsIPT9*), sorghum (*SbIPT7*) and maize (*ZmIPT1)* shared the colinear chromosome locations, but their Brachypodium ortholog (*BdIPT8*) was located in Chr. 1 (68,61Mb) instead of the colinearity region (Chr. 2, 58.81Mb) (Fig. 2), indicating that individual gene translocation of *BdIPT8* was happened in Brachypodium genome after its divergence from the common ancestor, probably resulting from the transposon activity (Bennetzen, 2000). Similar individual gene translocation of *IPTa* in rice (*OsIPT1* and *OsIPT2*) and Brachypodium (*BdIPT1 and BdIPT2*) seemed to happen in their genomes after Poaceae species split, because the ortholog in sorghum (*SbIPT1*) and maize (*ZmIPT2*) still showed micro-colinearity locations (Fig. 2).

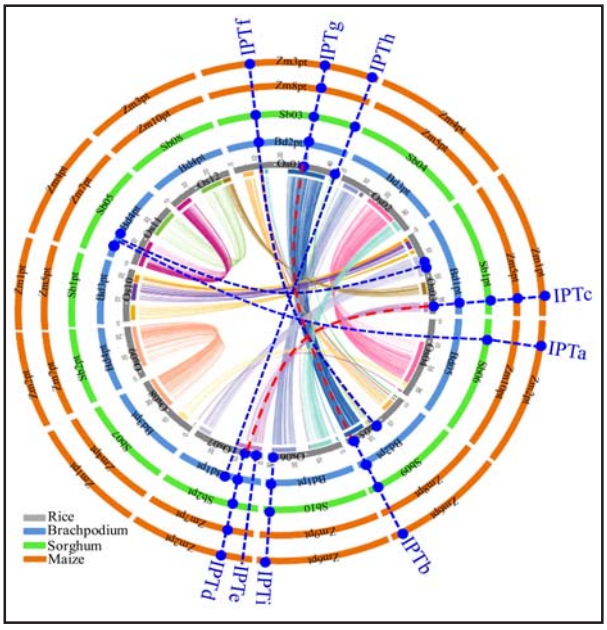

Fig. 2. Diagrammatic representation of *IPT* genes in Poaceae chromosomes. Duplicated segments of the rice genome originating from the ancestral whole genome duplication (WGD) are shown in the centre (adapted from Thiel *et al.,* 2009). The maize, sorghum and Brachypodium chromosomes are represented as concentric circles according to their macro-colinearity to the rice genome (adapted from Bolot *et al.,* 2009). The positions of *IPT* genes are shown with solid dots. Blue lines linked the *IPT* gene orthologs, and red line linked segmental duplication genes arising from WGD.

Gene Expansion of Poaceae *IPT* Family

To investigate the recent expansion of *IPT* genes, we first focused on the duplication events of rice members. Based on the genome assembly (Release v. 6.1), at least two segmental duplications of *OsIPT3/OsIPT8* and *OsIPT4/OsIPT5*, within the ortholog groups of *IPTb/IPTg* and *IPTc/IPTd,* respectively, were revealed to exist on rice chromosomes (Fig. 2), originated from the WGD of the putative ancestral cereal genome ~80 million years ago (Paterson *et al.,* 2004). From the comparative framework for rice, Brachypodium, sorghum and maize (Bolot *et al.,* 2009), the shared segmental duplications of the mentioned two rice gene pairs were extended to the relative chromosomes of the other three Poaceae species (Fig. 2). On the other hand, although *OsIPT1* and *OsIPT2* from *IPTa* ortholog group were located within regions of the intra-specific

duplication of rice genome, no paralogous gene was found in the corresponding chromosomal locations (Fig. 2), indicating one of the duplicates has been deleted (individual gene deletion) during rice genome evolution. By contrast, no duplicated chromosome regions of rice prokaryotic tRNA *IPT* (*IPTi* group, *OsIPT10*) and eukaryotic tRNA *IPT* (*IPTh* group, *OsIPT9*) regions was retained (Fig. 2), indicating that deletion of large genome blocks (block deletion) surrounded these two genes were happened in rice genome. From the comparative framework, the same individual gene deletions of *IPTa* and block deletions of *IPTh and IPTi* have been extended to the other three grasses (Fig. 2).

Gene Expression and Protein Properties of Poaceae *IPT* Genes

Different *IPT* gene members may play diverse functions *in planta* (Takei *et al.,* 2004; Brugiere *et al.,* 2008). To further understand these gene functions in Poaceae plants, we investigated *IPT* genes expression in rice and maize. The data revealed similar expression patterns among the orthologs cross Poaceae species, not only for the absolute expression level (data not shown), but also the organ specific expression manners (Fig. 3). For example, *IPTh* showed the highest expression levels in both rice and maize by comparing to any other paralogs, while *IPTg* showed rather low levels in all selected organs. Furthermore, transcripts of *IPTa* were more abundant in reproductive organs in both rice and maize (Fig. 3). In vegetable tissues, *IPTc and IPTd* showed the highest levels in root and *IPTi* in leaves. Interestingly, *IPT* gene of segmental duplicates showed similar expression patterns. For example, transcripts of *IPTb* and *IPTg* showed constitutive expression manners in plant organs, and *IPTc* and *IPTd* contained organ specific expressions with high RNA levels in root (Fig. 3).

Prediction of *IPT* proteins also revealed the similar isoelectric points (pI) and subcelullar localizations within orthologs cross Poaceae species. Members in *IPTb, c, d* and *g* groups contained alkaline pI and members in *IPTa* contained acid pI in both rice and maize (Table 3). Furthermore, most *IPTs* were predicted to chloroplast, except for a distinguishing mitochondrion localization of

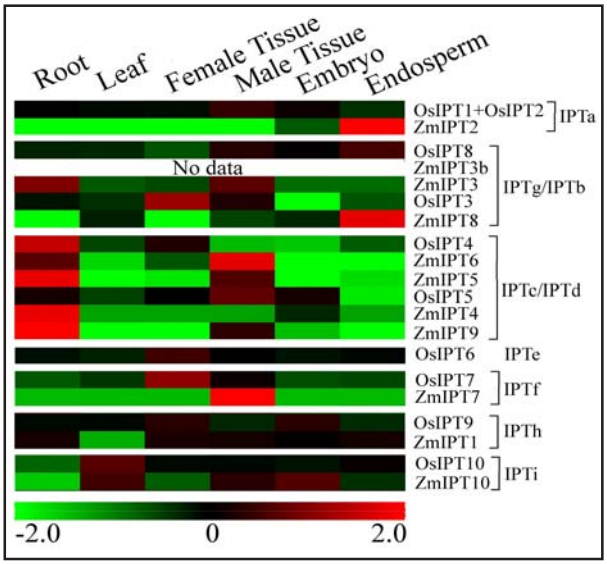

Fig. 3. Expression profiles of rice and maize *IPT* genes in different plant organs. Expression level of maize *IPTs* obtained from qPCR analyses in this work, and that of rice members were extracted from GENEINVESTIGATOR (*www. genevestigator. ethz.ch*). Relative gene expression data were gene-wise normalized by subtraction of the mean level of the six tissues.+represents the additive expression level of the both genes in each side. Colour scale represents the signal $\log_2$ value.

IPTb and *IPTi* members in both rice and maize (Table 3).

Regarding to the presence of cytokinin biosynthesis gene *IPT* in plants has been uncovered for several years (Kakimoto, 2001; Takei *et al.,* 2001; Miyawaki *et al.,* 2006), the function and evolution of *IPT* family genes in Poaceae plants have not been intensively studied yet. Taking the advantages of the well established gene assembles and comparative genome framework of rice, Brachypodium, sorghum and maize (Salse *et al.,* 2008; Bolot *et al.,* 2009), we carried out a comprehensive comparative genome analysis of *IPT* genes cross the Poaceae species. Our results highlighted several evolution features of *IPT* gene family in Poaceae crops.

Evolution and Expansion of Poaceae *IPT* Gene Family

After completion of sequencing of the Arabidopsis and rice genome, the complete set of *IPT* genes from both plants has been collected and assigned into three major groups referred as prokaryotic tRNA-, eukaryotic tRNA- and

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Table 3. Protein properties of rice, Brachypodium, sorghum and maize *IPTs*

Ortholog group	Gene name	Protein properties			[b]Subcellular localization	
		Length (aa)	MW (kD) [a]	pI[a]	Localization	Reliability
IPTa	*OsIPT1*	328	34.48	5.28	Other	0.68
	OsIPT2	325	34.62	5.68	Other	0.83
	BdIPT1	254	27.33	4.47	sp	0.66
	BdIPT2	197	21.21	6.76	Other	0.63
	SbIPT1	340	36.08	4.76	Other	0.55
	ZmIPT2	322	34.49	5.11	sp	0.67
IPTb	*OsIPT3*	360	38.29	7.80	mTP	0.78
	BdIPT3	358	37.11	6.99	cTP	0.75
	SbIPT2	314	32.76	9.23	mTP	0.55
	ZmIPT8	338	40.66	9.17	mTP	0.37
IPTc	*OsIPT4*	357	38.95	8.95	cTP	0.60
	BdIPT4	362	38.62	8.60	cTP	0.69
	SbIPT3	336	36.34	9.08	cTP	0.86
	ZmIPT6	338	36.49	9.11	cTP	0.83
	ZmIPT5	337	36.57	9.64	cTP	0.91
IPTd	*OsIPT5*	347	37.11	8.64	cTP	0.68
	BdIPT5	357	38.70	10.07	cTP	0.50
	SbIPT4	324	35.06	6.86	cTP	0.81
	ZmIPT4	347	37.24	8.30	cTP	0.88
	ZmIPT9	364	39.38	9.05	cTP	0.73
IPTe	*OsIPT6*[c]	235	25.69	6.07	Other	0.69
IPTf	*OsIPT7*	341	36.84	7.17	cTP	0.77
	BdIPT6	333	36.09	9.89	Other	0.77
	SbIPT5	368	39.34	6.26	Other	0.84
	ZmIPT7	352	37.96	5.58	Other	0.80
IPTg	*OsIPT8*	363	38.10	8.70	cTP	0.92
	BdIPT7	363	38.75	8.67	cTP	0.46
	SbIPT6	358	37.72	9.09	cTP	0.84
	ZmIPT3b	354	37.58	9.17	cTP	0.87
	ZmIPT3	348	36.61	9.52	cTP	0.91
IPTh	*OsIPT9*	462	52.07	5.85	cTP	0.57
	BdIPT8	472	52.60	6.59	cTP	0.89
	SbIPT7	469	52.31	6.16	cTP	0.67
	ZmIPT1	470	52.24	6.92	cTP	0.98
IPTi	*OsIPT10*	417	47.00	8.27	mTP	0.85
	BdIPT9	446	50.36	7.70	mTP	0.75
	SbIPT8[c]	340	NT	NT	NT	NT
	ZmIPT10	453	50.88	6.80	mTP	0.92

[a]The molecular weight (MW) and isoelectric point (pI) were predicted with pI/Mw software (http://expasy.org/tools/pi_tool.html).

[b]The subcellular localization/reliability (Loc/Rel) was calculated by TargetP (http://www.cbs.dtu.dk/services/TargetP) and assigned into four types : secretory pathway (sp), mitochondria (mTP), chloroplasmid (cTP) and no predication (other).

[c]Protein properties of *SbIPT8* (*SB10G301125*) were not tested because it was a truncated gene with 5' end missed.

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adenylate *IPT* (Takei *et al.,* 2001; Sakamoto *et al.,* 2006). The founding of prokaryotic tRNA *IPTs* solely presented in genomes of the basal land plant Physcomitrella and Selaginella suggested an ancient evolutionary emergence of this group members in plant (Frebort *et al.,* 2011). Here, our comprehensive phylogenic tree revealed a deep evolution origin of prokaryotic tRNA *IPT* in Poaceae plants, further supporting the most ancient members of *IPTs* in this group in response to cytokinin synthesis (Fig. 1). In addition, the similar constitutive expression patterns of the prokaryotic tRNA *IPT* might suggest that this group members took the function of cytokinin synthesis in whole plants in the early time (Fig. 3). Other plant *IPT* groups showed similar evolution distance to the prokaryotic tRNA *IPT* group than to that of each other groups, indicating that other *IPTs* might evolve individually from the prokaryotic tRNA *IPT* for developing more diverse functions in cytokinin synthesis processes which become more complex in higher plants (Fig. 1). The diverse expression patterns and distinct

subcellular localizations of the other group *IPT*s might also account for the complex cytokinin synthesis processes (Table 3; Fig. 3).

Conserved Function of Poaceae *IPT* Orthologs

Most gene families were expanded in Poaceae plants, partially as a result of the WGD (Shiu *et al.,* 2005; Feller *et al.,* 2011). The maize gene families could be further expanded by the genome allotetraploidization (Wei *et al.,* 2007; Bolot *et al.,* 2009). However, the members of *IPT* family in Poaceae did not considerably extend and remain constant to 8-11 members (Table 2), suggesting that the chromosome-specific genome rearrangements resulted in the deletion of most *IPT* duplicates. Most of *IPT* gene duplicates in Poaceae end up with paralogs to maintain a constant gene number, suggesting that *IPT* genes accounted for the important and conserved biological processes, since plants can be tolerant to a much higher gene dosage effect as compared to animals. The conserved biological functions of cytokinin dehydragenase (CKX) and cytokinin receptor (CHASE domain proteins, the first step for cytokinin signaling transduction) were also reflected by the constant family members within plant kingdom (Pils and Heyl, 2009; Gu *et al.*, 2010; Mameaux *et al.,* 2012; Tsai *et al.,* 2012).

The conserved biological functions of *IPT* genes could also be reflected by the conserved evolution features of the orthologs cross Poaceae plants. Firstly, the colinearity location among orthologs (Fig. 2) suggested that the orthologous members cross Poaceae were evolved from a common ancestor and might maintain the similar functions, since orthologs typically retained the similar functions (Tatusov *et al.,* 1997). Secondly, the same retention and deletion of the segmental duplicates in Poaceae plants (Fig. 2) indicated not only that the segmental duplication and deletion of *IPT*s happened in the ancestral cereal genome, and also that each Poaceae plants conservatively evolved these duplications and deletions. Thirdly, the similar gene expression patterns and protein properties between rice and maize orthologs (Table 3; Fig. 3) further indicated a conserved function of each ortholog cross Poaceae species.

Comparative phylogenetic analysis based on sequence similarity is a powerful method to identify homologous genes and to predict their physiological functions in target species. In the future, the cloning and characterization of *IPT* homologs from Poaceae genomes will be promoted due to the next generation of genome sequencing technologies (Holt *et al.,* 2008). However, the functional annotation of these genes and their respective products in some Poaceae plants, such as the important crops wheat and barley will be still difficult due to the rare of genetic materials and the difficulty of plant transformation. Our comparative analysis indicated a relatively conserved gene function of *IPT* orthologs cross Poaceae species. Thus, the molecular function of *IPT* orthologs can be intensively studied in the model species in which genetic tools are much more readily available. Subsequently, the results from the model species can be readily translated to other Poaceae species based on such comparative genome analysis.

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REFERENCES

Ashikari, M., Sakakibara, H., Lin, S., Yamamoto, T., Takashi, T., Nishimura, A., Angeles, E. R., Qian, Q., Kitano, H. and Matsuoka, M. (2005). Cytokinin oxidase regulates rice grain production. *Science* **309** : 741-45.

Barry, G. F., Rogers, S. G., Fraley, R. T. and Brand, L. (1984). Identification of a cloned cytokinin biosynthetic gene. *Proc. Natl. Acad. Sci. USA* **81** : 4776-80.

Bennetzen, J. L. (2000). Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol.* **42** : 251-69.

Bolot, S., Abrouk, M., Masood-Quraishi, U., Stein, N., Messing, J., Feuillet, C. and Salse, J. (2009). The 'inner circle' of the cereal genomes. *Curr. Opin. Plant Biol.* **12** : 119-25.

Brugiere, N., Humbert, S., Rizzo, N., Bohn, J. and Habben, J. E. (2008). A member of the maize isopentenyl transferase gene family, *Zea mays* isopentenyl transferase 2

(ZmIPT2), encodes a cytokinin biosynthetic enzyme expressed during kernel development. Cytokinin biosynthesis in maize. *Plant Mol. Biol.* **67** : 215-29.

Choi, J., Choi, D., Lee, S., Ryu, C. M. and Hwang, I. (2011). Cytokinins and plant immunity : old foes or new friends? *Trends Plant Sci.* **16** : 388-94.

Feller, A., Machemer, K., Braun, E. L. and Grotewold, E. (2011). Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant J.* **66** : 94-116.

Frebort, I., Kowalska, M., Hluska, T., Frebortova, J. and Galuszka, P. (2011). Evolution of cytokinin biosynthesis and degradation. *J. Exp. Bot.* **62** : 2431-52.

Golovko, A., Sitbon, F., Tillberg, E. and Nicander, B. (2002). Identification of a tRNA isopentenyltransferase gene from *Arabidopsis thaliana*. *Plant Mol. Biol.* **49** : 161-69.

Gu, R., Fu, J., Guo, S., Duan, F., Wang, Z., Mi, G. and Yuan, L. (2010). Comparative expression and phylogenetic analysis of maize cytokinin dehydrogenase/oxidase (CKX) gene family. *J. Plant Growth Regul.* **29** : 428-40.

Hirose, N., Takei, K., Kuroha, T., Kamada-Nobusada, T., Hayashi, H. and Sakakibara, H. (2008). Regulation of cytokinin biosynthesis, compartmentalization and translocation. *J. Exp. Bot.* **59** : 75-83.

Holt, K. E., Parkhill, J., Mazzoni, C. J., Roumagnac, P., Weill, F. X., Goodhead, I., Rance, R., Baker, S., Maskell, D. J. and Wain, J. (2008). High-throughput sequencing provides insights into genome variation and evolution in *Salmonella typhi*. *Nat. Genet.* **40** : 987-93.

Kakimoto, T. (2001). Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate : ATP/ADP isopentenyl-transferases. *Plant Cell Physiol.* **42** : 677-85.

Kasahara, H., Takei, K., Ueda, N., Hishiyama, S., Yamaya, T., Kamiya, Y., Yamaguchi, S. and Sakakibara, H. (2004) Distinct isoprenoid origins of cis- and trans-zeatin biosyntheses in Arabidopsis. *J. Biol. Chem.* **279** : 14049-54.

Mameaux, S., Cockram, J., Thiel, T., Steuernagel, B., Stein, N., Taudien, S., Jack, P., Werner, P., Gray, J. C., Greenland, A. J. and Powell, W. (2012). Molecular, phylogenetic and comparative genomic analysis of the cytokinin oxidase/dehydrogenase gene family in the Poaceae. *Plant Biotech. J.* **10** : 67-82.

Miyawaki, K., Tarkowski, P., Matsumoto-Kitano, M., Kato, T., Sato, S., Tarkowska, D., Tabata, S., Sandberg, G. and Kakimoto, T. (2006). Roles of Arabidopsis ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. *Proc. Natl. Acad. Sci. USA* **103** : 16598-16603.

Mok, D. W. and Mok, M. C. (2001). Cytokinin metabolism and action. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52** : 89-118.

Paterson, A. H., Bowers, J. E. and Chapman, B. A. (2004). Ancient polyploidization predating the divergence of the cereals, and its consequences for comparative genomics. *Proc. Natl. Acad Sci., USA* **101** : 9903-08.

Pils, B. and Heyl, A. (2009). Unraveling the evolution of cytokinin signaling. *Plant Physiol.* **151** : 782-91.

Qin, H., Gu, Q., Zhang, J., Sun, L., Kuppu, S., Zhang, Y. and Burow, M. (2012). Regulated expression of an isopentenyltransferase gene (*IPT*) in peanut significantly improves drought tolerance and increases yield under field conditions. *Plant Cell Physiol.* **52** : 1904-14.

Sakamoto, T., Sakakibara, H., Kojima, M., Yamamoto, Y., Nagasaki, H., Inukai, Y., Sato, Y. and Matsuoka, M. (2006). Ectopic expression of KNOTTED1-like homeobox protein induces expression of cytokinin biosynthesis genes in rice. *Plant Physiol.* **142** : 54-62.

Salse, J., Bolot, S., Throude, M., Jouffe, V., Piegu, B., Quraishi, U. M., Calcagno, T., Cooke, R., Delseny, M. and Feuillet, C. (2008). Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *Plant Cell* **20** : 11-24.

Shiu, S. H., Shih, M. C. and Li, W. H. (2005). Transcription factor families have much higher expansion rates in plants than in animals. *Plant Physiol.* **139** : 18-26.

Spichal, L. (2012). Cytokinins – Recent news and views of evolutionally old molecules. *Funct. Plant Biol.* **39** : 267-84.

Takei, K., Sakakibara, H. and Sugiyama, T. (2001). Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme in *Arabidopsis thaliana*. *J. Biol. Chem.* **276** : 26405-10.

Takei, K., Ueda, N., Aoki, K., Kuromori, T., Hirayama, T., Shinozaki, K., Yamaya, T. and Sakakibara, H. (2004). AtIPT3 is a key

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determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis. *Plant Cell Physiol.* **45** : 1053-62.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4 : Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.* **24** : 1596-99.

Tatusov, R. L., Koonin, E. V. and Lipman, D. J. (1997). A genomic perspective on protein families. *Science* **278** : 631-37.

Tsai, Y. C., Weir, N. R., Hill, K., Zhang, W., Kim, H. J., Shiu, S. H., Schaller, G. E. and Kieber, J. J. (2012). Characterization of genes involved in cytokinin signaling and metabolism from rice. *Plant Physiol.* **158** : 1666-84.

Vyroubalova, S., Vaclavikova, K., Tureckova, V., Novak, O., Smehilova, M., Hluska, T., Ohnoutkova, L., Frebort, I. and Galuszka, P. (2009). Characterization of new maize genes putatively involved in cytokinin metabolism and their expression during osmotic stress in relation to cytokinin levels. *Plant Physiol.* **151** : 433-47.

Wei, F., Coe, E., Nelson, W., Bharti, A. K., Engler, F., Butler, E., Kim, H., Goicoechea, J. L., Chen, M. and Lee, S. (2007). Physical and genetic structure of the maize genome reflects its complex evolutionary history. *PLoS Genet.* **3** : e123.

Zhao, L., Liu, T., An, X. and Gu, R. (2012). Evolution and expression analysis of the β-glucosidase (GLU) encoding gene sub-family in maize. *Genes Genom.* **34** : 179-87.

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