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Article

Genome-Wide Analysis of Oligopeptide Transporters Gene Family in *Medicago truncatula*

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Abstract: Oligopeptide transporters (OPTs) are a group of membrane localized proteins that have a broad range of substrate transport capabilities and contribute to numerous biological processes. However, limited information has been reported on OPTs in higher plants. In this study, a comprehensive analysis of the OPT gene family in *Medicago truncatula* was performed. A total of 26 OPT genes (*MtOPT01-MtOPT26*) have been identified in the *Medicago truncatula* genome. Phylogenetic analyses indicated that *MtOPTs* consisted of two distinct subgroups, 12 *MtOPTs* belonged to the peptide transport subgroup (PT-OPT) based on their predicted amino acid sequences containing the two highly conserved motifs (NPG and KIPPR) and 14 *MtOPTs* belonged to yellow stripe subgroup (YS-OPT). The *MtOPT*s distributed on each of 8 chromosomes in *Medicago truncatula*. Sequence analysis verified that *MtOPT*s significant similar to those in other plants. The copy number of *MtOPTs* was low and the multiply of *MtOPTs* was simple relatively. Gene structure analysis showed that most of the *MtOPT*s have various numbers of introns. The multiple of *MtOPT*s might play different biological roles which were supported by the fact that *MtOPT*s have a distinct tissue-specific expression pattern. The data obtained in this study will help to better understand the complexity of the *MtOPT*s gene family and provide new evidence for the function and evolution of the *OPT* gene family in higher plants.

Keywords: oligopeptide transporters (OPTs); *Medicago truncatula*; phylogenetic analysis; gene express

INTRODUCTION

The material transport of the organism is tightly related to biosynthesis, cell communication, nutrition metabolism, pathogen defense and a variety of physiological or biochemical activities [1]. Transport protein has the function of specifically carrying the molecules which is essential for growth and development and regulating the distribution of substance [2-4]. Peptide transport involves in the translocation of peptides (2–6 residues in length) across the cellular membrane with an energy- dependent manner. The OPT family have been found in bacteria, archaea and plants. OPT is a kind of peptide transport protein with transport various substrates ability belong to one of the three peptide transporter protein families in plant. Multiple OPTs have been identified in variety plant genome including Arabidopsis, rice, maize, poplar, grape, *thlaspi caerulescens* and *brachypodium distachyon*, from which OPTs in Arabidopsis and rice were well characterized [5-7]. Previous studies showed that the evolution relationship is very close among these OPTs in plant. According to the difference of protein motifs, OPT family divided into two divergent subgroups, the yellow stripe (YS-OPT) and the peptide transport (PT-OPT). PT-OPT shared the highly conserved motifs of NPG (Asn-Pro-Gly; encompassing a stretch of 18 amino acids) and KIPPR (Lys-Ile-Pro-Pro-Arg; 11–14 amino acids) while YS-OPT contains no [8].

In addition to peptide transport, OPTs have abilities to transport glutathione derivatives and metal complexes, and may be involved in stress resistance. Previous studies suggest distinct cellular roles for PT-OPTs, including nitrogen mobilization, seed germination, pollen, ovule and embryo development, glutathione transport, seed formation and metal transport [1, 9, 10]. Seven members of the *AtOPT* family transport tetra-and pentapeptides, presumably with proton cotransport [8]. *AtOPT3* appears to play a key role in early embryo development following fertilization. *BjGT1* (an OPT member from Indian mustard), *OsGT1* (an OPT homologs in rice) and *AtOPT6* transport glutathione derivatives and are induced by primisulfuron. These OPTs enable to uptake a non-protein-derived tripeptide glutathione (GSH and GSSG) and glutathione derivatives (GS conjugates) [11-13]. Heterologous expression in yeast indicates that AtOPT3 transport copper, manganese and iron [12]. In root tissue, *AtOPT2* express response to iron and zinc deficiency and *AtOPT3* response to iron, copper and manganese deficiency [9, 10]. *AtOPT6* and *AtOPT7* transport cadmium or cadmium–glutathione conjugates [13]. Arabidopsis PT-OPTs express in dividing areas of the plant. Furthermore, the expression of *AtOPT6* is sensitive to the herbicide primisulfuron to abscisic acid (ABA). AtOPT6 transport glutathione derivatives and is induced by primisulfuron. The expression of *AtOPT2* is induced by salt stress [11, 14]. The PT Clade of OPT was characterized in Rice. OsOPT1, OsOPT3, OsOPT4, OsOPT5, and OsOPT7 transport ferrous and/or ferric iron chelated to nicotianamine (NA), a nonpeptidyl metal-chelate. The expression of the *OsOPTs* was verified in roots, stems, leaves, hulls, pedicels, and embryos with different patterns [1]. Altogether, data showed that the substrate specificity and the physiological functions of OPTs may be diverse.

The YS subgroup includes the YS1 in *Zea mays* and yellow stripe–like (YSL) genes, described in Arabidopsis, rice [15-17]. Graminaceous plants acquire iron by secreting mugineic acid family phytosiderophores (PS) into the rhizosphere and taking up complexes of iron and PS. YS subfamily has capacity of transport metal chelated by NA or mugineic lysergic acid [16-19]. The first identified YSL transporter, ZmYS1 transport Fe^{3+}-PS and Fe^{2+}-NA complexes [17, 20]. The members similar to *ZmYS1* have been found in *Arabidopsis thaliana* and *Oryza sativa* genomes. Functional analysis showed that some YSL members also involved in the transport and homeostasis of other transition metals such as Zn, Ni, Mn and Cu [20, 21].

YSL members were different in their expression patterns, localization, and transport substrate specificity [22-24]. OsYSL2 transports Fe (II)-NA complex and Mn-NA complex [18]; OsYSL15 transport the Fe (III)-PS complex [23]. OsYSL18 transports Fe (III)-deoxymugineic acid (DMA) [25]. Different from other YSL members, OsYSL6 is required for tolerance to excess Mn in rice [26]. The expression of *HvYSL5* is strongly induced in the root by Fe deficiency and show diurnal rhythm [27]. AtYSL also transport Fe (III)-PS in yeast, although Arabidopsis does not synthesize PS. AtYSL1 and 3 are responsible for the mobilization of micronutrients such as Mn, Zn, Cu, and Fe from leaves and for loading of Fe-NA into the seeds [17, 28]. A study showed that AtYSL1 and 3 are able to transport Fe (II)-NA [29]. AtYSL2 transport Cu-NA and Fe (II)-NA complexes in yeast [15-17]. TcYSL3 of hyperaccumulator, *Thlaspi caerulescens*, is involved in the entry of Ni-NA into the symplastic transport in the roots for delivery to the xylem and unloading of the Ni-NA complexes from the xylem in the leaves [19]. All these findings indicated that YSLs had diverse functions in transporting of various metals. Both YS-OPT and PT-OPT involved in different aspects of metal transport which narrowed these two subfamilies [9].

Nitrogen, iron and phosphorus are the three nutrients most commonly limiting plant growth. OPTs appear to play important roles in acquisition of two of these key nutrients from the environment and subsequent transport around the plant [9]. However, compared with prokaryotes and animals, peptide transport received little attention in higher plants [30]. Medicago is an important forage crop which has high protein content and strong resistance. Although OPTs play important roles in the nitrogen utilization and metal transfer, its function in Medicago still did not clarify. The whole genome sequencing *of Medicago truncatula*, a model plant of leguminous spices in genomics research with a small genome, has completed [31]. Several gene families such as MAPKKK, WRKY were analyzed at genome-wide in *Medicago truncatula* [32, 33]. To provide basis for the research on the function of OPT in legume and conduce better understanding the transport of nutrition and metal in plant, genome-wide bioinformatics analysis were performed on the OPT family of *Medicago truncatula* in this study, The data will shed new light on the possible roles of the *MtOPT* family.

METHODS

Data Source of *Medicago truncatula*

Whole-genome sequencing data of *Medicago truncatula*, including gene transcription sequences, protein sequences, CDS sequences and their annotations (Version: Mt4.0v1), is from the JCVI [34]. (http://www.jcvi.org/medicago)

The identification and classification of excavated OPT genes

MtOPT genes domain information（PF02669）was downloaded by Pfam database [35] and protein sequence of *Medicago truncatula* (Operating parameters: -E0.01) was searched by HMMER (V3.0) software [36]. Sequence alignment between the excavated genes and OPT genes of Arabidopsis were conduct. *MtOPT* genes were classified according to the reported classification information of Arabidopsis OPT family. The isoelectric point, molecular weight and other sequences basic nature were analyzed by ExPAVY online ProtParam tool [37]. According to genome and cDNA sequence of *Medicago truncatula*, structural mapping and analysis of *MtOPT* gene family were conducted using GSDS online tool [38].

Phylogenetic analysis of *MtOPT* genes

Multiple sequence alignment with protein sequence *MtOPT* gene family were conducted by ClustalW2 software [39]. MEGA6.0 software [40] was used to analyze *MtOPT genes, AtOPT* genes (*Arabidopsis thaliana*) *and OsOPT* genes (*Oryza sativa*). Phylogenetic parameters are as follows. Constructing Method: Neighbor (neighbor-jioning, NJ); Genetic distance: Poisson distribution; Sampling frequency: 1000 (bootstrap: 1000 replication).

The chromosomal localization of *MtOPT* genes

Pairwise comparison analysis was according to *Medicago truncatula* genome sequence and CDS sequences obtained by BLAST online tools [41]. Gene duplication between each two *MtOPT* genes were defined when the consistency more than 85%. Combined with location information of MtOPT genes in *Medicago truncatula* genome, the CIRCOS software [42] was used to draw chromosomal localization analysis diagram.

Gene expression analysis of *MtOPT* genes

TopHat [43] and Cufflink [44] were used to analyze the gene expression of *MtOPT* morphology in dividing areas (roots, nodules, leaf, bud, carpel and flowers), according to the obtained transcriptome sequencing data of *Medicago truncatula*, SRA database of NCBI (http://www.ncbi.nlm.nih.gov, accession numbers: SRR350517-S350521, SRR350538 and SRR349692). Ggplot2 of R software (Version 3.1.0) were used for *MtOPT* gene expression cluster analysis.

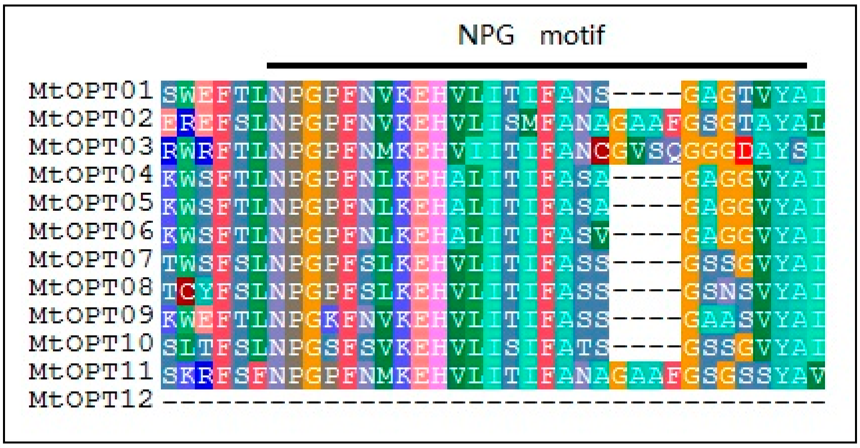
RESULTS

Identification of 26 *MtOPT* genes in *Medicago truncatula* genome

A total of 26 putative OPT gene homologues were identified in the *Medicago truncatula* genome by Pfam database and HMMER (V3.0) (Table 1). The predicated MtOPT proteins were confirmed by the OPT domains. All of the 26 putative MtOPT homologous belonged to the OPT gene family determined by OPT domain (SPYxEVRxxVxxxDDP) [45]. The amino acid number of most MtOPTs are from 552 (MtOPT12) to 773(MtOPT07) except that which of MtOPT25, 23 and 26 are 295, 235, and 123, respectively. All of the MtOPT proteins are hydrophobic with molecular weights range from 13 (MtOPT26) to 86 (MtOPT07) KDa. The isoelectric point (IP) of these amino acid ranged from 4.67 (MtOPT26) to 9.33 (MtOPT24). In accordance with the peptide transport (PT) clade contains the NPG and KIPPR motifs, 12 *MtOPT*s were clustered into PT subfamily and 14 *MtOPT*s were clustered into YS subfamily. The structure of NPG and KIPPR motifs in PT subfamily genes was shown in Figure 1 and 2.

Table 1 The26 MtOPT genes identified in the *Medicago truncatula* genome

Gene	Gene locus	AA	Molecular weight/D	Isoeletric point	Group
MtOPT01	Medtr3g080870	737	82900.8	7.51	PT
MtOPT02	Medtr3g111350	726	81794.9	8.93	PT
MtOPT03	Medtr4g133968	741	83411.2	9.25	PT
MtOPT04	Medtr5g096250	746	83568.9	8.91	PT
MtOPT05	Medtr5g096200	752	84063.5	8.55	PT
MtOPT06	Medtr5g096230	754	84231.8	8.95	PT
MtOPT07	Medtr7g092240	773	86408.0	7.92	PT
MtOPT08	Medtr7g092230	752	84040.4	8.85	PT
MtOPT09	Medtr8g061090	739	82596.7	8.27	PT
MtOPT10	Medtr7g092250	773	86273.2	8.93	PT
MtOPT11	Medtr4g092960	718	80399.8	8.85	PT
MtOPT12	Medtr2g009980	552	62203.8	8.99	PT
MtOPT13	Medtr5g091600	682	74437.7	8.96	YS
MtOPT14	Medtr3g063520	690	75447.8	8.92	YS
MtOPT15	Medtr6g077870	712	79020.3	8.66	YS
MtOPT16	Medtr3g063490	696	76218.4	8.89	YS
MtOPT17	Medtr3g092090	664	72805.7	8.78	YS
MtOPT18	Medtr0312s0020	673	74225.9	8.60	YS
MtOPT19	Medtr1g007540	673	74225.9	8.60	YS
MtOPT20	Medtr7g028250	679	74329.2	5.87	YS
MtOPT21	Medtr1g007580	673	73911.8	8.47	YS
MtOPT22	Medtr1g077840	680	75064.7	8.97	YS
MtOPT23	Medtr1626s0010	235	26735.3	8.53	YS
MtOPT24	Medtr4g133130	564	63088.9	9.33	YS
MtOPT25	Medtr5g091615	295	32929.5	9.21	YS
MtOPT26	Medtr0312s0050	123	13659.9	4.67	YS



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MtOPT01 SWEFTLNPGPFNVKEHVLITIFANS----GAGTVYAI
MtOPT02 EREFSLNPGPFNVKEHVLISMFANAGAAFGSGTAYAL
MtOPT03 RWRFTLNPGPFNMKEHVIITIFANCGVSQGGGDAYSI
MtOPT04 KWSFTLNPGPFNLKEHALITIFASA----GAGGVYAI
MtOPT05 KWSFTLNPGPFNLKEHALITIFASA----GAGGVYAI
MtOPT06 KWSFTLNPGPFNLKEHALITIFASV----GAGGVYAI
MtOPT07 TWSFSLNPGPFSLKEHVLITIFASS----GSSGVYAI
MtOPT08 TCYFSLNPGPFSLKEHVLITIFASS----GSNSVYAI
MtOPT09 KWEFTLNPGKFNVKEHVLITIFASS----GAASVYAI
MtOPT10 SLTFSLNPGSFSVKEHVLISIFATS----GSSGVYAI
MtOPT11 SKRFSFNPGPFNMKEHVLITIFANAGAAFGSGSSYAV
MtOPT12 -------------------------------------
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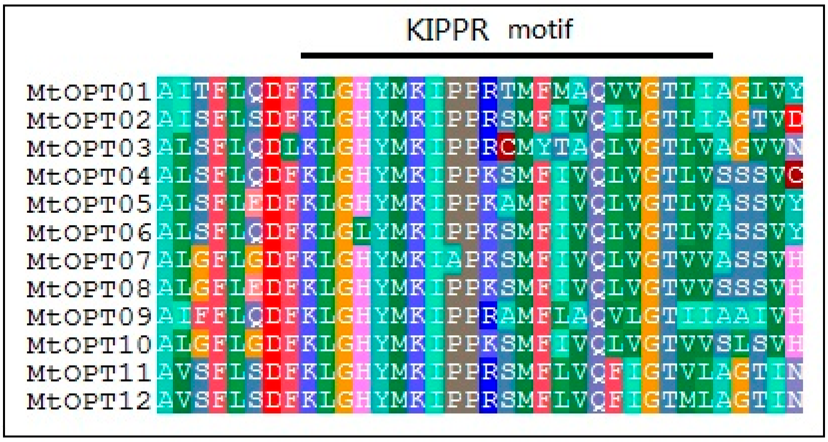
Fig.1 The NPF motifs of PT subfamily genes in MtOPT gene family



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MtOPT01 AITFLQDFKLGHYMKIPPRTMFMAQVVGTLIAGLVY
MtOPT02 AISFLSDFKLGHYMKIPPRSMFIVQILGTLIAGTVD
MtOPT03 ALSFLQDLKLGHYMKIPPRCMYTAQLVGTLVAGVVN
MtOPT04 ALSFLQDFKLGHYMKIPPKSMFIVQLVGTLVSSSVC
MtOPT05 ALSFLEDFKLGHYMKIPPKAMFIVQLVGTLVASSVY
MtOPT06 ALSFLQDFKLGLYMKIPPKSMFIVQLVGTLVASSVY
MtOPT07 ALGFLGDFKLGHYMKIAPKSMFIVQLVGTVVASSVH
MtOPT08 ALGFLEDFKLGHYMKIPPKSMFIVQLVGTVVSSSVH
MtOPT09 AIFFLQDFKLGHYMKIPPRAMFLAQVLGTIIAAIVH
MtOPT10 ALGFLGDFKLGHYMKIPPKSMFIVQLVGTVVSLSVH
MtOPT11 AVSFLSDFKLGHYMKIPPRSMFLVQFIGTVLAGTIN
MtOPT12 AVSFLSDFKLGHYMKIPPRSMFLVQFIGTMIAGTIN
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Fig. 2 The KIPPR motifs of PT subfamily genes in MtOPT gene family

Phylogenetic relationship of *MtOPT* genes

To further analysis the phylogenetic relationship, *MtOPT* gene family was compared with the *OPT* gene families of *Arabidopsis thaliana* and *Oryza sativa*. The comparison result supported the taxonomic status and the classification of the *MtOPT* gene family (Figure 3). The sequence of *MtOPT* gene family was highly conservative and consistent with the classification of At*OPT and OsOPT* gene families. Phylogenetic analysis showed genetic independence between PT and YS subfamilies, while the evolutionary relationship existed cross at *MtOPT24*. The sequence conservation in YS subfamily genes was higher than that in PT subfamily. The *MtOPT* genes could be further classified into 5 sections (I, II, III, IV and V). As Figure 4 shown, *MtOPT* 01, 02, 09, 11 and 12 were grouped into I; *MtOPT* 04, 05, 06, 07, 08 and 10 into II; *MtOPT* 03 and 24 into III; *MtOPT* 13, 14, 15, 16 and 25 into IV, and *MtOPT* 17, 18, 19, 20, 21, 22, 13 and 16 were grouped into V.

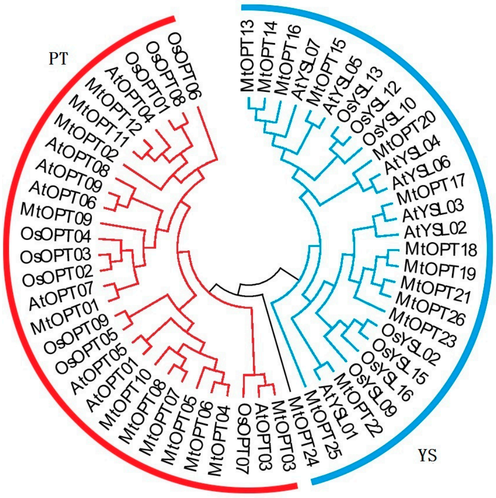

Fig. 3 The unrooted dendrogram of *MtOPT*, *AtOPT* and *OsOPT* gene family

The gene structure of *MtOPT*s

To obtain more information on the genomic organization of *MtOPT* genes, the intron boundary for the 26 *MtOPT*s were determined. The structure of *MtOPT*s was shown in Figure 4. Most of *MtOPTs* have introns except for *MtOPT26*, the shortest *MtOPT* gene. Introns in others *MtOPT* genes were from 1 to 8, and normally from 4 to 6. The most of 11 introns were found in *MtOPT24*. The number and length of introns were various in the whole *MtOPT* gene family, despite identical and similar in the genes belonging to the same subgroup.

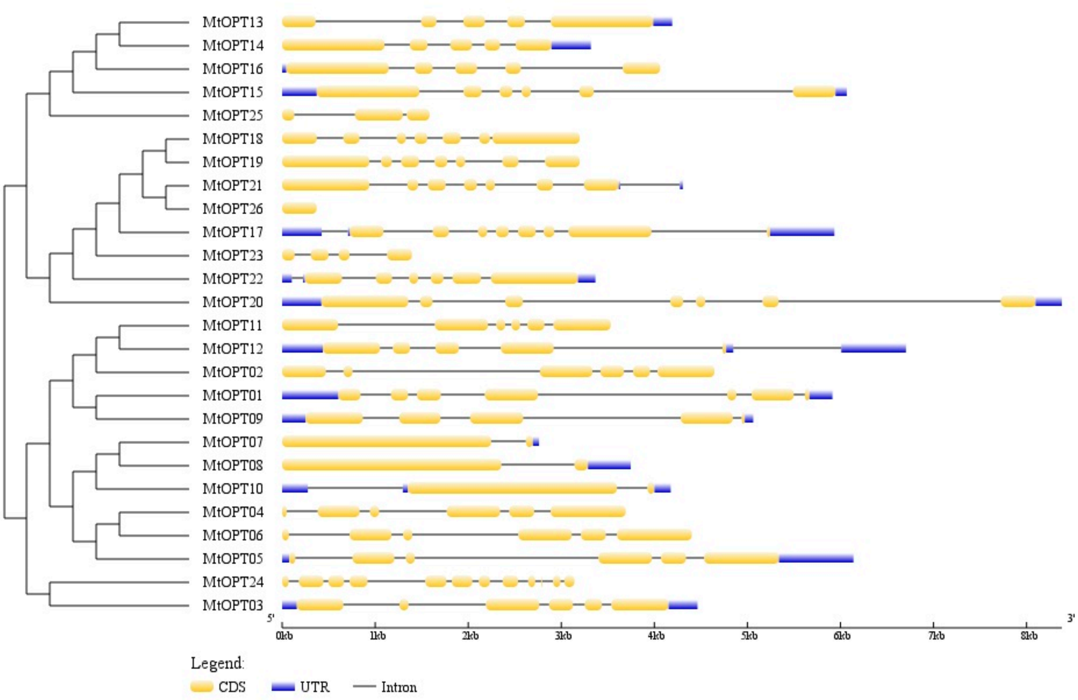

Fig. 4 The gene structure of *OPT* family genes in *Medicago truncatula*

Chromosome Location and Gene Duplication of *MtOPT* genes

The 26 *MtOPT* genes are distributed on 8 chromosomes of *Medicago truncatula* (Figure 5). From 1 to 5 *OPT* genes are distributed on every chromosome of *Medicago truncatula* without packing effect. Each of 5 *MtOPT* genes localized on chromosome 3 and 5; four *MtOPT* genes localized on chromosome 7 and three localized on chromosome 4; each of only 1 *MtOPT* gene localized on chromosome 1, 2 and 8. Multiple copy of *MtOPT* genes was found by gene sequences Pairwise alignments. In the PT subfamily, gene duplication was found in *MtOPt04*, *05* and *06* (chromosome 5) *and MtOPT07*, *08* and *10* (chromosome 7); and the duplication relation was found in the above 6 genes with *MtOPT11* (chromosome 2) and *MtOPT 12* (chromosome 4). Duplication was also found between *MtOPt01* (chromosome 3) and *MtOPT 09* (chromosome 8). In the YS-MtOPT subfamily, *MtOPt14* and *16* (chromosome 3) was found to be have duplication relation with *MtOPT13* and *25* (chromosome 5); *MtOPt15* on chromosome 6 also showed duplication relation with *MtOPt14* and *16*. However the replication of *OPT* genes is relatively simple in *Medicago truncatula* compared with other plants.

Fig. 5 Chromosome location and Gene duplication of *MtOPT* genes

Expression of *MtOPT* genes

Organ-specific of the 26 *MtOPT*s were obtained from SRA database extraction transcriptome data (Figure 6). Data showed that expression profile of the *MtOPT*s was different in various areas of the plant. Most of genes in PT-OPT subfamily expressed in all of the six areas (roots, nodules, leaf, bud, seedpod and flowers) and mostly expressed in nodule. YS-OPT subfamily genes mainly expressed in seedpod. Taken together, expression levels of YS-OPT subfamily genes was relative higher than those of PT-OPT subfamily genes.

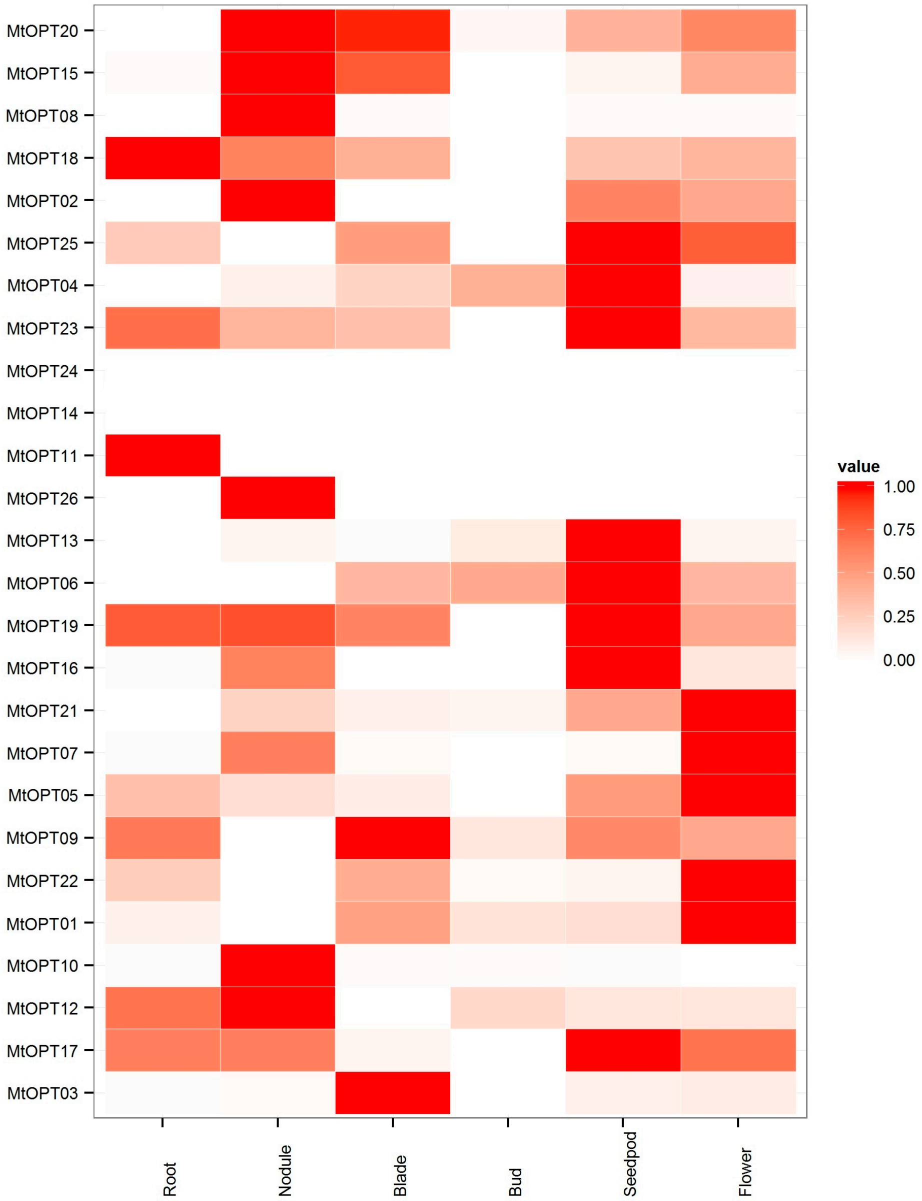

Fig. 6 The expression of *OPT* family genes in *Medicago truncatula*

DISCUSSION

To absorb and maintain a balance of potentially toxic metal ions, plants utilize poorly understood mechanisms involving a large number of membrane transporters and metal binding proteins with overlapping substrate specificities and complex regulation[9]. Higher plants contain a far greater number of OPT genes for these transporters than do other eukaryotes. This may be indicative of the relative importance of (oligo) peptides and their transport to plant growth and metabolism [11].

OPT genes belong to a small gene family in plant. Total of 26 OPT gene family members were identified in *Medicago truncatula* genome in this study. The *MtOPT* gene family is significantly larger than the OPT family in Arabidopsis and rice, 9 and 16 OPT gene family members, respectively. The amino acids Length encoded by *MtOPT* is generally between 500 and 800. Intron number is irregularity for *MtOPT29* contains 11 introns and *MtOPT26* contains no. Generally, the length in YS subfamily genes is less than that of the PT subfamily. This distinction may be caused by carrying different substrates，due to the transit of oligopeptide require more complex regulatory and transporter mechanisms. In evolution, PT subfamily and YS subfamily is independent, the gene sequences between two subfamilies have great difference, thought exist similarity in partial genes.

The OPT genes have been identified in rice and Arabidopsis genome suggest that the OPT family of transporters is larger and more diverse than previously recognized. The number of *MtOPT*s is larger than that of rice and Arabidopsis, nevertheless the diverse is not so obviously. The evolution of *MtOPT*s is consistent with *OsOPT*s and *AtOPT*s which have been further divided into two clusters [18].

OTP family transporter proteins differ in the transmission of physiological functions such as long distance distribution of nitrogen mobilization and heavy metal sequestration. These processes play important roles in development and growth of plant [5]. All of the *Arabidopsis* OPT family member can transport leu tetra-and pentapeptides with the exception of OPT2 and OPT3 [14, 30]. Contradictory evidence exists either in support of AtOPT6 being able to transport glutathione or against such glutathione transporting activity [10-12]. It is well known that chelate of iron and other metals such as Zn, Cu, Ni, Mn, etc. can be transported from rhizosphere surrounding to the root and transported in the intracellular simultaneously [45]. *AtOPT2* and *AtOPT3* are highly induced in response to metal (iron, copper and manganese) deficiencies. Yeast complementation experiments have shown that AtOPT3 can transport Cu, Mn, and possibly Fe [12]. AtOPT6 can mediate absorb glutathione derivatives and metal complexes; suggesting that it may also involve redox balance in the body. In addition, OPT transporter protein plays an important role in the maintenance of cellular homeostasis and adaptation to environmental stress [3, 4]. It was also shown that AtOPT3 is required for embryo development [10]. YS and PT subfamily were different in function, the report showing that rice PT-OPTs are capable of transporting NA-bound iron has narrowed capabilities gap between the PT and YS subfamilies [1-3]. AtOPT3 is likely involved in long distance movement of metal- chelate complexes in the plant while divergent in sequence from members of the YSL subfamily [10]. Due to all of titanium, PS and niacinamide are amino acid derivatives, these two types of transport protein subfamily belong to the same OPT big family.

According to the relative expression level of *MtOPT*s, All the *MtOPT*s studied express highly in root, flower, blade, seedpod, or nodules except *MtOPT14* and *24*. Most of the OPT genes express in vegetative tissues of and reproductive organs indicated that OPT gene family played an important role in entire life activities of *Medicago truncatula*. Chelate of metals can be transported from rhizosphere into root and transported in the intracellular simultaneously [45]. The presence of the *MtOPT* transcripts in root and blade suggests individual MtOPTs may have specific roles in the acquisition and redistribution of organic nitrogen and/or metal during plant growth. The expression in the reproductive organs, flower and seedpod, suggests the translocation function of individual MtOPTs in the plant development. Additionally, the transcript level of the gene family reveals that *MtOPT*s have complex functions. The expression of YS subfamily genes is significantly higher than that of PT subfamily genes in different parts. YS subfamily main in carpel tissues, followed by the roots and nodules indicating YS subfamily genes not only play an important role in the vegetative growth but also in reproductive organ development process; While PT subfamily expressed mainly in nodules which is specific in Leguminous plants may be related to nitrogen absorption in *Medicago truncatula*. In short, data obtained from the analysis will help to better understand the complexity of OPT family in *Medicago truncatula* and evolution of OPT gene family in higher plants.

OPT plays an important role in normal nutrition metabolism and metal stress response in plant.

Several of the plant OPT transporter gene families are fairly well described; however, the *MtOPT* family are not among them. This is the first report of *MtOPT* family. Moreover, the gene function and expression patterns need to be further understood.

CONCLUSION

In this study, we identified 26 OPT genes belonging to PT and YS distinct subfamilies from *Medicago truncatula*. The MtOPT gene family is larger than the OPT family in Arabidopsis and rice. The sequence of *MtOPT* gene family was highly conservative and consistent with the At*OPT and OsOPT* families though the multiply of *MtOPT*s was relatively simple. The *MtOPT*s were localized on all of the chromosomes in *Medicago truncatula* and have various introns in number and length. Most of *MtOPT*s genes showed distinct tissue-specific express in all of the six areas (roots, nodules, leaf, bud, seedpod and flowers) in *Medicago truncatula*. The transcript level of the *MtOPT* gene family supported that *MtOPT*s involved in the transport and homeostasis of important elements which are essential for growth and development of plant. The results should provide further understand on the systematic of OPT family and important theoretical basis in function of OPT genes in higher plants.

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REFERENCES

1. Vasconcelos M.W.; Li, G.W.; Lubkowitz, M.A.; Grusak, M.A. Characterization of the PT clade of oligopeptide transporters in rice. *Plant Genome*. **2008**, 1, 77-88.
2. Li, Z.S.; Gallet, O.; Gaillard, C.; Lemoine, R.; Delrot, S. The sucrose carrier of the plant plasmalemma. III. Partial purification and reconstitution of active sucrose transport in liposomes. *Biochim Biophys Acta*. **1992**, 1103, 259-267.
3. Delrot, S.; Atanassova, R.; Maurousset, L. Regulation of sugar, amino acid and peptide plant membrane transporter. *Biochim Biophys Acta*. **2000**, 1465, 281-306.
4. Lemoine, R. Sucrose transporters in plants: update on function and structure. *Biochim Biophys Acta*. **2000**, 1465, 246-262.
5. Williams, L.E.; J.K. Pittman.; J. L. Hall. Emerging mechanisms for heavy metal transport in plants. *Biochim Biophys Acta*. **2000**, 1465, 104-126.
6. Couturier, J.; Montanini, B.; Martin, F.; Brun, A.; Blaudez, D.; Chalot, M. The expanded family of ammonium transporters in the perennial poplar plant. *New Phytol*. **2007**, 174, 137-150.
7. Staceya, G.; Kohb, S.; Grangerc, C.; Beckerd, J.M. Peptide transport in plants. *Trends Plant Sci*. **2002**, 7, 257-263.
8. Lubkowitz, M. The OPT family functions in long-distance peptide and metal transport in plants. *Genet Eng(N Y)*. **2006**, 27, 35-55.
9. Wintz, H.; Fox, T.; Wu, Y.Y.; Feng, V.; Chen, W.; Chang, H.S.; Zhu, T.; Vulpe, C. Expression profiles of *Arabidops* is thaliana in mineral deficiencies reveal novel transporters involved in metal homeostasis. *J Biol chem*. **2003**, 278, 47644-47653.
10. Stacey, M.G.; Osawa, H.; Patel, A.; Gassmann, W.; Stacey, G. Expression analyses of *Arabidopsis* oligopeptide transporters during seed germination, vegetative growth and reproduction. *Planta*. **2006**, 223, 291-305.
11. Waterworth, W.M.; Bray, C.M. Enigma variations for peptides and their transporters in higher plants. *Ann Bot*. **2006**, 98: 1-8.

12. Zhang, M.Y.; Bourbouloux, A.; Cagnac, O.; Srikanth, C.V.; Rentsch, D.; Bachhawat, A.K.; Delrot, S. A novel family of transporters mediating the transport of glutathione derivatives in plants. *Plant Physiol*. **2004**, 134, 482-491.
13. Cagnac, O.; Bourbouloux, A.; Chakrabarty, D.; Zhang, M.Y.; Delrot, S. AtOPT6 transports glutathione derivatives and is induced by primisulfuron. *Plant Physiol*. **2004**, 135, 1378-1387.
14. Koh, S.; Wiles, A.M.; Sharp, J.S.; Naider, F.R.; Becker, J.M.; Stacey, G. An oligopeptide transporter gene family in *Arabidopsis*. *Plant Physiol*. **2002**, 128, 21-29.
15. DiDonato, R.J. Jr.; Roberts, L.A.; Sanderson, T.; Eisley, R.B.; Walker, E.L. *Arabidopsis* yellow stripe-like2 (YSL2): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant J*. **2004**, 39, 403-414.
16. Koike, S.; Inoue, H.; Mizuno, D.; Takahashi, M.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J*. **2004**, 39, 415-424.
17. Schaaf, G.; Schikora, A.; Häberle, J.; Vert, G.; Ludewig, U.; Briat, J.F.; Curie, C.; von Wirén N. A putative function for the *Arabidopsis* Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. *Plant Cell Physiol*. **2005**, 46, 762-774.
18. Yen, M.R.; Tseng, Y.H.; Saier, M.H Jr. Maize yellow stripe1, an iron phytosiderophore uptake transporter, is a member of the oligopeptide transporter (OPT) family. *Microbiology*. **2001**, 147, 2881-2883.
19. Gendre, D.; Czernic,P .; Conéjéro, G.; Pianelli, K.; Briat, J.F.; Lebrun, M.; Mari, S. TcYSL3, a member of the YSL gene family from the hyperaccumulator Thlaspi caerulescens, encodes a nicotianamine Ni/Fe transporter. *Plant J*. **2007**, 49, 1-15.
20. Roberts, L.A.; Pierson, A.J.; Panaviene, Z.; Walker, E.L. Yellow stripe1. Expanded roles for the maize iron-phytosiderophore transporter. *Plant Physiol*. **2004**, 135, 112-120.
21. Curie, C.; Cassin, G.; Couch, D.; Divol, F.; Higuchi, K.; Le, Jean, M.; Misson, J.; Schikora, A.; Czernic, P.; Mari, S. Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. *Ann Bot*. **2009**, 103, 1-11.
22. Murata, Y.; Ma, J.F.; Yamaji, N.; Ueno, D.; Nomoto, K.; Iwashita, T. A specific transporter for iron(III)-phytosiderophore in barley roots. *Plant J*. **2006**, 46, 563-572.
23. Inoue, H.; Kobayashi, T.; Nozoye, T.; Takahashi, M.; Kakei, Y.; Suzuki, K.; Nakazono, M.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. Rice OsYSL15 is an dron-regulated iron(III)-Deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. *J Bio Chem*. **2009**, 284, 3470-3479.
24. Ueno, D.; Yamaji, N.; Ma, J.F. Further characterization of ferric-phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley. *Experimental Botany*. **2009**, 60, 3513-3520.
25. Aoyama, T.; Kobayashi, T.; Takahashi, M.; Nagasaka, S.; Usuda, K.; Kakei, Y.; Ishimaru, Y.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. OsYSL18 is a rice iron(III)-deoxymugineic acid transporter specifically expressed in reproductive organs and phloem of lamina joints. *Plant Mol Biol*. **2009**, 70, 681-692.
26. Sasaki, A.; Yamaji, N.; Xia, J.; Ma, J.F. OsYSL6 is involved in the detoxification of excess manganese in rice. *Plant Physiol*. **2011**, 157, 1832-1840.
27. Zheng, L.; Fujii, M.; Yamaji, N.; Sasaki, A.; Yamane, M.; Sakurai, I.; Sato, K.; Ma, J.F. Isolation and characterization of a barley yellow stripe-like gene, HvYSL5. *Plant Cell Physiol*. **2011**, 52, 765-774.
28. Waters, B.M.; Chu, H.H.; DiDonato, R.J.; Roberts, L.A.; Eisley, R.B.; Lahner, B.; Salt, D.E.; Walker, E.L. Mutations in *Arabidopsis* yellow stripe-like1 and yellow stripe-like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiol*. **2006**, 141, 1446-1458.
29. Chu, H.H.; Chiecko, J.; Punshon, T.; Lanzirotti, A.; Lahner, B.; Salt, D.E.; Walker, E.L. Successful reproduction requires the function of *Arabidopsis* Yellow Stirpe-Like1 and Yellow Stripe-Like3 metal-nicotianamine transporters in both vegetative and reproductive structures. *Plant Physiol*. **2010**, 154, 197-210.
30. Koh, S.; Wiles, A.M.; Sharp, J.S.; Naider, F.R.; Becker, J.M.; Stacey, G. An oligopeptide

transporter gene family in *Arabidopsis*. *Plant Physiol*. **2002**,128, 21-29.

31. Wei, Z.W.; GAI, J.Y. Model legume: *Medicago truncatula*. *Acta Prataculturae Sinica*. **2008**, 17, 114-120.
32. Jiang, T.; Lin, Y.X.; Liu, X.; Jiang, H.Y.; Zhu, S.W. Genome-wide analysis of the WRKY transcription factor family in *Medicago truncatula*. *Acta Prataculturae Sinica*. **2011**, 20, 211-218.
33. Li, W.; Xu, H.; Liu, Y.; Song, L.; Guo, C.; Shu, Y. Bioinformatics analysis of MAPKKK family genes in *Medicago truncatula*. *Genes(Basel)* , **2016**, 7, 1-17.
34. Young, N.D.; Debellé, F.; Oldroyd, G.E. et al. The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature*, **2011**, 480, 520-524.
35. Finn, R.D.; Bateman, A.; Clements, J.; Coggill, P.; Eberhardt, R.T.; Eddy, S.R.; Heger, A.; Hetherington, K.; Holm, L.; Mistry, J.; Sonnhammer, E.; Tate, J.; Punta, M. Pfam: the protein families database. *Nucleic Acids Res*. **2014**, 42, D222-D230.
36. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Res*. **2011**, 39, W29-W37.
37. Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R.D.; Bairoch, A. ExPASy: the proteomics server for in depth protein knowledge and analysis. *Nucleic Acids Res*. **2003**, 31, 3784-3788.
38. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server.*Bioinformatics* **2015**, 31, 1296–1297.
39. Thompson, J.D.; Gibson, T.J.; Higgins, D.G. Multiple sequence alignment using ClustalW and ClustalX. Curr. Protoc. *Bioinformatics* **2003**.
40. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*, 2013, 30: 2725-2729.
41. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST : a new generation of protein database search programs. *Nucleic Acids Res*. **1997**, 25, 3389-3402.
42. Krzywinski, M.; Schein, J .; Birol, I .; Connors, J .; Gascoyne,. R; Horsman, D .; Jones, S.J.; Marra, M.A . Circos: An information aesthetic for comparative genomic. *Genome Res*. **2009**, 19, 1639-1645.
43. Traprell, C.; Pachter, L.; Salzberg, S.L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, **2009**, 25, 1105–1111.
44. Trapnell, C.; Williams, B.A.; Pertea, G.; Mortazavi, A.; Kwan, G.; van, Baren, M.J.; Salzberg, S.L.; Wold, B.J.; Pachter, L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol*, **2010**, 28, 511-515.
45. Lubkowitz, M.A.; Barnes, D.; Breslav, M.; Burchfield, A.; Naider, F.; Becker, J.M. Schizosaccharomyces pombe isp4 encodes a transporter representing a novel family of oligopeptide transporters. *Mol Microbiol*. **1998**, 28, 729-741.