Genome-wide Analysis of Phosphate Transporter Gene Family in *Zea mays* L.

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Phosphate transporters in maize

Several phosphate transporters (PTs) in maize, have been reported (Nagy et al., 2006; Willmann et al., 2013). In order to determine if there are any more members comprising the maize PT family, the mRNA and amino acid sequences of *Arabidopsis* and rice PT genes were employed for BLAST against the recently released maize genomic sequence database. Altogether, 40 genes representing distinct loci were annotated as 'transporters' (relating to phosphate uptake and transfer). Chromosomal organization analysis assigned the 40 genes to the 10 maize chromosomes: seven genes locate on chromosome 1 and 2; six genes locate on chromosomes 7 and 8; five genes locate on chromosomes 5; three genes locate on chromosomes 3; and other chromosomes with two genes each.

Phylogenetic analysis

A joint, un-rooted, phylogenetic tree was generated from alignments of the full-length protein sequences of the PT family members in maize (40 genes), rice (26 genes) and *Arabidopsis* (19 genes). Within this tree, the 40 PTs in maize were assigned into four clusters (I, II, III and IV); 13 maize PT members were grouped into Cluster I with their nine orthologous pairs from rice and nine orthologous pairs from *Arabidopsis*. Nine maize PT members were grouped together with their six rice and six *Arabidopsis* homologues, forming Cluster II. Cluster III contained 12 maize PT members, while the other maize PT family members were grouped together with sixrice and six Arabidopsis homologues, forming cluster IV.

Motif Analysis

Based on the protein sequence alignment analysis, 14 putative conserved motifs (21–100 amino acids in length) were identified in the ZmPT family. Among these 14 motifs, motifs 1, 2, 3, 4, 5 and 8 were identified in all Cluster I genes, with motif 2 appearing twice; motifs 12, 13 and 14 appeared in all Cluster II genes; motifs 6, 7, 9 and 11 appeared in all Cluster III genes (with the exception that gene GRMZM5G845775, which contained only motif 6); motifs 9 and 10 appeared in all Cluster IV genes (with the exception of AC233910.1_FG001 which contained only motif 10 and motif 10 appeared twice in all Cluster IV genes).

Protein architecture

As reported previously, the PT family exhibits high-sequence similarity and contains multiple putative trans-membrane segments (TMSs). The trans-membrane protein structure online prediction (http://www.ch.embnet.org/) tool was used to identify TMS in the maize PT family. In Cluster I, seven proteins contained 12 TMS', four proteins contained 13 TMS', one protein had 14 TMS' and another one had 9 TMS' and, 11 to 15 TMS' were identified on ZmPT proteins in Cluster II. In this study, 7 to 11 TMSs were identified on rice in Cluster III with an exception of one protein having only two TMS'. In Cluster IV, 5-6 TMS' were identified in 5 ZmPT proteins, with an exception of one protein containing only 3 TMS'.

Analysis of *cis* elements

By searching the PLACE database within the 2-kb upstream regions of the 40 ZmPT genes, 250 putative cis elements, with more than 6 bp length, were identified. Among these, 250 putative cis elements were identified and of those, the following 25 might be very important in most of the 40 ZmPT family members: ACGTATERD1; ARR1AT, BIHD1OS; CAATBOX1; CACTFTPPCA1; CGACGOSAMY3; CURECORECR; DOFCOREZM; DPBFCOREDCDC3; EBOXBNNAPA; GATABOX; GT1CONSENSUS; GTGANTG10; INRNTPSADB; MYBCORE; MYCCONSENSUSAT; NODCON2GM; OSE2ROOTNODULE; POLLEN1LELAT52; RAV1AAT; ROOTMOTIFTAPOX1; TAAAGSTKST1; TATABOX5; WBOXNTERF3; and WRKY71OS. Twenty of these 25 cis elements were found in all of the ZmPT genes. These main cis elements may potentially be involved in not only Pi response but also photosynthesis and rhizobium responses, multi-abotic- stress response and multi-hormonal signaling. Thus, it was indicated that transcriptional regulation of Pi transporter genes may be controlled by an array of physiological mechanisms.

Function of ZmPht1;6 in mediating Pi transportation

To analyze the function of ZmPht1;6 in mediating Pi transportation, membrane potential of the *Xenopus laevis*, oocytes injected with ZmPht1;6 mRNA were measured and recorded. Electrophysiological measurement showed that the plasma membrane potential became less negative when 10 mM NaH_2PO_4

was supplied in the external solution of oocytes injected with ZmPht1;6 mRNA. In contrast, water-injected oocytes did not show any response to 10 mM NaH_2PO_4. To further analyze the function of ZmPht1;6, the oocytes injected with Zmpht1;6 mRNA and water were incubated in 0.5 mM NaH_2PO_4 for 14 h to measure Pi content. The Pi content of Zmpht1;6-injected oocytes was more than 12 nmol and almost three times more than that of oocytes injected with water. The electrophysiological measurements and Pi contents of the ZmPht1;6- injected oocytes were similar to rice OsPT2-injected oocytes that were reported to be a low-affinity Pi transporter (Ai et al., 2009). This data suggested that ZmPht1;6 appears to mediate Pi uptake with low Pi affinity in the mM range.

Conclusion

Computational genome-wide analysis suggested that the PT family, in maize, is complex. In this study, ZmPht1;6 was validated as mediating Pi uptake with low Pi affinity in the mM range.

References

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