Research Note
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STUDIES ON SPATIO-TEMPORAL AND PHYTOHORMONAL EXPRESSION PATTERN OF *MTP2* PROTEIN AND ITS CHARACTERIZATION ACROSS DIFFERENT PLANT SPECIES

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Zinc is an essential micronutrient involved in a wide range of physiological processes of plants (Marschner, 1993). Even though it is required for plant growth, the excess amount of Zn causes toxic effects to plants. It is known that during Fe deficiency, the uptake of Zn is induced which leads to the accumulation of the excess amount of Zn in the plant cytoplasm. The plant has evolved an efficient mechanism in detoxifying the excess Zn uptake during Fe deficiency conditions, of which vacuoles are identified to be the main storage and detoxifying organelles for Zn excess in the cytoplasm (Gustin *et al.,* 2009). Zinc is normally available to the plants in its oxidized form Zn^{2+} from the soil through the mass flow and diffusion mechanism by roots (Rattan and Deb, 1981). Availability of Zn is high in low acidic pH wherein the high pH reduces its availability in soil and the root plasma membrane transporters reported for major uptake of Zn from the soil (Marschner, 1993).

Zinc uptake from the soil is mediated by the rhizosphere acidification process followed by solubilization. Plant roots uptake the zinc in the form of Zn^{2+} or the Zn-ligand complex (Von Wiren *et al.,* 1996). In the Zn^{2+} form of zinc uptake, the H^+ ions and organic acids are released by the plants to lowers the ph of the soil, which mediates the solubilization of Zn complexes in the soil. In Zn-ligand complexes, the phytosiderophores are effluxed into the soil which forms a stable complex with zinc ions. Nicotianamine (NA) was reported to be the major chelator, involved in the uptake of Zn ions from the soil (Trampczynska *et al.,* 2006; Haydon and Cobbett, 2007). Many genes have been reported to encode transporter proteins in the Zn uptake and transport mechanism. Among the reported zinc transporter families, *ZIP* (Zinc, Iron permease family/ZRT-IRT-like proteins) family (Grotz *et al.,* 1998), the *MTP* (Metal tolerance proteins) and, *HMA* (Heavy metal ATPases) family are the major ones. In Arabidopsis many *ZIP* proteins *i.e., ZIP1-4, ZIP7, ZIP11-12*, and *IRT3* are involved in zinc uptake from the soil (Kramer *et al.,* 2007), of which *IRT3* was well-studied plasma membrane transporter. The *ZIP* proteins are reported to be involved in the Zn detoxification and homeostasis mechanism process. *HMA* families of transporters are involved in the transport of metals in the plants and are known as P_{1B}-type ATPases. In Arabidopsis, eight *HMA*s have been identified, out of which *HMA1-HMA4* are belonging to Zn/Co/Cd/Pb subgroup (Cobbett *et al.,* 2003).

Apart from the above transporters *MTP1* and *MTP3* are reported to be involved in the sequestration of Zn ions into the vacuoles from the cytoplasm with the efflux of H^+ ions to the cytosol (Kobae *et al.,* 2004; Desbrosses-Fonrouge *et al.,* 2005). These belong to the cation diffusion facilitator family of proteins and are reported to be localized on the tonoplast membrane of the vacuole of the cell. To date, twelve members of *MTP* family transporters have been reported in Arabidopsis of which *MTP1* and *MTP3* were well studied. *MTP1* promoter activity is highest in young leaves, whereas *MTP3* expression is undetectable in shoots. Moreover, *MTP3* expression is very low under normal growth conditions and strongly increased in response to Fe deficiency and upon

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exposure of seedlings to excess Zn (Arrivault *et al.,* 2006). However, *MTP1* and *MTP3* have opposite effects on Zn accumulation.

Physiological Zn deficiency of *Arabidopsis thaliana* shoots results in increased root transcript levels of the membrane transport protein-encoding genes *METAL TRANSPORT PROTEIN2* (*MTP2*) and *HEAVY METAL ATPASE2* (*HMA2*), which are unresponsive to the local Zn status of roots. *MTP2* and *HMA2* act additively in the partitioning of Zn from roots to shoots. When compared to *MTP1* and *MTP3*, few studies were conducted on *MTP2* transporter following the Zn homeostasis pathway. Based on this background, the identification of *MTP2* transporter proteins in different crops, conserved motif sequence, evolutionary relationships, the expression pattern of MTP2 gene at different growth stages and to the plant hormone treatment were analyzed *insilico* in the present study, using online bioinformatics tools and plant databases.

The Uniprot ID, Q10LJ2 coding for the Oryza sativa japonica group *MTP2* protein sequence was downloaded from the UniProt database. The sequence was used as a query for the identification of similar proteins in other related crops with an E-value of 0 and more than 80% similarity. Similar sequences were identified in *Hordeum vulgare* (81.22%), *Aegilops tauschii* (82.11%), *Brachypodium distachyon* (81.89%), *Triticum dicoccoides* (82.68%), *Zea mays* (87.75%), *Oryza brachyantha* (88.72%), *Panicum virgatum* (86.47%), *Sorghum bicolor* (86.59%), *Panicum hallii* (87.10%), *Dichanthelium oligosanthes* (86.71%), *Setaria italica* (84.23%), and *Setaria viridis* (83.94%) crops with more than 80% similar identity (Table 1).

Table 1: Details of different crops with Protein IDs and similarity percentage downloaded from NCBI

Protein ID	Crop name	Similarity %
XP_015628702.1	Oryza sativa Japonica Group	100.00
KAE8781701.1	Hordeum vulgare	81.22
XP_020193507.1	Aegilops tauschii	82.11
XP_003557927.1	Brachypodium distachyon	81.89
XP_037430426.1	Triticum dicoccoides	82.68
XP_015689891.2	Oryza brachyantha	82.72
XP_039830393.1	Panicum virgatum	86.47
XP_021307383.1	Sorghum bicolor	86.59
PWZ53216.1	Zea mays	87.75
XP_025794726.1	Panicum hallii	87.1
OEL27878.1	Dichanthelium oligosanthes	86.71
XP_004984402.1	Setaria italica	84.23
XP_034573611.1	Setaria viridis	83.94

The conserved motifs among the above 13 *MTP2* protein sequences from different crops were identified using a MEME server with a minimum of 5 motifs and maximum width of 50. The first motif sequence identified was GTSLYLDVHIEVYPFLSVS AAHDIGETVRHQIQKEH NQVAEVFIHIDPSY, the second motif sequence was YRAAKAPRDKEHPYGH GKFESLGALGISSMLLVTSGGIAWHAFEVLQGVM, the third motif sequence, MILKAGIQTGYES VLELVDAAVDPSLLEPIKETILKVDGVKGCHRLRGRK, and the fourth motif sequence was LYWITKRAGEKE GSGLMKANAWHHRADAISSVVALVGVGGSILGL PLLDP.

Moreover, the sequence of the fifth motif discovered was IISSHFSKKMSLEHLMLHYVQG RVLLQVQVSMSPEILIRDAMEIAKQAEE and all the motifs identified were 50 amino acids in length (Figure 1). All the 13 protein sequences from the different crops have shared the five conserved motifs and their position in the above 13 crops was elucidated in Figure 2. The multiple sequence alignment and evolutionary analysis of *MTP2* protein in different crops were done using the CLUSTALW online tool. The phylogenetic tree was executed using the PhyML bootstrap method using the percent scoring method. The *MTP2* proteins showed 70-100% of similarity among the 13 proteins. The phylogenetic tree was divided into four main groups based on the tree topologies (Figure 3). In group, A four *MTP2* proteins of *Brachypodium distachyon, Hordeum vulgare, Triticum dicoccoides, Aegilops tauschii* have formed a cluster. In group B, *Oryza sativa Japonica* Group and *Oryza brachyantha* formed a cluster, wherein group C, *Sorghum bicolor,* and *Zea mays* formed a cluster. Two proteins of *Panicum sps*, two proteins of *Setaria sps,* and one protein of *Dichanthelium oligosanthes* formed a cluster in group D.

The RAP locus ID of *MTP2* of *Oryza sativa* was used as a query in the Ricexpro database for Spatio-temporal expression analysis. The overview of the expression pattern of *MTP2* protein in specific tissues and organs at various growth stages in the entire Spatio-temporal developmental cycle from the transplanting to harvesting was analyzed (Sato *et al.,* 2012). The expression profile of the *MTP2* gene in 48 samples of various tissues in three replicates was represented in Figure 4. The higher expression of *MTP2* protein was observed in ovary tissue, five days after flowering and followed by the root tissue at the vegetative stage at 12.00 AM. The lower expression of the protein in rice was noted in endosperm tissue at 7, 10, 14, 28, 42 days after flowering (Figure 4).

Apart from the above analysis, an overview of expression of *MTP2* gene in root and shoot of rice seedling treated with hormones namely, abscisic acid (ABA), gibberellic acid (GA3), indole-3-acetic acid (IAA), brassinolide (BL), trans-zeatin (tZ) and jasmonic acid (JA) was analyzed (Sato *et al.,* 2012). The expression pattern of *MTP2* in roots to different hormones was analyzed at 15min, 30min, 1h, 3h, and 6h of incubation period and in shoot samples at 1h, 3h, 6h, and 12hr of incubation with three replicates. The total RNA samples were labeled with Cy3 9mock treatment) and Cy5 (hormone treatment) and time course expression profile for the gene is shown as the log-ratio of signal intensity (log2 Cy5/Cy3) in Figure 5. From the figure, it is reported that at 6hr of abscisic acid, the expression of *MTP2* protein was upregulated in root tissue wherein it was found to be downregulated in shoot tissue. The expression of the gene was reported to be similar at 1h, 3h, and 6h gibberellic acid treatment of both root and shoot, but at 12h after treatment in the shoot, slight downregulation was recorded. In the case of IAA, tZ, and BL hormone treatment the expression pattern was the same in root and shoot tissues. The JA treatment induced the expression of *MTP2* protein at 6h in root wherein the shoots, the expression was induced at 12h of treatment.

The Spatio-temporal expression analysis of *MTP2* protein revealed the importance of the protein at the vegetative state of root and at ovary tissue when plants grown under normal conditions. Further, biotic and abiotic stresses are detected by the plant cell wherein the plant hormones act as a signaling molecule in activating the defense mechanism in them. The expression pattern studies of *MTP2* protein to different phytohormonal treatment results revealed the connection between the expressions of *MTP2* protein to plant hormones which indicated its downregulated expression towards JAs treatment. In the case of ABA treatment in roots, the expression was slightly upregulated at 3h of treatment, and in the shoot, the downregulation was recorded at 6h of treatment. Based on these insilico results, it is understood that, more research in identifying the protein interacting partners, protein structural studies for functional characterization is required.

Figure 1: Identified conserved motifs in *MTP2* proteins among 13 plant species

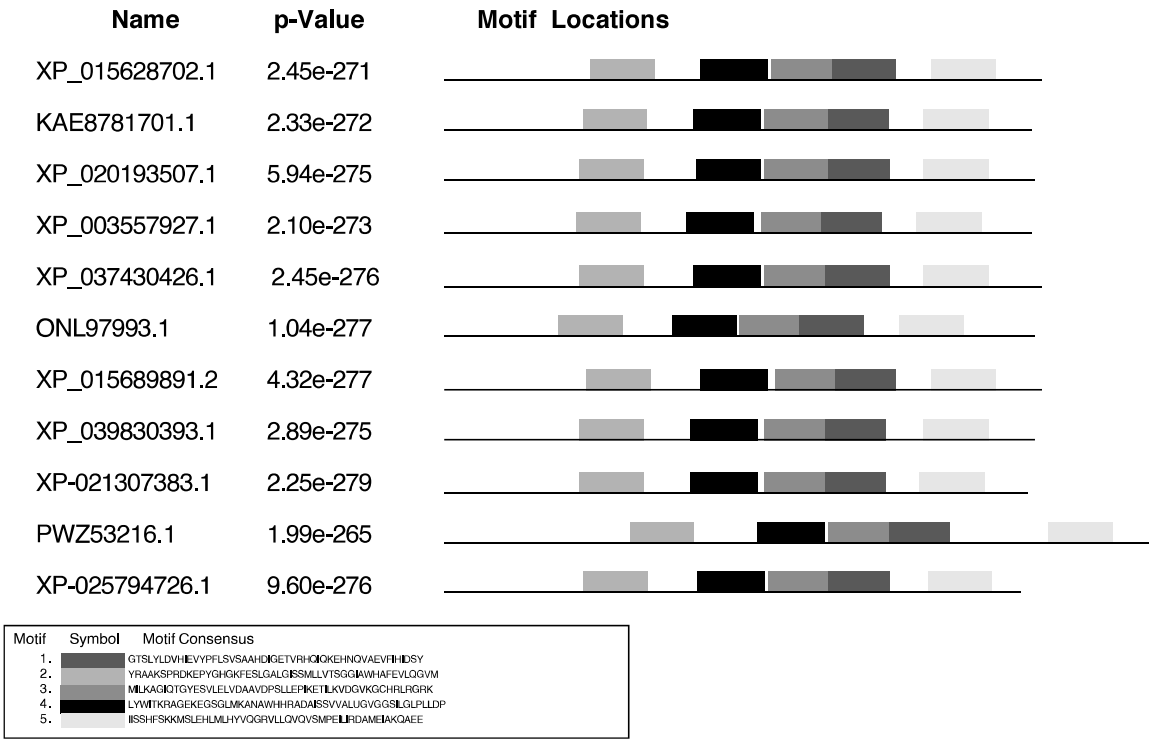

Figure 2. Motif locations identified in *MTP2* proteins among 13 plant species

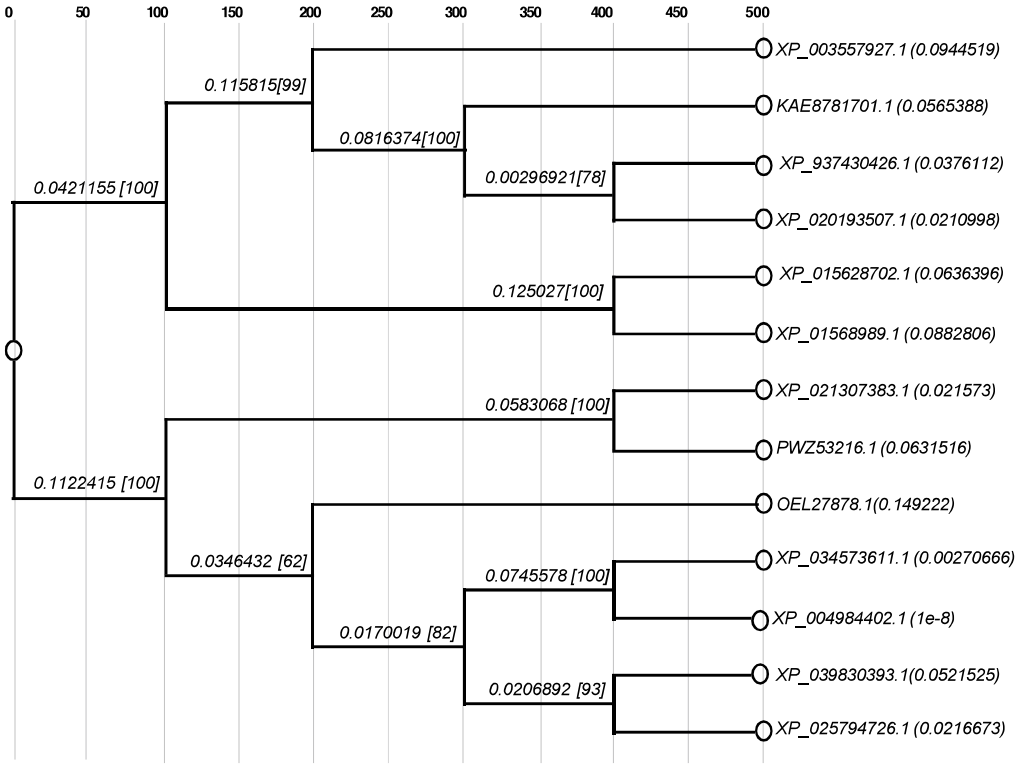

Figure 3. Phylogenetic tree constructed from *MTP2* of 13 plant species

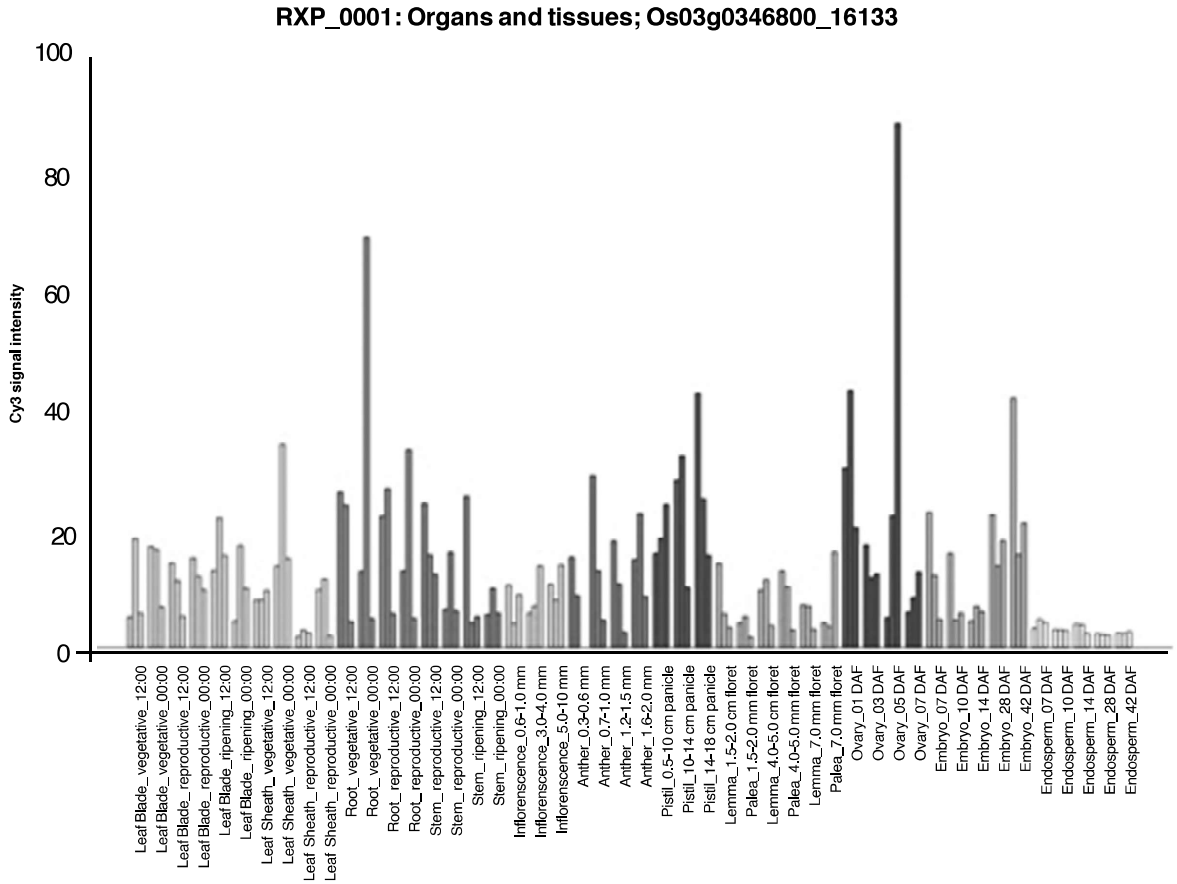

Figure 4. Graph indicating the expression of MTP2 in different developmental stages under normal growth conditions

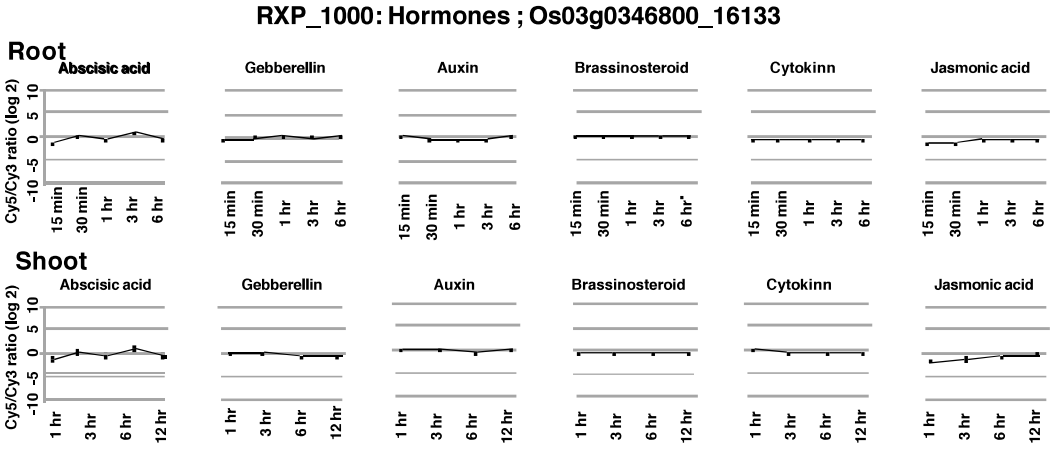

Figure 5. Graphs indicating the expression of *MTP2* protein in root and shoot tissues under plant hormonal treatment

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