Genome-Wide Survey and Expression Analysis of P$_{1B}$-ATPases in Rice, Maize and Sorghum

E Zhiguo[1], Li Tingting[1], Chen Chen[2], Wang Lei[1]

(*[1]Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China; [2]Key Laboratory of Plant Functional Genomics, Ministry of Education / Key Laboratory of Crop Genetics and Physiology of Jiangsu Province / College of Agriculture, Yangzhou University, Yangzhou 225009, China*)

Abstract: P$_{1B}$-type ATPase ion pumps that transport heavy metal ions across cellular membranes are essential for plant growth and development. To date, a genomic comparison overview of the family in rice, maize and sorghum is not yet available. In this study, a total of 31 heavy metal P$_{1B}$-type ATPase (HMA) genes were identified, including 9 in rice, 11 each from maize and sorghum. They were classified into two distinct subfamilies based on their sequence composition and phylogenetic relationship. Four pairs of HMA genes were expanded via gene duplication with tandemly duplicated. Comprehensive analyses were performed to investigate the expression profiles of HMA genes in various tissues by using quantitative real-time PCR. Some HMA members exhibited abundant and tissue-specific expression patterns. Moreover, most of the genes were found to be differentially expressed under the Cu/Cd treatment. This study will facilitate further studies on P$_{1B}$-type ATPase family and provide valuable hints for the functional validation in rice, maize and sorghum.

Key words: P$_{1B}$-type ATPase; rice; maize; sorghum; HMA gene family; expression pattern analysis

The transition metal elements widely exist in natural and agricultural environment (Xu et al, 2016). Some of them, such as copper (Cu), zinc (Zn) and manganese (Mn), are essential micronutrients for plant metabolism, but when present in excess, these and non-essential metals such as cadmium (Cd), plumbum (Pb) and mercury (Hg) can become extremely toxic (Williams et al, 2000). Thus, a finely tuned homoeostatic network to control cellular metal ion concentrations is necessary to plant growth and development. Ion homoeostasis in plants depends on the control of root uptake, root- to shoot-transport, and vacuolar sequestering and distribution of ions to various organs (Rascioa and Navari-Izzob, 2011). In plants, many transmembrane transporters involve in ion homoeostasis.

P$_{1B}$-ATPases, also known as heavy metal ATPases (HMAs), form a unique evolutionary branch of P-type ATPases, and can transport metallic cations (e.g. Cd^{2+}, Cu^{2+}, Ag^+, Zn^{2+} and Pb^{2+}) across membranes by utilizing the energy liberated from the ATP hydrolysis in plants (Axelsen and Palmgren, 2001; Mills et al, 2003; Hussain et al, 2004). The HMA members can be grouped into two distinct clades in phylogenetic analyses. One clade plays a role in Cu and Ag transport, while the second clade functions as a Zn/Co/Cd/Pb transporter (Axelsen and Palmgren, 2001). To date, multiple HMA genes have been cloned and identified in various crop species. In rice, OsHMA2 is localized to the plasma membrane and involved in xylem loading of Cd and Zn (Satoh-Nagasawa et al, 2012; Takahashi et al, 2012b; Yamaji et al, 2013). OsHMA5 is involved in loading Cu to the xylem of the roots and other organs (Deng et al, 2013).

http://dx.doi.org/10.1016/j.rsci.2018.06.004

OsHMA3 is located on the tonoplast, and responsible for vacuolar sequestration of Cd in the roots (Miyadate et al, 2011). Analogously, OsHMA4 functions to sequester Cu into root vacuoles, limiting Cu accumulation in the grains (Huang et al, 2016). In barley, HvHMA1 is involved in mobilizing organellar Zn and Cu, and plays a role in metal loading into grains (Mikkelsen et al, 2012). HvHMA2 functions as a Zn pump and plays a role in Zn transport from root to shoot (Mills et al, 2012). In wheat, TaHMA2 is localized to the plasma membrane and improves root-shoot Zn/Cd translocation (Tan et al, 2013).

Based on bioinformatics analysis, nine members (*OsHMA1*, *OsHMA2*, *OsHMA3*, *OsHMA4*, *OsHMA5*, *OsHMA6*, *OsHMA7*, *OsHMA8* and *OsHMA9*) are predicted in rice, which is consistent with previously reported (Williams et al, 2005). In addition, we identified 11 *ZmHMAs* and 11 *SbHMAs* in maize and sorghum genomes, respectively. As mentioned above, the total 31 HMA members in the three crops are divided into two groups, specifically, 11 members (*OsHMA1*, *OsHMA2*, *OsHMA3*, *ZmHMA1*, *ZmHMA2*, *ZmHMA3a*, *ZmHMA3b*, *SbHMA1*, *SbHMA2*, *SbHMA3a*, and *SbHMA3b*) fall into the Zn/Co/Cd/Pb subclass, and the other 20 members belong to Cu/Ag subclass. Furthermore, we investigated the phylogenetic relationship, chromosomal distribution, and transcript profiling of the 31 HMA members, the results suggested their functional diversifications and conservations.

MATERIALS AND METHODS

Plant materials and treatments with heavy metal stresses

In order to evaluate the expression profiles of HMA genes in various tissues at different development stages, we selected sequenced varieties Nipponbare (rice), B73 (maize) and BTX623 (sorghum) as the experimental materials. The seedlings of the three varieties were grown in the field during the normal growing season at (29 ℃–33 ℃)/(23 ℃–26 ℃) (day/night) with a photoperiod of 14 h. Five materials tested in the expression analysis were: (1) 20-day-old roots (young roots); (2) 20-day-old seedlings; (3) 60-day-old leaves (mature leaves); (4) 90-day-old culms; and (5) grains at 10 d after pollination stage.

For heavy metal stress treatments, seeds were immersed in water at 37 ℃ for 30 h, and then sown on a plastic net that was floated on nutrient solution in a growth chamber at 28 ℃ (light/dark as 14 h/10 h). Then, 14 day-old seedlings were treated with 50 μmol/L and 500 μmol/L Cu or Cd, and samples were collected after 24 h. All materials sampled were immediately frozen in liquid nitrogen and stored at -80 ℃ prior to RNA extraction.

Database screening and identification of OsHMAs, ZmHMAs and SbHMAs

To explore all the putative HMA members in the three grasses genomes, the two protein sequences of rice HMA (OsHMA2 and OsHMA3) were used as a query to search against the following database: RGAP 7.0 (http://rice.plantbiology.msu.edu/), Gramene (http://www. gramene.org/) and SbGDB (http://www. plantgdb.org/ SbGDB/). The Pfam (http://pfam.xfam.org/) and InterPro (http://www.ebi.ac.uk/interpro/) databases were used to confirm each predicted HMA protein sequence. After removing the redundant sequences, the remaining proteins which contained the characteristic heavy-metal-associated domain (IPR006121) were defined as putative heavy metal ATPases. The other information including chromosome location, exon/intron structure and the splicing variants were obtained from above three databases. The gene structures of the HMAs were analyzed using the GSDS (Gene Structure Display Server) website (http://gsds.cbi.pku.edu.cn/).

Sequence alignment and phylogenetic analysis

Multiple sequence alignment was performed using ClustalW program integrated in MEGA7.0 with default parameters. Moreover, to compare the evolutionary relationships of rice, maize and sorghum HMAs, the homodomains of OsHMAs, ZmHMAs and SbHMAs were aligned using CLC Genomics Workbench, and the result was exported as a tag image file.

The un-rooted phylogenetic trees of all HMA proteins were constructed with MEGA7.0 using both neighbor-joining (NJ) method and maximum likelihood (ML) method. The major parameters for NJ method were listed below: Bootstrap method (1000 replicates), Poisson model and complete deletion. Similarly, the parameters for constructing ML trees were as follows: Bootstrap method (1000 replicates), Jones-Taylor-Thornton (JTT) model, and Nearest-Neighbor-Interchange (NNI) method.

RNA isolation, cDNA synthesis and qRT-PCR analysis

To confirm the expression of HMA genes in rice,