Sequence Variation of Metallothionein (MT) Gene in Poaceae Family

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Sequence Variation of Metallothionein (MT) Gene in Poaceae Family

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Abstract. Several plant in Poaceae family can be used as a accumulator plant in phytoremediation technique to clean the contaminated area. Metallothionein (MT) is one of metal binding protein groups that regulate heavy metal absorption. MT has low molecular weight and has cysteine-rich residues. MT plays a role in essential metals homeostasis and also in toxic metals detoxification. Meanwhile, MT also functions as a chelator that can bind heavy metals. Due to no information about the functional domains of MT gene in plants, study the sequence variation of MT gene is required to find the conserved region, especially in Poaceae family, related to MT gene function in phytoremediation. The MT gene homology shows the gene genetic relatedness in interspecies. In this study, the MT gene sequences in Poaceae plants (Hordeum vulgare, Sorghum bicolor, and Setaria italica) were obtained from NCBI. T-cofe tool was used to align sequences for identifying regions similarity. BLAST was used to compare the sequence database and calculate significant of matches. The results indicated that MT gene in Poaceae plants have high homology in several regions. MT sequence of Chloris virgata had the sequence identity around 92% with Allium sativum which was grouped in Alliaceae family.

1. Introduction

Poaceae is one of the largest and nearly most ubiquitous monocotyledonous angiosperm, comprising of proximately 650 until 765 genera and more than 6000 species. These family members are also known as grasses. Grasses can be used in multiple ways for human’s life, for instance as a food (rice, wheat, and maize), as a building material, for making paper, and for making perfume (lemon grass) [1]. Grasses also has been known for their phytoremediation ability. Phytoremediation is defined as cleaning of pollutants by using plants including trees, grasses and aquatic plants [2].

Grasses are distinguished by a fast growth rate, well-developed root system, large biomass and a long-term growth cycle. In addition, they show a high tolerance to soil contamination with heavy metals and can accumulate large quantities of heavy metals in both roots and shoots [3]. Some of them have been recognized as potential accumulator plants, such as Hordeum vulgare [4], Sorghum bicolor [5], and Setaria italica [6].

The function in absorbing the heavy metals by the accumulator plant is regulated by many genes, by which, one is Metallothionein (MT) gene. Metallothionein is one of metal binding protein groups which is rich in amino acids cysteine (30% of total residues) and has a low molecular mass character [7]. MT was first discovered in 1957 by Margoshes & Vallee who isolated the MT from the horse's renal cortex and found that MT has a high affinity for Cd [8]. Since then, MT has been studied in
fungi, cyanobacteria, mammals, and also plants. Plant MTs can be divided into four types based on their amino acid residues, namely MT1, MT2, MT3, and MT4. Type 1 MTs comprise of six C-Xaa-C motifs (Xaa symbolize another amino acid) that are distributed equally among two domains. Type 2 MTs comprises of two cysteine-rich domains separated by a spacer of approximately 40 amino acid residues. The first pair of cysteines is existing as a C-C motif in amino acid positions 3 and 4 of these proteins. C-G-G-C motif is present at the end of the N-terminal cysteine-rich domain. Type 3 MTs comprise of only four Cys residues in the N-terminal domain. The consensus sequence for the first three is C-G-N-C-D-C. The fourth cysteine is not part of a pair of cysteines but is contained within a highly conserved motif, G-C-Xaa-K-K-G. The six Cys residues in the C-terminal cysteine-rich domain are arranged in C-Xaa-C motifs. Type 4 MTs have three cysteine-rich domains, each containing 5 or 6 conserved C residues, which are separated by 10 to 15 amino acids. Most of the cysteines are present as C-Xaa-C motifs [9].

MT can works against free radicals, such as hydrogen peroxide, superoxide, nitric oxide and hydroxyl radicals. It also functions for metal detoxification, and homeostasis [10]. Meanwhile, MT also functions as a chelator that can bind wide range of heavy metals. Its cystein-rich residue leads the chelating process by its group which has high affinity for heavy metal ions. Also, it has been known that metallothioneins play a role in absorbing excess metal because an increased concentration of metal ions will induce its response [11].

MT gene is a heavy-metal inducible gene. Its protein encoded regulates the plants to be easier to absorb and clean up the metal pollution from the environment [10, 12]. Therefore, metallothionein has been used as a biomarker of metal exposure [13, 14]. Certainly, study the character of this gene in metal accumulator plants is absolutely necessary to understand its function and regulation related to the resistant mechanism of accumulator plant under heavy metal rich conditions. However, there is no information about the functional domains of MT gene in plants. It should be noticed that there will be variation in specific gene or genomic region in a natural population. The level of this variation is not constant, but varies in different genes or gene region reflecting different functional characters or gene products. Thereby, study the sequence variation of MT gene is required to find out the gene conserved region, especially in Poaceae family, related to MT gene function for phytoextraction. The homology of MT gene can be utilized for showing the information about the genetic relatedness of the gene in interspecies.

2. Materials and Method

2.1. Sequence materials
DNA sequences used in this study were obtained from National Centre for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov). The sequences then were converted into FASTA format and grouped according to type of Metallothionein gene in Poaceae family.

2.2. Data analysis
Phylogenetic trees of MT sequences of Poaceae family were constructed based on the distance matrix with the maximum-likelihood (ML) method (www.phylogeny.fr). In order to analyze the ML tree, the bootstrap test was performed based on 1000 replications. To confirm the identity of MT sequence in Poaceae family, the sequences were subjected to BLAST algorithms search against the nucleotide sequences database (National Center for Biotechnology Information, NCBI), under an E-value threshold of <10^-3. T-coffee tool (tcoffee.crg.cat/) was utilized to align the sequences, therefore this tool identified the homology region between the sequences.

3. Results and Discussion
DNA sequence variation of MT gene, both within and between species in Poaceae family, provides the powerful information in determining interspecific phylogenetic distances. Also, the phylogenetic tree aims to identify the pattern of individual Poaceae plant in every MT gene type. In this study, the
phylogenetic tree was performed using the maximum-likelihood (ML) method. The ML test is generally used to figure out the evolutionary tree as the best tool to observe the variation in a group of sequences. This ML method was already used to construct the phylogenetic relationship of endosulfan-degrading bacteria [15], Hibiscus syriacus and other plant species based on the chloroplast genome sequence [16], plant annexins based on cDNA sequences contained within the 933-nt homologous tetrad core region [17], plant sulfotransferase enzymes [18], 16S rRNA gene sequences of Micromonospora species [19], the LYK group of LysM-receptor like kinases in Legumes [20].

Figure 1 shows that MT1A of Chloris virgata is grouped in the same cluster with MT1 of Eleusine indica, whereas MT1A of Sorghum bicolor and Setaria italica are gathered in the same cluster with Zea mays. Metallothionein 1 type BofHordeum vulgareis separately clustered with type MT1 of Aegilops tauschii subsp. tauschii. MT1B of Oryza sativa subsp. japonica is located in the same cluster with Oryza brachyantha, by which both are in the separate cluster with MT1A of Oryza sativa subsp. japonica. Overall, phylogenetic tree of MT1 in Poaceae plants presents a different cluster between MT1A and MT1B. BLAST data of MT1A sequence of Chloris virgata resulted in a high identity (90%) with Allium sativum which is being a member of Amaryllidaceae family, not Poaceae (Fig. 2).

Phylogenetic tree by using the maximum-likelihood (ML) method in MT2 gene sequence of Poaceae plants (Fig 3) indicates that MT2 is noticeably grouped in three clusters of MT2A, MT2B, and MT2C. Metallothionein gene type 2C of Sorghum bicolor, Setaria italica, Brachypodium distachyon, Hordeum vulgare, and Aegilops tauschii subsp. tauschii are separeated in a different cluster with MT2A gene of Oryza brachyantha, Setaria italica, Sorghum bicolor, and Zea mays. The results of multiple sequence alignment (MSA) presents that the homology region of MT2 gene sequence in Poaceae plants is identified in the area close to N-terminal domain (Fig. 4). This homologous region has around 70 base nucleotides in length which mostly contains of repeated codon for Cystein amino acids, namely TGT or TGC.

Figure 1. Phylogenetic analysis of 18 Poaceae plants based on nucleotide sequence variation of MT1 gene. Phylogenetic tree was performed using the maximum likelihood (PhyML) method. Branch support values are in red colour in above lines denoting branches.
Figure 2. BLAST of Chloris virgata MT1 sequence. The result shows a high similarity (90%) of Chloris virgata MT1 sequence with Allium sativum as a member of Amaryllidaceae.

Figure 3. Phylogenetic analysis of Poaceae plants based on nucleotide sequence variation of MT2 gene. Phylogenetic tree was performed using the maximum likelihood (PhyML) method. Branch support values are in red colour in above lines denoting branches.
**Figure 4.** Homology sequence of MT2 gene in Poaceae plants. The homologous region is located close to N-terminal domain of MT2 gene.

**Figure 5.** Phylogenetic analysis of Poaceae plants based on nucleotide sequence variation of MT3 gene. Phylogenetic tree was performed using the maximum likelihood (PhyML) method. Branch support values are in red colour in above lines denoting branches.

**Figure 6.** Phylogenetic analysis of Poaceae plants based on nucleotide sequence variation of MT4 gene. Phylogenetic tree was performed using the maximum likelihood (PhyML) method. Branch support values are in red colour in above lines denoting branches.
Only a few Poaceae plants used to construct phylogenetic relationship of MT3 gene (Fig.5). Metallothionein type 3 gene of *Oryza sativa subsp japonica* and *Oryza coarctata* bicolor are in-groups, whereas * Hordeum vulgare* and *Sorghum bicolor* are out-groups of *Oryza* plants. Figure 6 shows that separated clusters are presented in Metallothionein type 4A, 4B, and 4C between Poaceae plants. Interestingly, MT4 of *Hordeum vulgare* plants are outgroup of MT4A, MT4B, and MT4C in PhyML of Metallothionein genes in Poaceae plants.

When genes determining the genetic basis of a specific trait are known, there is a way to develop a molecular marker for a particular purpose. Genetic markers are ultimately based on the variation of gene sequence [21]. The knowledge of an adaptive genetic variation in plants is a basis to evolve a strategy to overcome heterogeneous conditions. Hence, the presence of MT genes variation in Poaceae plants is very important for the long-term persistence under metal contaminate area.

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