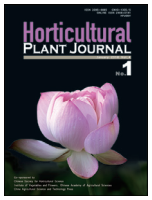
xxx. Horticultural Plant Journal, xxx (xx): xxx–xxx.

CSHS

Horticultural Plant Journal

Available online at www.sciencedirect.com
The journal's homepage: http://www.journals.elsevier.com/horticultural-plant-journal



Original article

Genome-wide identification and expression analysis of half-size *ABCG* genes in *Malus* x *domestica*

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Q2 Available online xxx

A B S T R A C T

Half-size adenosine triphosphate-binding cassette transporter subgroup G (*ABCG*) genes play crucial roles in regulating the movements of a variety of substrates and have been well studied in several plants. However, half-size *ABCG*s have not been characterized in detail in apple (*Malus* × *domestica* Borkh.). Here, we performed a genome-wide identification and expression analysis of the half-size *ABCG* gene family in apple. A total of 46 apple half-size *ABCG*s were identified and divided into six clusters according to the phylogenetic analysis. A gene structural analysis showed that most half-size *ABCG*s in the same cluster shared a similar exon–intron organization. A gene duplication analysis showed that segmental, tandem and whole-genome duplications could account for the expansion of half-size *ABCG* transporters in *M. domestica*. Moreover, a promoter scan, digital expression analysis and RNA-seq revealed that *MdABCG21* may be involved in root's cytokinin transport and that *ABCG17* may be involved in the lateral bud development of *M. spectabilis* 'Bly114' by mediating cytokinin transport. The data presented here lay the foundation for further investigations into the biological and physiological processes and functions of half-size *ABCG* genes in apple.

Keywords: ABCG gene; Apple; Duplication; Gene expression

1. Introduction

The ATP-binding cassette (ABC) transporters form a large family of transport proteins that catalyzes the active movements of a variety of substrates in organisms, ranging from bacteria to humans (Jeong et al., 2017). ABC transporters are involved in biological processes and are highly conserved in plants (Sugiyama et al., 2006). All of the ABC transporters share a similar molecular architecture that contains at least one nucleotide-binding domain (NBD) and several transmembrane domains (TMDs) (Jeong et al., 2017).

Plants are notable for their particularly large numbers of ABC Q3 proteins. Plant's ABC proteins are classified into eight subfamilies (ABCA–H) (Rajsz et al., 2016). The ABCG subfamily is composed of two major types of proteins: (i) full-size ABCG transporters, also known as the plant pleitropic drug-resistant-like subfamily, that contain two reverse-oriented NBD-TMDs; and (ii) half-size ABCG transporters, also known as the white-brown complex (WBC) subfamily, that contain one reverse-oriented NBD-TMD (Ding et al., 2013; Pang et al., 2013). Progress has been made in functionally characterizing the half-size ABCG transporters in *Arabidopsis*. A main function of ABCG transporters involves material transport. For example, *AtABCG11* (*WBC11*) and *AtABCG13* (*WBC13*) are required for the export of various cuticular lipids (Panikashvili et al., 2010, 2011). *ABCG9* (*WBC9*), *ABCG11* (*WBC11*) and *ABCG14* (*WBC14*), when physically interacting with each other, are required for vascular patterning (Le et al., 2013). *ABCG25* (*WBC25*) and *ABCG22* (*WBC22*) are directly or indirectly involved in abscisic acid (ABA) transport and responses (Kuromori et al., 2010a, 2010b), and *ABCG14* (*WBC14*) is involved in the acropetal translo-

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Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

https://doi.org/10.1016/j.hpj.2018.03.001

cation of root-synthesized cytokinins (Ko et al., 2014; Zhang et al., 2014).

Apple (*Malus* × *domestica* Borkh.) is an economically important fruit tree that is planted worldwide. Cellular transport is regulated by a complex network, and minimal information exists on the roles of half-size *ABCG* genes in mediating substrate transport in apple. In this study, we performed, for the first time, a wide-ranging analysis of the half-size *ABCGs* in *M. domestica*. A total of 46 half-size *ABCGs* were identified and subsequently investigated, including their phylogenetic relationships, chromosomal locations, gene duplication status, gene structures and expression profiles. The objectives of this study were: (1) to identify and characterize the half-size *ABCGs* in *M. domestica*; (2) to increase our understanding of the evolution and expansion of the half-size *ABCG* family in *M. domestica*; (3) to analyze the half-size *ABCG*'s expression profiles in *M. domestica*; and (4) to provide a basis for future studies on the structures and functions of the half-size *ABCGs* in *M. domestica*.

2. Materials and methods

2.1. Identification of half-size ABCGs in apple

To carry out a phylogenetic analysis of half-size ABCG transporters in *M. domestica* and *Arabidopsis thaliana*, we collected twenty-nine *Arabidopsis* protein family members in *Arabidopsis* Information Resource (http://www.Arabidopsis.org/index.jsp). Every sequence of AtABCG was used as a query to search the apple genome for homologs under the BLASTp algorithm, then the putative half-size ABCGs in the apple genome were confirmed by searching the National Center for Biotechnology Information's (NCBI's) protein collection database. Finally, the putative half-size ABCG protein sequences were used as queries to search the Pfam database (http://pfam.xfam.org/) to verify the presence of conserved domains. Apple sequences lacking the characteristic domains (NBD and TMD) were eliminated and the left apple proteins were used to search the nucleotide sequences by BLASTn.

2.2. Phylogenetic tree and gene structure building

The exon–intron layouts of apple half-size *ABCG* genes were produced using the online tool, Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) (Guo et al., 2007). The phylogenetic analysis was done with proteins using MEGA 5.0 under the following parameters: Neighbor Joining tree method, complete deletion with a bootstrap of 1000 repeats.

2.3. Analysis of chromosomal location and gene duplications

Information of the physical locations of all half-size *MdABCG* genes on chromosomes was obtained through BLASTn algorithm searches against the *Malus domestica* genome database in phytozome (http://www.phytozome.net/apple). Then all half-size *MdABCG* genes were mapped on the chromosome using the software MapDraw (Liu and Meng, 2003). The identification of half-size *ABCG* gene duplication events was also performed. Paralogous half-size *ABCG* gene pairs in *M. domestica* were recognized, on account of alignment results. The criteria described in previous studies (Yang et al., 2008) were adopted: shorter sequence covers over 70% of the longer sequence after alignment and the minimum identity of aligned regions is 70% (Ma et al., 2014). If homologous *ABCG* genes were located on the same chromosome, they were identified as tandem duplication genes; otherwise, they were segmental duplication genes.

2.4. Digital EST expression and RNA-seq expression analysis

The analysis of *MdABCGs* expression profiles was investigated at the transcriptional level. *MdABCGs* expression profiles were searched for using the BLAST program in the NCBI EST libraries with the following parameters: maximum identity > 90%, length > 200 bp and *E*-value < 10^{-10} (Ma and Han, 2016). The RNA-seq database of the lateral bud of *M. spectabilis* 'Bly114' got from our laboratory which has not released yet. The expression level was investigated based on RPKM (Reads per kilobases per million reads).

2.5. Plant material

Apple dwarfing rootstocks M9 seedlings planted in 2013 in Yangling, Shaanxi, and flowers, mature leaves, root tips, xylem, phloem of stems (5 cm long rootstock) were collected in 2016 used for qRT-PCR. Meantime lateral buds (diameter of 1–2 mm) were collected from *M. spectabilis* 'Bly114' obtained from apple germplasm nursery of the Institute of Agricultural Science of Qingdao for RNA-seq. Then the above materials were frozen with liquid nitrogen and stored in a –80 °C freezer (Song et al., 2017).

2.6. RNA extraction, cDNA synthesis and qRT-PCR analysis

Total RNA was extracted from the flowers, mature leaves, root tips, xylem, phloem of stems (5 cm long rootstock) using the cetyl trimethyl ammonium bromide (CTAB) method, followed by treatment with RNase-free DNase I (Invitrogen, Shanghai, China) to remove any residual genomic DNA. The first-strand cDNA was synthesized using a SYBR Prime Script RT-PCR Kit II (TaKaRa, Shanghai, China) according to the manufacturer's guidelines. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to analyze expression profile of the genes. qRT-PCR reactions were performed in a 20 μL volume with 1 μL cDNA which was diluted to 100 ng, and run in three independent biological replicates of each sample and three technical replicates for biological replicates. The qRT-PCR analysis was done by a SYBR Green qPCR kit (TaKaRa, Dalian, China), using Bio-Rad CFX Connect Real-Time PCR Detection System. *MdActin-3* gene (MDP0000912745) served as internal standards. The relative expression level of each gene was calculated with the $2^{-\Delta\Delta CT}$ method (Ma et al., 2014).

2.7. Promoter analysis

The 1000 bp long promoter sequences of *MdABCG17* and *MdABCG21* were obtained from apple_genome_contigs from GDR (https://www.rosaceae.org). The promoter sequences were then analyzed using the PLACE database in order to find the transcription factor (TF) binding motifs (Higo et al., 1999). TF binding motifs were assigned to the different processes based on the information in the PlantTF Database (http://planttfdb.cbi.pku.edu.cn/).

Please cite this article as: Juanjuan Ma et al., Genome-wide identification and expression analysis of half-size ABCG genes in Malus × domestica, Horticultural Plant Journal (2018), https://doi.org/10.1016/j.hpj.2018.03.001