RESEARCH ARTICLE

# Genomewide analysis of *ABCB*s with a focus on *ABCB1* and *ABCB19* in *Malus domestica*

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## Abstract

The B subfamily of ATP-binding cassette (ABC) proteins (ABCB) plays a vital role in auxin efflux. However, no systematic study has been done in apple. In this study, we performed genomewide identification and expression analyses of the *ABCB* family in *Malus domestica* for the first time. We identified a total of 25 apple ABCBs that were divided into three clusters based on the phylogenetic analysis. Most *ABCBs* within the same cluster demonstrated a similar exon–intron organization. Additionally, the digital expression profiles of *ABCB* genes shed light on their functional divergence. *ABCB1* and *ABCB19* are two well-studied auxin efflux carrier genes, and we found that their expression levels are higher in young shoots of M106 than in young shoots of M9. Since young shoots are the main source of auxin synthesis and auxin efflux involves in tree height control. This suggests that *ABCB1* and *ABCB19* may also take a part in the auxin efflux and tree height control in apple.

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## Introduction

Auxin plays an essential role in cell division and elongation (Campanoni and Nick 2005; Perrot-Rechenmann 2010), apical meristem dominance, vascular tissue differentiation (Mattsson *et al.* 2003), and other developmental and physiological processes (Balzan *et al.* 2014). Auxin gradients caused by the cell-to-cell or polar transport of auxin (PAT) provide the directional information required for the coordination of plant development and physiology (Forestan and Varotto 2010). PAT is controlled by the cellular influx and efflux of auxin through both membrane diffusion and carrier-mediated transport. To date, three types of auxin transporters have been reported: AUXIN RESISTANT 1/LIKE AUX1s (AUX1/LAXs) as auxin influx carriers, and PIN-FORMED (PIN) carriers and the B subfamily of ATP-binding cassette proteins (ABCBs), formerly called P-glycoproteins (PGPs)/multidrug-resistance (MDRs), as auxin efflux carriers (Shen *et al.* 2010; Cho and Cho 2013).

The ABCB family belongs to the ABC transporter superfamily. Full-size ABCB proteins have similar halves containing a transmembrane domain (TMD) and a nucleotide-binding domain (NBD). In *Arabidopsis*, 21 full-size ABCB proteins have been identified and divided into three clusters (Geisler and Murphy 2006; Kang *et al.* 2011). *AtABCB1* and its closest homologue *AtABCB19* are involved in polar auxin transport (Geisler *et al.* 2005; Sukumar *et al.* 2013). The overexpression of *AtABCB1* resulted in longer hypocotyls in *Arabidopsis* grown under dim light (Sidler *et al.* 1998). In *atpgp19* and *atpgp1*, as well as in the *atpgp19* double mutant, the polar auxin flow through stems was impaired by ~80% or more compared with the wild type and resulted in dwarfism (Noh *et al.* 2001; Geisler *et al.* 2005; Ye *et al.* 2013). The *ABCB1* orthologue has been cloned in *Sorghum* (*Dwarf3/SbPGP1*) and in maize (*Brachytic2/ZmPGP1*), and the mutants show reduced basipetal auxin transport and compact lower stalk internodes (Multani *et al.* 2003; Knöller *et al.* 2010). The *ABCB19* orthologue *OsABCB14* was cloned in rice and the knock down of *OsABCB14* confers decreased auxin concentrations and polar auxin transport rates (Xu *et al.* 2014). ABCBs probably interact with other regulatory proteins, such as PIN and the immunophilin-like TWISTED DWARF 1 (TWD1) to regulate auxin transport activities (Blakeslee *et al.* 2007; Wang *et al.* 2013), but ABCBs may mediate direct auxin transport (Geisler *et al.* 2005).

Apple (*Malus* × *domestica* Borkh.) is an important fruit crop that is grown worldwide. Recently, the full genome sequence of the domesticated apple was published (Velasco *et al.* 2010). This provides a useful genomic tool for the study of apple *ABCBs*. In this study, for the first time we performed a comprehensive analysis of the ABCB family in

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*M. domestica*. A total of 25 *Malus* ABCBs were identified and subsequently subjected to a systematic analysis, including phylogenetic relationships, chromosomal location, gene duplication status, gene structure and digital expression profiling. Since *ABCB1* and *ABCB19* play an important role in PAT, the low auxin transport ability in apple dwarfing rootstocks may be involved in the polar indole 3-acetic acid (IAA) transport disability (Van Hooijdonk *et al.* 2010; Cho *et al.* 2014), we also performed a tissue-specific expression analysis of *ABCB1* and *ABCB19* in apple. We found that *ABCB1* and *ABCB19* are expressed higher in young shoots of M106 than in young shoots of M9 suggesting that *ABCB1* and *ABCB19* may also participate in PAT which probably had relation with dwarfing stem in apple. Thus, our analysis of the *ABCB* gene family will contribute to future studies on the functional characterization of ABCB proteins and give some hint on dwarfing character research in apple.

## Materials and methods

### *Multiple sequence alignment and phylogenetic tree building*

To perform a phylogenetic analysis of ABCB transporters in apple and *Arabidopsis thaliana*, we collected 21 *Arabidopsis* family members from The Arabidopsis Information Resource (http://www.Arabidopsis.org/index.jsp). Every sequence of *A. thaliana* was used as a query in the basic local alignment search tool (BLAST)P algorithm to search the apple genome for homologues, and then the putative ABCBs in apple genome were confirmed by searching the National Center for Biotechnology Information's (NCBI's) protein collection database. Finally, ABCB protein sequences were used as queries to search the PROSITE database to rule out the presence of partial ABCB transporters. The intron–exon structures of apple *ABCB* genes were produced using the online tool Gene Structure Display Server (Guo *et al.* 2007). Transmembrane domains were predicted using the online tool TMHMM Server v.2.0.

Multiple amino acid sequences were aligned using the ClustalX program ver. 2.0. The phylogenetic analysis was performed using MEGA 5.0 with the following parameters: neighbour-joining tree method, complete deletion and a bootstrap of 1000 repeats.

### *Analysis of chromosomal location and gene duplications*

Information on the physical locations of all *MdABCB* genes on chromosomes was obtained through BLASTN searches against the *M. domestica* genome database in phytozome (http://www.phytozome.net/apple). All *MdABCB* genes were then mapped on the chromosome using the software MapInspect. The recognition of *ABCB* gene duplication events was also carried out. Paralogous *ABCB* gene pairs in *M. domestica* were identified on the basis of alignment results. The criteria described in previous studies (Yang *et al.* 2008) were adopted: shorter sequences cover over 70% of the longer sequence after alignment and the minimum identity of the aligned regions is 70% (Ma *et al.* 2014).

### *Digital and expressed sequence tag (EST) expression analyses*

*MdABCB*s expression profiles were analysed at the transcriptional level. *MdABCBs* expression patterns were searched for using the BLAST programme in the NCBI *M. domestica* EST libraries with the following parameters: maximum identity >95%, length >200 bp and E value $<10^{-10}$ (Giorno *et al.* 2012).

### *Expression analysis of MdABCBs in microarray*

The microarray data of gene expression in apple during rootstock–scion interactions (GSE4762) was downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) using the GSE series accession number GSE24523. The sequences of identified *MdABCB* containing genes were used as queries to blast against the probe platform (GPL3715) to discover corresponding unigene IDs that were used in the microarray data.

### *Plant material*

Leaves, young shoots, roots, and xylem and phloem of stems from apple rootstock M106 (vigorous) and M9 (dwarf) were collected. The above materials were cut into pieces and immediately placed in an ultra-low temperature freezer.

### *RNA extraction, purification and cDNA synthesis*

Total RNA was extracted from frozen samples according to the cetyl trimethyl ammonium bromide method. RNase-free DNase I (Invitrogen, Carlsbad, USA) was used to remove any residual genomic DNA. The first-strand cDNA was synthesized from 1 $\mu$g of total RNA in a volume of 20 $\mu$L using a SYBR Prime Script RT-PCR Kit II (Takara, Dalian, China).

### *qRT-PCR*

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to analyse gene expression. Quantitative real-time PCR was performed using the Bio-Rad CFX Connect Real-Time PCR Detection System. cDNAs were diluted to 100 ng and run in three technical replicates, with 1 $\mu$L template in a total reaction volume of 20 $\mu$L. PCR amplification conditions were as follows: 95°C for 5 min, then 40 cycles of 94°C for 20 s, 58°C for 20 s, and 72°C for 10 s. A melting curve analysis was performed to determine whether a single product was amplified. The apple actin gene was used as an internal standard in the analysis. The relative expression level of each gene was calculated according to the $2^{-\Delta\Delta CT}$ method.

### Hierarchical clustering analysis

Gene expression levels were grouped by a two-way hierarchical clustering method using the TIGR MeV ver. 4.0.9 software package (Saeed *et al.* 2003).

## Results

### Genomewide identification of Malus ABCB genes

The nomenclature of putative *Malus ABCB* genes were assigned based on their *A. thaliana* homologues. We identified a total of 25 *Malus* ABCBs with a repeated TMD–NBD structure comprised of 7–13 transmembrane helices and length varying between 1145 and 1726 amino acids (table 1).

### Phylogenetic analysis of MdABCBs

To investigate the evolution of ABCBs, an unrooted phylogenetic tree was generated by using the 25 *M. domestica* ABCBs, and 21 *A. thaliana* ABCBs. The result of this analysis is shown in figure 1. Based on an already published *Arabidopsis* ABCB category (Geisler and Murphy 2006) these MdABCBs were clustered into three distinct groups: MdABCB1, MdABCB1b, MdABCB2, MdABCB13a, MdABCB13b, MdABCB19, MdABCB19b, MdABCB20a and MdABCB20b in cluster I; MdABCB2-like, MdABCB2b-like, MdABCB9, MdABCB9b, MdABCB9-like, MdABCB11, MdABCB11-like, MdABCB11b, MdABCB11c, MdABCB11e and MdABCB11d in cluster II; and MdABCB15-like, MdABCB15b-like, MdABCB15c, MdABCB15c-like and MdABCB15d-like in cluster III.

Because of the importance of *ABCB1* and *ABCB19* in PAT, we also developed a phylogenetic tree for ABCB1 and ABCB19 in plants. We found that eudicot ABCB1 and ABCB19 cluster together while monocot ABCB1 and ABCB19 cluster together (figure 2).

### Chromosomal location and gene duplication

To determine the chromosomal distribution of the *ABCB* genes in *M. domestica*, the physical locations of the *MdABCB* genes on the chromosomes were obtained through BLASTN searches in Genome Database for Rosaceae (GDR) (http://www.rosaceae.org/tools/ncbi_blast) against the *M. domestica* genome database. Among the 25 *MdABCB* genes, a total of 24 genes were distributed across 13 of the 17 *M. domestica* chromosomes, while one, *MdABCB11*-like was unanchored (figure 3). Generally speaking, the number of *MdABCB* genes on each chromosome appeared to be uneven, ranging from zero to four genes per chromosome. Apple chromosome 17 had the greatest number of *MdABCB* genes, containing four *MdABCB* genes, followed by three on chromosomes 9, 10 and 13. *MdABCB9*, *MdABCB9b* and *MdABCB9*-like are closely located on chromosome 10, while chromosomes 7, 14 and 15 contain no *MdABCB* genes.

Given their importance in the amplification of gene families, potential duplication events in the MdABCB family

**Table 1.** The B subfamily of the ATP-binding cassette (ABC) proteins (ABCB) in apple.

| Gene name | Gene locus | Chromosome | Coding sequence size (bp) | Exon |
|---|---|---|---|---|
| *MdABCB1* | MDP0000183294 | chr12:14650249..14656002 | 4065 | 10 |
| *MdABCB1b* | MDP0000231597 | chr4:6671970..6677802 | 3864 | 11 |
| *MdABCB2* | MDP0000286528 | chr6:4573732..4581090 | 3786 | 11 |
| *MdABCB2*-like | MDP0000399961 | chr17:12915072..12923786 | 5178 | 11 |
| *MdABCB2b*-like | MDP0000248803 | chr9:17738404..17744362 | 4629 | 9 |
| *MdABCB9* | MDP0000753748 | chr10:26299913..26307086 | 3717 | 12 |
| *MdABCB9b* | MDP0000292611 | chr10:26289833..26298328 | 3696 | 12 |
| *MdABCB9*-like | MDP0000257503 | chr10:26278626..26287262 | 3435 | 13 |
| *MdABCB11* | MDP0000849544 | chr17:24692300..24697940 | 3888 | 12 |
| *MdABCB11*-like | MDP0000166916 | Unanchored (chr9) | 3891 | 12 |
| *MdABCB11b* | MDP0000318119 | chr13:13132894..13138488 | 3885 | 12 |
| *MdABCB11c* | MDP0000302716 | chr13:13162520..13168388 | 3900 | 12 |
| *MdABCB11d* | MDP0000123768 | chr8:19916509..19922040 | 3774 | 13 |
| *MdABCB11e* | MDP0000924533 | chr13:11515102..11520460 | 3885 | 12 |
| *MdABCB13a* | MDP0000124006 | chr12:21005460..21011214 | 3738 | 9 |
| *MdABCB13b* | MDP0000149035 | chr8:753373..757874 | 3741 | 9 |
| *MdABCB15*-like | MDP0000383809 | chr9:2465415..2475159 | 4245 | 7 |
| *MdABCB15b*-like | MDP2000521460 | chr17:2819356..2829387 | 4860 | 7 |
| *MdABCB15c* | MDP0000244899 | chr16:5275688..5280699 | 3807 | 7 |
| *MdABCB15c*-like | MDP0000863169 | chr3:25021559..25026060 | 3744 | 7 |
| *MdABCB15d*-like | MDP0000260433 | chr2:5248205..5254505 | 3807 | 9 |
| *MdABCB19* | MDP0000320690 | chr9:3021773..3028630 | 3723 | 10 |
| *MdABCB19b* | MDP0000265271 | chr17:3338032..3347970 | 3882 | 11 |
| *MdABCB20a* | MDP0000295837 | chr1:17301063..17308468 | 4224 | 11 |
| *MdABCB20b* | MDP0000203626 | chr1:17079103..17086292 | 4278 | 12 |

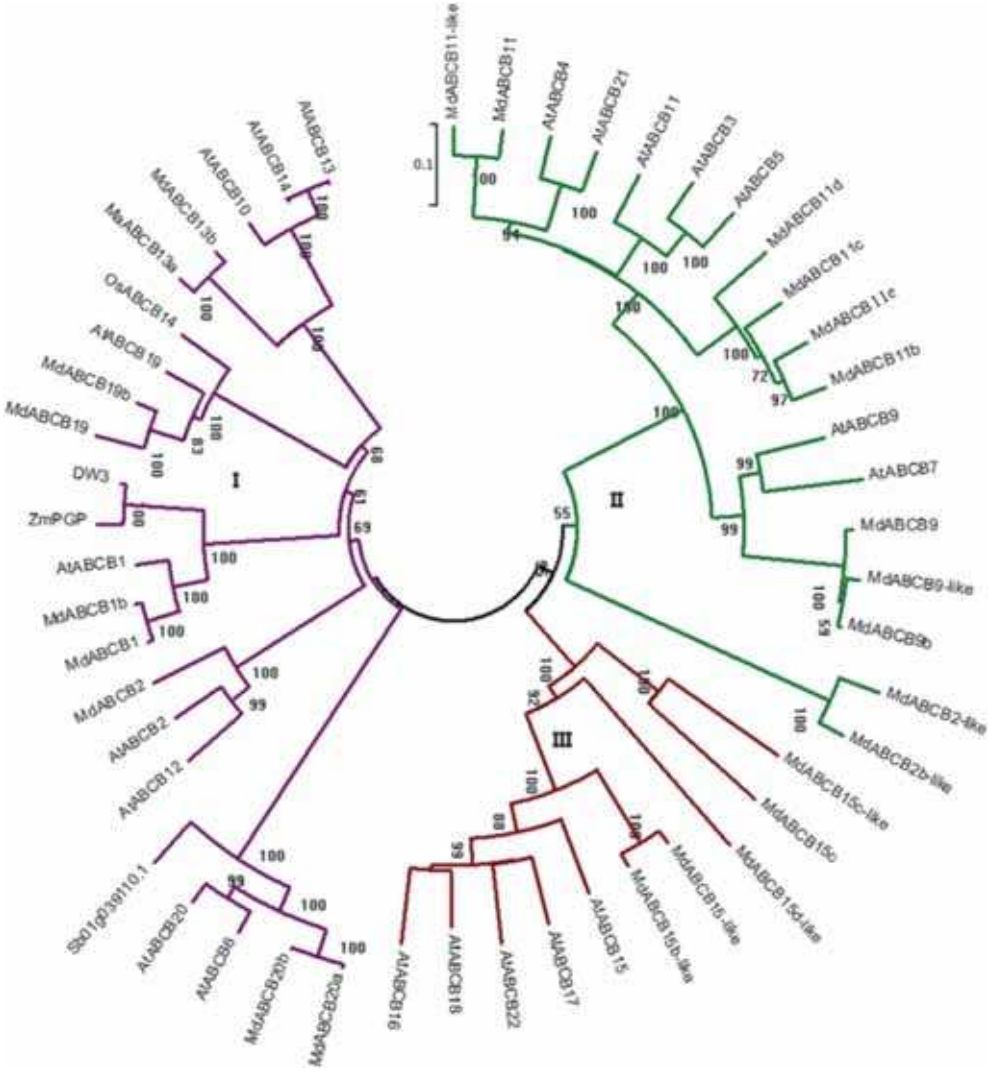


**Figure 1.** A phylogenetic tree showing predicted relationships among the B subfamily of the ATP-binding cassette (ABC) proteins (ABCB) in *M. domestica* and *A. thaliana*. We classified 25 apple ABCB transporter proteins and 21 *Arabidopsis* ABCB transporter proteins into three clusters. Each cluster is marked in a different colour.

were analysed. Based on protein sequence identities, seven pairs of putative paralogous MdABCBs were identified, accounting for 50% of the entire MdABCB family. These gene pairs have high degrees of protein sequence identities. For instance, the sequence of MdABCB13a covers 99.9% of that of MdABCB13b after alignment, and the identity of the aligned region is 93.34%. Among these paralogous gene pairs, three pairs are located on different chromosomes, whereas no traceable duplication events could be determined for the other gene pair since one gene of this pair was anchored on an unmapped scaffold (figure 3).

### *Gene structures and digital expression of MdABCB genes*

Exon–intron structures of the 25 *MdABCB* genes were generated based on their corresponding genome sequences and coding sequences (figure 4), and the *MdABCB* genes have 7–13 exons. As expected, most *ABCB* genes within the same cluster demonstrated very similar exon–intron distribution patterns in terms of exon length and intron number. For example, most *ABCB* genes in cluster III have seven exons.

The expression pattern of a gene is generally correlated with its function; hence, we analysed expression of *MdABCB*s by counting the number of ESTs per tissue in the NCBI EST libraries. This resulted in the assignment of *MdABCB*s to eight groups on the basis of the tissue and organ types in which *MdABCB*s were present (table 2). *MdABCB2*-like, *MdABCB2b*-like, *MdABCB11d*, *MdABCB15c*, *MdABCB15c*-like and *MdABCB15d*-like may be pseudogenes since no corresponding ESTs were found in any organ. *MdABCB9*, *MdABCB9b* and *MdABCB9*-like were expressed only in fruit. Other *ABCB*s were expressed in more than one tissues.

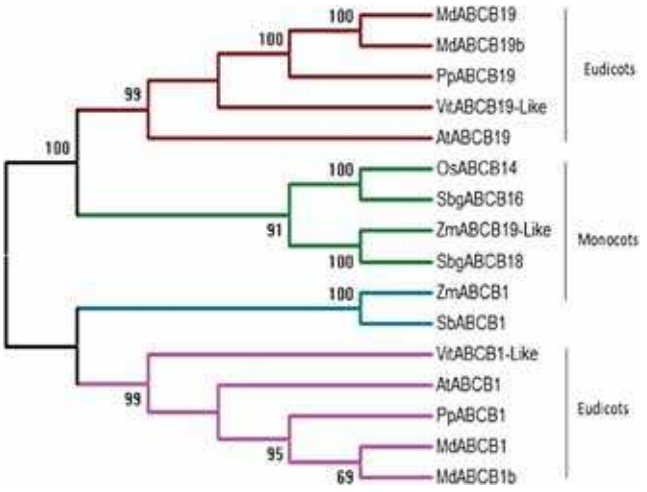


**Figure 2.** A phylogenetic tree of ABCB1 and ABCB19 in plants. The GenBank accession numbers are as follows: SbABCB1(AY3-7289), SbgABCB18 (XP_002448624.1) and SbgABCB16 (XP_002-447959.1) in *Sorghum bicolor*; ZmABCB1 (AY366085), Zm ABCB19-like (XP_008663648.1) in *Zea mays*; VitABCB19-like (XP_002283051.2), VitABCB1-like (XP_002266505.1) in *Vitis vinifera*; OsABCB14(NP_001052982.1) in *Oryza sativa*. The GDR accession numbers in peach are as follows: PpABCB1 (ppa00-0269m) and PpABCB19 (ppa000359m).

For example, *MdABCB1* and *MdABCB1b* were expressed in leaf, root, flower, fruit, shoot and bud. Interestingly, *MdABCB15*-like is the only *MdABCB*s that expressed in xylem indicating that *MdABCB15*-like may take a role in this tissue.

Since *ABCB1* and *ABCB19* are well studied, to investigate the expression of *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* in the entire plant, the expression patterns of *MdABCB1* and *MdABCB1b* containing probes from a microarray during rootstock–scion interactions were found using a BLAST search against the probe sequences (GPL3715). *MdABCB1* and *MdABCB1b* both have a highest expression level in Gala/M111(vigorous), but show lower level in Ambrosia/B9, Melrose/B9 (dwarf), Gala/M9 (dwarf) (figure 5).

***Tissue-specific expression of ABCB1 and ABCB19***

Owing to the fact that *ABCB1* and *ABCB19* are well-studied *ABCB*s involved in polar auxin transport, we searched for the tissue-specific expression of *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b*. Primer sequences for the quantification of transcripts by RT-PCR are provided in table 3. Leaves, young shoots, roots, and the xylem and phloem of stems from the apple rootstock M106 (vigorous) and M9 (dwarf) were used. Here, *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* all showed high expression levels in young shoots of M106, *MdABCB1* and *MdABCB19* had second expression peaks in roots of M106 (figure 6a). Unlike the expression profile in young shoots of M106, *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* all showed a low expression levels in young shoots of M9 (figure 6b), while *MdABCB19* had a higher expression in roots of M9 than M106 (figure 6b).

## Discussion

In plants, some members of the *ABCB* subfamily have been described in auxin transport (Geisler and Murphy 2006; Lewis *et al.* 2009; Cho and Cho 2013). Both size and composition of the *ABCB* family have been analysed and characterized in different plant species (Carraro *et al*. 2012). The present study for the first time investigates this gene family in the economically relevant domesticated apple and shows that its genome contains 25 full-length *ABCB* genes. This number is similar to the 24 loci encoding *ABCB* genes found in rice (Garcia *et al.* 2004). Among the 25 apple *ABCB* genes, three pairs, *MdABCB1*, *MdABCB1b*; *MdABCB13a*, *MdABCB13b*; and *MdABCB19*, *MdABCB19b*, with similar structures and expression features are probably duplications produced by a whole genome

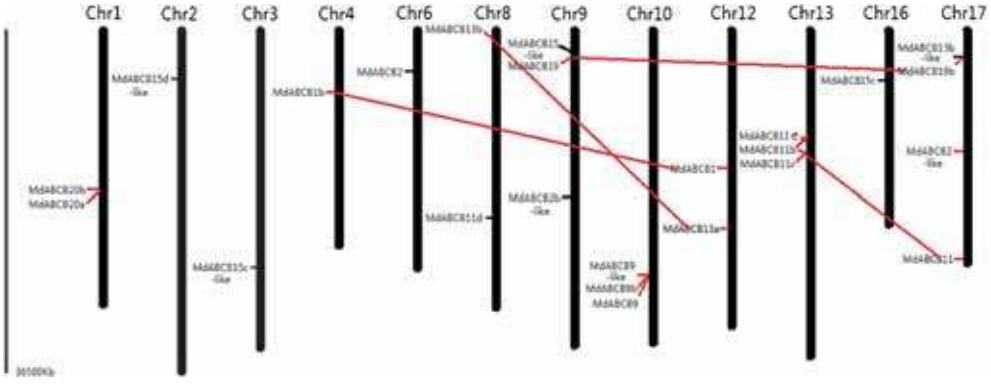


**Figure 3.** Chromosomal distribution and gene duplication of *ABCB*s in *M. domestica*. The scale is in kilobases (kb). The chromosome numbers are indicated at the top of each chromosome. The paralogous *ABCB* genes are connected with a red line.

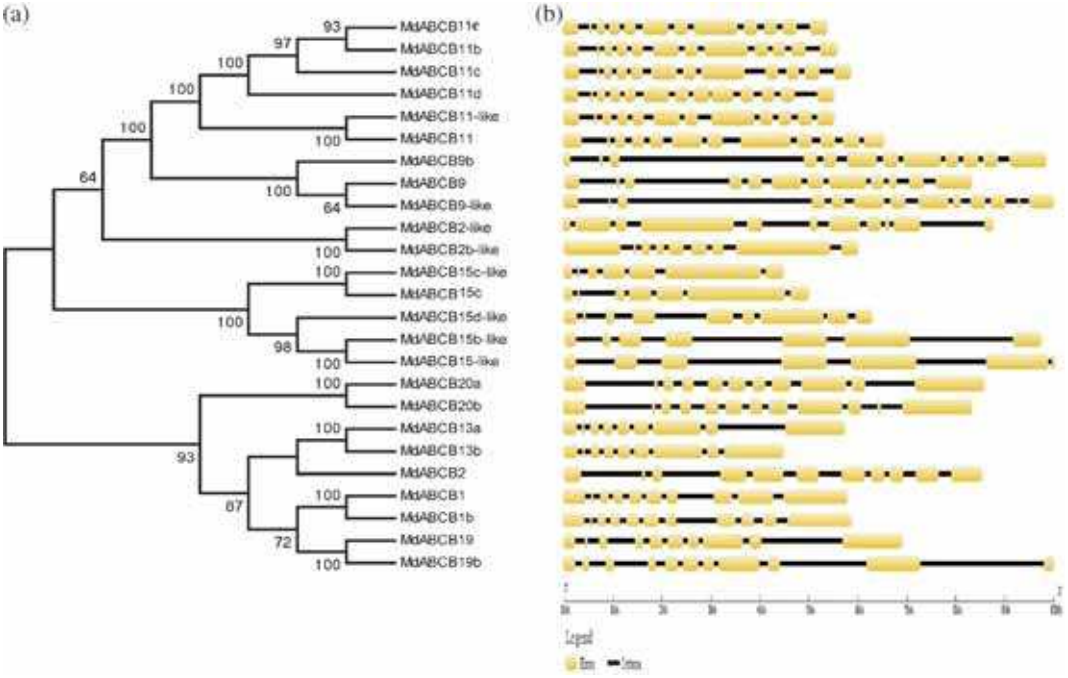


**Figure 4.** Phylogenetic analysis, gene structure of ABCB family in *M. domestica.* (a) The phylogenetic tree of all ABCBs in *M. domestica*. (b) The exon/intron organization of the *ABCB* genes of *M. domestica*.

**Table 2.** Digital expression of *ABCB*s in apple.

| Gene name | Leaf | Root | Flower | Fruit | Shoot | Seed | Bud | Xylem |
|---|---|---|---|---|---|---|---|---|
| *MdABCB1* | + | + | + | + | + | | + | |
| *MdABCB1b* | + | + | + | + | + | | + | |
| *MdABCB2* | + | | | | + | | | |
| *MdABCB2*-like | | | | | | | | |
| *MdABCB2b*-like | | | | | | | | |
| *MdABCB9* | | | | + | | | | |
| *MdABCB9b* | | | | + | | | | |
| *MdABCB9*-like | | | | + | | | | |
| *MdABCB11* | + | + | + | + | | | | |
| *MdABCB11*-like | + | + | + | + | | | | |
| *MdABCB11b* | + | + | | | | | + | |
| *MdABCB11c* | + | + | | | | | + | |
| *MdABCB11d* | | | | | | | | |
| *MdABCB11e* | + | + | | | | | + | |
| *MdABCB13a* | + | | + | + | | | | |
| *MdABCB13b* | + | | | | | | | |
| *MdABCB15*-like | | + | | | | + | | + |
| *MdABCB15b*-like | | | | + | | + | | |
| *MdABCB15c* | | | | | | | | |
| *MdABCB15c*-like | | | | | | | | |
| *MdABCB15d*-like | | | | | | | | |
| *MdABCB19* | + | + | | + | + | | | |
| *MdABCB19b* | + | + | + | | + | | | |
| *MdABCB20a* | + | | | | | | + | |
| *MdABCB20b* | + | | | | | | + | |

+, expressed; blank space, not expressed.

duplication event. An apple genome sequence comparison revealed that 1–7, 2–8, 2–15, 3–11, 4–12, 5–10, 6–14, 9–17 and 13–16 are homologous chromosome pairs (Velasco *et al.* 2010). Whole genome duplication events provide gene families with the opportunity to grow during evolution and are followed by gene loss, with some gene

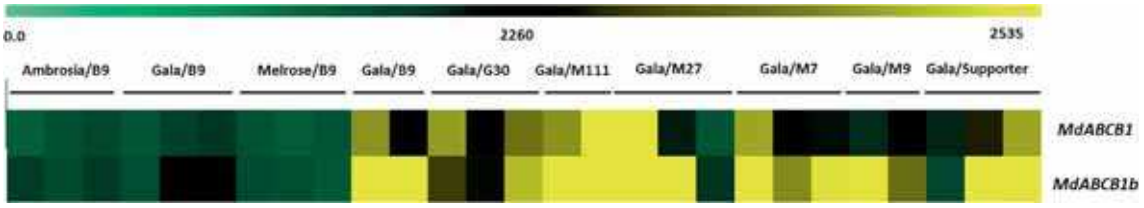


**Figure 5.** Heatmap representation for expression patterns of *ABCB1* across different rootstock–scion interactions in apple.

**Table 3.** Primers used for qRT-PCR.

| Primer name | Sequences (5′–3′) | Production size (bp) |
|---|---|---|
| *MdABCB1F* | 5′-ATCTCCACCATGTTTGCCGT-3′ | 153 |
| *MdABCB1R* | 5′-TTCCTGCTTCGCTATTCCGG-3′ | |
| *MdABCB1bF* | 5′-TGGAAGACGTTGAAGTTGCG-3′ | 185 |
| *MdABCB1bR* | 5′-TGGTAGCTTCGTCAAGGAGG-3′ | |
| *MdABCB19F* | 5′-ACCTCCAGATTCCACTACGC-3′ | 218 |
| *MdABCB19R* | 5′-GCGCCAGGGTTAATGTTTCT-3′ | |
| *MdABCB19bF* | 5′-ATGTACATTGGAGTCGGGCT-3′ | 206 |
| *MdABCB19bR* | 5′-TTTCACATCAGCAGCATCCG-3′ | |
| *MdActin-F* | 5′-TGACCGAATGAGCAAGGAAATTACT-3′ | 155 |
| *MdActin-R* | 5′-TACTCAGCTTTGGCAATCCACATC-3′ | |

pairs being maintained while others are not (Sémon and Wolfe 2007).

To date the best-characterized *ABCB*s are *AtABCB1* and *AtABCB19*. *AtABCB1/PGP1* was the first plant MDR-like gene cloned from *Arabidopsis* and a new allele, designated as *atpgp1-2*, had shorter hypocotyls and a dwarf phenotype under long-day conditions (Geisler *et al.* 2005; Ye *et al.* 2013). *Atpgp19* and *atpgp1* and the *atpgp19* double mutant, conferred dwarfism (Noh *et al.* 2001; Geisler *et al.* 2005; Ye *et al.* 2013). Our phylogenetic analysis showed that *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* clustered together with *AtABCB1* and *AtABCB19*, which are known as IAA transporters with a high specificity for IAA (Zazímalová *et al.* 2010). Futher, eudicot *ABCB1* and *ABCB19* genes cluster together, while monocot *ABCB1* and *ABCB19* genes cluster together, and in *populus* as many as 10 distinct *ABCB* gene clades that contain a clear monocot/dicot split with strong support (Carraro *et al.* 2012).

Clearly, there is much to learn regarding the role of *ABCB1* and *ABCB19* in IAA transport which play a role in tree height control in *M. domestica.* Since tissue-specific expression profiles and the expression patterns from a microarray and EST database for the *MdABCB* genes are very frequently used to predict the functions of unknown genes in specific species. *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* all have expression in shoot by EST analysis. *MdABCB1* and *MdABCB1b* both have a highest expression level in Gala/M111 (vigorous), but show lower level in Ambrosia/B9, Melrose/B9 (dwarf), Gala/M9 (dwarf) (figure 5). Further, *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* are all expressed higher in young shoots of M106 (vigorous) than in young shoots of M9 (dwarf) (figure 7). Considering that the low auxin-transport ability in dwarfing rootstocks may be the main reason for the short stem (Van Hooijdonk *et al.* 2010) and young shoots are the main source of IAA synthesis, *MdABCB1*, *MdABCB1b*, *MdABCB19* and *MdABCB19b* may be involved in PAT which play a role in tree height control in *M. domestica* as well, although each gene may take part in different apple species.

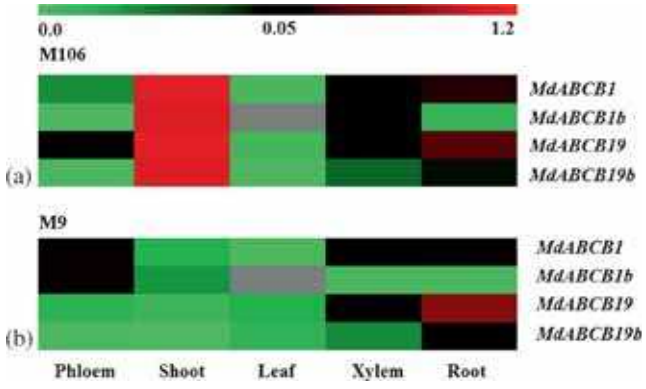


**Figure 6.** Heatmap representation for expression patterns of *ABCB1* and *ABCB19* across different tissues from M106 and M9. The expression profile data of these genes in leaves, young shoots, roots, and xylem and phloem of stems from the apple rootstock M106 and M9 were obtained using qRT-PCR. The vertical axis represents the relative expression amount. Apple actin-3 was used as internal reference.

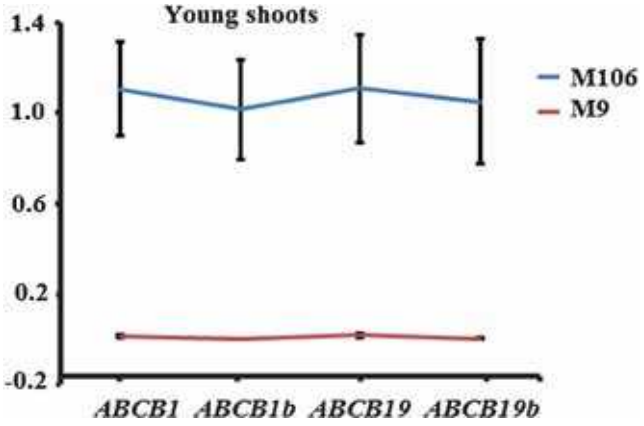


**Figure 7.** The expression profiles of *ABCB1* and *ABCB19* in young shoots between M106 and M9. The vertical axis represents the relative expression amount. Apple actin-3 was used as internal reference.

## Conclusions

Following genome analysis, a total of 25 apple *ABCB*s were identified and divided into three clusters. The *MdABCB*s within clusters demonstrated similar exon–intron organization. The digital expression of *MdABCB* genes shed light on their functional divergence, and *ABCB1* and *ABCB19* with higher expression in young shoots of M106 than M9 may also be involved in the auxin efflux which take part in height control in apple tree.

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