



Research article

Comprehensive genomic analysis of the *TYROSINE AMINOTRANSFERASE* (*TAT*) genes in apple (*Malus domestica*) allows the identification of *MdTAT2* conferring tolerance to drought and osmotic stresses in plants

Haibo Wang^{a,b,1}, Qinglong Dong^{a,1}, Dingyue Duan^a, Shuang Zhao^a, Mingjun Li^a, Steve van Nocker^{a,c}, Fengwang Ma^{a,**}, Ke Mao^{a,*}

^a State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling, 712100, China

^b Shandong Institute of Pomology, Tai'an, 271000, China

^c Department of Horticulture, Michigan State University, East Lansing, 48824, USA



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ABSTRACT

Tyrosine aminotransferase (TAT, EC 2.6.1.5) is the first key enzyme that catalyzes the reversible interconversion of tyrosine and 4-hydroxyphenylpyruvate in the tyrosine-derived pathway for syntheses of important secondary metabolites and compounds. Although plant *TAT* genes have been proposed to be important in response to abiotic stress, there is little information about *TAT* genes in woody perennial tree species, especially in economic fruit trees. Based on *TAT* domain searching, sequence homology screening and phylogenetic analysis, we identified four *TAT*s in apple genome. Then, we carried out a detailed phylogenetic analysis of *TAT* genes from multi-species, focusing on apple (*Malus domestica*). The result showed that the *TAT* family comprises three major classes corresponding to genes from angiosperms, mammals, and bacteria. Angiosperm *TAT* genes could be further divided into six subclasses. Analysis of intron-exon structure revealed that the typical *TAT* gene contains six introns and seven exons, with exons of similar size at each exon location. Promoter analysis showed that the 5'-flanking region of apple *MdTAT*s contain multiple *cis*-acting elements including those implicated in light, biotic stress, abiotic stress, and hormone response. *MdTAT*s were expressed to various levels in all apple structures and organs evaluated, and showed distinct expression patterns under water deficit stress. Ectopic expression of *MdTAT2* in *Arabidopsis* or over-expression of *MdTAT2* in apple callus tissue conferred enhanced tolerance to drought and osmotic stress. Collectively, these results suggest a role for *TAT* genes in drought and osmotic stresses and provide valuable information for further research of *TAT* genes and their function in plants.

1. Introduction

Tyrosine aminotransferase (TAT, EC 2.6.1.5) is a key enzyme in the tyrosine-derived pathway in rosmarinic acid biosynthesis (Petersen and Simmonds, 2003). It catalyzes the reversible transamination of tyrosine and 2-oxoglutarate, yielding glutamate and 4-hydroxyphenylpyruvate (Riewe et al., 2012). In plants, 4-hydroxyphenylpyruvate is a precursor for biosynthesis of various secondary metabolites and compounds including plastoquinone, tocopherols, rosmarinic acid and

benzylisoquinoline alkaloids (Huang et al., 2008; Lee and Facchini, 2011; Riewe et al., 2012; Wang et al., 2015).

Previous studies showed that *TAT* belongs to a multigene family (Riewe et al., 2012; Wang et al., 2015). In the *Arabidopsis* genome, there are eight putative *TAT* genes (Prabhu and Hudson, 2010; Riewe et al., 2012), four of which (*AtTAT1*, *AtTAT2*, *COR13*, and *SUR1*) have been demonstrated to have *TAT* enzyme activity. *TAT* was shown to be the target of 5-benzylloxymethyl-1,2-isoxazolines derivatives and the herbicide cinmethylin (Grossmann et al., 2012; Riewe et al., 2012).

* Corresponding author. State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling, Shaanxi, 712100, China.

** Corresponding author. State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling, Shaanxi, 712100, China.

E-mail addresses: wanghaibo992@126.com (H. Wang), dong19850412@163.com (Q. Dong), 1448633011@qq.com (D. Duan), 812972738@qq.com (S. Zhao), limingjun@nwsuaf.edu.cn (M. Li), vannocke@msu.edu (S. van Nocker), fwm64@sina.com (F. Ma), maoke2002@nwfau.edu.cn (K. Mao).

¹ These authors have contributed equally to the work.

TAT in mammals and fungi is understood in considerable detail because it has been subjected to comprehensive biochemical and structural analysis (Blankenfeldt et al., 1999; Lee and Facchini, 2011; Mehere et al., 2010; Schneider et al., 2008; Sobrado et al., 2003). However, TAT in plants has not been well studied. In *Arabidopsis*, and in *Chenopodium quinoa* and *Amaranthus caudatus* cell cultures, TAT has been implicated as the first enzyme in tocopherol biosynthesis, and is induced by methyl jasmonate (MeJA), wounding, and coronatine (Antognoni et al., 2009; Holländer-Czytko et al., 2005; Lee and Facchini, 2011; Lopukhina et al., 2001). Additionally, TAT activity was found in MeJA treated *Salvia miltiorrhiza* hairy root cultures and in rosmarinic acid-producing *Coleus blumei* and *Anchusa officinalis* cell suspension cultures (De-Eknamkul and Ellis, 1987; Lee and Facchini, 2011; Xiao et al., 2009). Moreover, overexpression of a TAT gene from *Perilla frutescens*, designated *PfTAT*, resulted to increased rosmarinic acid yield (Lu et al., 2013). In plants, rosmarinic acid and tocopherols are capable of scavenging free radicals, and enhance protection against a variety of abiotic and biotic environmental stressors (Lee and Facchini, 2011). These findings suggested that TAT genes might participate in regulating tolerance of plants to biotic and abiotic stress.

Water scarcity, including drought conditions potentially exacerbated by climate change, is an increasing challenge for production of agricultural crops (Breshears et al., 2005). Apple (*Malus domestica*) is one of most economically important horticultural crops worldwide. In China, which is the primary producer of apples in the world by volume, apple production is centered on the Loess Plateau. Although the climate of this area is usually ideal for apple production, production is often negatively affected by severe drought (Yan et al., 2015). Thus, in China and elsewhere, a primary breeding goal for apple cultivar improvement is drought tolerance (Wang et al., 2018). Based on this, and the previous findings about the responses of plant TAT genes to various abiotic stresses (Antognoni et al., 2009; Holländer-Czytko et al., 2005; Lee and Facchini, 2011; Lopukhina et al., 2001), here, we carried out the first thorough analysis of the TAT genes in apple (*MdTATs*) and their potential function in tolerance to abiotic stresses. Our results lay the foundation for future multifunctional analysis of these genes and improvement of apple tolerance to abiotic stresses.

2. Materials and methods

2.1. Plant materials and treatments

Young roots, stems, and fully expanded leaves, as well as flowers and mature fruit (70 mm, 150 days after bloom), were collected from 5-year-old 'Golden Delicious' apple (*Malus domestica*) trees grafted onto *M. hupehensis* rootstock. Samples used for examining the effect of drought stress were harvested from 3-month-old apple ('Golden Delicious') trees on *M. hupehensis* rootstocks. These plants were grown in pots (height, 320 mm; diameter, 300 mm) in a greenhouse, and treatments began when the plants were approximately 500 mm tall. To induce water deficit, irrigation was withheld from plants for up to 10 d while the designated control plants continued to receive normally scheduled irrigation (Shao et al., 2014). Our sampling schedule involved harvesting mature leaves at the middle nodes on the 0, 2, 4, 6, 8, and 10 d of the water deficit period. All harvested material was frozen immediately in liquid N₂ and stored at −80 °C.

Seedlings of *Arabidopsis thaliana* L. (Heyn), accession Columbia ('Col'), were used for genetic transformations and assays of osmotic and drought tolerance. They were cultured in a growth chamber under 16-h light photoperiods at 23 °C. For the drought tolerance assay, water was withheld from 4-week-old plants for 20 d before they were re-watered. Survival rates were scored on the 3rd day after re-watering began, and evaluated based on approximately 80 plants per line. Well-watered plants were used as the negative control. Relative electrolyte leakage (REL), malondialdehyde (MDA), and proline were measured on the 15th day after the start of the treatment. For the osmotic stress assay, 5

day-old seedlings, grown on Murashige and Skoog (MS) agar plates, were vertically plated on MS agar medium supplemented with 0 or 200 mM mannitol. Root lengths and fresh weights were measured on the 12th day after transfer. All experiments were repeated three times.

For phenotyping transgenic 'Orin' apple callus during a normal growth period or in response to simulated drought stress, wild-type (WT) and two *MdTAT2* transgenic apple callus lines were used. WT and transgenic 'Orin' apple callus tissues were obtained and cultured based on the methods of Hu et al. (2012) and Wang et al. (2017). Callus portions (0.5 g) were maintained on MS medium for 10 d after sub-culturing, and then transferred to MS medium, or MS medium supplemented with 150 mM mannitol for 15 days.

2.2. Identification of apple TAT genes

Computational identification of apple TAT genes utilized two draft genome sequences (v1.0 and GDDH13) as maintained by the GDR database (<http://www.rosaceae.org/>). The HMM (Hidden Markov Model) file PF00155 (Aminotran_1_2) was obtained from the Pfam database (<http://pfam.xfam.org/>; version 31.0) and was used to search the apple genome via HMMER software (version 3.1b2) with the default E-value (0.05). Protein sequences of the eight *Arabidopsis* TAT proteins were obtained from the TAIR database (<http://www.arabidopsis.org/>). Sequences with very atypical sizes (> 650aa, or < 300aa) were omitted from the HMM screening results, and the remaining protein sequences were subjected to phylogenetic analysis (MEGA 7) with eight AtTAT protein sequences (Prabhu and Hudson, 2010; Riewe et al., 2012), resulting in the recognition of original putative MdTAT proteins from apple. The reliability of the candidate protein sequences was confirmed by ensuring that conserved TAT domain was present in each candidate MdTAT protein; for this purpose, we used NCBI Conserved Domain Search (CDD: <http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and the Pfam database (Pfam31.0: <http://pfam.xfam.org/>) (Tian et al., 2015). Putative MdTAT proteins were annotated using blastp and the NCBI nr protein database (<https://www.ncbi.nlm.nih.gov/>). Finally, after omitting proteins that were annotated as other types of aminotransferase, the final candidate protein sequences were defined as MdTATs. To confirm this result, the eight AtTAT proteins were also used as query sequences to search the predicted apple proteins in the GDR database (http://www.rosaceae.org/tools/ncbi_blast) using blastp method.

2.3. Sequence alignments and phylogenetic analysis

Multiple sequence alignments were carried out on 11 full-length TAT amino acid sequences using DNAMAN 6.0.3.99 with default parameters. The phylogenetic tree of apple TAT representatives was constructed with MEGA 7 software using the neighbour-joining (NJ) method together with amino acid sequences of TAT proteins from various species (Table S1). These parameters were used in the NJ method: bootstrap (1000 replicates), complete deletion, and amino: p-distance (Wang et al., 2014).

2.4. Structure model analysis

MdTAT protein structure homology modeling analysis and prediction were performed using the online SWISS-MODEL website (<http://swissmodel.expasy.org/>) (Arnold et al., 2006). Based on the results of this analysis, we modeled the proposed 3-D structure in Protein Data Bank (PDB ID: 3DYD and 4IX8) (Moreno et al., 2014), and used molecular visualization software (RasTop 2.2: <http://www.geneinfinity.org/rastop/>) to present the model (Tian et al., 2015).

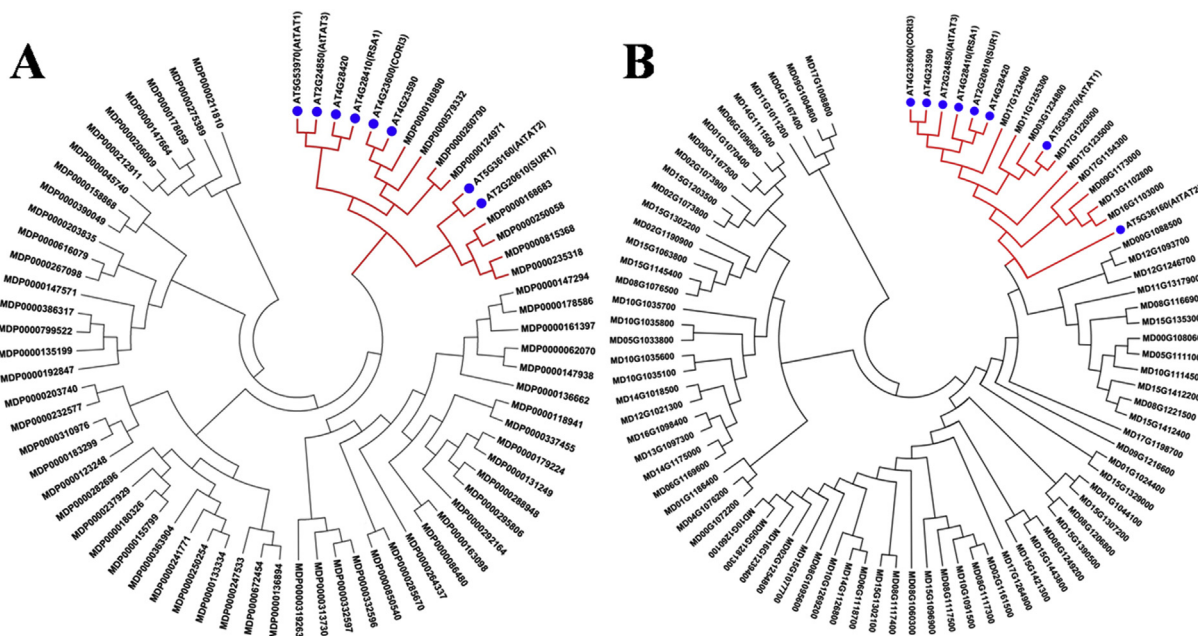


Fig. 1. The phylogenetic tree of putative apple TAT proteins in apple genome (A) v1.0, (B) GDDH13 and 8 AtTAT proteins.

Table 1
MdTAT genes in apple genome.

Gene name	Gene ID	Accession	Chromosome location (v1.0)	ORF (bp)	Size (aa)	Mol. Wt (kDa)	pI
	(v1.0/GDDH13)	number					
<i>MdTAT1</i>	MDP0000260790	KY359351	chr17:19251204..19254203	1746	581	65.246	4.64
	MD17G1234900 ✓		chr17:28374537..28377973	1272	423	46.798	5.329
<i>MdTAT2</i>	MDP0000124971	KY359352	chr17:19290605..19293537	1020	339	37.944	6.34
	MD17G1235000 ✓		chr17:28380025..28389789	1281	426	47.249	5.687
<i>MdTAT3</i>	MDP0000180890	KY359353	chr11:29256496..29259258	1251	416	46.276	5.832
	MD11G1255300		chr11:36818082..36820041	885	294	33.214	6.90
<i>MdTAT4</i>	MDP0000579332	–	chr3:27933400..27935304	921	306	34.532	6.351
	MD03G1234800		chr3:32047258..32061426	1458	485	53.883	7.306

The genes marked in bold are selected for further research.

2.5. Intron-exon structure, chromosomal location, and gene-duplication analysis

Genomic DNA sequences (apple genome v1.0) and chromosomal locations of TATs in apple and the intron-exon structure of TATs from 24 species were obtained from the Plaza 3.0 website (<http://bioinformatics.psb.ugent.be/plaza/>) (Table S2; Proost et al., 2015). Structural depictions of TAT genes, including exon and intron numbers and locations were created using an online gene structure display server (GSDS: <http://gsds.cbi.pku.edu.cn/>). The method of Dong et al. (2018) was used for investigating segmental and tandem duplication events.

2.6. Cis-acting element prediction in promoters

Identification of putative cis-acting elements in apple TAT gene promoters was carried out for the 1500 bp genomic region upstream of the translational start codon (ATG). The promoter sequences and primers of *MdTAT* genes are listed in Table S3 and Table S5, respectively. Putative cis-acting elements in the promoters were predicted according to the Plant CARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

2.7. Cloning of MdTATs and quantitative real-time PCR (qRT-PCR) analysis

RNA was extracted from previously frozen apple tissues using a CTAB-based method and from *Arabidopsis* leaves, with Trizol reagent (Thermo-Fisher Scientific) (Dong et al., 2018). Two micrograms of total RNA were used as a template for synthesizing first-strand cDNA. For cloning *MdTAT*, the source was fully expanded leaves of ‘Golden Delicious’ apple. Oligonucleotide primers are listed in Table S5. The 5′- and 3′-untranslated regions (UTRs) were obtained with a Rapid Amplification for cDNA Ends kit (TaKaRa, Dalian, China).

For qRT-PCR assays, reverse transcription was performed with 1 µg of total RNA from each sample, followed by PCR-amplification using 1 µL of the product as template. qRT-PCR assays were conducted in 20-µL reaction mixtures containing 10 µL of SYBR® Premix Ex Taq™ (TaKaRa), using an iQ5 instrument (Bio-Rad, Hercules, CA, USA) as described previously (Dong et al., 2018). Thermal cycling included an initial 3 min at 95 °C; then 40 cycles of 10 s at 95 °C, 30 s at 58 °C, and 15 s at 72 °C; followed by 3 min at 72 °C; and then 81 gradient temperature cycles from 55 °C to 95 °C with a 0.5 °C increment of each cycle for 7 s. Three biological replicates were utilized for each assay, and Δ Ct values were calculated using *MdMDH* as the endogenous control (Perini et al., 2014). Relative quantification was calculated according to the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) and dissociation curve analysis was performed for determining the specificity of the

MdTAT1MVEKESKKK.....	WNEGNNFHGEQKTSPPITVIRGILYIMIRSLN...	KDDDRPIPLGHGDSAFFCFRTISAAAEAVVDALRSARYNSISSTV	89
MdTAT2MEMGVEWNGQKK.....	WQFRGN...EELN.TASISVIRGALTIMKNLRNPAGDQRP	ITMLGRGDETEFRSFWITPAVDAVADALHSQFNISQPTGG	92
MdAT3MENRTSD.....LQAA...PVDTTSTTITIKGILSLILLQNDVEND...	GRRLISLGMGDSPTAFSCFFTHVQSBAVVDALQSKFNQAPTVG	82
At2g20610	MSEQPHANLAVPAKTEKPTITQTNG...	...DKAAKASTVIRGLVIMLFNCG...	...KDVNTILPLGHGDSVYPCFRTICIEAEAVVDVLRSGKNSYGRGAG	109
At2g24850MASNGVINCANAN.....VWRKGN...	...GATSDAAVTLRLKAFGMFNCT...	92
At4g23590MASQCCVD.....WQFSGS...	...DAEKAQASLIGYSSEIFSLCD...	98
At4g23600MATLKCID.....WQFSGS...	...EAAKDAASLSGYSYLSALYALCD...	78
At4g28410MSQHMLLLPSFETDEYDERKTTDHGGTGGSVWRKGN...	...KAAKEAASVSMKGTILARLFCCS...	...KDVKTILPLGHGDSVYPCFRTICIEAEAVVDVLRSGKNSYGRGAG	111
At4g28420DISVWRKGS...	...DNAAKASSVIMRVIVYKLFDEC...	...LDVKFELLPLAHGDSVYPCFRTICIEAEAVVDVLRSGKNSYGRGAG	103
At5g36160MGENGAKR.....WNEGAN...	...EVVERSNTLIRYNTNLINCLD...	85
At5g53970MENG.....ATTSTITIKGILSLIMESITTEDEGGKRVISLGMGDS	DTLYSCFRTITQVSLQAVSDSLSNKFFHGSSTV	76
Consensus	e	w f g a a t r g l l c	kplilg gds cfrt ae avvdalrsg nsy p g	
MdAT1	ILPARRAHAGYSHLIPYNLSPDLYVLVCGSNATFVLEALR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	208
MdAT2	VIPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	211
MdAT3	LPCTRRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	201
At2g20610	ILPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	228
At2g24850	VFKARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	212
At4g23590	ILPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	197
At4g23600	LAAARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	197
At4g28410	ILPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	230
At4g28420	ILPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	222
At5g36160	VPVARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	204
At5g53970	LPARRAHAGYSLRQNLKLSANDVLAGCTAIEIVSVLAR	PGANILLPRGHEFYDVRAACSNLNFHYDILLPEKGEVDL	EAVALSLENVAMVINPFGNPGNVSQHIEK	195
Consensus	arravaeylndrlp kl ddvft t gc gaie	lar p anillprgpf y r a levr dlpe	weidd vealadentvamvinnpncgnvys hlmk	
MdAT1	IARAKKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	328
MdAT2	IARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	331
MdAT3	IARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	321
At2g20610	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	348
At2g24850	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	332
At4g23590	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	317
At4g23600	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	317
At4g28410	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	350
At4g28420	VARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	342
At5g36160	IARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	324
At5g53970	IARTANKILGLIIVAEVYEQITESTNFEVNAHAATVPVITL	SISKRWIPGWRILWVTOPTAALKNSGLIERITECVLTTPATF	QCAIIPQLEKTEEFYSNIIAIIIRCAD	315
Consensus	ae a klglmividevy fg pvpmpg f siyvitllsiskgw vpgwr	aw dp g f t v i ldit dpat iqaa p ilekt eff k lk vd		
MdAT1	ICFDRLQIEPITCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	423
MdAT2	LLYEMVKEIPCLTCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	426
MdAT3	ICCDRIEIPCLTCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	416
At2g20610	LVCDRILQIEPITCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	461
At2g24850	LSCEKRLQIEPITCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	445
At4g23590	FGYSLKNIETITCYMKPEKACTFLWTETDPLHFVEDIEDHDFOR	KLAKENLVLPGLAFQGNWIRSIDMETPRLEDAFBRILKSCBRHS	SVIVASSLKDVGYN.....	424
At4g23600	FGYSLKNIETITCYMKPEKACTFLWTETDPLHFVEDIEDHDFOR	KLAKENLVLPGLAFQGNWIRSIDMETPRLEDAFBRILKSCBRHS	SVIVASSLKDVGYN.....	422
At4g28410	FAFDALKIDIPCLTCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	447
At4g28420	LVCDRILQIEPITCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	449
At5g36160	ICYELMKIPCLTCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	420
At5g53970	ICCDRIEIPCLTCEKPEKSGMSFMVKNLELLEDIDGDMFCIKL	EEESVVVLPVAVGMKNWRTTACDHSCLEDGLGKMAKCGRHA	KKH.....	414
Consensus	d lk lplcltp kpe c l kl lsiledi dd drc klakeenlv lpg a glkmw	rti e ledaf rik f rha k		

Fig. 2. Alignment of the deduced amino acid sequences of TAT proteins from *Arabidopsis* and apple. Motif 1 is the aminotransferases family-I pyridoxal-phosphate attachment site, and Motif 2 is the highly conserved residue Arg.

amplifications.

2.8. Genetic transformation of MdTAT2 into arabidopsis and apple callus

Primers containing *Sall* and *Bam*HI digestion sites (Table S5) were used to amplify the coding region of *MdTAT2*. PCR was performed based on the parameters of Tan et al. (2015): Denaturation at 95 °C for 10 min; 30 cycles of denaturation at 95 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 120 s; extension at 72 °C for 10 min. The cDNA was purified through agarose gel electrophoresis, digested with *Sall* and *Bam*HI, and ligated into the *Sall* and *Bam*HI sites of binary vector pBI121 (Clontech, Palo Alto, USA) under the control of the CaMV35S promoter. The resulting constructs were introduced into *Agrobacterium tumefaciens* strain EHA105 by electroporation.

Transformation of *Arabidopsis* used the floral dip method (Clough and Bent, 1998). Transgenic seeds (T_1) were sown on an MS medium with 50 mg L⁻¹ kanamycin for transgene selection. Then, kanamycin-resistant plants were confirmed by PCR. T_3 homozygous lines were obtained after selecting transgenic lines at a 3:1 segregation ratio. *MdTAT2* presence and expression in those plants was analyzed by PCR and qRT-PCR, respectively. These PCR identifications were carried out using the primers in Table S5 and the method as described by Tan et al. (2015).

Transformation of apple callus followed the protocol described by Hu et al. (2012) and Wang et al. (2017). The key steps were as follows. 'Orin' apple callus was cultured on solid MS medium containing 1.0 mg L⁻¹ 2,4-D, 1.0 mg L⁻¹ 6-BA, 30 g L⁻¹ sucrose and 7 g L⁻¹ agar in the dark at 25 ± 1 °C, and subcultured at 2-week intervals. After

subculturing three times, ~2.0 g callus was transferred into suspension medium (the same ingredients as solid medium but without agar). Cultures were maintained on a rotary incubator (110 r min⁻¹) and subcultured three times at 2-week intervals. Suspension cultures in exponential growth phase were mixed with *Agrobacterium* culture and rotated gently for 10 min at 25 °C for transformation. After 2 d co-cultivation on solid medium, the callus was washed three times with sterile water containing 400 mg L⁻¹ cefotaxime, and then transferred to a selection medium supplemented with 300 mg L⁻¹ cefotaxime and 30 mg L⁻¹ kanamycin for transgene selection. After 3–5 serial subcultures, resistant callus showing stable growth was subjected to PCR and qRT-PCR for analysis of the transgene.

2.9. Measurements of relative electrolyte leakage (REL), malondialdehyde (MDA) and proline

REL in *Arabidopsis* leaves was evaluated with the method of Lutts et al. (1996). REL in apple callus was measured by the method of Liu et al. (2006), with slight modifications as follows. Callus (0.1 g) was washed with distilled water three times, incubated in 10 ml of deionized water, and then kept at room temperature for 12 h. Electrolyte conductivity (E1) was measured by a conductivity meter (Mettler Toledo Inlab 738, Zurich, Switzerland). The samples were then boiled for 30 min to induce complete leakage, followed by cooling at room temperature for 12 h. Then, final electrolyte conductivity (E2) was measured. The relative conductivity was calculated as formula REL (%) = E1/E2 × 100. MDA level was determined by the thiobarbituric acid (TBA) method as described by Heath and Packer (1968). Free

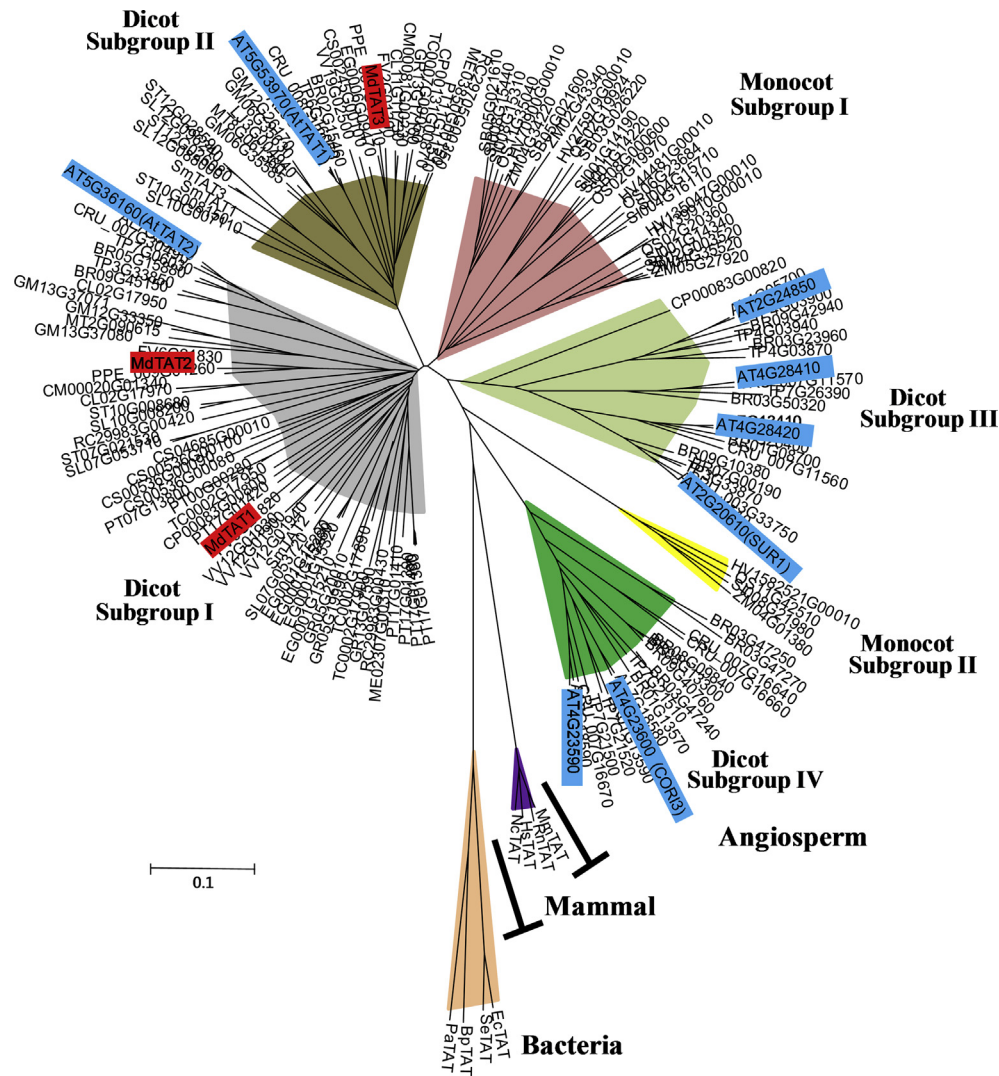


Fig. 3. Phylogenetic tree of TAT proteins in various species. The unrooted phylogenetic tree was constructed using MEGA software (version 7) with the neighbour-joining (NJ) method. GenBank Accession numbers of the above proteins are shown below: *Mus musculus* (MmTAT, NP_666326.1), *Homo sapiens* (HsTAT, NP_000344.1), *Neovison vison* (NvTAT, AAD45375.1), *Rattus norvegicus* (RnTAT, AAA42203.1), *Escherichia coli* (EcTAT, NP_418478.1), *Bordetella petrii* (BpTAT, CAP43123.1), *Pseudomonas aeruginosa* (PaTAT, AAD45270.1), *Salmonella enterica* (SeTAT, CAD09232.1). The remaining TAT protein sequences were obtained from PLAZA 3.0 Dicots and PLAZA 3.0 Monocots databases, see Table S1 for details.

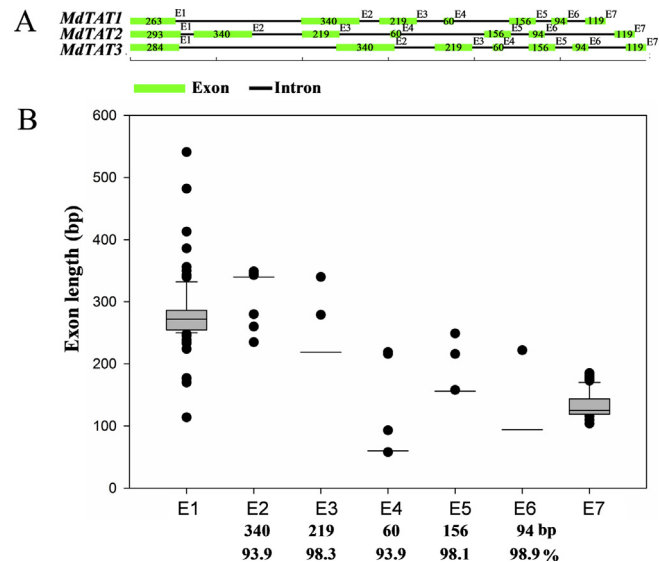


Fig. 4. Analysis of the (A) *MdTAT* gene structures and (B) exon-length distributions of predicted *TAT* genes in 25 plant species. Exon size values were determined using the vertical box plot depiction in the SigmaPlot 12.0 program. The long black line shows the mean value of the exon length. The details of exon length of *TAT* genes in 25 plant species are shown in Table S2.

Table 2
The *cis*-acting elements of three promoters in apple *TAT* genes.

<i>Cis</i> -acting elements	Response Stress	<i>MdTAT1</i>	<i>MdTAT2</i>	<i>MdTAT3</i>
ABRE	ABA		1/1	
ARE	Hypoxia	2/0	1/0	1/0
CGTCA	MeJA	1/1	2/0	
ERE	Ethylene	1/2		
HSE	Heat	0/2	2/2	
LTR	Chilling		3/0	0/2
MBS	Drought	1/0	1/1	1/0
TCA	SA			1/2
TC-rich repeat	Defence	0/1		0/2
W-box	Pathogen	1/0		
WUN	Wound			0/1

Sequences and functions for ABRE (ACGT-containing ABA response element), ARE (anaerobic response element), CGTCA (*cis*-acting regulatory element involved in the MeJA-responsiveness), ERE (ethylene-responsive element), HSE (heat shock element), LTR (*cis*-acting element involved in low-temperature responsiveness), MBS (MYB binding site involved in drought-inducibility), TCA (salicylic acid response element), TC-rich repeat (*cis*-acting element involved in defense and stress responsiveness), WUN (wound-responsive element) elements or W-box (elicitation; wounding and pathogen responsiveness/binding site of WRKY type transcription factors) were obtained from PlantCARE. The number of *cis*-acting element in plus strand and minus strand of each promoter is distinguished with an oblique line. Blank space indicates no corresponding *cis*-acting element in either strand of a promoter.

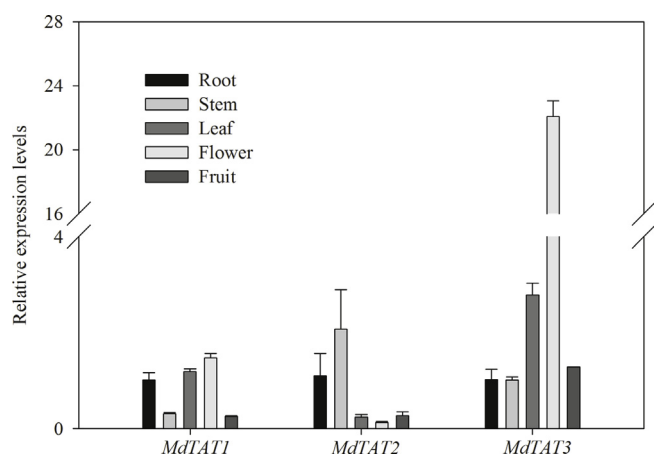


Fig. 5. Expression analysis of apple TAT genes in various apple structures using qRT-PCR. *MdMDH* primers were used as the internal standard for each gene. The error bar of each value indicated standard deviation. To investigate *MdTATs* expression patterns, the full-length cDNA, 5'-UTR and 3'-UTR sequences of *MdTAT* genes were isolated by RT-PCR. Based on these results, specific primers of *MdTATs* were designed and used in qRT-PCR assay (Table S5).

proline concentration was measured using the sulfosalicylic acid-acid ninhydrin method of Bates et al. (1973). For each transgenic or WT line, physiochemical measurements were repeated three times.

2.10. Statistical analysis

Data are shown as mean values and standard deviation (SD). The IBM SPSS Statistics version 20 software (SPSS Inc., Chicago, USA) was used for the statistical analysis. Student's *t*-test was used to evaluate differences in *MdTATs* expression levels between drought and control treatments, and in morphological and physiochemical phenotypes between transgenic lines and WT under different abiotic stresses at significance levels of $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***)

3. Results

3.1. Identification of TAT genes in the apple genome

We combined a hidden Markov model-based search algorithm (HMMER) targeting the conserved TAT domain, with sequence homology screening (blastp) and phylogenetic analysis (MEGA 7), resulting in the identification of eight original putative *MdTAT* proteins in apple genome v1.0 (Fig. 1A) and nine in GDDH13 (Fig. 1B).

The further confirmation of conserved domain and functional annotation were performed. Finally, four TAT genes in apple genome v1.0 and four TAT genes in genome GDDH13 were identified. These genes in different genome exhibited one to one correspondence according to their chromosome location (Table 1), suggesting four members in apple TAT gene family. These genes were designated *MdTAT1-MdTAT4*

(Table 1). Among them, MD17G1234900 (*MdTAT1*), MD17G1235000 (*MdTAT2*) identified in apple genome GDDH13, and MDP0000180890 (*MdTAT3*) in genome v1.0 showed similar peptide length (416–426 amino acids), molecular weight (~46–47 kDa), and isoelectric point (pI; ~5.3–5.8) (Table 1). These were consistent with the structure of TAT proteins in other plants such as *Arabidopsis*. However, the calculated peptide length and pI of either MDP0000579332 in apple genome v1.0 or MD03G1234800 in apple genome GDDH13, which was designated *MdTAT4*, was strikingly different from others (Table 1). Because of the possibility that the structure of *MdTAT4* was mis-annotated in the apple genome, this gene was omitted in further investigations.

3.2. Cloning of the full-length MdTAT genes

We amplified full-length cDNAs of *MdTAT1*, *MdTAT2*, and *MdTAT3* by RT-PCR for further analysis. Alignment of peptide sequences of the three *MdTAT* proteins with eight *AtTAT* sequences showed that *MdTAT* proteins were highly homologous to *AtTAT* proteins (Fig. 2). The three *MdTATs* contain Motif 1 (the aminotransferases family-I pyridoxal-phosphate attachment site) and Motif 2 (the highly conserved residue Arg) both of which are required for TAT activities conserved in plant species (Lu et al., 2013).

3.3. Phylogenetic analysis of MdTAT proteins

To examine the evolutionary relationship among TAT proteins, we constructed an unrooted phylogenetic tree of three TAT proteins (*MdTAT1*, *MdTAT2* and *MdTAT3*) identified in apple genome in this study (Table 1) and other TAT proteins from various species (Table S1). The resulting phylogenetic tree distinguished three major groups corresponding to proteins from angiosperm, mammal, and bacteria (Fig. 3). Further analysis revealed that the angiosperm group could be divided into six subgroups: Dicot Subgroup I, II, III, and IV, and Monocot Subgroup I and II. Among the *MdTAT* and *AtTAT* proteins, *MdTAT1*, *MdTAT2*, and At5g36160 (*AtTAT2*) could be unambiguously classified in Monocot Subgroup I, *MdTAT3* and At5g53970 in Monocot Subgroup II, At2g24850, At4g28410, At4g28420, and At2g20610 (*SUR1*) in Monocot Subgroup III, and At4g23590 and At4g23600 (*COR13*) in Monocot Subgroup IV (Fig. 3).

3.4. Structural analysis of MdTAT proteins

The web server SWISS-MODEL was used to perform protein structure homology modeling alignment and analysis, and the RasTop software was used to build 3-D models of the *MdTAT* proteins. In SWISS-MODEL, the *MdTAT1*, *MdTAT2*, and *MdTAT3* protein homology models were built and evaluated using the homologous template 3DYD, 3DYD, and 4IX8.pdb, respectively (Fig. S1). The obtained 3D model of apple TAT proteins showed that *MdTAT1* and *MdTAT2* structure closely matched that of human TAT (PDB ID: 3DYD.A, RMSD = 2.30 Å and 36.39% sequence identity for residues 39–420; RMSD = 2.30 Å and 35.79% sequence identity for residues 44–423), whereas *MdTAT3*

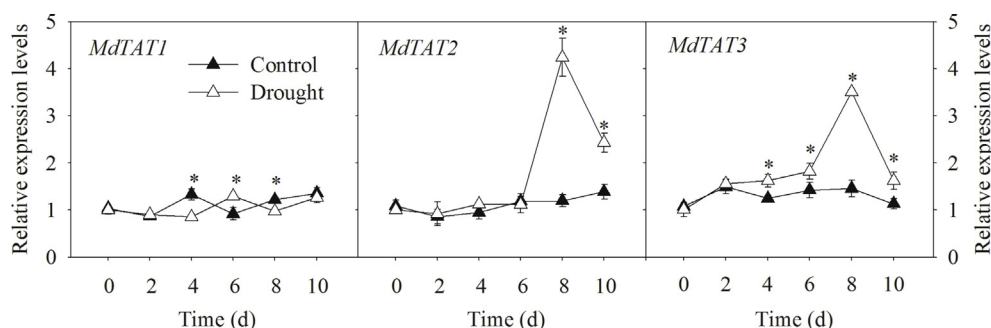


Fig. 6. Analysis of apple TAT genes expression under drought stress using qRT-PCR. *MdMDH* primers were used as the internal standard for each gene. The error bar of each value indicates standard deviation. Data were different from control values at significance levels of $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***) under drought treatment, according to Student's *t* tests.

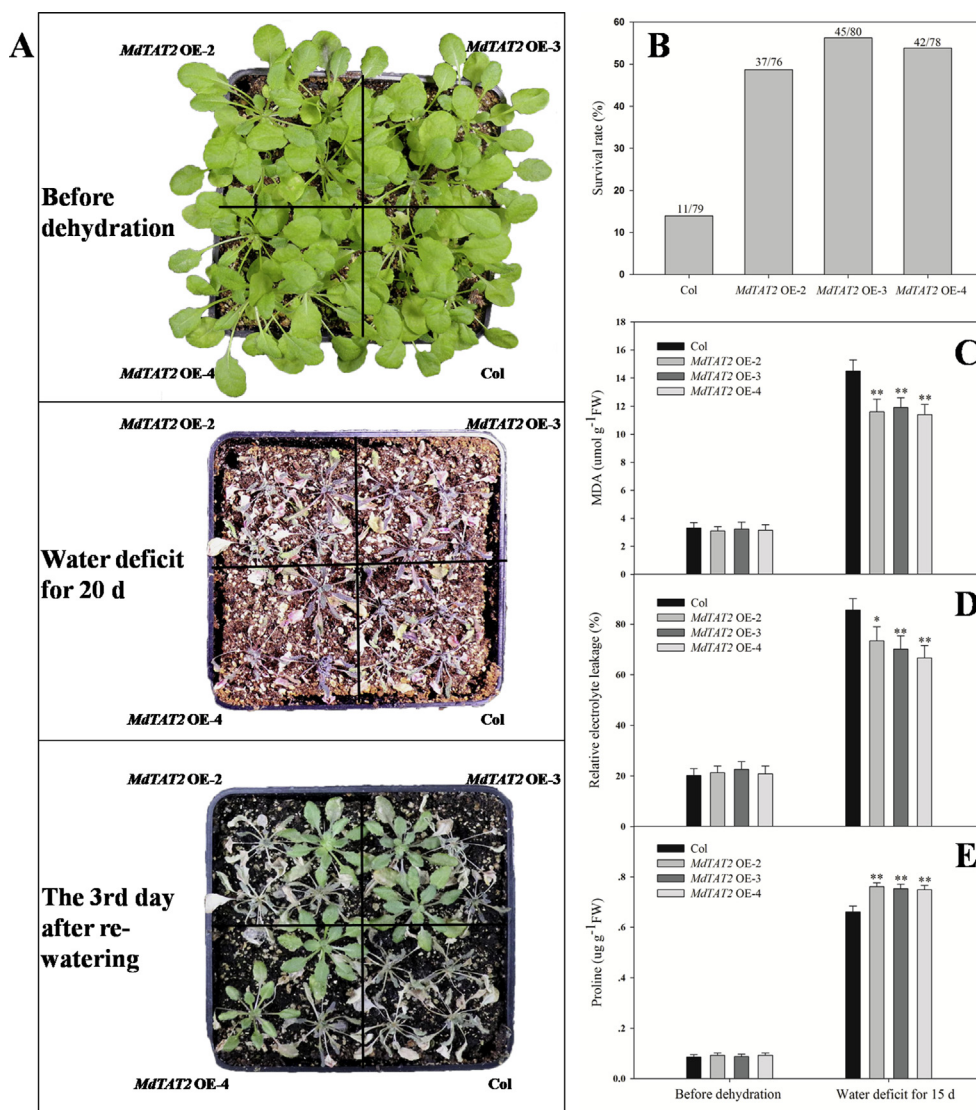


Fig. 7. Comparison of drought tolerance of 'Col' and transgenic *Arabidopsis* conferred by *MdTAT2*. (A) Growth phenotypes of 4-week-old soil-grown plants of 'Col' and transgenic lines (OE-2, -3 and -4) treated with natural drought for 20 d, and then re-watered. (B) Survival rates of 'Col' and three transgenic lines were investigated on the 3rd day of re-watering. Number of survival plants and total plants of each line on the survival rate plot. (C) MDA accumulation, (D) REL level and (E) proline concentration of each line was measured on the 15th day of natural drought. Physiochemical results were shown as means and SDs from three independent experiments. Data of transgenic lines were different from 'Col' values at significance levels of $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***), according to Student's *t* tests.

structure most closely matched that of *Leishmania infantum* TAT (PDB ID: 4IX8.A; RMSD = 2.35 Å and 30.99% sequence identity for residues 36–414).

To better understand the gene structure diversity of apple TAT proteins, the intron-exon organization in the coding sequences of apple TAT genes was analyzed. As shown in Fig. 4A, *MdTAT1*, *MdTAT2*, and *MdTAT3* contained seven exons and six introns. Whereas the length of the first exon showed different sizes (263, 293, and 284 bp, respectively), the second to seventh exon in these genes exhibited the same size: 340, 219, 60, 156, 94, and 119 bp, respectively. We then analyzed the exon-intron structures of 115 TAT genes in 25 plant species, including these three *MdTATs* (Table S2). The results showed that the lengths of the second to sixth exon in most TAT genes were similar size, specifically 340, 219, 60, 156, and 94 bp, respectively. The TAT genes whose exon exhibit certain size at a specific exon location (second to sixth exon) account for 93.9%, 98.3%, 93.9%, 98.1%, and 98.9% of total genes tested, respectively (Fig. 4B).

3.5. Cis-acting elements analysis in the *MdTAT* promoters

As a first step to understand how *MdTAT* genes might be regulated, we analyzed potential cis-acting elements in the promoter regions, 1500 bp genomic DNA sequences upstream of the start codon (ATG). In addition to putative cis-acting elements involved in light-response (Table

S4), we identified eleven classes of putative cis-acting elements associated with response to various stresses including hypoxia, heat, chilling, drought, pathogens, and wounding, or stress-associated hormones such as Salicylic acid (SA), Methyl jasmonate (MeJA), Absciscic acid (ABA), or ethylene (Table 2; Table S4). These cis-acting elements could play distinct and important roles in the response to diverse stresses.

3.6. Expression profiles for *MdTAT* genes in various plant structures and under drought stress condition

We analyzed the relative mRNA accumulation levels of *MdTAT* genes in the flowers, fruit, stems, roots, and leaves using qRT-PCR (Fig. 5). *MdTATs* were expressed in each of these structures, but to different levels. *MdTAT1* and *MdTAT3* exhibited strongest expression in the flower and leaf (Fig. 5). Strong expression of *MdTAT1* was also detected in the root (Fig. 5). Additionally, *MdTAT2* exhibited higher expression in the stem and root than in other organs (Fig. 5).

To investigate the potential response of the *MdTAT* genes to drought stress, we subjected three-month-old 'Golden Delicious' trees to water deficit conditions for progressive time periods. *MdTAT2* and *MdTAT3* were obviously induced by drought stress, especially on the 8th day of water deficit treatment. Compared with control plants, their expression levels increased 3.55 and 2.41 times, respectively (Fig. 6). On the 10th day of treatment, these two genes were still induced by drought stress,

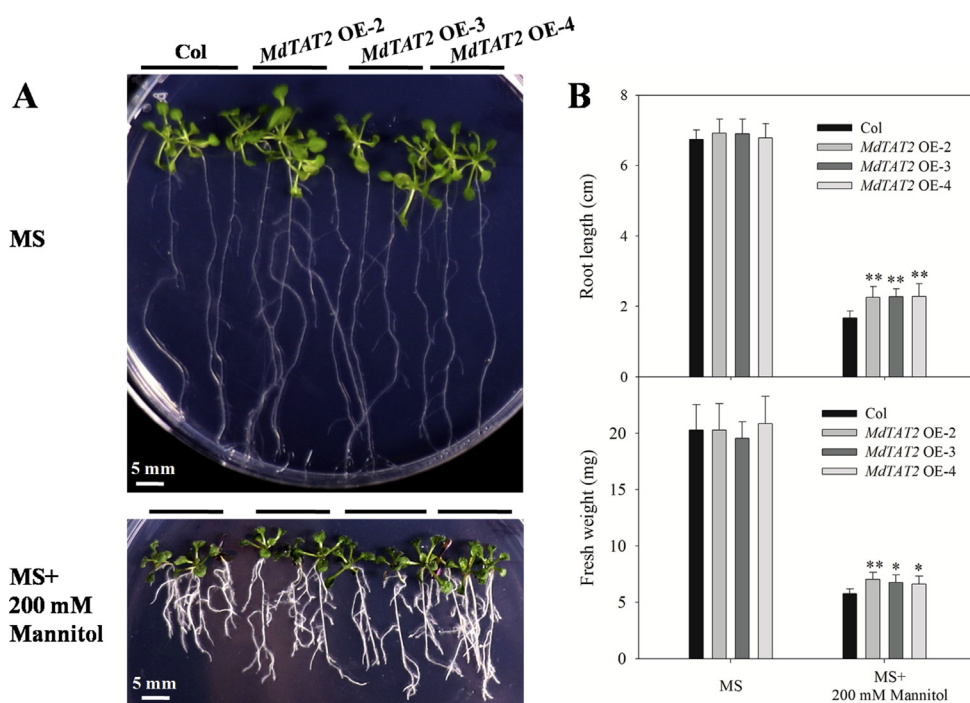


Fig. 8. Effects of osmotic stress on growth of 'Col' and transgenic *Arabidopsis* seedlings (*MdTAT2* OE-2, -3 and -4). (A) Growth phenotypes of 5d-old 'Col' and transgenic seedlings on MS medium, or MS medium supplemented with 200 mM mannitol for 12 d. (B) Root lengths and fresh weights of 'Col' and transgenic seedlings treated with 200 mM mannitol for 12 d. Results were means of 18 seedlings per line per experiment and their SDs from six independent experiments. Data of transgenic lines were different from 'Col' values at significance levels of $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***), according to Student's *t* tests.

with more expression increment of *MdTAT2*. However, the response of *MdTAT1* to water deficit differed significantly from *MdTAT2* and *MdTAT3*. The expression level of *MdTAT1* in plants under water deficit stress was the same as the control before the first two days of treatment (Fig. 6). As the stress continued, the expression of *MdTAT1* was suppressed by water deficit on the 4th and 8th day. However, on the 6th day of water deficit treatment, the expression level of this gene was slightly up-regulated compared with the control (Fig. 6).

3.7. Overexpression of *MdTAT2* in *Arabidopsis* enhanced tolerance to drought stress

Because the expression of *MdTAT2* was induced more significantly than other two *MdTATs* under drought stress (Fig. 6), we chose *MdTAT2* for further analyzing its biological functions of influencing drought tolerance in *Arabidopsis*. The full-length open reading frame (ORF) of *MdTAT2* was engineered under control of a 35S promoter segment, and introduced into *Arabidopsis* (Fig. S2). To assess the influence of *MdTAT2* to promote drought tolerance when expressed in transgenic *Arabidopsis*, we carried out soil drought tolerance experiments (Fig. 7A). Plants were grown under optimal conditions, and then water was withheld for 20 d. After 20 d of natural drought, leaves of the tested lines were purplish and showed severe wilting. However, three transgenic *Arabidopsis* lines overexpressing *MdTAT2* (*MdTAT2* OE-2, -3 and -4) exhibited obviously stronger growth (Fig. 7A). After water was restored, more plants of transgenic lines recovered growth than 'Col' (Fig. 7A and B). About 50% of the transgenic plants survived this treatment, compared to only 14% for wild type 'Col' (Fig. 7B). In addition, we evaluated MDA accumulation, REL levels and proline concentrations in leaves of the three transgenic lines under water deficit for 15 d (Fig. 7C, D and E). The significantly lower MDA accumulation and REL level, and higher proline concentration further support the results of phenotypic comparisons and survival rates between transgenic lines and 'Col'. The morphological, survival and physiochemical differences between transgenic lines over-repressing *MdTAT2* and 'Col' under water deficit, indicated that this gene conferred drought tolerance in *Arabidopsis*.

3.8. Overexpression of *MdTAT2* in *Arabidopsis* and apple callus enhanced tolerance to osmotic stress

To subsequently determine the potential for *MdTAT2* to promote osmotic stress tolerance when expressed in transgenic *Arabidopsis*, we examined growth of wild type 'Col' and transgenic seedlings on non-modified media or media supplemented with 200 mM mannitol. On control medium lacking mannitol, elongation of the primary root and plant mass increase were similar between the transgenic and 'Col' plants. However, in medium supplemented with 200 mM mannitol, the primary root lengths of three transgenic lines were significantly longer than 'Col', and the final plant mass of the transgenic lines was greater than that of 'Col' (Fig. 8A and B). These results suggested that ectopic expression of *MdTAT2* in *Arabidopsis* alleviated the negative effects of osmotic stress.

The similar transgenic methods were used to introduce *MdTAT2* into 'Orin' apple callus (Fig. S2), and to assess the influence of this gene on tolerance to osmotic stress in apple (Fig. 9A). The wild type (WT) and *MdTAT2* overexpressing apple callus explants were maintained on 150 mM mannitol for 15 d. We observed no significant difference between WT and *MdTAT2* OE lines in terms of fresh weights or physiochemical indices when grown on control MS medium (Fig. 9). However, we found that the two transgenic lines grew faster and reached a greater final mass than WT on media supplemented with mannitol (Fig. 9A and B). Under mannitol treatment, though the MDA, REL and proline levels for both WT and two transgenic lines OE-1 and OE-2 were increased than normal (MS) conditions, there was significantly less MDA, lower REL level, and more proline found in these transgenic lines compared with WT (Fig. 9C, D and E).

Taken together, our data illustrate the potential of this gene for improving tolerance to osmotic stress in plants.

4. Discussion

TAT enzymes catalyze the reversible interconversion of tyrosine and 4-hydroxyphenylpyruvate, which is the first step in the tyrosine-derived pathway (Huang et al., 2008; Lee and Facchini, 2011; Riewe et al., 2012). In recent years, there has been extensive research on TAT proteins in higher plants, yet the mechanism of action and biological

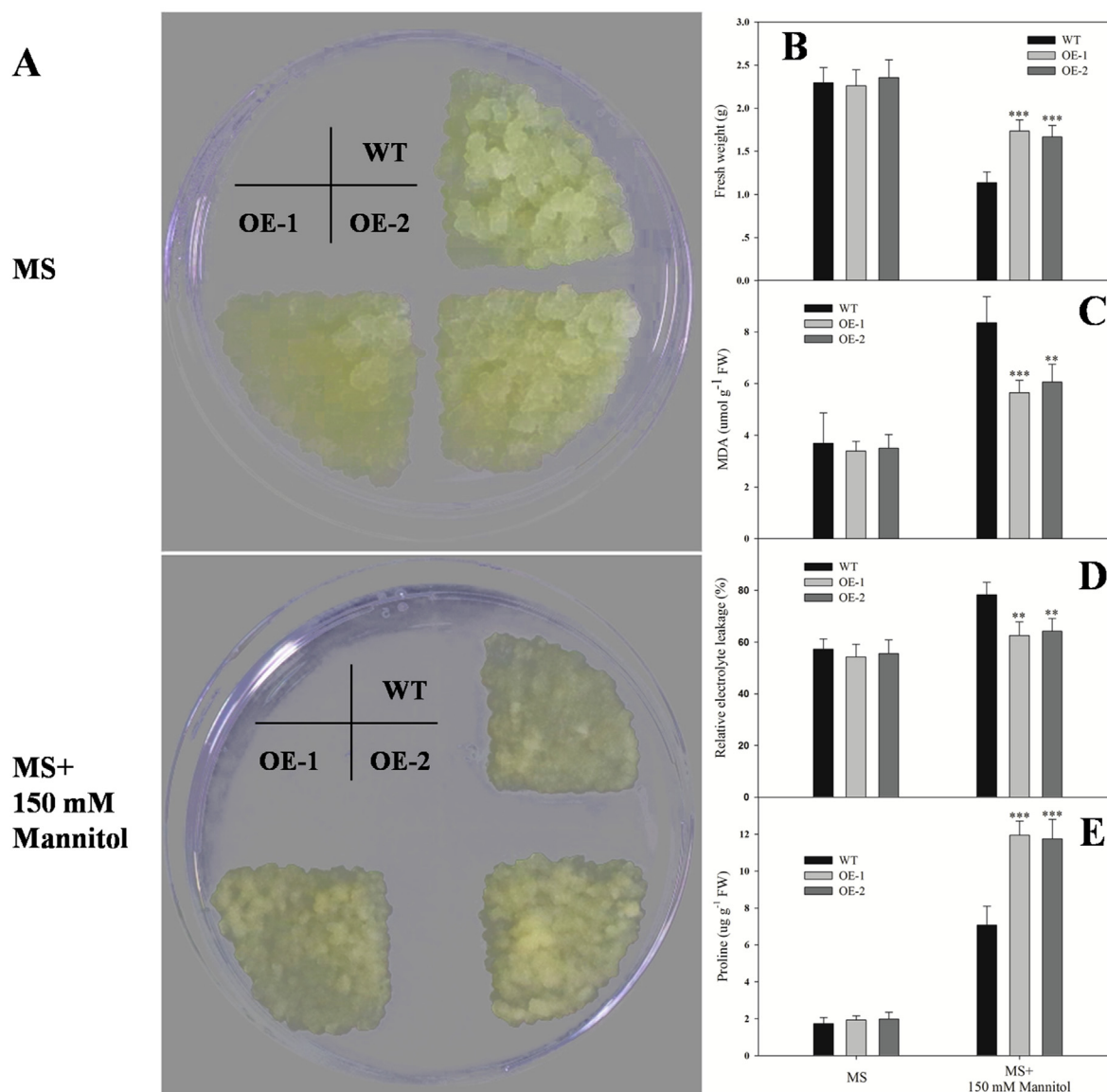


Fig. 9. Effects of osmotic stress on growth of callus derived from WT and *MdTAT2* overexpressing lines. (A) Morphology of callus on MS medium, or MS medium supplemented with 150 mM mannitol, for 15 d. (B) Fresh weight, (C) MDA accumulation, (D) REL level and (E) proline concentration of each line was measured after stress treatment. Results were means of six biological repetitions per line per experiment and their SDs from six independent experiments. Data of transgenic lines were different from WT values at significance levels of $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***), according to Student's *t* tests.

function(s) of TAT proteins remains poorly understood. In this study, we carried out a genomic census of TAT genes in apple, as well as a preliminary assessment of their potential activities and function.

TAT genes have been found to be present as multi gene families in *Salvia miltiorrhiza* (Wang et al., 2015) and *Arabidopsis* (Prabhu and Hudson, 2010; Riewe et al., 2012). There have been no previous reports of TAT genomic organization in woody perennial plants such as apple. We found that the number of TAT genes differed substantially among species. For example, 19 and 13 TAT genes were found in the PLAZA 3.0 Dicots database for *Brassica rapa* and *Populus trichocarpa*, respectively, while only one TAT gene was found in *Lotus japonicas*. The genomic abundance of TAT genes may reflect elaborations of the biochemical pathways in which these genes participate (Tian et al., 2015). In this study, we identified four TAT family genes in apple based on the earlier apple genome v1.0 (Velasco et al., 2010) and the new genome GDDH13 (Daccord et al., 2017) for the thorough identification of this gene family. The apple genome v1.0 has been used widely in genome-wide genes investigation, for example, the *MYB* (Cao et al., 2013), *SnRK* (Shao et al., 2014), *YTH* (Wang et al., 2014), *MAD-box* (Tian et al.,

2015), *WRKY* (Gu et al., 2015), *AGO* (Zhou et al., 2016), *bHLH* (Mao et al., 2017), *VQ* (Dong et al., 2018), etc. Recently, the new apple genome based on one doubled-haploid 'Golden Delicious' line 'GDDH13' was sequenced, the quality of apple genome annotation had been greatly improved (Daccord et al., 2017). The combined application of both genomes in this study would help to identify TAT gene family more accurately and comprehensively.

Previous studies suggested that segmental, tandem, and whole-genome duplications are critical for both the diversification of gene function and the rearrangement and expansion of genomes (Dong et al., 2018; Tian et al., 2015). Whole-genome duplication events have occurred in apple (Velasco et al., 2010) and some gene families have undergone segmental, tandem, and whole-genome duplications, for example, the apple *MYB*, *MADS-box* and *WRKY* gene family (Cao et al., 2013; Gu et al., 2015; Tian et al., 2015). In this study, we detected that two *MdTAT* genes (*MdTAT1* and *MdTAT2*) were close to each other in their chromosome locations (Table 1), suggesting they present in tandem copies within the same chromosome. This result indicated that tandem duplication might have led to the expansion of the apple TAT

gene family.

Based on the phylogeny of TAT proteins among various species, the TAT genes have been divided into angiosperm, mammal and bacteria groups (Lu et al., 2013; Wang et al., 2015). In this study, we constructed a phylogenetic tree from TAT protein sequences from 30 plant species, and discovered that these could be unambiguously classified into six distinct clades - Dicot Subgroup I, II, III, and IV, and Monocot Subgroup I and II (Fig. 3). Exon-intron structural diversity can provide important evidence for phylogenetic relationships (Tian et al., 2015). Our analysis of exon length and number of TAT gene family members in various species indicate that the typical TAT gene contains six introns and seven exons, and has exons of similar size at each exon location (Fig. 4B; Table S2). Taken together, these results suggest that the genes of the plant TAT family, in particular dicotyledonous and monocotyledonous TAT genes, are highly conserved.

A previous study showed that *At4g23600* (*COR13*) transcript levels were increased in response to methyl 12-oxophytodienoic acid, MeJA, or wounding, and that COR13 protein levels were increased by octadecanoids, coronatine, or wounding (Lopukhina et al., 2001). The *At2g24850* protein catalyzes the initial step in tocopherol biosynthesis, - the conversion of tyrosine to p-hydroxyphenylpyruvate. The *At2g24850* gene was induced by methyl-12-oxophytodienoic acid and MeJA, coronatine, wounding, the herbicide oxyfluorfen, or UV light (Lee and Facchini, 2011; Sandorf and Holländer-Czytko, 2002). RNA expression and enzymatic activities of *At5g53970* (*AtTAT1*) was reported to be induced under conditions leading to oxidative stress, and in developing seeds and senescing leaves (Holländer-Czytko et al., 2005; Riewe et al., 2012). In addition, *S. miltiorrhiza* TAT gene (*SmTAT*) expression was positively regulated by ABA, MeJA, SA, and UV-B (Huang et al., 2008). These studies indicated the responses of TAT genes in plants to various abiotic and biotic stresses. In this study, cis-acting element analysis of the *MdTATs* promoter showed that the 5'-flanking region of *MdTATs* contained elements implicated in transcriptional response to light, ABA, MeJA, ethylene, SA, hypoxia, heat, chilling, drought, defense, pathogen and wound response (Table 2). We found that *MdTAT2* and *MdTAT3* expression was positively induced by drought stress, suggesting a role for these genes in drought tolerance. We showed the ectopic expression of *MdTAT2* in *Arabidopsis* facilitated growth either under mannitol treatment or soil drought stress, and obtained similar results for apple callus over-expressing *MdTAT2* under mannitol treatment. In addition, the improved physiochemical indices (MDA, REL and proline) in *Arabidopsis* and apple callus associated with over-expressing *MdTAT2* suggests that *MdTAT2* confers tolerance to drought and osmotic stresses in plants and suggests a new function for *MdTAT* genes in response to abiotic stresses.

Taken together, the results in this study provide new evidences that *MdTAT* genes participate in response to abiotic stresses in plants. Future work should elucidate the cellular and molecular mechanisms, providing bases for increasing resilience of apple to abiotic stress under production conditions.

Conflicts of interest

The authors declare that they have no competing interests.

Author contributions

FM, HW, and KM conceived and designed the study. HW, QD, DD, SZ, and ML performed the analyses. HW, QD, KM and NS drafted the manuscript. FM and KM supervised the process of this research. FM provided financial support for the study. All authors critically revised and provided final approval of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2018.10.033>.

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Abbreviations

- ABA:** Absciscic acid
REL: Relative electrolyte leakage
MeJA: Methyl jasmonate
MDA: Malondialdehyde
MS: Murashige and Skoog
ORF: Open reading frame
qRT-PCR: Quantitative real-time PCR
SA: Salicylic acid
TAT: Tyrosine aminotransferase
WT: Wild type