Saudi Journal of Biological Sciences 28 (2021) 5693–5703

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Comparative genomic analysis of *MYB* transcription factors for cuticular wax biosynthesis and drought stress tolerance in *Helianthus annuus* L.

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ARTICLE INFO

Article history:
Received 3 May 2021
Revised 19 May 2021
Accepted 2 June 2021
Available online 10 June 2021

Keywords:
Sunflower
MYB
Gene expression
Genome-wide identification
Drought
Wax biosynthesis

ABSTRACT

Sunflower is an important oil-seed crop in Pakistan, it is mainly cultivated in the spring season. It is severely affected by drought stress resulting in lower yield. Cuticular wax acts as the first defense line to protect plants from drought stress condition. It seals the aerial parts of plants and reduce the water loss from leaf surfaces. Various myeloblastosis (MYB) transcription factors (TFs) are involved in biosynthesis of epicuticular waxes under drought-stress. However, less information is available for MYB, TFs in drought stress and wax biosynthesis in sunflower. We used different computational tools to compare the *Arabidopsis* MYB, TFs involved in cuticular wax biosynthesis and drought stress tolerance with sunflower genome. We identified three putative *MYB* genes (*MYB16, MYB94* and *MYB96*) in sunflower along with their seven homologs in *Arabidopsis*. Phylogenetic association of MYB TFs in Arabidopsis and sunflower indicated strong conservation of TFs in plant species. From gene structure analysis, it was observed that intron and exon organization was family-specific. MYB TFs were unevenly distributed on sunflower chromosomes. Evolutionary analysis indicated the segmental duplication of *the MYB* gene family in sunflower. Quantitative Real-Time PCR revealed the up-regulation of three *MYB* genes under drought stress. The gene expression of *MYB16*, *MYB94* and *MYB96* were found many folds higher in experimental plants than control. The present study provided the first insight into MYB TFs family's characterization in sunflower under drought stress conditions and wax biosynthesis TFs.

1. Introduction

Sunflower (*Helianthus annuus*) is a member of Compositae family and is indigenous to North America (Schilling and Heiser, 1981; Blackman et al., 2011). This crop is mainly cultivated for production of edible and after cotton, sunflower is the second most important oil-seed crop of Pakistan (Ahmad et al., 2020). *H. annuus* exhibits considerable variation in genome size therefore, much of this variation is attributable to differences between ploidy levels. Indeed, Helianthus contains diploid (2n = 2x = 34), tetraploid (2n = 4x = 68) and hexaploid species (2n = 6x = 102) (Rieseberg et al., 1990). A high-quality sunflower highly repetitive reference genome (https://sunflowergenome.org/), approximately 3.6 gigabases has been reported (Badouin et al., 2017) but lacks extensive characterization in terms of genes and proteins. The production of sunflower is compromised due to various abiotic stresses, especially drought (Riaz et al., 2020). Wax on plant leaf surfaces acts as the first line of defense and protects plants from water loss through transpiration (Serrano et al., 2014). Epicuticular wax is the mixture of very long chain (VLC) lipids derived from fatty-acids which are made due to acyl-CoA elongase activities. Currently, the mechanism of wax biosynthesis is well studied; however the roles of different proteins involved in the process are not fully understood (Pascal et al., 2013). Cuticular waxes also provide resistance to plants against insects, pathogens and bacteria (Zeisler-Diehl et al., 2018), protect plants from ultraviolet

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.06.009
1319-562X/

radiations (Barnes et al., 1994; Gordon et al., 1998), decrease deposition of water on plant surfaces, and reduce dust, pollens and other pollutants on plant surfaces (Kerstiens et al., 1996). Presence of wax is a characteristic of making plants adaptable to drought stress (Johnson et al., 1983). High water losses have been associated with the quantity of wax present of leaf surfaces (Hall and Jones, 1961; Lai et al., 2007) and plants having thin wax coating on surfaces transpire more water through stomata.

Transcription factors (TFs) have vital roles in regulation of gene expression (Latchman, 1997). A typical TF contains two domains, i.e., DNA binding domain and transcriptional activation/repression domain (Riechmann et al., 2000). The two domains along with other proteins function to activate/repress transcription in response to internal/external signal (Riechmann et al., 2000). MYB (myeloblastosis) TFs family is among the largest TF families in plants (Peng et al., 2016; Mondal et al., 2018; Yang et al., 2019; Li et al., 2020). They regulate various cellular processes, including hormonal signal transduction, plant development, biotic and abiotic stresses response, heavy metal stresses, differentiation and metabolism (Peng et al., 2016; Mondal et al., 2018; Ashrafi-Dehkordi et al., 2018; Baldoni et al., 2015). Being largest family, MYB TFs are categorized in four classes, i.e. 1R, 3R, R2R3, and 4RMYB (Roy et al., 2016). All the MYB TFs involved in cuticular wax biosynthesis are the members of R2R3-MYB subfamily of TFs. In Arabidopsis, MYB TFs involved in cuticular wax biosynthesis under drought are *AtMYB16*, *AtMYB30*, *AtMYB41*, *AtMYB94*, *AtMYB96*, *AtMYB106*, and *AtMYB116* and a TF *SlMYB12* has been reported in tomato (Cominelli et al., 2008; Seo et al., 2011; Oshundiya et al., 2014).

AtMYB16 and *AtMYB106* are two paralogous TFs involved in wax biosynthesis process, with cooperation of WIN1/SHN1 genes in *Arabidopsis thaliana* and *Tornia fournieri* (Folkers et al., 1997; Jakoby et al., 2008; Gilding et al., 2010; Oshima et al., 2013). Expression of these genes by fusion to repressor domain using RNAi, negatively influence the cuticular wax formation (Oshima et al., 2013; Hiratsu et al., 2003). Previous research indicated that *AtMYB30* encodes four enzymes that are involved in acyl-CoA elongation complex and responsible for VLCFAs synthesis (Raffaele et al., 2008). Expression of *AtMYB41* gene is very low in ordinary conditions however under abscisic acid, salinity and drought stress, its expression is highly enhanced. Overexpression of *AtMYB41* gene increases cuticular permeability, biosynthesis and transportation of lipids and cuticular metabolism in transgenic *Arabidopsis* (Cominelli et al., 2008). Initially, in Arabidopsis, *AtMYB96* was considered as drought regulator and was integrated with ABA and auxin signals. Overexpression of *AtMYB96* gene has confirmed the drought tolerance in Arabidopsis and its knockout mutant has proved more drought sensitivity than wild type plants (Seo et al., 2011). Transcriptional activation of *AtMYB96* under drought-stress and role of wax-biosynthesis has been confirmed later on (Oshundiya et al., 2014). They also reported that wax deposition was increased on Arabidopsis leaves and stem during the activation of myb96-1D mutant. Over-expression of *AtMYB96* gene was also responsible for enhanced drought tolerance in oilseed crop (Lee et al., 2014). Over-expression of *AtMYB94* has also shown its involvement in wax biosynthesis (Lee and Such, 2015).

Expression of *AtMYB94* tends to increase ~9-fold under drought conditions. Further, it has been observed that *AtMYB94* TF activates other wax biosynthesis genes, i.e. *CER2*, *KCS2*, *WSD1*, *FAR3* and *ECR* by directly binding with their promoters (Lee and Such, 2015). Similar TF *ZmMYB94* has also been reported in maize which is involved in cuticular wax deposition in young seedlings and regulates the pattern of epicuticular wax biosynthesis on epidermis of maize young leaves (La Rocca et al., 2015). Overexpression of *EsWAX1* in Arabidopsis stimulates the expression of other wax associated genes like *AtKCS1*, *AtKCR1* and *AtCER1* and increases the wax accumulation (Zhu et al., 2014). In wheat, a MYB TFs (*TaMYB31*) has been reported which is involved in up-regulation of wax-biosynthesis under drought conditions (Zhao et al., 2018). Another drought and cuticular wax related TF *SlMYB12* belonging to this family has been discovered in tomato having potential role in tomato fruit cuticular wax biosynthesis (Adato et al., 2009). The aim of present research was genome-wide identification and characterization of MYB TFs involved in cuticular wax biosynthesis in sunflower by using computational biology tools. This research revealed functional homology at genomic and proteomic levels in sunflower about MYB transcription factors.

2. Materials and methods

2.1. Plant materials and growth conditions

Sunflower genotypes FH 331, FH 639, FH 634, FH 629, FH 630, FH 631, FH 639, FH 649, FH 583 and Hysun 33 were cultivated under controlled conditions in growth chambers. Uniform agronomic practices were applied for all the genotypes. Their temperature, humidity and light intensity were maintained as 25 ± 2 °C, 40%, 650 μmol m-2 s-1 respectively. Thirty days old seedlings were subjected to drought treatments by withholding water until the plants tends to wilt. Leaves of 45 days old treated and control plants were collected from identical position for biochemical analysis of wax components. Data was recorded from five different plants of the same cultivar and then averaged.

2.2. Determination of sunflower wax chemical composition

Chemical composition of epicuticular wax components was determined by using gas chromatography. Six major wax components (alkanes, esters, aldehydes, acids, alcohols and unknown traces) were determined based on chemical compound classes. Forty- five days old leaves were cut and immersed in hersan for 60 s. Solution was concentrated at 40 °C. Free radical of hydroxal and carboxal groups in solution was converted into their ethers and esters. Wax constitutes were identified by their dectron impact microscopic spectra.

2.3. Retrieval of protein sequences

Sequences of already reported cuticular wax and drought related Arabidopsis MYB TFs were retrieved from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). Plant transcription database (http://planttfdb.cbi.pku.edu.cn/index.php) was used for further confirmation of these sequences. BLASTp tool of NCBI was used to find similar proteins in sunflower.

2.4. Physio-chemical properties and sub cellular localization of MYB TFs

To find the physiochemical properties of MYB TFs in both plant species, web tool (http://web.expasy.org/cgi-bin/protparam/protparam) was used (Gasteiger et al. 2005). Amino acid length (a.a), protein molecular weight (MW), theoretical isoelectric point (PI) of MYB TFs was also calculated. Subcellular localization of MYB TF proteins in sunflower and Arabidopsis were found described by (Chou et al., 2010; Mooney et al., 2011) using web-server Plant-mPLoc http://www.csbio.sjtu.edu.cn/bioinf/plant/.

Table 1
Means and Standard error of various wax components under normal and drought conditions.

Varieties	Alkane		Aldehyde		Fatty acids		Primary Alcohol		Wax esters		Unknown Traces	
	Normal	Stress	Normal	Normal	Normal	Normal	Stress	Stress	Stress	Stress	Normal	Stress
Hysun 33	0.68 ± 0.02hij	1.50 ± 0.03 cd	0.29 ± 0.01 k	4.83 ± 0.07efg	0.21 ± 0.01 h	0.49 ± 0.02de	0.40 ± 0.01gh	0.10 ± 0.00i	7.40 ± 0.20ab	0.10 ± 0.00 l	0.49 ± 0.01 h-k	0.50 ± 0.01hij
FH-331	0.56 ± 0.02j	0.72 ± 0.02 g-j	0.28 ± 0.01 k	4.69 ± 0.11fgh	0.35 ± 0.02ef	0.56 ± 0.02c	0.72 ± 0.01a	0.45 ± 0.01d	6.03 ± 0.13c	0.45 ± 0.01ij	0.56 ± 0.01fgh	0.72 ± 0.02 cd
FH-583	0.54 ± 0.02j	0.88 ± 0.05e-h	0.60 ± 0.02 fg	3.60 ± 0.09jk	0.30 ± 0.01 fg	0.48 ± 0.01def	0.64 ± 0.01b	0.40 ± 0.01de	4.80 ± 0.08efg	0.72 ± 0.02d	0.48 ± 0.02ijk	0.64 ± 0.02e
FH-606	0.63 ± 0.03ij	1.08 ± 0.04e	0.56 ± 0.02fgh	4.34 ± 0.08ghi	0.35 ± 0.00ef	0.56 ± 0.01c	0.36 ± 0.01hi	0.54 ± 0.02c	6.03 ± 0.07c	0.54 ± 0.01gh	0.56 ± 0.01fgh	0.45 ± 0.01jkl
FH-629	0.55 ± 0.01j	1.04 ± 0.04ef	0.30 ± 0.01 k	3.15 ± 0.05 k	0.20 ± 0.01 h	0.40 ± 0.01gh	0.24 ± 0.01 k	0.24 ± 0.01gh	5.60 ± 0.05 cd	0.40 ± 0.01j	0.40 ± 0.01 lm	0.48 ± 0.02ijk
FH-630	0.88 ± 0.03e-h	2.31 ± 0.04a	0.56 ± 0.01fgh	5.20 ± 0.16def	0.40 ± 0.02de	0.32 ± 0.02ij	0.33 ± 0.01ij	0.55 ± 0.01c	6.93 ± 0.06b	0.55 ± 0.01gh	0.64 ± 0.02e	0.33 ± 0.01 *m*
FH-631	0.91 ± 0.03efg	1.35 ± 0.02d	0.70 ± 0.01de	3.92 ± 0.07ij	0.63 ± 0.02b	0.42 ± 0.01fgh	0.54 ± 0.02 cd	0.63 ± 0.03b	5.31 ± 0.10de	0.63 ± 0.02ef	0.42 ± 0.02kl	0.54 ± 0.01ghi
FH-634	0.84 ± 0.03fgh	1.65 ± 0.05c	0.49 ± 0.01hi	4.13 ± 0.09hij	0.56 ± 0.02c	0.28 ± 0.01jk	0.44 ± 0.02efg	0.66 ± 0.01ab	6.93 ± 0.15b	0.55 ± 0.02gh	0.63 ± 0.01ef	0.77 ± 0.01c
FH-639	0.81 ± 0.02ghi	1.92 ± 0.09b	1.17 ± 0.02a	4.86 ± 0.14efg	0.72 ± 0.02a	0.45 ± 0.01efg	0.48 ± 0.01def	0.36 ± 0.01ef	7.92 ± 0.11a	0.72 ± 0.02d	0.99 ± 0.03a	0.60 ± 0.02efg
FH-649	0.72 ± 0.03 g-j	1.54 ± 0.06 cd	1.04 ± 0.02b	4.72 ± 0.12 fg	0.40 ± 0.01de	0.24 ± 0.01 k	0.33 ± 0.01ij	0.66 ± 0.01ab	6.93 ± 0.12b	0.88 ± 0.02c	0.88 ± 0.02b	0.66 ± 0.02de

Means sharing similar letter in a column are statistically non-significant (P > 0.05).

2.5. Multiple sequence alignment

Amino acid sequences of identified MYB TF proteins retrieved from NCBI were aligned by using ClustalW in MEGA6 (Tamura et al., 2013) and an unrooted phylogenetic tree was constructed by neighbor-joining method (Saitou & Nei 1987) at 1000 bootstrap value. The phylogenetic tree was constructed to investigate the evolutionary roots of MYB TFs in *Arabidopsis* and sunflower.

2.6. Gene structure analysis and identification of conserved motifs

cDNA and genomic sequences of sunflower MYB TFs were retrieved from NCBI and Arabidopsis from TAIR. The Online Gene Structure Display Server (GSDS 2.0) (http://gsds.cbi.pku.edu.cn/index.php) was used to visualize the gene structure by comparing each cDNA sequence with the corresponding genomic sequence. Online MEME version 4.8.2 tool (http://meme.nbcr.net/meme/intro.html) was used to identify the conserved motifs in *A. thaliana* and *H. annuus*. Default parameters used were; any number of repetitions, the optimum number of motifs = 20 and maximum motif width was ranged from 6 to 250.

2.7. Chromosomal mapping of MYB TFs

All the *MYB* genes of sunflower and Arabidopsis were mapped on chromosomes of respective plant species. We used an online tool "Map gene 2chromosome v2" (http://mg2c.iask.in/mg2c_v2.0/) by preparing the inputs files containing the following information i.e., Gene ID, start and end position of gene, chromosome ID of gene and respective chromosomal sequence length. Default parameters of the tool remained unchanged.

2.8. Synteny analysis and gene ontology

To determine the evolutionary relationship among MYB TFs, the protein sequences of sunflower and Arabidopsis were submitted to the online Synteny analysis tool Circoletto (tools.bat.infspire.org/circoletto). Gene Ontology of cuticular wax responsive MYB TFs was predicted by Blast2GO program (Götz et al., 2008) using amino acid sequences with default parameters.

2.9. Expression profiling of MYB genes in sunflower

Total RNA was isolated from sunflower plants after 10 days of drought stress by following the methods of Jaakola et al. (2001) with some modifications. RNA was quantified by a nano-drop, ND-1000 (NanoDrop Technologies, Inc.) spectrophotometer. An online tool primer3 (https://frodo.wi.mit.edu/) was used to design the primers from already reported gene of MYB family having role in epicuticular wax biosynthesis under drought. cDNA was synthesized using Olig (dT) primer and the M−MLV reverse-transcriptase enzyme (ThermoFisher Scientific, USA) following the vender's protocol. Gene expression was carried out by real-time PCR using SYBER Green qPCR Master-Mix (ThermoFisher Scientific, USA) in CFX96 Real Time PCR System (BIO-RAD, USA). The reaction was set-up in a total volume of 20 µl, containing 10 µl of qPCR Master-Mix, 0.1 µM of each of primers and 100 ng of sample cDNA. The reaction was denatured to 95 °C for 30 s, followed by 44-cycles at 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s. Fold gene expression was estimated by $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001). The variation in gene expression was analyzed by using *Actin* gene as the reference. Following is the list and sequences of primers for *MYB* and *Actin* genes (Supplementary Table 1).

3. Results

GC–MS was used to determine the biochemical components of epicuticular waxes in sunflower leaves in normal and treated plants. The results displayed significant difference in wax constituents under normal and drought conditions (Table 1). Among the genotypes highest amount of alkane under normal conditions were noted in FH-631 (0.91 μg/cm^2) followed by FH-634 (0.84 μg/cm^2) while under drought FH-630 showed highest amount of alkane (2.31 μg/cm^2) lacking behind FH-639 (1.92 μg/cm^2). Results indicated that aldehyde concentration was higher in regularly watered plants and low in stress subjected plants. Highest aldehyde concentration was observed in FH-639 (1.17 μg/cm^2) during normally grown plants followed by FH-649 (1.04 μg/cm^2). Under limited supply of water FH-649 exhibited (0.88 μg/cm^2) concentration of aldehydes. Average fatty acid concentration of sunflower genotypes was noted (0.58 μg/cm^2). Primary alcohol was the major components of epicuticular waxes in sunflower genotypes with the average of 5.37 μg/cm^2. FH-639 showed highest amount (7.92 μg/cm^2) of primary alcohol among all the genotypes followed by Hysun-33 (7.4 μg/cm^2). Average concentration of esters was measured 0.44 μg/cm^2 whereas, FH-639 showed dominant class of wax esters (0.72 μg/cm^2) during normal growth conditions while lowest wax esters were observed in Hysun33 (0.1 μg/cm^2) under drought stress.

Sunflower genome has been recently sequenced, few studies in genome wide study are undertaken for wax and drought responsive MYB, TFs in this important oil seed crop. In A. thaliana, six already reported genes encoding wax biosynthesis under drought stress (*AtMYB16*, *AtMYB41*, *AtMYB94*, *AtMYB96*, *AtMYB106*, and *AtMYB116*) and their homologous genes were subjected to BLAST to find the similar sequences in H. annuus. Physiochemical properties for wax response of *A. thaliana* and Sunflower MYB, TFs proteins were determined (Tables 2 and 3). From the results, it was concluded that cuticular wax related MYB TFs were distributed on 1st, 3rd, 4th and 5th chromosomes of *A. thaliana* and no genes was located on 2nd chromosome. Distribution of Exonic regions in plants have got much attention in genomic research as they play important role in transcription and translation of protein sequences to perform different functions. In Arabidopsis genome, exons were ranged from 3 to 5. Amino acid length of MYB, TFs was varied and was in the range of 198 to 388. The value of isoelectric point (PI) in *Arabidopsis* gene was ranged from 4.29 to 10.11 and molecular weight was in the range of 21923.1 Da to 43557.84 Da (Table 2). In sunflower, 10 wax and drought responsive MYB TFs and their homologs were reported which were unevenly distributed throughout the genome. These genes were located on chromosome 6, 7, 8, 12, 15, 16 and 17. Numbers of exons were counted 3 in all the MYB genes. Length of amino acids sequences varied from 228 to 375. PI value indicated that among ten MYB TFs, four were basic in nature and six were acidic. PI value was varied from 5.42 to 9.76. Subcellular location indicated their presence in the nucleus (Table 3).

3.1. Multiple sequence alignment and phylogenetic analysis of MYB TFs

MYB TF protein sequences of sunflower, Arabidopsis, maize and soybean were aligned by ClustalW online program. A phylogenetic tree was constructed by MAGA 6 software through neighbor-joining method (Fig. 1). On the bases of un-rooted phylogenetic tree, MYB TF proteins were divided in four clades. Genes in the same cluster were showing homology between MYB TF of sunflower and Arabidopsis. Seven members were present in 1st clad three in 2nd, eleven in 3rd and four in 4th clad. This classification represented the same pattern as reported in Chickpea by (Azeem et al. 2018; Waqas et al. 2019).

Table 2
Physiochemical properties of wax responsive MYB TFs in *Arabidopsis*.

S. No.	Gene	Gene ID	Locus tag	Ch. No.	Exon	Amino acids	Protein M.W. (Da)	P. I.	Sub-Cellular location
1	MYB16-1	831383	AT5G15310.1	5	3	247	36943.4	7.02	Nucleus
2	MYB16-2	831383	AT5G15310.2	5	3	326	35727.9	7.32	Nucleus
3	MYB16-3	831383	AT5G15310.3	5	3	246	25756.53	10.11	Nucleus
4	MYB16-4	831383	AT5G15310.4	5	3	315	28442.5	10.61	Nucleus
5	MYB41-1	828927	AT4G28110.1	4	3	282	31651.2	5.90	Nucleus
6	MYB41-2	828927	AT4G28110.2	4	3	198	21923.1	4.29	Nucleus
7	MYB94-1	823914	AT3G47600.1	3	3	333	37143.5	6.78	Nucleus
8	MYB94-2	823914	AT3G47600.2	3	3	327	36607.34	8.02	Nucleus
9	MYB96-1	836367	AT5G62470.1	5	3	352	39031.3	5.84	Nucleus
10	MYB96-2	836367	AT5G62470.2	5	3	351	39031.34	5.65	Nucleus
11	MYB106-1	821209	AT3G01140.1	3	5	345	38574.90	8.43	Nucleus
12	MYB106-2	821209	AT3G01140.2	3	5	388	43557.84	8.67	Nucleus
13	MYB116-1	839118	AT1G25340.1	1	4	283	32691.7	6.26	Nucleus
14	MYB116-2	839118	AT1G25340.2	1	4	308	35618.9	6.26	Nucleus
15	MYB116-3	839118	AT1G25340.3	1	4	278	32145.55	5.60	Nucleus

Table 3
Physiochemical properties of wax responsive MYB TFs in Sunflower.

S. No.	Gene	Gene ID	Locus tag	Ch. No.	Exon	Amino acids	Protein M.W. (KD)	P. I.	Subcellular location
1	MYB16-1	110871830	HannXRQ_Chr08g0210621	8	3	327	36987.94	9.25	Nucleus
2	MYB16-2	110896476	HannXRQ_Chr12g0362221	12	3	304	34466.24	9.55	Nucleus
3	MYB41-1	110864867	HannXRQ_Chr06g0171131	6	3	331	37418.68	5.42	Nucleus
4	MYB41-2	110917164	HannXRQ_Chr16g0517211	16	3	308	34944.33	6.32	Nucleus
5	MYB41-3	110925496	HannXRQ_Chr17g0559401	17	3	286	32340.55	6.24	Nucleus
6	MYB41-4	110911865	HannXRQ_Chr15g0482591	15	3	355	37008.67	5.63	Nucleus
7	MYB41-5	110924219	HannXRQ_Chr17g0559711	17	3	325	40368.54	5.54	Nucleus
8	MYB106-1	110921145	HannXRQ_Chr17g0545981	17	3	375	41358.85	6.23	Nucleus
9	MYB106-2	110868689	HannXRQ_Chr07g0199311	7	3	228	26227.44	9.67	Nucleus
10	MYB106-3	110897003	HannXRQ_Chr12g0378731	12	3	307	34943.36	8.63	Nucleus

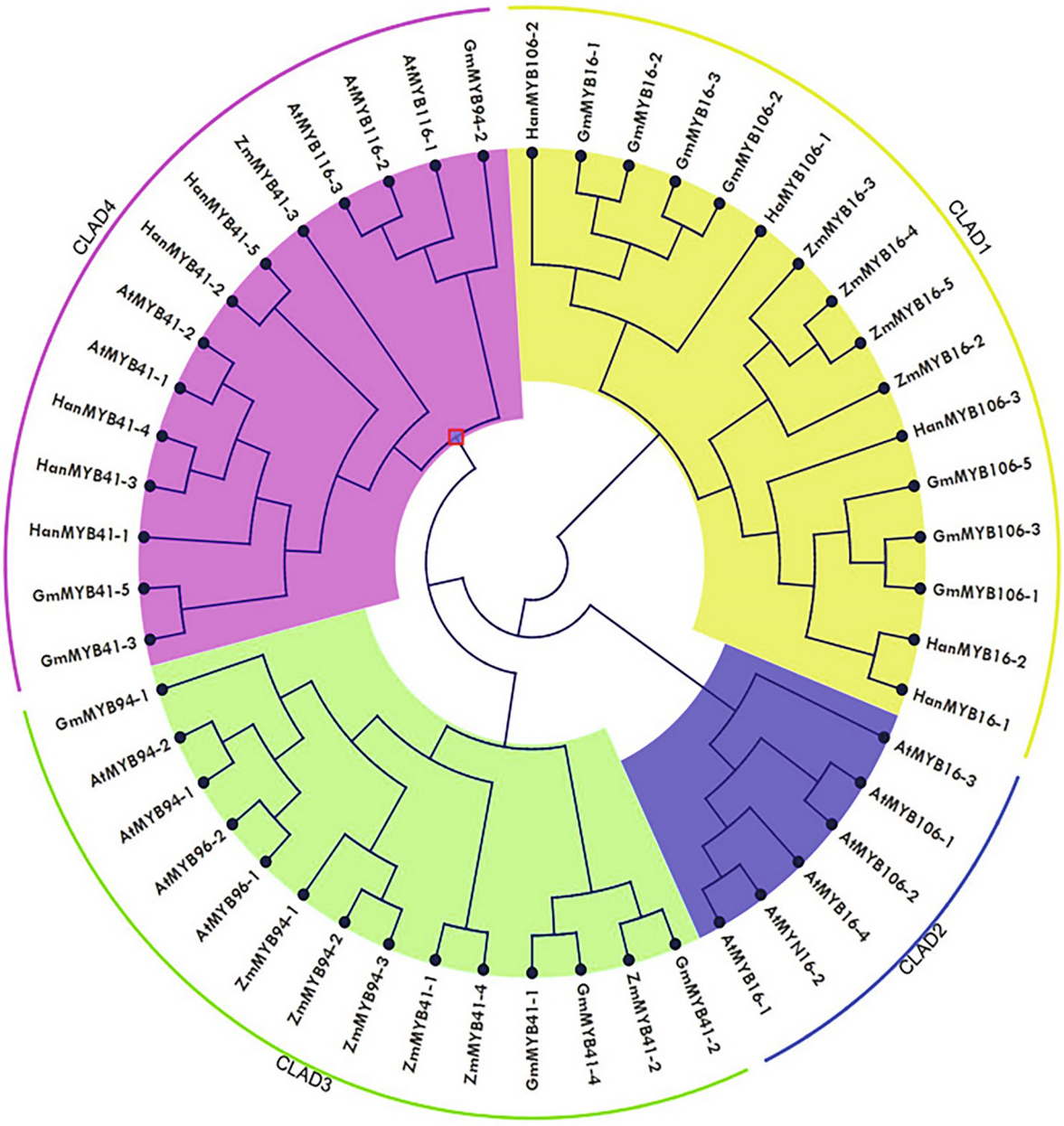

Fig. 1. Phylogenetic analysis of MYB TFs in different plants, (a) sunflower and Arabidopsis, (b) Arabidopsis, sunflower, maize and soybean. Different clads were marked with different background colors to make them easily visible.

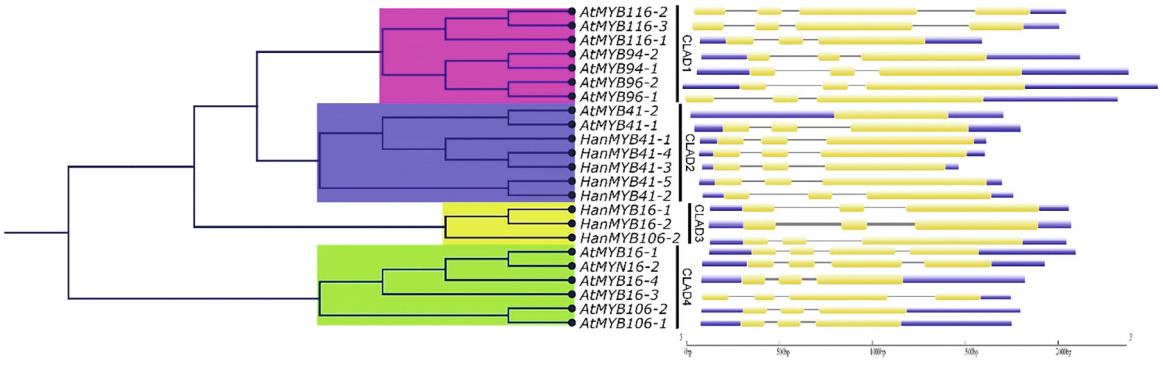

Fig. 2. Phylogenetic association and gene structure analysis of Arabidopsis and sunflower wax biosynthesis MYB TFs. Yellow color indicating the exons, black lines between exons are representing introns and blue bars are showing downstream/upstream.

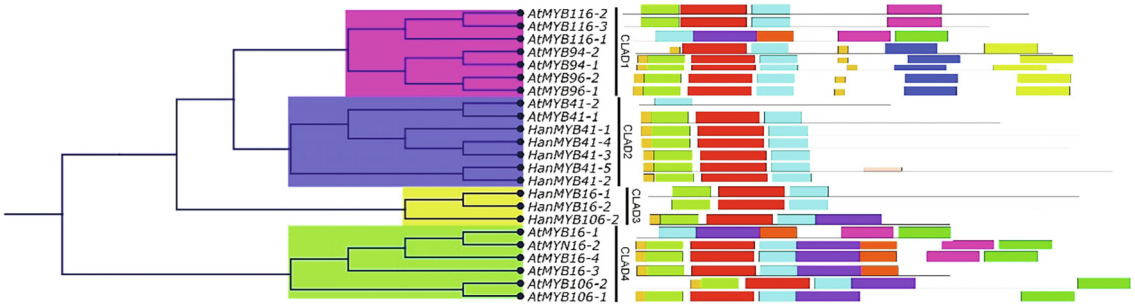

Fig. 3. Phylogenetic association and conserved motif analysis of Arabidopsis and sunflower MYB TFs. Each motif is separated with a different color.

3.2. Gene structure of MYB transcription factors

In this study, a significant difference was observed in gene structure among the MYB TF members due to variation in number and location of introns and exons. The number of introns varied from 1 to 4. A gene *AtMYB41-1* has no intron in its genome which may be lost during the evolution from the ancestral sequence. Most of the exons present in same clad were conserved. The exons present in 1st and 2nd clad showed same position and size but difference in intron length indicating that they are evolutionary close to each other and have similarity in functions (Fig. 2).

3.3. Conserved motif analysis of MYB transcription factors

Protein motifs of MYB TFs were analyzed by using online tool MEME (http://meme.nbcr.net/meme/cgi-bin/meme.cgi). We observed seven diverse motifs in both plant species. It was also noted that expression pattern of these motifs was diverse. However, motif 1, 2 and 3 showed regular expression pattern whereas motif 4, 5, 6 and 7 rarely showed expression across the species under study (Fig. 3).

3.4. Comparison of chromosomal mapping of MYB genes in Arabidopsis and sunflower

Chromosomal mapping determines the exact location of a gene on chromosome, approximate distance between specific genes and strengthen the linkage between genes. Mapping of Arabidopsis chromosomes was carried out by online tool available at TAIR. The results showed that in Arabidopsis, *MYB* genes were distributed on four chromosomes at different locations, however no gene was located on chromosome number 2 (Fig. 4a). According to the sunflower chromosomal mapping results, *MYB* genes were present on 6, 7, 8, 12, 15, 16 and 17th chromosomes (Fig. 4b). According to genomic size, genes were laying between 25 kb and 45 kb.

3.5. Evolutionary relationship of sunflower and Arabidopsis MYB genes by Synteny analysis

A comparative Synteny analysis was performed between sunflower and Arabidopsis MYB TF proteins to determine the evolutionary association of these genes in both plant species. 10

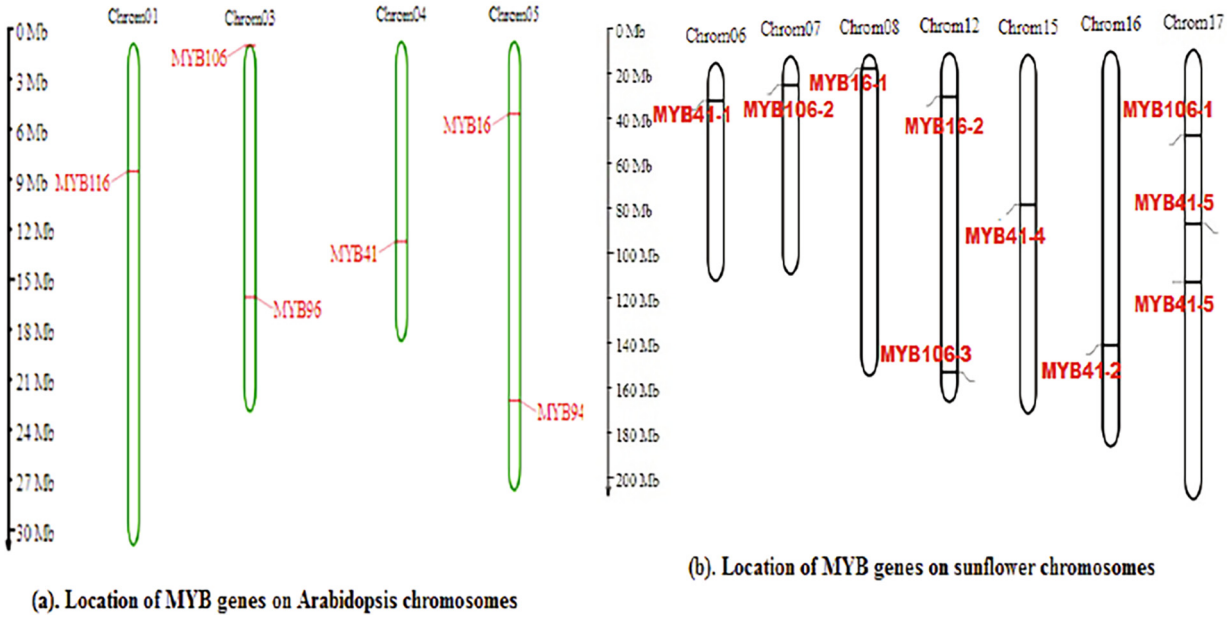

Fig. 4. Chromosomal mapping of MYB genes on Arabidopsis and Sunflower genome.

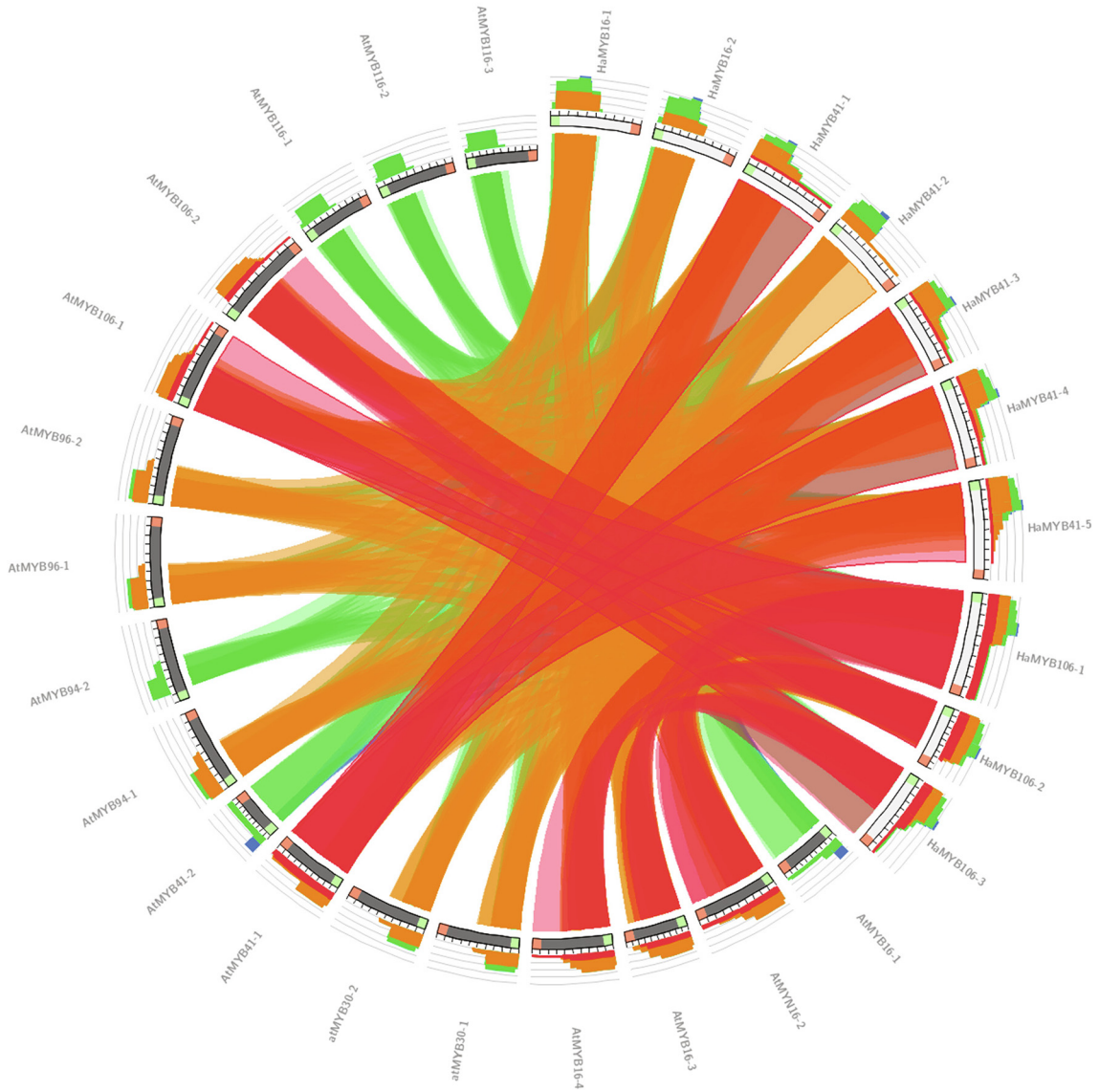

Fig. 5. Evolutionary relationship between Arabidopsis and sunflower genome MYB TFs. Colored lines crossing at different points in circle indicates that they have same evolutionary origin.

Sunflower and 15 Arabidopsis proteins were used to perform the synteny analysis. The results indicated that MYB proteins of both species were closely associated with each other. It was observed that there were some genes having greater similarity than the other genes (Fig. 5). A gene *HanMYB106* dissect *AtMYB106-1, AtMYB106-2, HanMYB106-2, HanMYB106-3, AtMYB16-1, AtMYB16-2, AtMYB16-3* and *AtMYB16-4* indicated that they have same evolutionary origin and recently been separated. Similar evolutionary pattern was shown by *AtMYB41-1* gene which was dissecting *HanMYB-41–1, HanMYB-41–2, HanMYB-41–3, HanMYB-41–4 and HanMYB-41–5.*

3.6. Gene ontology

About thirty-nine different biological process were involving *MYB* genes. Enrichment of biosynthesis process indicated the role of these genes in wax biosynthesis. The other major processes include regulation of metabolism, cellular metabolic process, primary metabolic process, nitrogen compound metabolic process, cellular and organ development process (Fig. 6). This information indicated their diverse role in nature.

3.7. Expression analysis of MYB genes in sunflower

Different levels of *MYB16, MYB94 and MYB96* expression were found in sunflower leaves after drought stress, when qPCR was performed. *MYB16, MYB94* and *MYB96* transcripts were detected in sunflower with diverse expression levels (Fig. 7). It was noted that transcription levels of *MYB16, MYB94* and *MYB96* were many folds higher in drought subjected genotypes as compared to control, suggesting their role in wax production during water stress. They were up-regulated under drought stress as compared to control plants. *MYB16* showed 4–5 times more expression under stress conditions, *MYB94* showed 2.5–4.5 times more expression while *MYB96* exhibited 3.5–5.5 times up-regulation in gene expression when compared with control. Moreover, it was also observed that the expression of three genes (*MYB16, MYB94* and *MYB96*) was different in different genotypes indicating the genetic variation in the population.

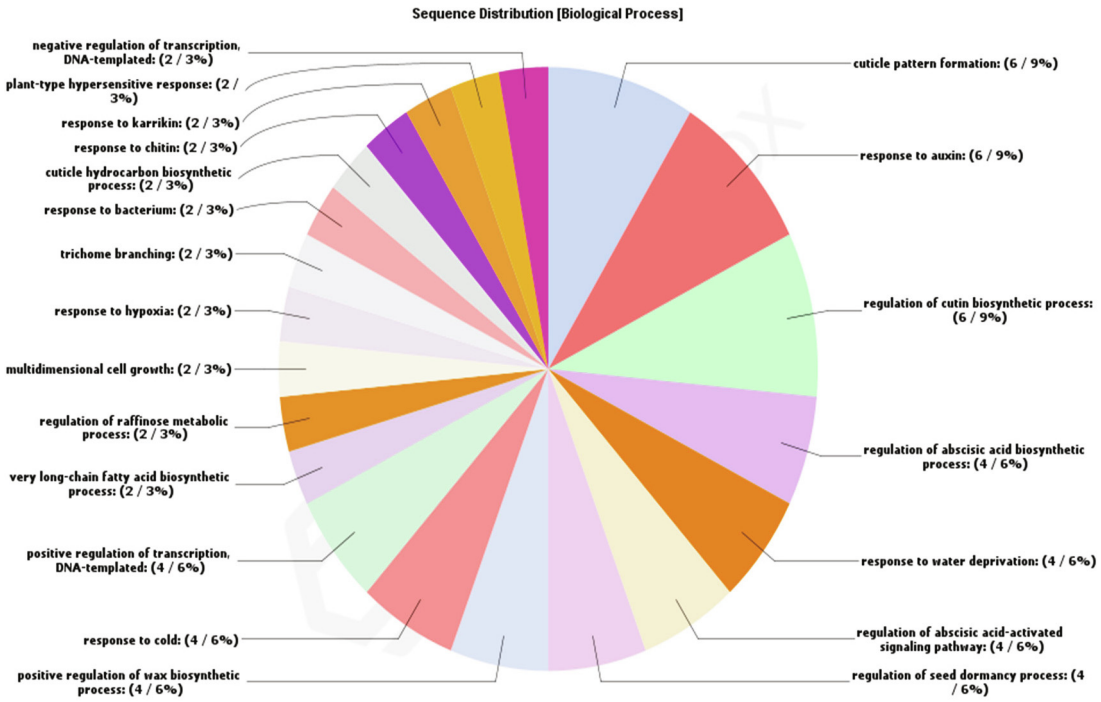

Fig. 6. Gene ontology of MYB TFs proteins in Arabidopsis and sunflower indicating their diverse roles in various vital processes.

4. Discussion

Sunflower is mainly cultivated for its seeds which are used to extract the edible oil along with other products e.g., husk, food cake and ornamental uses. Crop productivity is inherently sensitive to environmental changes (Ali et al., 2016) and their performance is affected badly under biotic and abiotic stress conditions. Cuticular wax is the natural gift for plants to save them during biotic and abiotic stress conditions (Wójcicka, 2015). Cuticular wax acts as a first line of defense to protect the plants from environmental damage (Schilling and Heiser, 1981). In past, it has been reported that cuticular wax decreases plant water loss by reducing transpiration rate, stomatal conductance and maintain the osmotic adjustment and leaf water potential. Cuticular wax also protect the plants form ultraviolet radiations, heat stress and cold injury. In addition to abiotic stresses, role of cuticular wax has also been observed against biotic stresses such as fungal and pathogen interactions. They protect plants from ultraviolet radiations, insects, pathogens, salinity high and low temperature (Xue et al., 2017). Major function of cuticular wax is to protect plants from drought stress by controlling gaseous exchange and non-stomatal water loss (Lee and Shu, 2015).

In the present study, sunflower leaf epicuticular wax chemical composition consists of alkanes, acids, esters, aldehydes, alcohols and unknown classes. The dominant wax class found in sunflower under drought was primary alcohol followed by alkanes, aldehydes, fatty acids, wax esters and some unknown residues. Our results were agreed with who reported alcohol as a dominant wax compositional class with 63%, 75–89% and 24.47% concentrations to total wax load in barley, maize and pear leaves respectively. However these results contradicted with (Bondada et al., 1996; Jenks et al., 1995) who observed alkane as a dominant class on leaves and stem of Arabidopsis, and cotton with 58%, 42.65% of total wax constituents respectively. The alcohol is the second dominant class with 24% following alkane in Arabidopsis leaf epicuticular wax. Cameron et al. (2006) also contradicted our results who observed alkane as a dominant wax chemical compositional class with 80 ± 2% following by 4% alcohol to total wax load in tobacco tree (*Nicotiana glauca* L.) leaves. The adult leaves of Arabidopsis have wax esters (42%) as dominant wax class. The epicuticular wax chemical composition and their percentage to total wax load varies among plant not only in plant species but even also within plant organs. The chemical wax composition depends on plant species and genotypes. The variation in wax chemical composition and concentration bring resistance to various biotic and abiotic stresses in plants. Variation in wax composition depends on environmental conditions, degree of a specie to tolerance/susceptibility the drought stress. From the previous research it can be concluded that plants subjected to drought stress enhances alcohol and alkane as compared to normally grown plants (Cameron, 2005). MYB TFs comprise one of the largest transcription factor families in plants (Peng et al. 2016; Mondal and Roy 2018; Yang et al., 2019). They are involved in various cellular processes, including hormonal signal transduction, plant development, biotic and abiotic stresses response, heavy metal stresses, differentiation and metabolism (Ashrafi-Dehkordi et al., 2018; Baldoni et al., 2015; Zhang et al., 2018; Cao et al., 2020). In Arabidopsis, number of MYB TFs is involved in cuticular wax biosynthesis under drought including *AtMYB16, AtMYB30, AtMYB41, AtMYB94, AtMYB96, AtMYB106,* and *AtMYB116* and *SlMYB12* have been reported in tomato (Cominell et al., 2008; Seo et al., 2011; Oshundiya et al., 2014).

Comparative genomics and phylogenetic analysis revealed that the Arabidopsis and sunflower MYB TFs have the same evolutionary roots. On the bases of un-rooted phylogenetic tree, MYB TF proteins were divided in four clades. Proteins in the same cluster were showing homology between MYB TFs of sunflower and Arabidopsis. Seven members were present in 1st clad three in 2nd, eleven in 3rd while four in 4th clad. This classification represented the same pattern as reported in Chickpea (Azeem et al., 2018; Waqas et al., 2019). Moreover, distribution pattern of intron/exon is an important tool for comparative genomic study to gain the insight about a gene family, because this pattern supports the evolutionary association of a gene with its ancestors (Waqas et al., 2019; Cao et al., 2017). Similar results were already published (Li et al.,

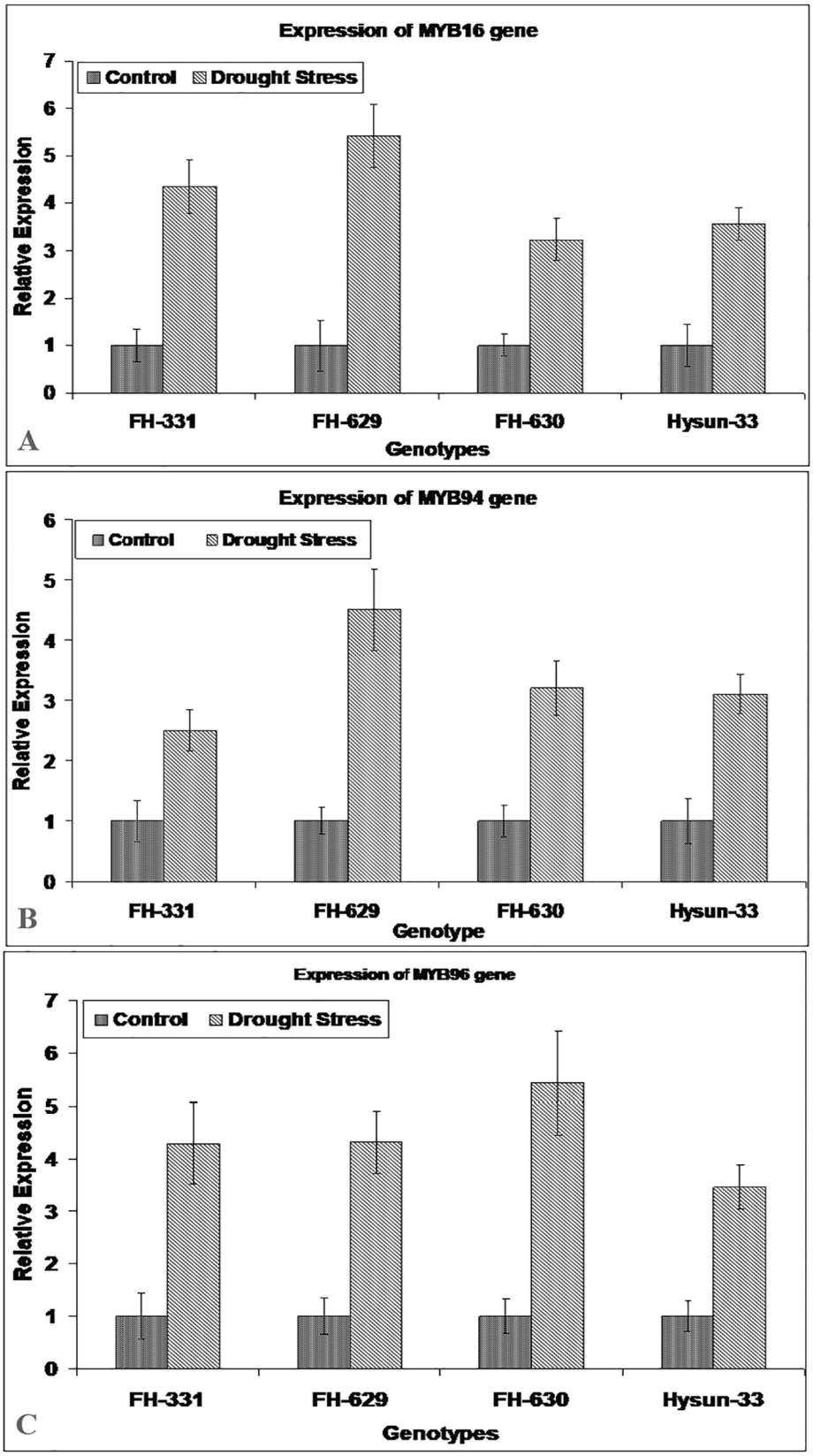

Fig. 7. Comparison of relative gene expression of three *MYB* genes in sunflower subjected to drought stress, (A) relative expression of *MYB16* gene, (B) relative expression of *MYB94* gene and (C) relative expression of *MYB96* gene. The error bars represent the mean ± SD of three biological replicates.

2016) who reported that homologous MYB protein laying in same clad or subclass have same evolutionary origin. Differences in gene structure of the same class members might be due to the differences in evolutionary history and these proteins may have new functional characteristics (Yang et al., 2019). The results revealed the diversity in functions of MYB TFs. Presence of phylogenetic specific pattern of conserved domains has been reported by (Azeem et al., 2018; Waqas et al., 2019). This pattern of conserved motifs indicated the recent common origin of MYB TFs (Du et al., 2013; Tan et al., 2020). Furthermore, presence of conserved motifs supports the evidence of functional conservation and gene duplication events in plants (Waqas et al., 2019). Conserved motifs also determine the diversity of domain architecture which has been utilized to maintain beyond the core components of MYB domain and have an important role for the functioning of MYB related proteins (Du et al., 2013; Tan et al., 2020).

AtMYB16 and *AtMYB106* are two paralogous genes involved in regulation of cuticular wax biosynthesis with the cooperation of *WIN1*/*SHN1* genes in *A. thaliana* and *T. fournieri* (Folkers et al., 1997; Jakoby et al., 2008; Gilding et al., 2010; Oshima et al. 2013). Expression of these genes by fusion to repressor domain using RNAi, negatively influence the cuticular wax formation (Oshima et al., 2013; Hiratsu et al., 2003). Previous research indicated that *AtMYB30* encodes four enzymes that are involved in acyl-CoA elongation complex, which is responsible for VLCFAs synthesis (Raffaele et al., 2008). Expression of *AtMYB41* gene is very low in ordinary conditions however under abscisic acid, salinity and drought stress its expression is highly increased. Overexpression of *AtMYB41* gene increases cuticular permeability, biosynthesis and transportation of lipids and cuticular metabolism in transgenic Arabidopsis (Cominelli et al., 2008). Initially in Arabidopsis, *AtMYB96* was considered as drought regulator which was thought to be integrated with ABA and auxin signals. Overexpression of *AtMYB96* gene has confirmed the drought tolerance in Arabidopsis and its knockout mutant have proved more drought sensitive than wild type plants (Seo et al., 2011). Our results also showed the same pattern and *HaMYB96* was upregulated under drought stress conditions. Upregulation of of *AtMYB94* and *AtMYB96* under limited water supply has already been reported in Arabidopsis (Oshundiya et al., 2014; Lee et al., 2016). Transcriptional activation of *AtMYB96* under drought stress and cuticular wax biosynthesis has been confirmed by (Seo et al., 2011). Overexpression of *AtMYB96* gene has been proved to enhance drought tolerance in emerging oil-seed crops (Lee et al., 2014).

Overexpression of *AtMYB94* has also confirmed its involvement in wax biosynthesis as it enhanced the accumulation of cuticular wax and reduced the rate of stomatal transpiration in Arabidopsis. Its expression tends to increase ~ 5-fold under drought conditions. Further, it has been observed that *AtMYB94* TF activates other wax biosynthesis genes, i.e. *CER2*, *KCS2*, *WSD1*, *FAR3* and *ECR* by directly binding with their promoters (Lee et al., 2015). Similar TF *ZmMYB94* has been reported in maize which is involved in cuticular wax deposition in young seedling and regular the pattern of epicuticular wax biosynthesis on epidermis of maize young leaves (La Rocca et al., 2015). In wheat a TF, *TaMYB31* has been reported which is involved in up-regulation of wax biosynthesis under drought conditions (Zhao et al., 2018). Similarly, *MYB16* has been characterized for cuticle formation in vegetative organs under drought stress (Oshima et al., 2013).

5. Conclusions

MYB, TFs were widely studied in other crop species like *Arabidopsis*, *Glycine* max, Gossypium *hirsutum*, *Zea mays* and Triticum aestivum but few studies were available in sunflower. Recent release of sunflower genome provided the opportunity to determine structural and functional diversity of MYB, TFs in Sunflower. Different computational tools were used to identify and characterize *MYB* genes in sunflower. In current study, three *MYB* genes and their seven homologs were identified having role in wax biosynthesis. Gene expression analysis revealed that *MYB16, MYB94* and *MYB96* were up-regulated under drought stress which strengthened the hypothesis. In future, this study may serve as foundation to further characterize the role of *MYB* gene family in wax biosynthesis by regulating stress responsive genes.

6. Key message

The identified MYB TFs in sunflower genome are unevenly distributed. Among, identified TFs the up-regulation of *MYB16*, *MYB94* and *MYB96* under drought condition displayed their involvement for plant resistance for abiotic stresses and was biosynthesis.

Author contributions

HMA and MR conceived the idea and performed experimental work. MR supervised the experiment. HMA, SF, FA, TS, MI and SAB analyzed the data and wrote the manuscript. SAK, SHQ, MR, SA, FMP and SF provide with technical assistance and critically analyzed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

The authors gratefully acknowledge the financial support from Higher Education Commission, Government of Pakistan. Secondly, FMP and SA thank the Chilean National Fund for Scientific and Technological Development (FONDECYT) grant number 1201973.

Acknowledgments

The authors acknowledge Mr. Fida Hussain, Oilseed Research Institute, Ayyub Agriculture Research Institute, Faisalabad, Pakistan for providing seeds of sunflower cultivars.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.06.009.

References

Adato, A., Mandel, T., Mintz-Oron, S., Venger, I., Levy, D., Yativ, M., Domínguez, E., Wang, Z., De Vos, R.C.H., Jetter, R., Schreiber, L., Heredia, A., Rogachev, I., Aharoni, A., 2009. Fruit-surface flavonoid accumulation in tomato is controlled by a SLMYB12-regulated transcriptional network. PLoS Genet. 5, e1000777.

Ahmad, H.M., Mahmood-ur-Rahman, F.A., Shaheen, T., Irshad, M.A., 2020. Genome-wide analysis of long chain acyl Co-A synthetase (LACS) genes in sunflower (*Helianthus annuus*) suggests their role in drought stress. Int. J. Agric. Biol. 24, 863–887.

Ali, Z., Shabbir, M., Qadeer, A., Muhammad Ahmad, H., Qasim, M., Aziz, O., 2016. Performance evaluation of gladiolus varieties under diverse climatic conditions. Plant Gene Trait 7, 1–9.

Ashrafi-Dehkordi, E., Alemzadeh, A., Tanaka, N., Razi, H., 2018. Meta-analysis of transcriptomic responses to biotic and abiotic stress in tomato. Peer J 7, e4631.

Azeem, F., Ahmad, B., Atif, R.M., Ali, M.A., Nadeem, H., Hussain, S., Manzoor, H., Azeem, M., Afzal, M., 2018. Genome-wide analysis of potassium transport-related genes in chickpea (*Cicer arietinum* L.) and their role in abiotic stress responses. Plant Mol. Biol. Report 36, 451–468.

Badouin, H., Gouzy, J., Grassa, C.J., Maurat, F.S., Cottret, E.S., Briere, L., Owens, C.L., Carrere, G.L., Mayjonade, S., et al., 2017. The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. Nature 546, 148–152.

Baldoni, E., Genga, A., Cominelli, E., 2015. Plant MYB transcription factors: their role in drought response mechanisms. Int. J. Mol. Sci. 13, 15811–15851.
Barnes, J., Paul, N., Percy, K., Broadbent, P., McLaughlin, C., Mullineaux, P., Creissen, G., Wellburn, A., 1994. Effects of UV-B radiation on wax biosynthesis. In: Air Pollutants and the Leaf Cuticle. Springer, Berlin Heidelberg, pp. 195–204.
Blackman, B.K., Scascitelli, M., Kane, N.C., Luton, H.H., Rasmussen, D.A., Bye, R.A., Lentz, D.L., Rieseberg, L.H., 2011. Sunflower domestication alleles support single domestication center in Eastern North America. PNAS 108, 14360–14365.
Bondada, B.R., Oosterhuis, D.M., Murphy, J.B., Kims, K.S., 1996. Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract and boll. Environ. Exp. Bot. 36, 61–69.
Cameron, K.D., 2005. Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol. 140, 176–183.
Cameron, K.D., Teece, M.A., Smart, L.B., 2006. Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol. 140, 176–183.
Cao, Y., Li, K., Li, Y., Zhao, X., Wang, L., 2020. MYB transcription factors as regulators of secondary metabolism in plants. Biology 11, 346.
Cao, Y., Han, Y., Meng, D., Li, G., Li, D., Abdullah, M., Jin, Q., Lin, Y., Cai, Y., 2017. Genome-wide analysis suggests the relaxed purifying selection affect the evolution of *WOX* genes in *Pyrus Bretschneideri*, *Prunus Persica*, *Prunus Mume*, and *Fragaria Vesca*. Front. Genet. 8, 78.
Chou, K.C., Shen, H.B., 2010. A new method for predicting the subcellular localization of eukaryotic proteins with both single and multiple sites: Euk-MPLoc 2.0. PLoS One 5, e9931.
Cominelli, E., Galbiati, M., Tonelli, C., 2008. Integration of water stress response: cell expansion and cuticle deposition in Arabidopsis Thaliana. Plant Signal Behav. 3, 556–557.
Du, H., Wang, Y.B., Xie, Y., Liang, Z., Jiang, S.J., Zhang, S.S., Huang, Y.B., Tang, Y.X., 2013. Genome-wide identification and evolutionary and expression analyses of MYB-related genes in land plants. DNA Res. 20, 437–448.
Folkers, U., Berger, J., Hülskamp, M., 1997. Cell morphogenesis of trichomes in arabidopsis: differential control of primary and secondary branching by branch initiation regulators and cell growth. Development 124, 3779–3786.
Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M.R., Appel, R.D., Bairoch, A., 2005. Protein identification and analysis tools on the ExPASy server. In: The Proteomics Protocols Handbook. Humana Press, pp. 571–607.
Gilding, E.K., Marks, M.D., 2010. Analysis of purified glabra3-shapeshifter trichomes reveals a role for NOECK in regulating early trichome morphogenic events. Plant J. 64, 304–317.
Gordon, D.C., Percy, K.E., Riding, R.T., 1998. Effects of u.v.-B radiation on epicuticular wax production and chemical composition of four picea species. New Phytol. 138, 441–449.
Götz, S., García-Gómez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talón, M., Dopazo, J., Conesa, A., 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 36, 3420–3435.
Hall, D.M., Jones, R.L., 1961. Physiological significance of surface wax on leaves. Nature 191, 95–96.
Hiratsu, K., Matsui, K., Koyama, T., Ohme-Takagi, M., 2003. Dominant repression of target genes by chimeric repressors that include the EAR Motif, a repression domain, in Arabidopsis. Plant J. 34, 733–739.
Jaakola, L., Pirttilä, A.M., Halonen, M., Hohtola, A., 2001. Isolation of High Quality RNA from Bilberry (*Vaccinium Myrtillus* L.) Fruit. Appl. Biochem. Biotechnol.-Part B Mol. Biotechnol. 19, 201–203.
Jakoby, M.J., Falkenhan, D., Mader, M.T., Brininstool, G., Wischnitzki, E., Platz, N., Hudson, A., Hülskamp, M., Larkin, J., Schnittger, A., 2008. Transcriptional profiling of mature arabidopsis trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106. Plant Physiol. 148, 1583–1602.
Jenks, M.A., Tuttle, S.D., Eigenbrode, K.A., 1995. Feldmann Leaf epicuticular waxes of the eceriferum mutant in Arabidopsis. Plant Physiol. 108, 369–377.
Johnson, D.A., Richards, R.A., Turner, N.C., 1983. Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness1. Crop Sci. 23, 318.
Kerstiens, G., 1996. Signalling across the divide: a wider perspective of cuticular structure-function relationships. Trends Plant Sci. 1, 125–129.
La Rocca, N., Manzotti, P.S., Cavaiuolo, M., Barbante, A., Vecchia, F.D., Gabotti, D., Gendrot, G., Horner, D.S., Krstajic, J., Persico, M., Rascio, N., Rogowsky, P., Scarafoni, A., Consonni, G., 2015. The maize fused leaves1 (*Fdl1*) gene controls organ separation in the embryo and seedling shoot and promotes coleoptile opening. J. Exp. Bot. 66, 5753–5767.
Lai, C., Kunst, L., Jetter, R., 2007. Composition of alkyl esters in the cuticular wax on inflorescence stems of arabidopsis thaliana cer mutants. Plant J. 50, 189–196.
Latchman, D.S., 1997. Transcription factors: an overview. Int. J. Biochem. Cell Biol. 29, 1305–1312.
Li, S., Wang, X., He, S., Li, J., Huang, Q., Imaizumi, T., Qu, L., Qin, G., Qu, L.J., Gu, H., 2016. *CFLAP1* and *CFLAP2* are two BHLH transcription factors participating in synergistic regulation of AtCFL1-mediated cuticle development in arabidopsis. PLoS Genet. 12, e1005744.
Li, P., Wen, J., Chen, P., Guo, P., Ke, Y., Wang, M., Liu, M., Tran, L.S.P., Li, J., Du, H., 2020. Myb superfamily in *Brassica napus*: evidence for hormone-mediated expression profiles, large expansion, and functions in root hair development. Biomole 10, 1–17.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4), 402–408.
Lee, S.B., Kim, H., Kim, R.J., Suh, M.C., 2014. Overexpression of arabidopsis MYB96 confers drought resistance in *Camelina sativa* via cuticular wax accumulation. Plant Cell Rep. 33, 1535–1546.
Lee,S.B.,Suh,M.C.,2015.Advancesintheunderstandingofcuticularwaxesinarabidopsis thaliana and crop species. Plant Cell Reports. Springer Verlag, 557–572.
Lee, S.B., Kim, H.U., Suh, M.C., 2016. *MYB94* and *MYB96* additively activate cuticular wax biosynthesis in arabidopsis. Plant Cell Physiol. 57, 2300–2311.
Mondal, S.K., Roy, S., 2018. Genome-wide sequential, evolutionary, organizational and expression analyses of phenylpropanoid biosynthesis associated MYB domain transcription factors in arabidopsis. J. Biomol. Struct. Dyn. 36, 1577–1601.
Mooney, C., Wang, Y.H., Pollastri, G., 2011. SCLpred: protein subcellular localization prediction by N-to-1 neural networks. Bioinfor 27, 2812–2819.
Oshima, Y., Mitsuda, N., 2013. The MIXTA-like transcription factor MYB16 Is a major regulator of cuticle formation in vegetative organs. Plant Signal Behav. 8, e26826.
Oshundiya, F.O., Olowe, V.I.O., Sowemimo, F.A., Odedina, J.N., 2014. Seed yield and quality of sunflower (Helianthus Annuus L.) as influenced by staggered sowing and organic fertilizer application in the humid tropics. Helia 37, 237–255.
Pascal, S., Bernard, A., Sorel, M., Pervent, M., Vile, D., Haslam, R.P., Napier, J.A., Lessire, R., Domergue, F., Joubès, J., 2013. The arabidopsis Cer26 Mutant, like the Cer2 mutant, is specifically affected in the very long chain fatty acid elongation process. Plant J. 73, 733–746.
Peng, X., Liu, H., Wang, D., Shen, S., 2016. Genome-wide identification of the jatropha curcas MYB family and functional analysis of the abiotic stress responsive gene *JcMYB2*. BMC Genomics 17, 251.
Raffaele, S., Vailleau, F., Léger, A., Joubès, J., Miersch, O., Huard, C., Blée, E., Mongrand, S., Domergue, F., Roby, D., 2008. A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in arabidopsis. Plant Cell 20, 752–767.
Riaz, A., Iqbal, M.S., Fiaz, S., Chachar, S., Amir, RM., Riaz, B., 2020. Multivariate analysis of superior *Helianthus annuus* L.genotypes related to metric traits. Sanis Malays. 49, 461–470.
Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C.Z., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K., Yu, G.L., 2000. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science 290, 2105–2110.
Rieseberg, L.H., Seiler, G.J., 1990. Molecular evidence and the origin and development of the domesticated sunflower (Helianthus annuus, Asteraceae). Econ. Bot. 44, 79–91.
Roy, S., 2016. Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signal Behav. 11, e1117723.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Schilling, E.E., Heiser, C.B., 1981. Infrageneric classification of Helianthus (Compositae). Taxon 30, 393–403.
Seo, P.J., Lee, S.B., Suh, M.C., Park, M.J., Park, C.M., 2011. The MYB96 transcription factor regulates cuticular wax biosynthesis under drought conditions in arabidopsis. Plant Cell 23, 1138–1152.
Serrano, M., Coluccia, F., Torres, M., L'Haridon, F., Métraux, J.P., 2014. The cuticle and plant defense to pathogens. Front. Plant Sci. 5, 274.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tan, L., Ijaz, U., Salih, H., Cheng, Z., Htet, N.N.W., Azeem, Ge Y.F., 2020. Genome-wide identification and comparative analysis of MYB transcription factor family in musa acuminata and musa balbisiana. Plants 9, 413.
Waqas, M., Azhar, M.T., Rana, I.A., Azeem, F., Ali, M.A., Nawaz, M.A., Chung, G., Atif, R.M., 2019. Genome-wide identification and expression analyses of WRKY transcription factor family members from chickpea (Cicer Arietinum L.) reveal their role in abiotic stress-responses. Genes Genomics 41, 467–481.
Wójcicka, A., 2015. Surface Waxes as a Plant Defense Barrier towards Grain Aphid. Acta Biol. Cracoviensia Ser. Bot. 57, 95–103.
Xue, D., Zhang, X., Lu, X., Chen, G., Chen, Z.H., 2017. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Front. Plant Sci. 8, 621.
Yang, K., Li, Y., Wang, S., Xu, X., Sun, H., Zhao, H., Li, X., Gao, Z., 2019. Genome-wide identification and expression analysis of the MYB transcription factor in moso bamboo (*Phyllostachys edulis*). Peer J 1, e6242.
Zeisler-Diehl, V., Müller, Y., Schreiber, L., 2018. Epicuticular wax on leaf cuticles does not establish the transpiration barrier, which is essentially formed by intracuticular wax. J. Plant Physiol. 227, 66–74.
Zhao, Y., Cheng, X., Liu, X., Wu, H., Bi, H., Xu, H., 2018. The wheat MYB transcription factor TaMYB31 is involved in drought stress responses in arabidopsis. Front. Plant Sci. 9, 1426.
Zhang, T., Zhao, Y., Wang, Y., Liu, Z., Gao, C., 2018. Comprehensive analysis of Myb gene family and their expressions under abiotic stresses and hormone treatments in *Tamarix hispida*. Front. Plant Sci. 9, 1303.
Zhu, L., Guo, J., Zhu, J., Zhou, C., 2014. Enhanced expression of *esWAX1* improves drought tolerance with increased accumulation of cuticular wax and ascorbic acid in transgenic arabidopsis. Plant Physiol. Biochem. 75, 24–35.