



Integrated Morphological, Physiological and Molecular Analysis of the Drought Response in Cultivated and Wild *Prunus* L. Subgenera *Cerasus* Species

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Abstract

Knowledge about the genetic potential of wild *Prunus* species is important for recognizing gene pools in germplasm and developing effective strategies for germplasm conservation and breeding. In this study, cultivated sweet cherry (*Prunus avium* L.) and relative wild *Cereus* subgenus genotypes belonging to *P. microcarpa* Boiss and *P. incana* (Pall.) Batsch. species were assayed in controlled drought conditions performing an integrated morphological, physiological, genomic and transcriptomic analysis of the stress response. These analyses were performed under well-watered and water-deficient conditions analysing morphology of leaves, gas exchange parameters and chlorophyll content differently affected by drought, especially under long-term water deficiency. From a genomic point of view, these genotypes were characterized using simple sequence repeat (SSR) markers. Finally, in the analysis of the transcriptional responses, drought tolerance candidate genes were analysed by quantitative PCR (qPCR). From a morphological point of view, *P. microcarpa* showed less leaf area, height and diameter in comparison to *P. incana* and mainly *P. avium*. The highest rate of photosynthesis in genotypes differently was observed in the control treatment and the lowest rate viewed during stress. The analysed genes showed different responses to drought in the genotypes, and more detailed analysis techniques to explain the molecular mechanisms of drought tolerance are needed in these genotypes. Our results provide a significant contribution to the understanding of how *P. incana* and *P. microcarpa* respond to drought stress, which may help to explain molecular and physiological mechanisms associated with the response to the drought of cherries. Additionally, these results show that wild cherries can be considered as a genetic source of drought resistance in breeding programs.

Keywords *Prunus* · Cherry · Drought · Microsatellites · Genomics · qPCR · Transcriptomics · Breeding

Key Message

Our study starts up for the first time the evaluation of drought tolerance of wild cherry species *Prunus incana* (Pall.) Batsch. and *P. microcarpa* Boiss in comparison with the cultivated one (*P. avium* L.) to the understanding of how they respond to drought stress which may help to illuminate the molecular mechanisms associated with the drought response of *Prunus*.

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Introduction

In the last few decades, the biggest challenge especially in agriculture sector that the world faces is climate change and global warming. Following climate change, drought has become likely the most important constraint limiting the productivity crop and finally food security worldwide. In some countries, climate change has been so high that it can compensate for improvements in yield resulting from technology, fertilization and other factors through negative effects on plant growth and reproduction. Reduce precipitation and change of rainfall patterns causes repeated droughts around the world (Lesk et al. 2016; Lobell et al. 2011; Yordanov et al. 2000).

To study drought response, several parameters and their relations such as stomatal conductance, net photosynthetic

rate leaf turgor synthesis of abscisic acid or water-use efficiency have been studied in fruit trees (Martínez-García et al. 2020). On the other hand, extensive studies suggest that plant strategies to reduce drought effects include those that enable plants to avoid and tolerate low water potentials. In the avoid strategy, water loss and water uptake maintain balanced and preserved the plant water status by tapping ground water with deep roots, stomatal closure and small leaves. With the onset of drought, plants tolerate water stress through osmotic or elastic adjustment or the accumulation of osmoprotective substances such as cyclitols (Pirasteh-Anosheh et al. 2016; Roychoudhury et al. 2013). Therefore, activation of these processes enables maintenance of cellular homeostasis through lipid and carbohydrate metabolism.

Based on the current comprehension from drought-responsive genes that included regulatory and effector genes, the identification of these genes and understanding their functional are necessary for improvement of drought tolerance in crops of economic importance (Shinozaki and Yamaguchi-Shinozaki 2007). In *Prunus* species, several water-deficit resistance genes dependent and independent of abscisic acid (ABA) biosynthesis have been identified mainly transcription factors (TF) such as *basic helix-loop-helix (bHLH)* (Bianchi et al. 2015). Due to ABA multiple roles in seed germination and dormancy infliction (Rodríguez-García et al. 2009), fruit ripening (Teribia et al. 2016) and induction of drought resistance (Balint and Reynolds 2016; Li et al. 2012) in plants, each of these genes is expected to have specific functions. ABA receptors promote drought resistance in *Arabidopsis* and rice by limiting the loss of transpiration water and creating similar reactions to summer dormancy, such as old leaves senescence and growth prevention in young tissues in continuous drought conditions (Zhao et al. 2016; Lau et al. 2021). *HD-Zip* (homeobox-leucine zipper) genes have been detected in a vast variety of plant species and have a numerous functional range. Chen et al. (2010) showed that the 12 *HD-Zip* genes are responsive to the drought and salt stressors. *ATHB-12* is one of the *HD-Zip* families that is induced by water-deficit and acts as a negative primary regulator of the ABA response mechanism in *Arabidopsis*. In addition, *AFP3/ABI* (ninja-family protein/five-binding protein) regulates stress response by the downregulation of ABA responses (García et al. 2008).

Given the ABA role in abiotic stresses, it is important that its receptors play important role in responses to these stresses. In apple (*Malus domestica* Borkh.), the activation of the ABA signal pathway mediated by γ -aminobutyric acid (GABA) improves drought resistance (Liu et al. 2021; Yao et al. 2020). In addition, it is known that several TF families including *bHLH*, *MYB*, *WRKY*, *bZIP*, *AUX/IAA*, *dehydration-responsive element binding protein (DREB)* and *peroxidase (Pd)* are involved in drought stress in plants

(Alimohammadi et al. 2013; Feng et al. 2017). However, few previous studies have performed on the function of these genes under drought stress in cherry. Recently, Xu et al. (2023) evidenced the role of ABA-related genes and transcription factors (*PavWRKY* and *PavMYB*), in response to drought of cherry rootstock.

On the other hand, *Prunus* is one of the genera belonging to the Rosaceae family with high diversity and economic significance. It is native to temperate regions of the Northern Hemisphere specially Europe and Asia (Mozaffarian 2002). While most commercial cultivars are sensitive to drought, wild genotypes grow well in water-limited areas and play an important role in the ecological environment. Research on molecular mechanisms underlying the drought tolerance of these plants is scarce, and we can almost say that wild species of cherries have not been investigated in relation to the molecular basis of their drought stress tolerance (Mozaffarian 2002). Although *P. incana* and *P. microcarpa* two wild species are resistant to drought, so far, no research has been conducted on their resistance to water scarcity (Nazari et al. 2012). Only recent evidence in in vitro assays has been described (Sevgin 2021). These wild species not only can be used as a source of new genes or alleles, but also have the potential to breeding of rootstocks for dwarfing, cold and drought-tolerant.

Iran is one of the countries of origin of *Cerasus* subgenus plants, and there has been no study on this subgenus about drought tolerance; in this study, we investigated the integrated morphological, physiological and transcriptional response of wild cherries to drought stress in order to provide a comprehensive analysis of adaptation in cultivated cherry (*P. avium* L.) and wild *P. microcarpa* Boiss and *P. incana* (Pall.) Batsch. subgenera *Cerasus* species under control and drought stress. The information obtained may provide new insights into the underlying molecular mechanisms of the response to drought stress in *Prunus* species.

Material and Methods

Plant Materials

In this study, different species were assayed including the cultivated *P. avium* L. ['Avi-Ala'] and the wild species *P. microcarpa* Boiss ['Mic-Kor'] and *P. incana* (Pall.) Batsch ['Inc-Kho']. One accession of *P. mahaleb* L. ['Mah'] was also assayed as out group in the phylogeny analysis (Table 1; Fig. 1). Seeds from the different accessions collected in different parts of Iran were stratified and germinated. Seedlings were grown under irrigation in glasshouse with 48–55% relative humidity and day/night temperature ~ 30/18 °C. One-year-old seedlings were incorporated in this study. Thirty pots of each species were randomly selected and divided

Table 1 Comparison of means of quantitative morphological traits (leaf area, height and diameter) of the studied control genotypes from *Cerasus* subgenus species (*P. avium*, *P. microcarpa* and *P. incana*). Five biological replicates were assayed to evaluate each physiological parameter. Values with different letters indicated statistically different values

Genotype	Species	Origin	Leaf area (mm ²)	Height (cm)	Diameter (mm)
Avi-Ala 1	<i>Prunus avium</i>	Qazvin (Alamut))	867.26 dce	56.66 jhik	3.56 j
Avi-Ala 2	<i>Prunus avium</i>	Qazvin (Alamut)	824.39 ij	53.33 jlk	3.89 ijhg
Avi-Ala 3	<i>Prunus avium</i>	Qazvin (Alamut)	835.823 higj	53.66 jlk	4.57 ef
Avi-Ala 5	<i>Prunus avium</i>	Qazvin (Alamut)	833.51 higj	52.66 lk	3.73 ij
Avi-Ala 7	<i>Prunus avium</i>	Qazvin (Alamut)	834.79 higj	45.00 mn	4.13 ijhgf
Avi-Ala 8	<i>Prunus avium</i>	Qazvin (Alamut)	851.73 hdgfe	42.33 n	4.57 ef
Avi-Ala 11	<i>Prunus avium</i>	Qazvin (Alamut)	920.73 a	168.00 a	5.49 ba
Avi-Ala 12	<i>Prunus avium</i>	Qazvin (Alamut)	868.82 dce	75.00 d	5.73 ba
Avi-Ala 13	<i>Prunus avium</i>	Qazvin (Alamut)	816.71 j	66.00 e	4.56 ef
Avi-Ala 14	<i>Prunus avium</i>	Qazvin (Alamut)	839.85 higfj	61.00 fhcg	3.70 ij
Avi-Ala 16	<i>Prunus avium</i>	Qazvin (Alamut)	827.80 hij	53.33 jlk	3.71 ij
Avi-Ala 18	<i>Prunus avium</i>	Qazvin (Alamut)	859.07 dgfe	46.66 mn	3.77 ijh
Avi-Ala 20	<i>Prunus avium</i>	Qazvin (Alamut)	890.40 bc	64.33 feg	4.41 egf
Avi-Ala 22	<i>Prunus avium</i>	Qazvin (Alamut)	874.46 dc	44.33 mn	4.33 ehgf
Avi-Ala 23	<i>Prunus avium</i>	Qazvin (Alamut)	826.194 hij	45.33 mn	4.27 ehgf
Avi-Ala 24	<i>Prunus avium</i>	Qazvin (Alamut)	819.71 j	133.66 b	5.87 a
Mean values	<i>Prunus avium</i>		856.86	70.51	4.44
Mic-kor 1	<i>Prunus microcarpa</i>	Kurdistan	15.80 l	29.33 o	5.17 bdc
Mic-Kor 2	<i>Prunus microcarpa</i>	Kurdistan	14.94 l	12.66 p	2.73 k
Mic-Kor 3	<i>Prunus microcarpa</i>	Kurdistan	17.07 l	76.66 d	4.40 egf
Mean values	<i>Prunus microcarpa</i>		15.93	39.55	4.12
Inc-Kho	<i>Prunus incana</i>	West Azerbaijan	226.08 k	53.00 jlk	4.73 edc

into two groups, one group was use for drought treatment, and the other was used as control. The drought treatment was applied by withholding water and stopping irrigation

during 15 days, while the control treatment was continued irrigation (Fig. 2). This period of 15-day no watering used as drought treatments to compare control vs non-irrigated

Fig. 1 Map of Iran with the geographical locations of collecting sites of *Prunus avium* accessions and the three related species (*P. microcarpa*, *P. incana* and *P. mahaleb*) assayed in this study (see codes in Table 1)

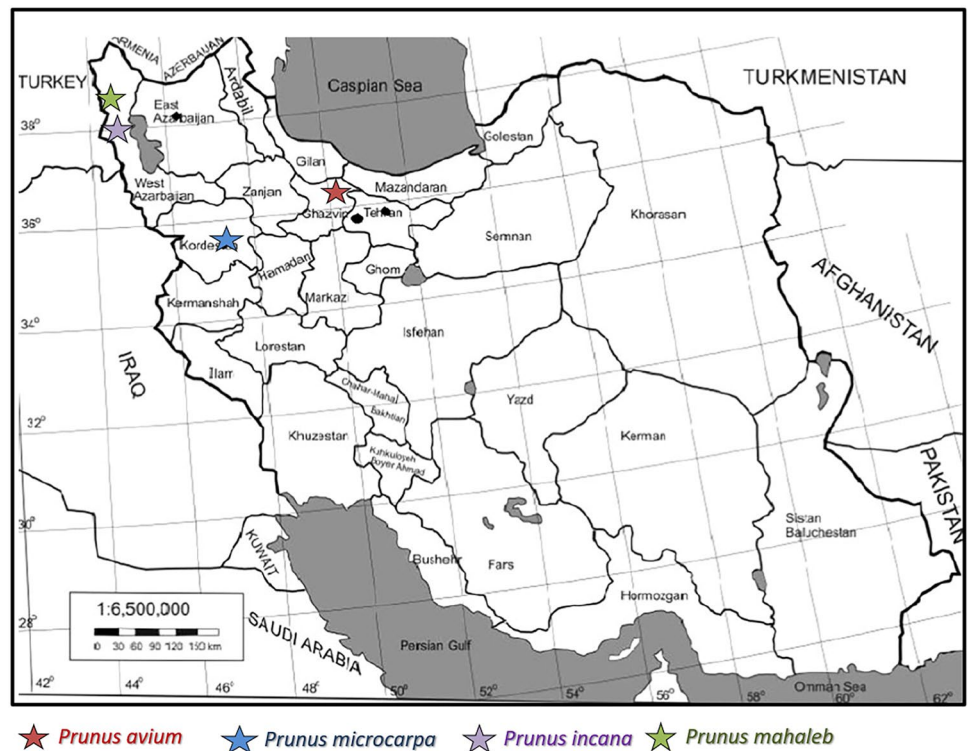
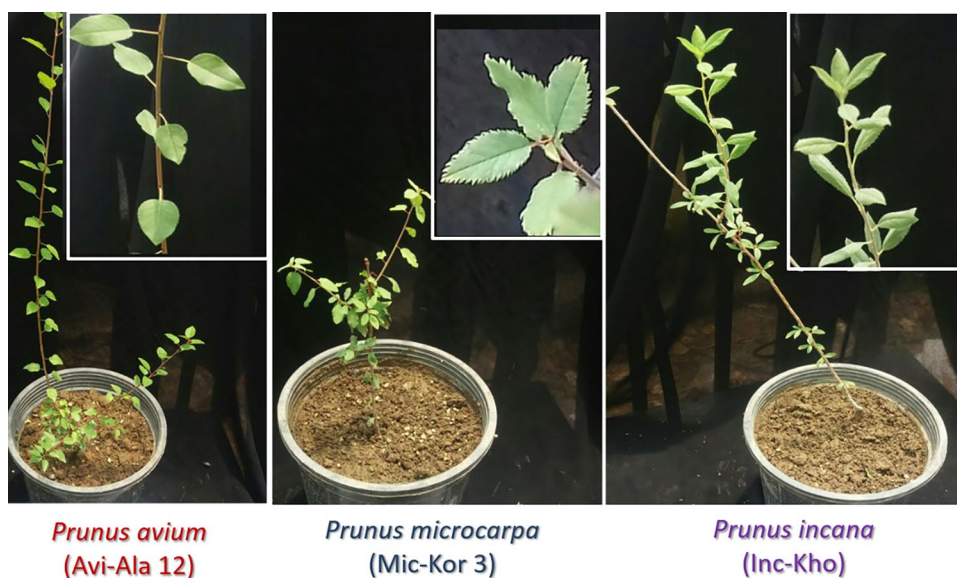


Fig. 2 Overview of the assayed whole plants and detail of leaves showing the studied morphological traits (leaf area, height and diameter) of the studied genotypes from cultivated and wild *Cerasus* subgenus species in control conditions in pots



seedlings will allow the monitoring of the response to water stress of seedlings as described before by Centritto (2005) and Ćereković et al. (2013) and Bnikkou et al. (2021).

Evaluation of Morphological and Physiological Parameters

Five biological replicates were assayed to evaluate each morphological and physiological parameter. Morphological parameters included leaf area (mm^2), height (cm) and diameter (mm). In addition, all physiological measurements were performed after 0, 7 and 15 days at 09:00 to 11:00 during the process of drought stress assaying five replications per assayed accession. The net photosynthetic rate (P_n) was measured using a portable photosynthesis (LICOR 6400, LICOR Inc., Lincoln, NB, USA). Chlorophyll index was measured using a Minolta SPAD-502 m (Karimpour et al. 2021). The SPAD-502 m is a non-destructive measuring device initially developed for the chlorophyll content of leaves widely used to optimize the timing and quantity of fertilizer to improve crop yield. Chlorophyll content is in general one indicator of plant health also affected by other physiological factors including growth regulators, photorespiration and oxidative stress (Ling et al. 2011). At the same time, plant stress meter was used to measure chlorophyll fluorescence. After a dark-adapted period (20 min) with dark leaf clip, the minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum photochemical efficiency (F_v/F_m) were measured (Turner 1988). Relative water content (RWC) was measured as per previously published method and calculated using the following equation: $\text{RWC} (\%) = [(FW - DW)/(TW - DW)] \times 100$.

DNA Extraction and SSR Analysis

DNA was isolated by CTAB method from several seedlings from *Prunus avium*, *Prunus microcarpa* and *Prunus incana* and *Prunus mahaleb* used as out group using a modified procedure of the described by Doyle and Doyle (1989). The genomic DNA was quantified at 260 nm, and its purity was measured at 260/280 nm absorbance ratio using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific). Isolated DNA was analysed using a set of 20 SSR codominant markers specific to *Prunus* species (Table 2). PCRs were performed in 15 μl mix containing approximately 5 ng of genomic DNA, 0.2 μM of each primer, 1 $\times$ Taq buffer (Bio-labs), 1 mM MgCl_2 , 0.2 mM of dNTPs mix and 1 unit of Taq polymerase (Biolabs). The amplification program was carried out according to the following: an initial melting step at 94 $^{\circ}\text{C}$ for 4 min followed by 35 cycles (of 95 $^{\circ}\text{C}$ for 30 s, annealing temperature (53.8, 55 and 57 $^{\circ}\text{C}$) for 30 s and 72 $^{\circ}\text{C}$ for 1 min) and then by a final elongation step at 72 $^{\circ}\text{C}$ for 7 min and hold at 10 $^{\circ}\text{C}$. Amplified products were resolved by electrophoresis in TBE buffer using 3% Meta-Phor agarose gel with a 1 kbp DNA ladder as a molecular standard. Polymorphic alleles were scored as present (1) or absent (0). The band scoring was analysed with the ImageJ gel analysis software (Wayne Rasband, National Institutes of Health, USA). The genetic diversity characterization was estimated by the number of heterozygote alleles per locus SSRs, and farther genetic information of the codominant SSR markers was determined by the observed heterozygosity (H) and by power of discrimination (PD) (Kloosterman et al. 1993). In addition, the mean character difference distances were calculated for all pairwise comparisons with the (ImageJ gel analysis software), which was used to construct

Table 2 SSR markers assayed, number of alleles detected, observed heterozygosity and power of discrimination (PD)

Markers	Reference	Repeat motif	Size (bp)	Ta (°C)	Allele (no.)	Heterozygosity	PD
PceGA34	Downey and Iezzoni (2000)	-	155	60	7	0.364	0.744
UDAp471	Messina et al. (2004)	-	157	56	7	0.727	0.826
UDAp-412	Messina et al. (2004)	(AG) ₉ (N) ₇ (AG) ₁₈	141	56	6	0.273	0.810
UDAp456	Messina et al. (2004)	-	167	56	7	0.727	0.826
UDP98-025	Testolin et al. (2000)	(CA) ₁₉	135	57–63	4	0.500	0.694
UDP98-410	Testolin et al. (2000)	(AG) ₂₃	146	57–63	7	0.308	0.805
UDP98-416	Testolin et al. (2000)	(AG) ₁₈	106	57–63	3	0.111	0.370
UDP-008	Cipriani et al. (1999)	(CA) ₂₃	165	55–63	2	0.000	0.180
BPPCT-034	Dirlewanger et al. (2002)	(GA) ₁₉	228	57–60	5	0.200	0.680
BPPCT-012	Dirlewanger et al. (2002)	(CT) ₁₃ CC(CT) ₇	164	57–60	5	0.222	0.765
BPPCT-007	Dirlewanger et al. (2002)	(AG) ₂₂ (CG) ₂ (AG) ₄	149	57–60	3	0.308	0.473
BPPCT030	Dirlewanger et al. (2002)	(AG) ₂₅	175	57–60	4	0.091	0.446
PACITA10	Lopes et al. (2002)	(CT) ₂₆	175	46	3	0.100	0.340
PACITA23	Lopes et al. (2002)	(AC) ₂ (AG) ₁₈	146	50	5	0.417	0.681
PACITA6	Lopes et al. (2002)	(GA) ₂₃	218	50	5	0.727	0.645
CPSCT005	Mnejja et al. (2004)	(CT) ₁₆	175	62	4	0.615	0.627
CPSCT044	Mnejja et al. (2004)	(GT) ₈ (AT) ₄ CA	200	62	1	0.000	0.000
CPPCT006	Aranzana et al. (2002)	(CT) ₁₆	190	57–60	3	0.167	0.569
CPPCT023	Aranzana et al. (2002)	(CT) ₉	166	55	1	0.000	0.000
CPPCT-008	Aranzana et al. (2002)	(CT) ₆ ...(CT) ₇	161	59	4	0.083	0.514

UPGMA dendrograms (cluster analysis) (Nei and Li 1979) depicting the phonetic relationship among different ecotypes. Relative support for the branches in each dendrogram was assessed by UPGMA bootstrap analysis (2000 replicates). Finally, association studies of SSR polymorphisms were carried out with mixed linear model (MLM) considering both Q and K matrices as covariates in TASSEL software (Yu and Buckler 2006).

RNA Extraction and qRT-PCR Analysis

Total RNA was extracted from leaves of selected seedlings ‘Avi-Ala 12’ (sensitive), ‘Mic-Kor 3’ (tolerant) and ‘Inc-Kho’ (tolerant) by the method of Le Provost et al. (2007). The most important genes related to the drought response previously described in different *Prunus* species including *PdDREB2c*, *BHLH71*, *PdP40*, *ATHB-12*, *ABI* and *AUX_1AA* were analysed by qPCR (García et al. 2008; Li et al. 2012; Balint and Reynolds 2016; Yao et al. 2020; Liu et al. 2021). Several housekeeping reference genes were assayed using two as internal controls: *RNA polymerase II (RP11)* and *ubiquitin 10 (UBQ10)* (Tong et al. 2009). Specific primers for all genes were designed based on *Prunus* sequences using Primer3 software (Table 3). CDNA was synthesized using SSIII Reverse Transcriptase (Thermo Fisher Scientific). To investigate the expression pattern of candidate genes in samples after 15 days under drought stress compared to that in control samples, real-time qPCR experiments were executed

with One-Step Plus Real-Time PCR system (Applied Biosystems) assaying three biological replicates and two technical replicates. Primers designed on almond (*P. dulcis* (Mill.) D.A. Webb) sequences were validated by the standard curve method. For all real-time qPCR reactions, a 10 µl mix was

Table 3 Specific/degenerate primers used for amplification of genes related to drought response from the assayed cultivated and wild cherry species. *RNA polymerase II (RP11)* and *Ubiquitin 10 (UBQ10)* have been assayed as housekeeping reference genes

Gene	Oligonucleotides
<i>PdDREB2c F</i>	5'-TTTTCCTCACTGCCCTTGATGCTGC-3'
<i>PdDREB2c R</i>	5'-ACTGAGAATGGTGAAGGCCGA-3'
<i>BHLH71 F</i>	5'-GTTGGAGTTTCTGTGCTT-3'
<i>BHLH71 R</i>	5'-GTTTGTGTGTGTAGGGTG-3'
<i>PdP40 F</i>	5'-TTT CTG GGG AGG GTT TGC TTC CAT C-3'
<i>PdP40 R</i>	5'-TCT CCC TTC GTC CCA GTA AAT GGT C-3'
<i>ATHB-12 F</i>	5'-CTGCAACTGAATAACCAAGG-3'
<i>ATHB-12 R</i>	5'-TACAGCATACTCAGAGCC-3'
<i>ABI F</i>	5'-CCTCGCCTTTCTCCATTA-3'
<i>ABI R</i>	5'-CCATCCCAAGTAAACCAAG-3'
<i>AUX_1AA F</i>	5'-TCTCCACCAACGCCTTCT-3'
<i>AUX_1AA R</i>	5'-ACAACAACCTACTCCTCTCC-3'
<i>RP11 F</i>	5'-TGAAGCATACACCTATGATGATGAAG-3'
<i>RP11 R</i>	5'-CTTTGACAGCACCAGTAGATTCC-3'
<i>UBQ10 F</i>	5'-AAGGCTAAGATCCAAGACAAAGAG-3'
<i>UBQ 10 R</i>	5'-CCACGAAGACGAAGCACTAAG-3'

made including 5 µl Power SYBR® Green PCR Master Mix (Applied Biosystems), 20 ng of cDNA and 0.5 µl of each primer (5 µM). The experiments were employed in the following conditions: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The melting temperature of these experiments was set to 60–95 °C and rising in 0.3 °C/s. Each biological sample was implemented in duplicate. RPII and actin were used as reference genes for data normalization, and the levels of relative expression were calculated by the method proposed by Pfaffl (2001). Data following a normal distribution were subjected to ANOVA single factor ($p \leq 0.05$) to test for significant differences between treatments and genotypes in terms of gene expression.

Results

Evaluation of Morphological and Physiological Parameters

According to the results of vegetative trait evaluation, *P. avium*, *P. microcarpa* and *P. incana* control genotypes showed high diversity in terms of morphological leaf traits (Fig. 2). Interestingly, ‘Mic-Kor 2’ had significantly the smallest leaf area, diameter and the lowest height with short nodes among genotypes that can be interesting for breeders. The largest leaf area and the highest height belonged to the ‘Avi-Ala 11’, and the largest trunk diameter was found in the ‘Avi-Ala 24’ genotypes (Table 1). In terms of an overview of genotypes, from a morphological point of view, ‘Mic-Kor’ showed less leaf area, height and diameter in comparison to ‘Inc-Kho’ and mainly ‘Avi-Ala’. In addition, Mic-Kor genotypes tended to recumbent growth habit with more leaf serration than other genotypes (Fig. 2). For ‘Inc-Kho’ and ‘Mic-Kor’, the leaves have pubescence on the lower surface which was easily visible with the naked eye. Finally, ‘Inc-Kho’ on the contrary with ‘Mic-Kor’ has longer and darker leaves.

On the other hand, compared means showed that the physiological parameters differently affected by drought, especially under long-term water deficiency. After 15 days of drought stress, all physiological parameters in the genotypes were significantly reduced compared to controls (Fig. 3). But *P. incana* and *P. microcarpa* drought-tolerant genotypes showed higher photosynthetic stability in contrast with the more drought susceptible *P. avium* genotypes. This effect was obviously in the appearance of *P. avium* genotypes as opposed to *P. incana* and *P. microcarpa* genotypes. ‘Avi-Ala 12’ showed the greatest reduction in photosynthesis rate (AN) and ‘Inc-Kho’ and ‘Mic-Kor 3’ the minimal. This is the highest photochemical efficiency (Fv/Fm) observed in ‘Avi-Ala 7’

and ‘Avi-Ala 2’. ‘Inc-Kho’ and ‘Mic-Kor 2’ also showed high photochemical efficiency versus Avi-Ala 1 and 3 that showed greatest reduction.

RWC is considered as an important criterion of plant water status. The leaf RWC decreased significantly in drought-stressed plants at 15 days of experiment in comparison with the control plants. But significant differences were observed between genotypes, so that ‘Avi-Ala 18’ reached a minimum value of RWC (47.17%) in front of ‘Inc-Kho’ that showed the maximum (71.48%) (Fig. 3).

Leaf Chl concentration was significantly affected by drought after 15 days of stress (Fig. 2). So ‘Inc-Kho’, ‘Mic-Kor 2’, ‘Mic-Kor 3’ and ‘Avi-Ala 2’ and ‘Avi-Ala 18’ showed the most leaf Chl concentration than the other genotype. ‘Avi-Ala 14’ showed the lowest leaf Chl concentration. The general aspects of the whole plants of studied genotypes from wild *Cerasus* subgenus species after the drought stress treatment including cultivated *P. avium* and wild *P. microcarpa* and *P. incana* corroborated this reduction Chl concentration in *P. avium* (Fig. 3).

Genomic Characterization

The evaluation of the 20 SSR markers in the 4 studied *Prunus* species generated a total of 86 alleles (Table 2). High level of polymorphism was detected among the studied species, and thus, they were useful for fingerprinting study in *Cerasus* subgenera germplasm (Fig. 4). The most of the amplified allele sizes ranged between 100 and 180 bp. Some of these alleles in different species were shared. The highest number of alleles (7) was observed at PceGA34, UDAp471, UDAp456 and UDP98-410 loci. The lowest number of alleles was obtained in the CPDCT044 and CPPCT023 loci (Table 2).

‘Inc-Kho’ within species showed the highest number of alleles with 10 alleles, while the least number of alleles was showed in Avi-Ala 1 with 4 alleles. Notably, ‘Inc-Kho’ was placed in a group with ‘Mah-Urm’. Within the species, the most polymorphic samples were those corresponding to ‘Inc-Kho’ and ‘Mic-Kor’ species which were polymorphic in all the SSRs and showed more polymorphisms than other species (Fig. 4).

According to the results, CPPCT-008 showed the lowest heterozygosity value (0.08) and UDAp471, UDAp456 and PACITA6 the highest (0.72). ‘Avi-Ala 22’ and ‘Avi-Ala 23’ genotypes were showed the highest and lowest heterozygosity, respectively. Conforming to genetic matrix distance, ‘Avi-Ala 11’ with ‘Avi-Ala 14’ and ‘Avi-Ala 16’ with ‘Inc-Kho’ were, respectively, the closest and the furthest genotypes (Fig. 4). However, no SSR markers showed a good degree of linkage with the drought response of the assayed genotypes and the evaluated gene expression.

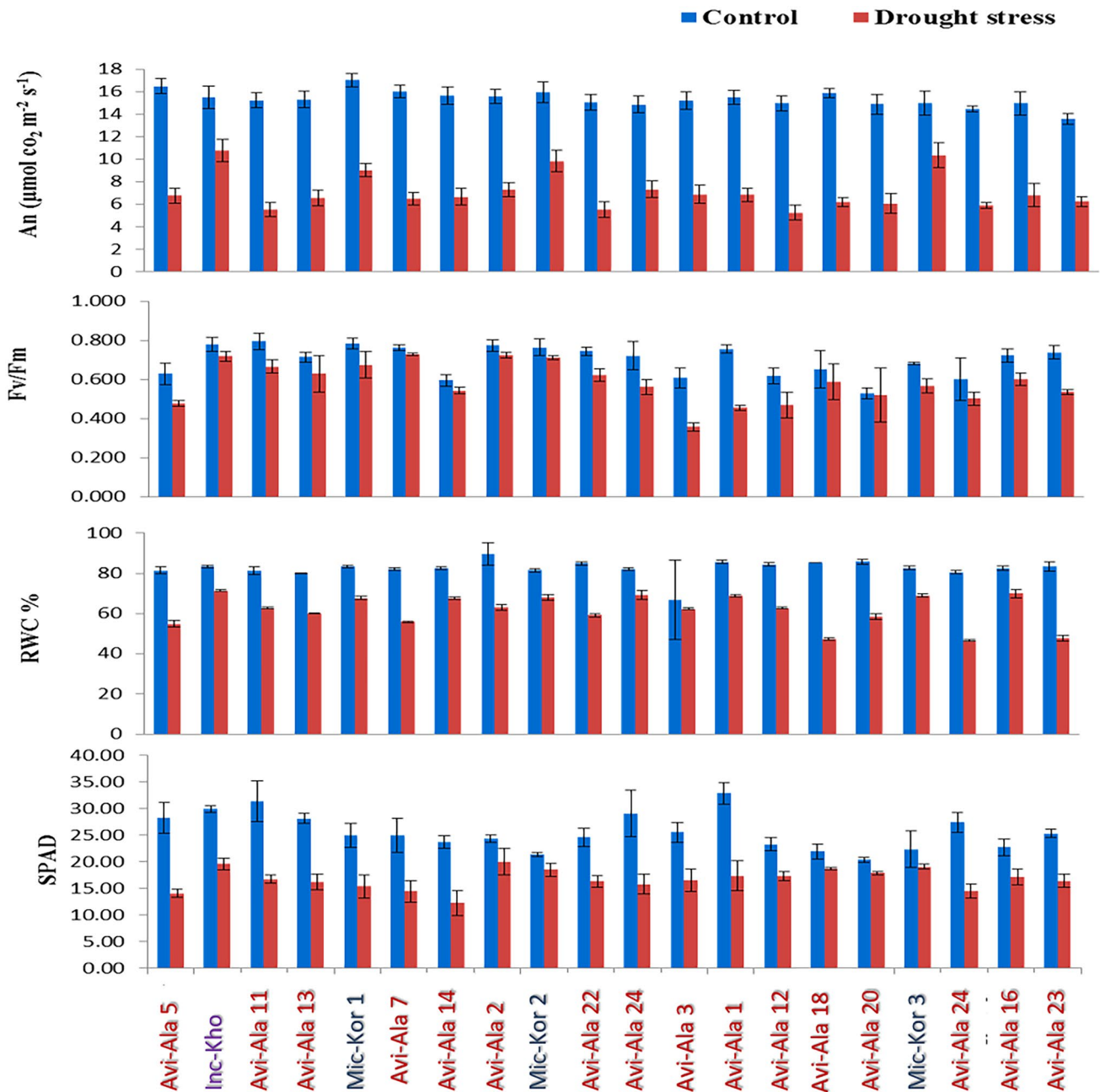


Fig. 3 Photosynthesis rate (An), maximum photochemical efficiency (Fv/Fm), relative water content (RWC) and SPAD in control and drought-stressed of studied *P. avium* accessions and the related species (*P. micro-*

carpa and *P. incana*) assayed in this study after 15 days of drought. Five biological replicates were assayed to evaluate each physiological parameter. Standard deviations are indicated with vertical bars

Transcriptomic Analysis

The assayed genes related to drought response *auxin-responsive protein IAA1-like (AUX_IAA)*, *PdDREB2c*, *BHLH71*, *PdP40*, *ATHB-12* and *ABI* were analysed by qPCR (Fig. 5). *AUX/IAA* expression in drought-treated leaves decreased compared to control levels in all genotypes, significantly in *P. incana* after 15 days of treatment. *DREB2C* expression in

sensitive genotype (*P. avium* ‘Avi-Ala 12’) had no significant difference with control but in the tolerant genotype (*P. microcarpa* ‘Mic-Kor 3’) has notably increased. *P. incana* had been meaningfully reduced in the treatment. *AFP3* (ninja-family protein) levels in sensitive genotype (*P. avium* ‘Avi-Ala 12’) significantly increased compared to control. *P40* (*peroxidase 40*) belongs to the peroxidase family that plays a key role in oxidative metabolism. Unlike Mic-Kor

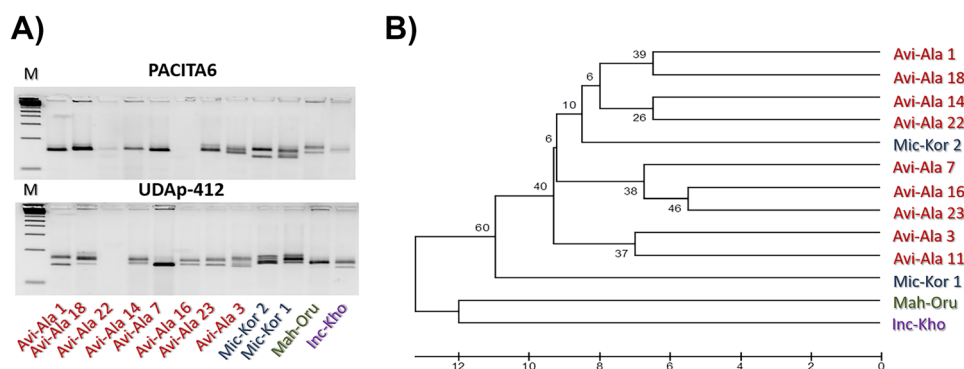


Fig. 4 **A** Methaphor[®] agarose gels showing SSR polymorphism of the assayed UDAP-412 and PACITA6 markers in the cultivated and wild cherry samples analysed. M, molecular ladder assayed 1 kb DNA ladder. **B** Dendrogram of the assayed accessions of *Prunus*

avium and the three related species (*P. microcarpa*, *P. incana* and *P. mahaleb*) (see codes in Table 1) based on UPGM original tree analysis of SSR polymorphisms

3, P40 expression levels in Inc-Kho significantly decreased under drought stress; *ATHB-12* (*Arabidopsis thaliana homeobox-leucine zipper protein*) expression did not significantly varied with respect to control in any of the genotypes studied. *ATHB12* expression decreased non-significantly in all genotypes. *BHLH71* levels in sensitive genotypes (*P. avium* ‘Avi-Ala 12’) significantly decreased compared to the control. Its expression increased in tolerant species (*P. incana* ‘Inc-Kho’) towards the control (Fig. 5).

Discussion

Evaluation of Morphological and Physiological Parameters

Iran is one of the origins of cultivated cherries (*P. avium*) and has a significant share of their production in the world (258,691 hg/ha). Due to climatic conditions and the strong germplasm of these plants in Iran, most rootstocks used in Iran originated from Mazard, with significant difference in resistance to abiotic and biotic stresses versus commercial varieties. Meanwhile, *P. incana* and *P. microcarpa* are unknown to some extent, and no study has yet been conducted on the resistance of these species to environmental stresses as the valuable source of genes for future breeding programs (Nazari et al. 2012). To face environmental stresses and climate change, native genotypes with interesting physiological traits need to be investigated and protected for development of improved rootstock varieties. In this regard, we appraised various Iranian cherries in their native habitats for resistance to drought stress. Morphological data in this study showed that *P. incana* and *P. microcarpa* can be used in breeding programs as dwarf and drought-resistant rootstocks in sweet and sour cherry in agreement with the recent in vitro results published by Sevgin (2021). *P. incana*

and *P. microcarpa* species are able to grow in rocky and dry soils having small leaves with many pubescence which indicate their resistance to drought conditions previously reported by Mozaffarian (2002).

Plants can successfully use complex physiological and molecular strategies to cope with environmental pressures. In this study, physiological measurements confirmed that plants are specifically affected by water stress and that plants provide physiological responses to deal with it. Photosynthetic processes, including Pn and chlorophyll, decreased drastically as the drought continued; similar results on *Prunus* response to water stress have been reported by several studies (Jiménez et al. 2013; Escobar-Gutiérrez et al. 1998). Photosynthesis is the most important plant metabolism for energy supply. It can be said that stomatal conductance is the most important factor affecting photosynthesis than during the drought period; its reduction can be ascribed to variability in soil moisture. With decreasing Gs and chlorophyll, photosynthesis decreases during drought stress in plants, according to the findings of other studies (Wang et al. 2015; Monakhova and Chernyadev 2002).

The effect of drought stress on photosynthesis could be direct by photosynthetic metabolism and Calvin cycle enzyme activity or indirect by increased oxidative stress. In wild almonds (*P. mongolica* Maxim.), low expression of transcripts related to photosynthetic routines resulted in reduced efficiency of photosystem I, photosystem II, light-harvesting chlorophyll protein complex and ultimately reduced photosynthesis (Wang et al. 2015; Fahad et al. 2017). Damage to photosynthetic systems and reduced leaf chlorophyll can also be due to reduced CO₂ uptake due to drought stress. RWC was found at the lowest level in seedlings after 15 days of water stress. Leaf RWC indicates metabolic activity in tissues that its reduction due to water stress can be attributed to the unavailability of water in the soil and/or the inability of root systems to compensate for water

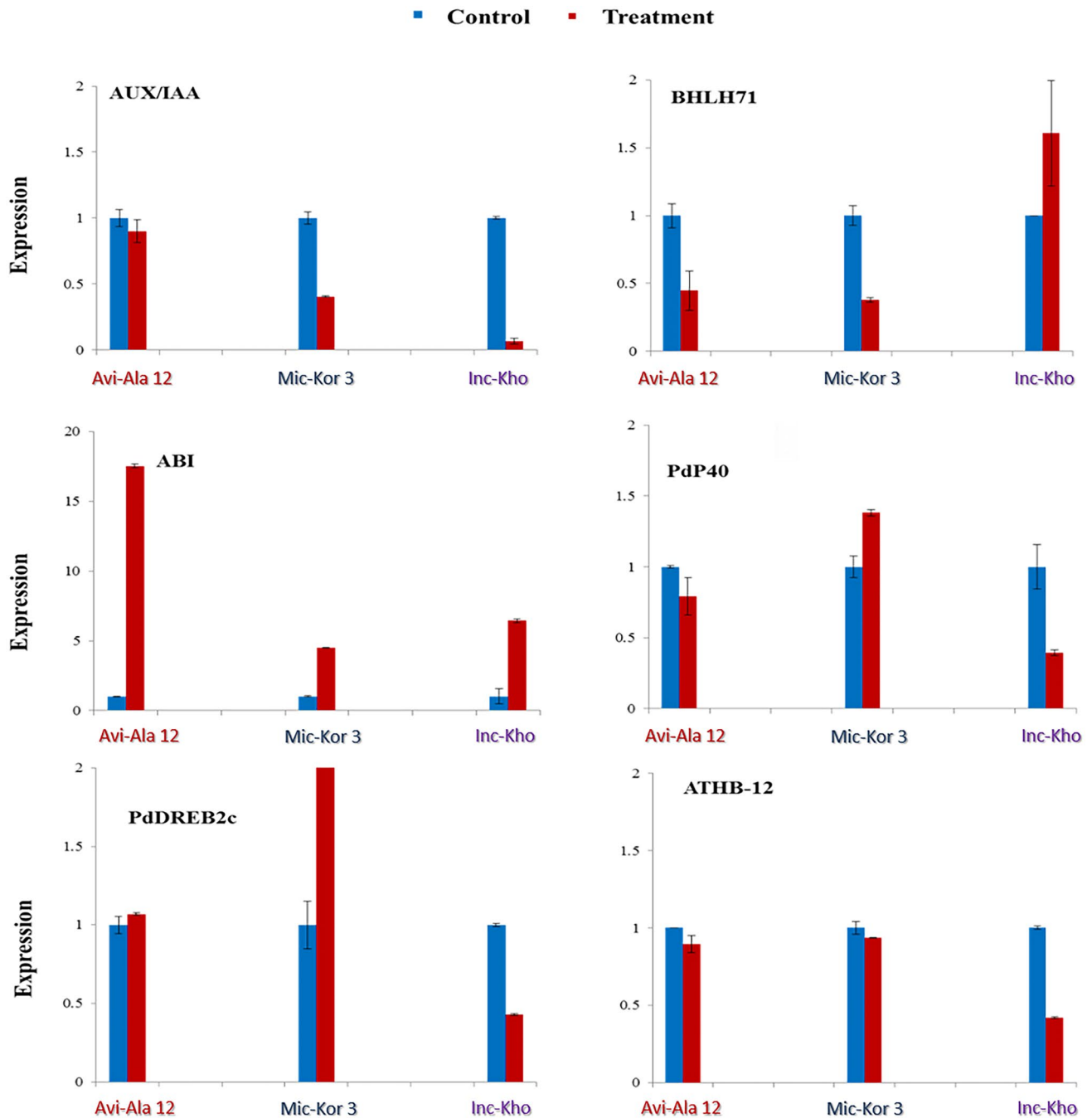


Fig. 5 qPCR analysis of candidate genes in genotypes of 'Avi-Ala 12' (sensitive) and 'Mic-Kor 3' and 'Inc-Kho' (tolerant) after 15 days under drought stress compared to control (see codes in Table 1). Three

biological replicates and two technical replicates were assayed. Standard deviations are indicated with vertical bars

lost through transpiration (Martínez-García et al. 2020; García et al. 2007; Shalhevet 1993; Gadallah 2000). In all genotypes, a decrease in Fv/Fm indicates photochemical damage in photosystem II and reduces absorption, which can be caused by a disturbance in the photosynthetic electron transfer system or damage to the thylakoid membranes (García-Sánchez et al. 2007). The reduction in the number of

chloroplasts and their deformation due to drought in tobacco has been reported by Yang et al. (2017).

In Japanese plum (*Prunus salicina* L.), the decrease in shoot growth was also more pronounced in more drought-tolerant cultivars in comparison with the less tolerant. The application of moderate water stress caused an intense drop in the midday water potential and RWC in stressed

trees of assayed cultivars (Hajlaoui et al. 2022). In addition, regulated deficit irrigation (RDI) caused a reduction in gas exchange parameters (photosynthetic assimilation and stomatal conductance) in the three assayed plum cultivars (Hajlaoui et al. 2022; Hamdani et al. 2023) in agreement with the drought response found in our tolerant and sensitive wild and cultivated cherry species.

Genomic Characterization

SSR markers have been selected as important markers for studies of genetic diversity in sweet cherry (Stanys et al. 2012). In this study, SSR was applied to evaluate the genetic variability and relationships of selected genotypes.

The observed diversity of germplasm was good and can greatly aid in breeding programs and rootstock selection. Geographical diversity and possibly open pollination of these genotypes have led to high genetic diversity in wild species of this subgenus. *P. incana* placed in a group and *P. microcarpa* genotypes were grouped separately with *P. avium* genotypes which were probably due to pollination between them. Contrary to assumption, all genotypes of *P. avium* were not included in a same cluster. This could be because of the different locations of the collection and probably cross-pollination. In addition, in most cases, tested species indicated 2–4 alleles in each example according species which is consistent with findings of Khadivi-Khub et al. (2014).

This close genetic relationship allows the use of this wild species in the breeding of the cultivated *P. avium* species as has been described in almond (*P. dulcis*) and plum (*P. salicina*) (Paudel et al. 2019; Hajlaoui et al. 2022).

Regarding the linkage of SSR markers with the drought response of the assayed genotypes and the evaluated gene expression, the lack of results indicated the need to increase number of DNA markers in these association studies with the use of the most abundant single-nucleotide polymorphism (SNP) markers (Salazar et al. 2017).

Transcriptomic Analysis

On the other hand, the results of transcriptomic analysis were remarkable. The reduction of IAA (involved in auxin signal transduction) was more pronounced in ‘Inc-Kho’ under drought stress. One of the plants’ responses to drought is to reduce the synthesis of growth-promoting hormones such as auxin and cytokinin. By reducing these hormones, cell division and enlargement are reduced, and thus, the plant survives by diminishing growth in drought conditions (Wang et al. 2015). The AUX/IAA family has various functions in regulating plant growth and development such as root development, shoot growth and fruit ripening (Luo et al. 2018). It seemed that the inhibition of response to the auxin hormone IAA (indole-3-acetic Acid) is an adaptive

survival strategy to reduce lateral root emergence as their maintenance requires metabolic investment that may slow the axial root elongation in deep soil. This is important for the acquisition of water which availability is higher in deep soils. During drought stress, the auxin receptor *TIR1* (*transport inhibitor response1*) levels are kept low by the upregulated miR393 resulting in a decrease *auxin response factors* (ARFs) mediated from AUX/IAA heterodimerization, which, as growth slows, may increase the plant’s tolerance to stress (Singh et al. 2017).

DREB2C expression in sensitive species (‘Avi-Ala 12’) had no significant difference with control but in the tolerant genotype (‘Mic-Kor 3’) has notably increased. ‘Inc-Kho’ had been meaningfully reduced in the treatment. This could be because of the different mechanisms of species to confronting with drought stress taking in account that *P. incana* and *P. microcarpa* genotypes showed higher photosynthetic stability in contrast with *P. avium* genotypes. *DREB* family plays important role in adjusting plant responses to abiotic stresses. Lee et al. (2010) showed that *DREB2C* interacts with *ABF2*, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. AFP3 (ninja-family protein/ABI five-binding protein 3) levels in sensitive species (‘Avi-Ala 12’) significantly increased compared to control. *AFP3* regulates stress response by the downregulation of ABA responses and stress responses (Zhao et al. 2016). AFPs are caused by ABA and dehydration stresses in seedlings. But their induction time is different. In addition, they negatively regulate jasmonic acid signalling as a part of repressor complex. Previous studies have shown different roles of some of them, such as seedling growth, germination control, carbohydrate metabolism, root growth and flowering (Huang and Wu 2007; Lopez-Molina et al. 2003). Recently, in agreement with our results, Xu et al. (2023) also evidenced the role of ABA-related genes in response to drought of cherry rootstock.

PdP40 belongs to the peroxidase family that plays a key role in oxidative metabolism. Unlike ‘Inc-Kho’ and ‘Avi-Ala 12’, its levels in ‘Mic-Kor 3’ had been significantly increased under drought stress that it might be due to differences in drought management mechanisms in genotypes. Plants have different enzymatic and non-enzymatic antioxidant systems to respond to environmental stresses that control the overproduction of reactive oxygen species under stress (Mittler 2002; Moller 2001). Drought stress like other stresses induces production reactive oxygen species (ROS) in plants. Some drought-resistant plant produces secondary metabolites such as antioxidant enzymes under stress conditions that scavenge reactive oxygen species (ROS) (Foyer et al. 1997; Jogawat et al. 2021). Recently, antioxidant enzyme genes (*PavGST* and *PavPOD*) were identified as differentially expressed by using RNA-Seq in relation to drought resistance in cherry rootstock (Xu et al. 2023),

highlighting the role of antioxidant enzymes under stress conditions that scavenge ROS (Fig. 6).

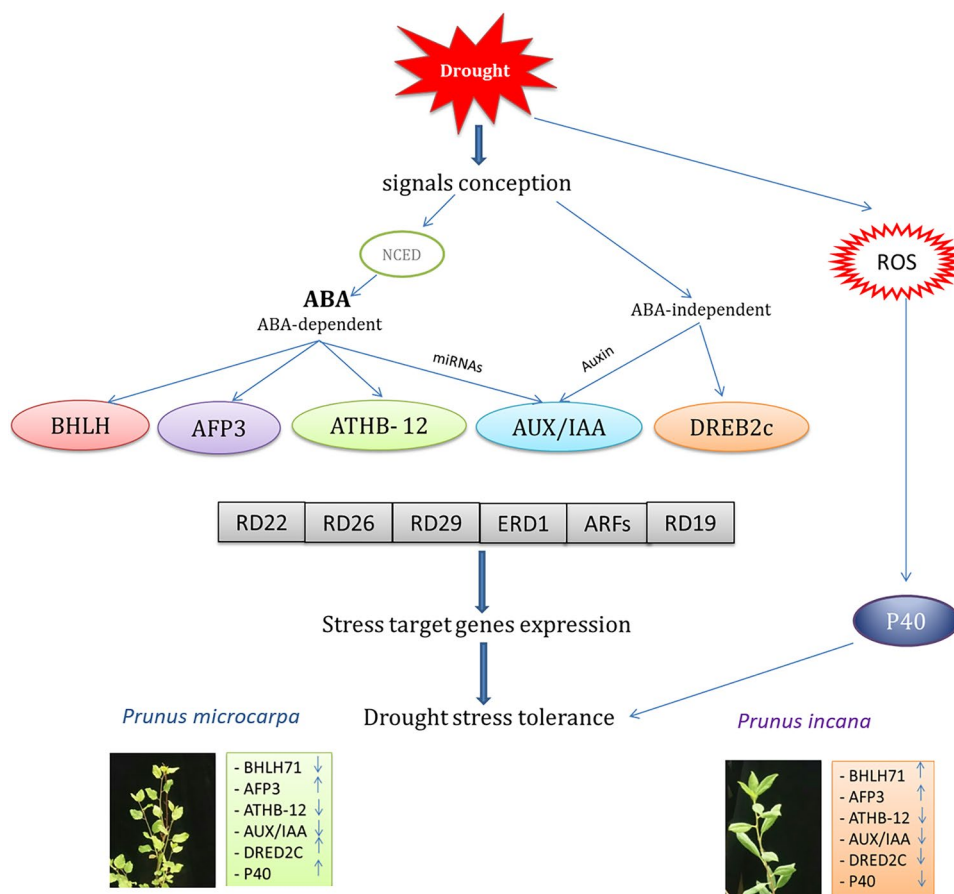
BHLH71 levels in sensitive species ('Avi-Ala 12') significantly decreased compared to control. Its expression had been increase in tolerant species ('Inc-Kho') towards control. Filiz and Kurt (2021) reported that 72% of the BHLH family found under drought stress in potatoes were upregulated that some of them are thought to be involved in root hair development under drought stress. According to studies, *BHLH71* may be involved in the differentiation of stomatal guard cells (Nadeau 2009). In apple, most of the *BHLH TFs* are crucial regulators of drought stress by involving in ABA signalling pathways (Mao et al. 2017). Xu et al. (2023) also evidenced the role of transcription factors (*PavWRKY* and *PavMYB*), in response to drought of cherry rootstock.

Finally, *ATHB-12* expression had not been significantly different towards control in all three genotypes. There was a non-significant decrease in *ATHB12* expression in all three genotypes during drought stress. In *Arabidopsis thaliana*, leaf growth was promoted by *ATHB12* mainly along the cell expansion stage, and suppression of its expression leads to reduced leaf growth and development (Hur et al. 2019). In our study, the reduced expression of *ATHB12* in drought treatments especially in 'Inc-Kho' was due probably to the

interaction with ABA signalling. It has been shown that there is an interaction between ABA signalling and *ATHB12* and *HTHB7*, due to their downregulation by ABI (Olsson et al. 2004). Valdés et al. (2012) also reported that *ATHB12* transcription factor which is an ABA-induced gene was induced by drought conditions and ABA signal in *Arabidopsis*. According to studies, HD-Zip family I is involved in regulating growth and stress responses. The overexpression of the *Oshox22* increases the ABA content and reduces the drought tolerance in plants (Zhang et al. 2012). However, no studies have been performed on the *ATHB12* under drought stress in wild cherry cultivars.

Our study starts up the evaluation of drought tolerance of *P. incana* and *P. microcarpa* for the first time and put out a significant portion to the understanding of how their response to drought stress which may help to illuminate the molecular mechanisms was associated with the drought response of *Prunus*. With the base of a different phenotype response to drought, *P. microcarpa* showed less leaf area, height and diameter in comparison to *P. incana*; a differential gene expression response was observed (Fig. 6). In order to better understand the relationships between genes during drought stress, biochemical analysis is also required that we will be doing in the near future. The future implementation

Fig. 6 Schematic representation of the drought tolerance response at biochemical and molecular level in wild cherry species *Prunus microcarpa* and *P. incana*



of new omics approaches across time points will help the dissection of this response in cultivated and wild *Prunus* L. subgenus *Cerasus*, determining when gene express or switch off in response to drought.

Conclusions

P. incana and *P. microcarpa* are widely established in mountains of Iran and show extreme tolerance to drought. No research has been conducted to studies the genomic resources of these species and the molecular mechanisms underlying their drought tolerance. Therefore, to check the mechanisms that authorize these plants to maintain growth in extremely dry environments, the response of these wild seedlings to drought stress was analysed using physiological and molecular approaches. After 15 days of drought stress, although the all physiological parameters in the genotypes were significantly reduced compared to controls, *P. incana* and *P. microcarpa* genotypes showed higher photosynthetic stability in contrast with *P. avium* genotypes. Due to the changes in the levels of hormones (such as auxins and abscisic acid) and their roles in closing the stomata and reducing growth for promoting plant survival in the arid environments, measuring their level is recommended in this study. Expression analysis of candidate genes related to drought stress in studied *Prunus* species showed that depending on the gene, the expression pattern can change between genotypes in response to drought stress. In this study, our hypothesis of high tolerance of *P. incana* and *P. microcarpa* to drought stress in comparison with the cultivated sweet cherry species (*P. avium*) was confirmed, and this study showed an overview of the function of the studied genes in these wild genotypes under drought stress. However, the genotypes showed opposite trends and different expression in the studied genes. So that contrary to our expectations, wild species showed low expression in some of the studied genes, including *ATHB12*.

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Author Contribution SJ and ASP did the qPCR experiments. SJ, JAS and PJMG did the SSR experiment. KA and PMG designed the experiments. SJ, KA, NB and PMG wrote the paper.

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high nutraceutical values’ (Nut4Drought) of the ARIMNET-2 European Program.

Data Archiving No data archiving.

Data Availability Data available on request from the authors. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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References

- Alimohammadi A, Shiran B, Martínez-Gómez P, Ebrahimie E (2013) Identification of water-deficit resistance genes in wild almond *Prunus scoparia* using cDNA-AFLP. *Sci Hort* 159:19–28
- Aranzana MJ, García-Mas J, Carbó J, Arús P (2002) Development and variability analysis of microsatellite markers in peach. *Plant Breed* 121:87–92
- Balint G, Reynolds AG (2016) Abscissic acid and its catabolites in leaves and berries of chardonnay are affected by water status. *Plant Growth Reg J* 35:1126–1140
- Bnikkou S, Laknifli A, Majourhat K, Jalili S, Hernández M-Gómez P, Martínez-García PJ (2021) Molecular characterization using SSR markers and biochemical analysis of Moroccan and Spanish argan [*Argania spinosa* (L.) Skeels] ecotypes under water stress and rewatering. *Biologia* 76:799–808
- Bianchi VJ, Rubio M, Trainotti L, Verde I, Bonghi C, Martínez-Gómez P (2015) *Prunus* transcription factors: breeding perspectives. *Front Plant Sci* 6:443
- Centritto M (2005) Photosynthetic limitations and carbon partitioning in cherry in response to water deficit and elevated CO₂. *Agri Ecosys Enviro* 106:233–242
- Čereković N, Pagter M, Kristensen HL, Pedersen HL, Brennan R, Petersen KK (2013) Effects of drought stress during flowering of two pot-grown blackcurrant (*Ribes nigrum* L.) cultivars. *Sci Hort* 162:365–373
- Chen H, Hwang JE, Lim CJ, Kim DY, Lee SY, Lim CO (2010) Arabidopsis DREB2C functions as a transcriptional activator of HsfA3 during the heat stress response. *Biochem Biophys Res Comm* 401:238–244
- Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. *Theor Appl Genet* 99:65–72
- Dirlwanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity

- analysis in peach and sweet cherry (*Prunus avium* L.). *Theor Appl Genet* 105:127–138
- Downey SL, Iezzoni AF (2000) Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *J Am Soc Hort* 125:76–80
- Doyle JJ, Doyle JL (1989) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Escobar-Gutiérrez AJ, Zipperlin B, Carbonne F, Moing A, Gaudillière JP (1998) Photosynthesis, carbon partitioning and metabolite content during drought stress in peach seedlings. *Funct Plant Biol* 25:197–205
- Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A (2017) Crop production under drought and heat stress. *Plant Res Manag Opt* 8:1–16
- Feng Y, Liang C, Li B, Wan T, Liu T, Cai Y (2017) Differential expression profiles and pathways of genes in drought resistant tree species *Prunus mahaleb* roots and leaves in response to drought stress. *Sci Hort* 226:75–84
- Filiz E, Kurt F (2021) Expression and co-expression analyses of WRKY, MYB, bHLH and bZIP transcription factor genes in potato (*Solanum tuberosum*) under abiotic stress conditions: RNA-seq data analysis. *Potato Res* 2:1–21
- Foyer CH, López-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. *Physiol Plant* 100:241–254
- Gadallah MAA (2000) Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. *Arid Environ* 44:451–467
- García AL, Marcellis L, García-Sánchez F, Nicolas N, Martínez V (2007) Moderate water stress affects tomato leaf water relations in dependence on the nitrogen supply. *Biol Planta* 51:707–712
- García ME, Lynch T, Peeters J, Snowden C, Finkelstein R (2008) A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. *Plant Mol Biol* 67:643–658
- García-Sánchez F, Versluesen JP, Gimeno V, Botía P, Perez-Perez JG (2007) Responses to flooding and drought stress by two citrus rootstock seedlings with different water-use efficiency. *Physiol Planta* 130:532–542
- Hajlaoui H, Maatallah S, Guizani M, Boughattas NEH, Guesmi A, Ennajah M, Dabbou S, Lopez-Lauri F (2022) Effect of regulated deficit irrigation on agronomic parameters of three plum cultivars (*Prunus salicina* L.) under semi-arid climate conditions. *Plants* 22:1545
- Hamdani A, Hssaini L, Bouda S, Charafi J, Boutagayout A, Razouk R (2023) Agronomic and physiological response of various Japanese plums (*Prunus salicina* L.) to severe water stress. *Vegetos*. <https://doi.org/10.1007/s42535-022-00544-7>
- Huang MD, Wu WL (2007) Overexpression of TMAC2, a novel negative regulator of abscisic acid and salinity responses, has pleiotropic effects in *Arabidopsis thaliana*. *Plant Mol Biol* 63:557–569
- Hur YS, Kim J, Kim S, Son O, Kim WY, Kim GT, Ohme-Takagi M, Cheon CI (2019) Identification of TCP13 as an upstream regulator of ATHB12 during leaf development. *Genes* 10(9):644. <https://doi.org/10.3390/genes10090644>
- Jiménez S, Dridi J, Gutiérrez D, Moret D, Irigoyen JJ, Moreno MA, Gogorcena Y (2013) Physiological, biochemical and molecular responses in four *Prunus* rootstocks submitted to drought stress. *Tree Physiol* 33:1061–1075
- Jogawat A, Yadav B, Lakra N, Singh AK, Narayan OP (2021) Cross-talk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiol Planta* 172:1106–1132
- Karimpour S, Davarynejad GH, Zaki-Aghl M, Safarnejad MR, Martínez-Gómez P, Rubio M (2021) Rapid assessment of sanitary and physiological state of thermotherapy-treated apple shoots by chlorophyll content evaluation. *Eur J Hort Sci* 86:205–211
- Khadivi-Khub A, Zamani Z, Fattahi R, Wünsch A (2014) Genetic variation in wild *Prunus* L. subgen. *Cerasus* germplasm from Iran characterized by nuclear and chloroplast SSR markers. *Trees Struct Funct* 28:471–485
- Kloosterman AD, Budowle B, Daselaar P (1993) PCR- amplification and detection of the human DIS80 VNTR locus. Amplification conditions and application in forensic analysis. *Integr J Legal Medec* 105:257–264
- Lau SE, Hamdan MF, Pua TL, Saidi NB, Tan BC (2021) Plant nitric oxide signaling under drought stress. *Plants* 10:360
- Le Provost G, Herrera R, Paiva JA, Chaumeil P, Salin F, Plomion CA (2007) Micromethod for high throughput RNA extraction in forest trees. *Bio Res* 40:291–297
- Lee SJ, Kang JY, Park HJ, Kim MD, Bae MS, Choi HI, Kim SY (2010) DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. *Plant Physiol* 153:716–727
- Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. *Nature* 529:84–87
- Li Q, Li P, Sun L, Wang Y, Ji K, Sun Y, Leng P (2012) Expression analysis of β -glucosidase genes that regulate abscisic acid homeostasis during watermelon (*Citrullus lanatus*) development and under stress conditions. *Plant Physiol J* 169:78–85
- Ling Q, Huang W, Jarvis P (2011) Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. *Photosynth Res* 107:209–214
- Liu C, Wang H, Zhang X, Ma F, Guo T, Li C (2021) Activation of the ABA signal pathway mediated by GABA improves the drought resistance of apple seedlings. *Int J Mol Sci* 22:12676
- Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. *Science* 333:616–620
- Lopes MS, Sefc KM, Laimer M, Machado ADC (2002) Characterization of microsatellite loci in apricot. *Mol Ecol Notes* 2:24–26
- Lopez-Molina L, Mongrand S, Kinoshita N, Chua NH (2003) AFP is a novel negative regulator of ABA signaling that promotes ABI5 protein degradation. *Genes Dev* 17:410–418
- Luo J, Zhou JJ, Zhang JZ (2018) Aux/IAA gene family in plants: molecular structure, regulation, and function. *Int J Mol Sci* 19:259
- Mao K, Dong Q, Li C, Liu C, Ma F (2017) Genome wide identification and characterization of apple bHLH transcription factors and expression analysis in response to drought and salt stress. *Front Plant Sci* 8:480
- Martínez-García PJ, Hartung J, Pérez de los Cobos F, Martínez-García P, Jalil S, Sánchez-Roldán JM, Rubio M, Dicenta F, Martínez-Gómez P (2020) Temporal response to drought stress in several *Prunus* rootstocks and wild species. *Agronomy* 10:1383
- Messina R, Lain O, Marrazzo MT, Cipriani G, Testolin R (2004) New set of microsatellite loci isolated in apricot. *Mol Eco Notes* 4:432–434
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7:405–410
- Mnejja M, García-Mas J, Howad W, Badenes ML, Arús P (2004) Simple-sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. *Mol Ecol Notes* 4:163–166
- Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Ann Review Plant Biol* 52:561–591
- Monakhova OF, Chernyadev II (2002) Protective role of karolin-4 in wheat plants exposed to soil drought. *App Biochem Microbiol* 38:373–380
- Mozaffarian V (2002) Studies on the flora of Iran, new species and new records. *Pak J Bot* 34:391–396

- Nadeau JA (2009) Stomatal development: new signals and fate determinants. *Curr Opin Plant Biol* 12:29–35
- Nazari SA, Zamani Z, Fatahi MR, Sofla HS (2012) Morphological characterization of *Prunus incana* Pall. by multivariate analysis. *Plant Syst Evol* 298:29–35
- Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction. *Proc Nat Acad Sci USA* 76:5269–5273
- Olsson A, Engström P, Söderman E (2004) The homeobox genes ATHB12 and ATHB7 encode potential regulators of growth in response to water deficit in Arabidopsis. *Plant Mol Biol* 55:663–677
- Paudel I, Gerbi H, Wagner Y, Zisovich A, Sapir G, Brumfeld V, Klein T (2019) Drought tolerance of wild versus cultivated tree species of almond and plum in the field. *Tree Physiol* 40:454–466
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45
- Pirasteh-Anosheh H, Saed-Moucheshi A, Pakniyat H, Pessarakli M (2016) Stomatal responses to drought stress. In: Ahmad P (ed) *Water stress and crop plants: a sustainable approach*, 1st edn. John Wiley & Sons, Hoboken, NJ, USA, pp 24–40
- Rodríguez-Gacio MDC, Matilla-Vázquez MA, Matilla AJ (2009) Seed dormancy and ABA signaling: the breakthrough goes on. *Plant Signal Behav* 4:1035–1048
- Roychoudhury A, Paul S, Basu S (2013) Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. *Plant Cell Reprod* 32:985–1006
- Salazar JA, Pacheco I, Shinya P, Zapata P, Silva C, Ruiz D, Martínez-Gómez P, Infante R (2017) Genotyping by sequencing for SNP-based linkage analysis and identification of QTLs linked to fruit quality traits in Japanese plum (*Prunus salicina* Lindl.). *Front Plant Sci* 8:476
- Sevgin N (2021) Drought stress responses of *Prunus microcarpa* C. A. Mey. subsp. *tortosa* rootstocks under in vitro conditions. *Legume Res* 44:LR–589
- Shalhevet J (1993) Plants under salt and water stress. *Plant Adapt Enviro Stress* 1:133–154
- Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. *J Exp Bot* 58:221–227
- Singh R, Parihar P, Singh S, Singh MPVB, Singh VP, Prasad SM (2017) Micro RNAs and nitric oxide cross talk in stress tolerance in plants. *Plant Growth Regul* 83:199–205
- Stanys V, Frercks B, Siksnianiene JB, Stepulaitiene I, Gelvonauskiene D, Staniene G, Bobinas C (2012) Identification of sweet cherry (*Prunus avium* L.) cultivars using AFLP and SSR markers. *Zemdirbyste* 99:437–444
- Teribia N, Tijero V, Munné-Bosch S (2016) Linking hormonal profiles with variations in sugar and anthocyanin contents during the natural development and ripening of sweet cherries. *New Biotech* 33:824–833
- Testolin R, Marrazzo T, Cipriani G, Quarta R, Verde I, Dettori MT, Sansavini S (2000) Microsatellite DNA in peach (*Prunus persica* L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. *Genome* 43:512–520
- Tong Z, Gao Z, Wang F, Zhou J, Zang Z (2009) Selection of reliable reference genes for gene expression studies in peach using real-time PCR. *BMC Mol Biol* 10:71
- Turner NC (1988) Measurement of plant water status by the pressure chamber technique. *Irrig Sci* 9:289–308
- Valdés AE, Övernäs E, Johansson H, Rada-Iglesias A, Engström P (2012) The homeodomain-leucine zipper (HD-Zip) class I transcription factors ATHB7 and ATHB12 modulate abscisic acid signalling by regulating protein phosphatase 2C and abscisic acid receptor gene activities. *Plant Mol Biol* 80:405–418
- Wang J, Zheng R, Bai S, Gao X, Liu M, Yan W (2015) Mongolian almond (*Prunus mongolica* Maxim): the morpho-physiological, biochemical and transcriptomic response to drought stress. *PLoS ONE* 10:e012442
- Xu Y, Wang Y, Xu J, Zhengxin L, Manzoor MA, Mao J, Zhang X, Liu R, Whiting MD, Jiu S, Zhang C (2023) Strigolactone and salicylic acid regulate the expression of multiple stress-related genes and enhance the drought resistance of cherry rootstocks. *Sci Hort* 313:111827
- Yang H, Zhao L, Zhao S, Wang J, Shi H (2017) Biochemical and transcriptomic analyses of drought stress responses of LY1306 tobacco strain. *Sci Rep* 246:1–10
- Yao T, Zhang J, Xie M, Yuan G, Tschaplinski TJ, Muchero W, Chen JG (2020) Transcriptional regulation of drought response in *Arabidopsis* and woody plants. *Front Plant Sci* 8:11
- Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica* 38:171–186
- Yu JM, Buckler ES (2006) Genetic association mapping and genome organization of maize. *Curr Opin Biotech* 17:155–160
- Zhang S, Haider I, Kohlen W, Jiang L, Bouwmeester H, Meijer AH, Ouwerkerk PB (2012) Function of the HD-Zip I gene Oshox22 in ABA-mediated drought and salt tolerances in rice. *Plant Mol Biol* 80:571–585
- Zhao Y, Chan Z, Gao J, Xing L, Cao M, Yu C, Zhu JK (2016) ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proc Natl Acad Sci USA* 113:1949–1954

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