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GENE EXPRESSION ANALYSES OF SOME TRANSPOSONS, PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES IN MELON CULTIVARS EXPOSED TO DROUGHT STRESS

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ABSTRACT

As sessile organisms, plants are exposed to a wide variety of abiotic and biotic stress. In this case, plants put very complex molecular mechanisms into action against these adverse environmental conditions. Especially at the gene level, it alarms many genes (transcription factors, promoters, miRNA, lncRNA, structural genes, transposons etc.). Transposons, which are among these genes, have recently been studied for their functions. However, it is not known exactly how the plants show the transposons activity, especially in abiotic stress situations. In this respect, expression situations of diverse transposons in plants are still very curious. In this study, various transposon genes belonging to different plant species (tobacco-specific *Tto-1*, *Tto-5* and *Tnt-1*, *Excoecaria agallocha*-specific *EARE1*, rice-specific *Tos17*, barley-specific *BARE1*, melon-specific *Reme1*, *Triticum*-specific *Ttd1a*) were used to measure horizontal transfer activities and the expression levels of these transposons were measured by q-RT PCR in sensitive and resistant melon accessions treated with drought stress. Additionally, chlorophyll content, glutathione reductase (GR) activity and some morphometric data were measured in these melon accessions under drought stress. Sensitive and resistant accessions showed significant differences in terms of both gene expression and physiological parameters, indicating the possible role of transposons in drought conditions.

Key words: Drought, Gene Expression, Melon, Transposon, q-RT PCR.

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INTRODUCTION

Most eukaryotic genomes mainly consist of non-coding regions including introns, intergenic DNA and repetitive elements. High copy number of transposable elements account for large part of repetitive elements in eukaryotic genomes. Transposable elements (TEs) are mobile genetic elements capable of changing their position within the genome. With the strategy of horizontal transfer, TEs can jump among organisms or species via a transfer vector such as a tick or virus (Ivancevic *et al.* 2018). Retrotransposons have an inherent ability to jump or transfer between species as they do within genomes (Peccoud *et al.* 2017). In the light of more than 700 genome data from plants, fungi and animals, it is known that horizontal transfer is not limited to certain types of retrotransposons.

TEs can be divided into two main classes based on their transposition mechanisms. While Class 1 elements known as retrotransposons transpose via a 'copy and paste' mechanism, Class 2 elements (DNA transposons) move as DNA through a 'cut-and-paste' mechanism (Bourque *et al.* 2018). Both classes can be subdivided into subclasses based on mechanism of chromosomal integration. Class 1 elements commonly called as retrotransposons are the predominant TEs in plant genomes and often contain Long Terminal Repeats (LTR). The abundance of TEs vary in size. Some TEs including short interspersed nuclear elements (SINEs) can be as short as 80 bp while other TEs such as helitrons can be as long as 15 kb. Helitrons are known that one of three groups of eukaryotic class 2 replaceable elements (TEs). For instance, TEs make up 85% of maize genome (Schnable *et al.* 2009), 20% of *Arabidopsis thaliana* genome (Slotkin and Martienssen 2007), 60% of *Gylcine max* genome (Oliver *et al.* 2013) and two third of human genome (de Koning *et al.* 2011; Karakülah 2018).

The epigenetic mechanism can cause a dynamic process, especially such as the hypersensitivity reaction (HR) in plants, and also cause the modifications of chromatin structure and also have a powerful influence on plant morphology, contributing to the adaptation of plants to stress (Thiebaut *et al.* 2019). In nature, plants are constantly subjected to both biotic and abiotic stress negatively affecting plant growth and productivity. In contrast to animals, plants cannot move to avoid stressful environmental conditions; instead, they have established several mechanisms based on plasticity or enough genetic diversity for adaptation (Chevin *et al.* 2010). TEs induced changes are a potentially rich source of variation because their insertions affect coding regions or cause genomic rearrangements through mutations (Casacuberta and González 2013). The expression level or expression pattern of plant genes can be altered by TEs through

several mechanism including disruption of cis-regulatory sequences, alteration of chromatin structure or providing novel regulatory information (Elbarbary *et al.* 2016; Mita and Boeke 2016).

TEs have been reported to be activated under adverse environmental conditions, and this activation is often considered as evidence for involvement of TEs in response to the stress factors (Casacuberta and González 2013; Chénais *et al.* 2012; Negi *et al.* 2016). Several recent studies have revealed molecular mechanism behind the activation of TEs under stress. The involvement of some TE families in stress responses mainly occurs by altering gene expression and mobilization (Bucher *et al.* 2012; Huang *et al.* 2017; Hummel *et al.* 2017). Many stresses responsive gene in *Arabidopsis* have TEs in their promoters. For example, expression of the tobacco *Tnt1* element can be induced under biotic and abiotic stress conditions (Grandbastien *et al.* 2005) or activation of mPing DNA transposon element of rice may take happen as a response to cold or salt stress (Yasuda *et al.* 2013). The ONSEN retro transposable element of *Arabidopsis thaliana* is activated by heat stress (Cavrak *et al.* 2014). In some cases, activation of TEs can be followed by repression of TEs (Secco *et al.* 2015). It is very complicated to determine which one of the silencing or activation mechanisms will be activated. For example, cold responsive TEs in *Arabidopsis thaliana* were analyzed in ten different ecotypes. For seven ecotypes a big part of TEs (65-80%) showed increased level of expression under stress compared to non-stress conditions. On the contrary, for the other three ecotypes, the majority of TEs (63-79) showed reduced expression level in stress compared to non-stress conditions (Barah *et al.* 2013)(Walsh *et al.* 2013) . The aim of this study is to observe some morphological, biochemical and molecular changes in two different melon accessions exposed to drought stress. Expression profiles of transposon genes belonging to different plants in melon accessions exposed to drought stress and whether these genes have inter-species transferability are important purposes of this study.

MATERIALS AND METHODS

Plant Material and Stress Treatment: The seeds of two melon (*Cucumis melo* L.) accessions, 45 (drought-susceptible, DS) and 8 (drought-tolerant, DT) were obtained from a collection of melon accessions preserved at the department of Horticulture, Siirt University. Surface-sterilized uniform seeds were planted in drainage free plastic pots with 15 cm height and 10 diameters. Each pot was filled with air dried, sieved (0.5 mm) and uniformly mixed peat loam soil. Plants were raised in a greenhouse with controlled environment: relative humidity 60%-70%, temperatures were 42/22 °C (day/night) and photoperiod (14 h). The seedlings were thinned at 2 true leaves stage and 3 plants per pot were kept. The experiment was performed in triplicate using three plants per cultivar. When the seedlings reached to four leaf stages (28 days-old), the control group was irrigated with a 4-day interval and stress groups were not irrigated. Plant samplings and measurements were carried out at the 21st day of the drought treatment.

Determination of Physiological Parameters: The chlorophyll content of the leaves was determined according to the method suggested by (Arnon 1949). Accordingly, 200 mg fresh leaf samples were homogenized in 80% acetone and filtered, and the final volume of solution was completed to 10 ml with acetone. The absorbance of the solution was taken at 645 nm and 663 nm for chlorophyll a and b against the solvent (80% acetone) blank. Calculations were made using the following formulas by (Lichtenthaler and Wellburn 1983)

Chlorophyll a = 12,7 x A663 – 2,69 x A645

Chlorophyll b = 22,9 x A645 – 4,68 x A663
(A: measured absorbance value).

Glutathione reductase (GR) activity was determined based on the glutathione-dependent oxidation of NADPH, as described by (Cakmak and Marschner 1992). Accordingly; 0.1 ml 0.5 mm oxidized glutathione (GSSG), 0.1 ml 0.12 mM NADPH, enzyme extract and 50 mM phosphate buffer (Ph = 7.6) containing 0.1 mM EDTA were added to the reaction medium and absorbance at 340 nm was recorded.

Determination of leaf relative water content (RWC) was conducted according to the method offered by (Sánchez *et al.* 2004). Right after harvest, fresh leaves were weighed to determine fresh weight and then leaves were kept in pure water for 4 hours and turgor weights were determined after this period. The dry weight (DW) was determined by drying the leaves in an oven at 65°C for 48 h. RWC of each leaf was calculated according to the following formula.
(Fresh Weight-Dry Weight) / (Turgor Weight-Dry Weight) X 100

Morphological parameters: In order to determine fresh and dry weight, randomly selected 3 plants were weighed in analytical balance and their fresh weight was determined. Then; same samples were air-dried and placed in an oven at 65°C for 48 hours. Dry samples were weighed in analytical balance and dry weight was determined.

Three plants per sub plot in total of three plants per treatment and three fruits per plant were randomly selected and used to measure some growth parameters including mean fruit weight, shell thickness according to Sari *et al.* (2018). Stem diameter was determined in mm (± 0.1) with the help of numerical compass.

Gene expression analyses (RNA isolation, cDNA synthesis and q-RT PCR analysis): Total RNA isolations from control and stress-treated leaves were performed using Trizol (Invitrogen) reagent. Accordingly, after the leaf tissues were milled, they were homogenized in sterile tubes containing 1 ml of TRIzo1 reagent. After, the samples leave five minutes at room temperature (RT), 0.2 ml of chloroform was added per 1 ml of TRIzol reagent. Then the caps of the tubes were well closed and mixed by shaking vigorously 15 sec. After incubation at room temperature (RT) for 2-3 min, tubes were centrifuged at 15000 rpm 20 min under the 4^0C. Then, supernatant was transferred to a new tube and 0.5 ml isopropyl alcohol was added and samples were leave at RT for 10 min. then it was centrifuged at 14000 rpm at 4^0C for 10 min. After centrifugation, the supernatant carefully removed. Then, 70% ethanol equal to the equal volume of TRIzol reagent was added on the pellet and the pellet floats. Then, it was centrifuged at 10000 rpm at 4^0C for 5 min. After centrifugation, supernatant carefully removed and pellet leave for dry and dissolve the RNA in 30 µL sterile water and quality of RNA samples were checked with 2% agarose gel and nano-drop spectrophotometer. Then RNA samples were stored at -80^0C.

For the cDNA synthesis from total RNA; cDNA Synthesis Kit |(Sigma-Aldrich) was used. According to manufacturer recommendations: 1 µl of 50 pmol/µl oligo dT(20) primer, 1-5 µg total RNA and 10 mM dNTP mixture were transferred to nuclease-free sterile tubes and the final volume was completed to 12 µl with sterile water. The mixture was kept at 65°C for 5 min and then placed on ice. Next, 2 µl 0.1 M DTT, 1 µl RNase inhibitor and 4 µl first chain buffer were added and the contents were mixed gently, then incubated at 42°C for 2 min. Next, 1 µl (200 units) Superscript III reverse transcriptase enzyme was added. The prepared mixture was incubated: 90 minutes at 50°C, the reaction was hold at 70°C for 15 minutes in PCR and then terminated.

The Picoreal (Thermo) qPCR system was used to measure the expression profile of transposons. Primers were designed using the Primer 3 program (Rozen and Skaletsky 2000). The primers used in this analysis are described in Supplementary Table S1. The qPCR reaction was performed as previously described (Inal *et al.* 2014). Briefly, 0.1 µl of gene-specific primer, 2 µl of cDNA and 10 µl of SYBR Green I Master Mix (Thermo) were mixed and the final volume was completed to 20 µl with dH2O. In order to obtain a stronger modeling result for normalization, 18S rRNA were used as reference genes. For data analysis, the 2–ΔΔCt method, which was widely used in previous studies, was used (Livak and Schmittgen 2001).

Statistical Analysis: The drought experiment was conducted using a completely randomized experimental design with two factors (drought and cultivar). Treatments had five replications, with five explants each. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $p \leq 0.05$. All statistical analyses were performed using the JMP8 software package. Student-T analysis test was used to compare the cultivars and treatments.

RESULTS AND DISCUSSION

The effects of drought stress on various growth parameters of the two melon accessions are presented in Table 1. Accordingly; average fruit weight, stem diameter, plant fresh and dry weight were reduced under drought treatment as compared to control plants whereas outer shell thickness slightly differed (Table 1). Drought stress negatively affected all above-mentioned characteristics related to fruit. Our findings are in consistent with the results of previous studies by (Gonzalez-Dugo *et al.* 2007; Ismail 2010), reporting drought-induced decrease in yield related parameters under drought conditions. Under severe water deficiency, negative changes in growth parameters and yield components may be due to infrequent application of water resulting in a lack of moisture in active crop root zone, inadequate moisture conservation, and poor nutrient utilization, which affects net assimilation, thereby decreasing the production and allocation of carbohydrates to the epigeous plant parts including the fruits (Huang *et al.* 2011). No significant difference was observed among growth parameters as a result of LSD test indicated on Table 1.

Table 1. Effect of the drought stress and cultivars interaction on plant growth parameters (LSD test, P=0.05).

Parameter	Control		Treatment	
	8	45	8	45
Fruit weight (g)	1447.0	883.7	809.3	677.7
Stem diameter (mm)	12.6	14.2	11.9	13.1
Shell thickness (mm)	3.16	2.86	4.28	4.38
Plant fresh weight (g)	241.0	181.7	204.0	151.9
Plant dry weight (g)	89.2	75.1	73.3	50.6
Relative water content (%)	77.8	64.7	68.5	55.7

---*NS*: nonsignificant

We observed that RWCs were ranged from 55,68 to 77,81% in two cultivars. While the highest value was detected for control plants of cultivar 8 (77,81%), the lowest value was recorded for plants of cultivar 45 under drought stress (55,68%) (Table 1). RWC is accepted as an strong indicator of plant water statues and high RWC indicates plant tolerance to drought stress (Sade *et al.* 2015). Therefore, decreases in RWC of accessions could be correlated with plants' ability to absorb water from the soil as an indicator of their tolerance.

The chl *a* and *b* content of both cultivars were decreased with different levels. Drought tolerant cultivar 8 reduced chl *a* and *b* content with 5,16 and 93,41% respectively while decrease in drought sensitive cultivar 45 was 49,31 and 61,81%, respectively (Figure 1(a)). There is a strong link between chlorophyll content and drought resistance of plants. Change in total chlorophyll content depends on duration and severity of stress and developmental stage of plant. For many species, no-change or decrease in leaf chlorophyll content under drought induced conditions has been noted (Anjum *et al.* 2011). As expected, the chlorophyll *a* content of the resistant cultivar changed less than the susceptible cultivar. The decrease in chlorophyll b content was sharper compared to the chlorophyll a content. Reduction in chl *a* and *b* content is seen as a part of drought response mechanism to decrease capacity of absorbing and conversion of light energy by chloroplasts (Cui *et al.* 2004; Pastenes *et al.* 2005).

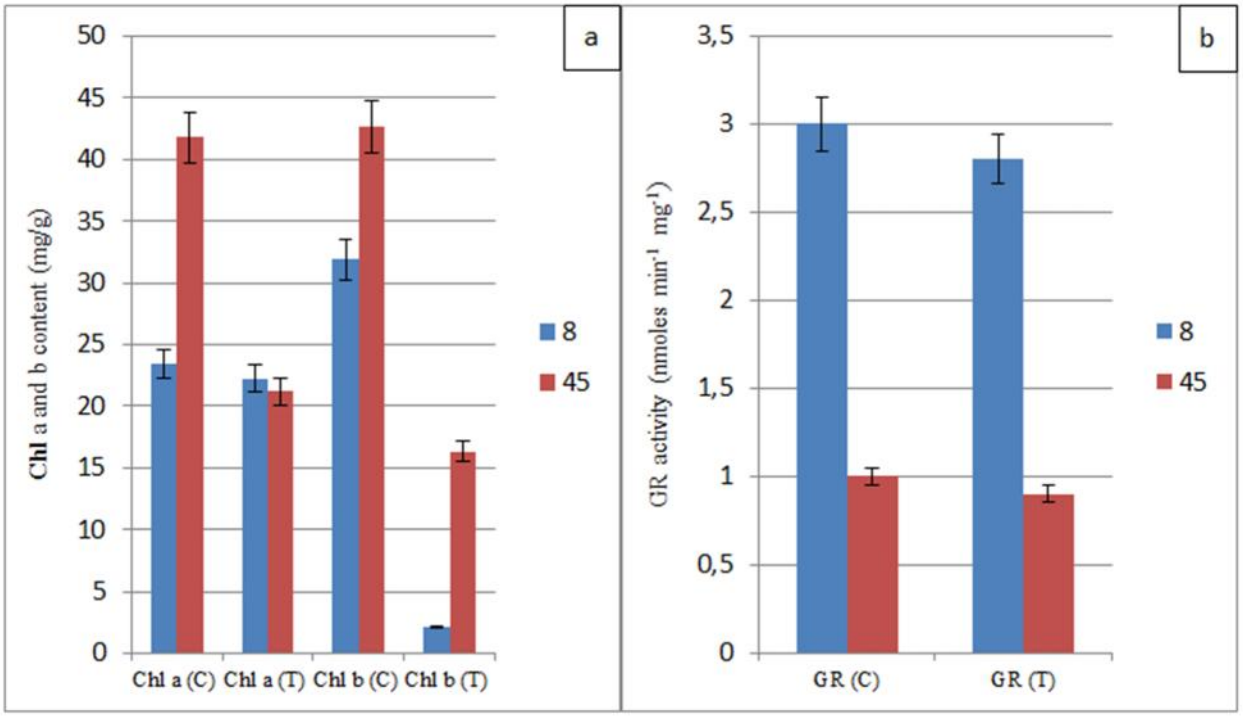

Fig. 1 Chlorophyll *a* and *b* content (a) and glutathione reductase activity (b) (C: Control, T: Treatment).

In the present study, no significant difference in GR activity was observed (Figure 1 (b)). The effects of drought stress reported in the literature on GR activity are contradictory and can vary depending on plant tolerance and the form of stress (Contour-Ansel *et al.* 2006). Drought stress leads to oxidative stress and generation of reactive oxygen species (ROS) which are highly reactive and toxic. Under favorable conditions, there is a balance between formation and consumption of ROS. If this balance is disturbed, ROS could attack lipids, proteins, carbohydrates and nucleic acids, impairing normal cell functions and perturbing cellular integrity and finally leading to cell death (Gill *et al.* 2012). In order to maintain vital cellular functions, plants have possess various antioxidant enzymes including glutathione reductase (GR) (Noctor and Foyer 1998). GR has a key role in maintaining the supply of reduced glutathione (Pastori *et al.* 2000).

q-RT PCR expression profile of transposons: Expression levels of *Ttd1a*, *BARE1*, *Tnt1*, *EARE-1, Tto1*, *Tto5*, *Tos17*, *Reme1* retrotransposons in two different melon accessions (45 (drought-susceptible, DS) and 8 (drought-tolerant, DT)) exposed to drought stress were analyzed by q-RT PCR. The changes in the gene profile obtained from the analysis result are shown graphically (Figure 2) below and discussed.

Gene expression level for Tto1 retrotransposon increased in treatment groups of both accessions

compared to control groups. Expression of the *Tto1* element could be activated by viral attacks, wounding, salicylic acid and jasmonate (Hirochika *et al.* 1996; Takeda *et al.* 1998). In *Tto1*, a repeated 13-bp motif which shares characteristic with motif found in the several defense related genes and these similarities explain molecular background of induction of *Tto1* by stress (Grandbastien 1998).

When susceptible melon accession 45 and drought resistant accession 8 were compared under drought stress, higher level of *Ttd1a* retrotransposon genes expressions was found in the susceptible accession. *Ttd1a* activity under stress and light stress have been shown to be regulated by transcription factors able to bind the promoter of *Ttd1a* (Woodrow *et al.* 2011). Likewise, in our case, it seems likely that *Ttd1a* was involved in defense responses by creating polymorphism through mobilization. As a result, it was predicted that *Ttd1a* gene plays an important role in the drought resistance mechanism of melon. In many previous studies, it has been found that retrotransposon in plants under abiotic stress shows a dynamic expression profile (Woodrow *et al.* 2010).

Reme1 gene expression exhibited variable expression between cultivars and treatment groups. Compared to the control groups, the expression level decreased in the resistant cultivar while it increased in the susceptible cultivar (Ramallo *et al.* 2008). There are very few studies in the literature when we can compare our results. Our results are in contradiction with earlier findings (Ramallo *et al.* 2008). who has reported that no detectable change was observed in the levels of *Reme1* transcripts in melon leaves exposed to wounding or water stress, however upon UV light treatment, a significant increasing was recorded. The differences between the two studies may have resulted from the resistance of the study material and the parameters used in this study (duration and intensity of stress).

Another retrotransposon analyzed in the context of this study is *BARE1*. The expression profiles of BARE1, *Tnt1* and *Tos17*, were found to be similar with little differences (Figure 1). It was found that the expression level of these genes was significantly higher in control groups of both resistant and susceptible accessions than treatment groups. That great variations in copy number of *BARE1* was observed between different habitats and the presence of *BARE1* promoter of ABA (abscisic acid)-response elements was found in the genes induced by water stress (Suoniemi *et al.* 1996). These result indicates that this transposon is stress-induced (Kalendar *et al.* 2000). Therefore, the *BARE1* transposon is known one of the clearest examples of stress-inducible variation in TEs.

Transcriptional activation of *Tnt1* by biotic and abiotic stress factors including wounding, freezing, salicylic acid, $CuCl_2$ or oxidative stress was reported in *Arabidopsis*, tobacco and tomato (Hernández-Pinzón *et al.* 2009; Mhiri *et al.* 1997). It has been reported that specific responses to different stress conditions originate from sequence variation in the 3′ untranslated region of *Tnt1* (Beguiristain *et al.* 2001). Our results are in complete agreement with (Fox *et al.* 2018) who found drought induced transcription of *Tnt1*-94 in *Pinus halepensis* during recovery in which *Tnt1–94* was the most dominant retrotransposon.

To the best of our knowledge, there is no study in the literature to evaluate and compare *Tos17* activity in terms of drought stress. But, it is known that *Tos17*, a member of *copia*-like retrotransposon, is one of the few active LTR-retrotransposons in plants and could be induced by tissue culture (Hirochika *et al.* 1996) and suppressed by DNA methylation (Cheng *et al.* 2015). Furthermore, there is a correlation between its transcription and transposition (Cheng *et al.* 2015). Specific epigenetic, developmental or environmental factors could affect the LTR_RT life cycle and thereby LTR-TR transcription (Ito *et al.* 2011; Mirouze *et al.* 2009) and this seems to be the most potential scenario for the differences recorded between control and treatment groups.

The *Tto 5* and *EARE 1* genes also showed a similar expression profile. Both genes showed high expression in resistant and sensitive accessions exposed to drought stress. While the *Tto5* gene expression level was found to be more in the treated group of the sensitive accession, the *EARE1* gene was found to exhibit a higher gene expression profile in the drought exposed group of the resistant accession (Figure 1). It has been reported that *EARE 1* expression was slightly upregulated upon cold treatment and wounding. Moreover, 5′ LTR of *EARE-1* contains some TATA-boxes and CAAT-boxes involved in stress (Huang *et al.* 2017). Similar expression profile was observed in our study (Huang *et al.* 2017). (Todorovska 2007) observed that *Tto 5* retrotransposons was activated after viral attack. In addition, another important point that the transposon genes in current study belong to different plant species, so it was determined for the first time with this study that different origin transposon genes were expressed in different melon accessions. In other words, the transfer of transposon genes between species has once again been experimentally achieved with this study. It has been previously suggested that transposon genes can be transferred between species in many species other than melon.

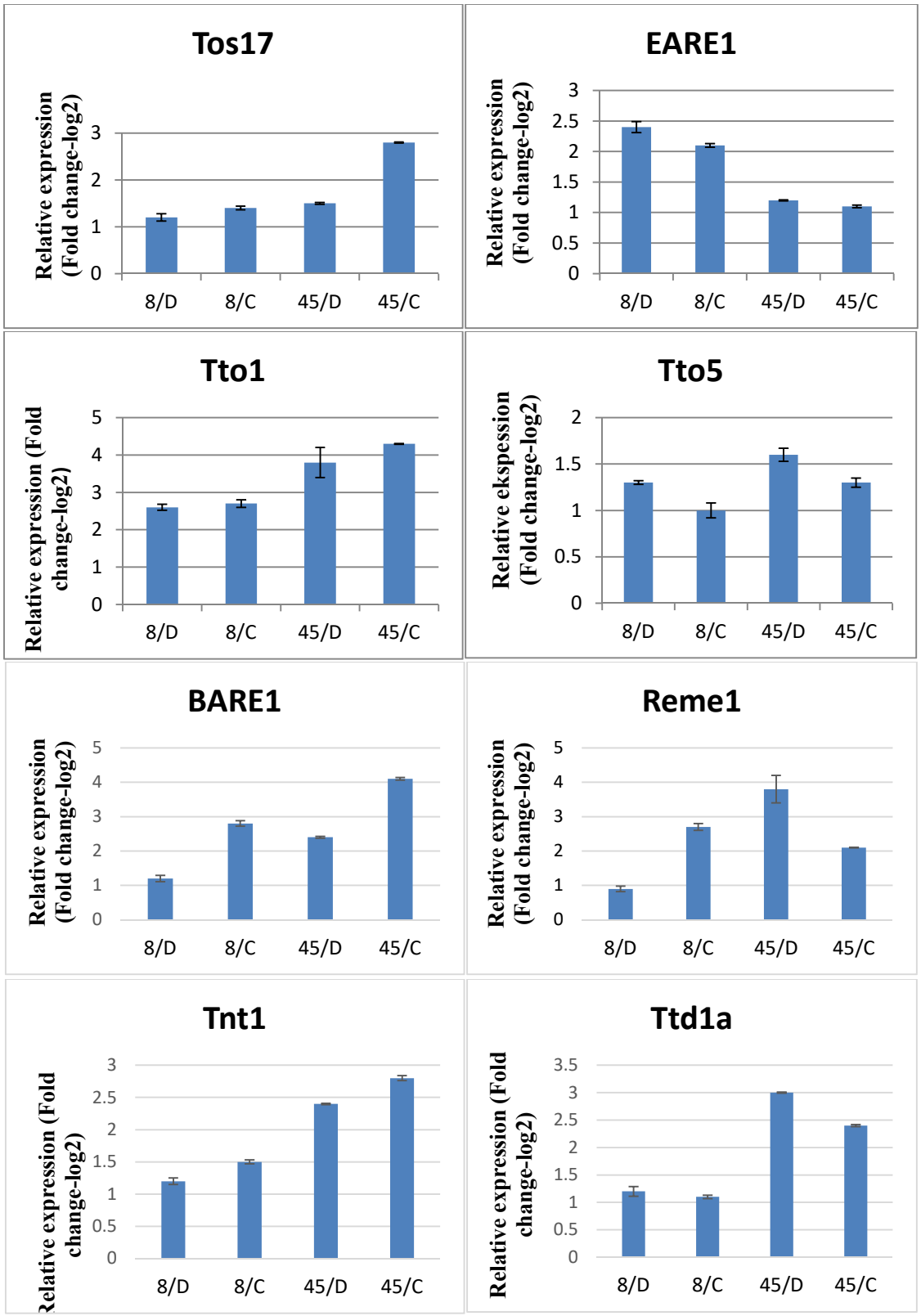

Figure 2 Gene expression results in melon seedlings grown under drought conditions. X axis: Cultivars/Treatment, Y axis: Relative Gene Expression (Fold change-log2); (D: Drought treatment, C: Control).

Conclusion: Results of current study showed important contributions to how different physiological, biochemical, molecular and physiological mechanisms work against drought stress of melon plant. In addition, another important aim of the current study is that the different transposon elements which originating from different plant species (*Tto-1, Tto-5, Tnt-1* tobacco origin, *EARE1 Excoecaria agallocha* origin , *Tos17* for rice origin, *BARE1* for barley origin, *Reme1* for melon origin , *Ttd1a* for *Triticum* origin) showed expression profile in different melon accessions. It has once again been confirmed that the chlorophyll and glutathione reductase enzymes are important molecules especially under drought stress in plants. In this study, transposons that were identified and characterized in different species were investigated in melon accessions. Further studies are needed for the inter-species transfer and the expression of these transposons under stress conditions.

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REFERENCES

Anjum, S. A., X.-y. Xie, L.-c. Wang, M. F. Saleem, C. Man, and W. Lei (2011). Morphological, physiological and biochemical responses of plants to drought stress. African J. Agricultural Research. 6(9): 2026-2032.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology. 24(1): 1.

Barah, P., N. D. Jayavelu, J. Mundy, and A. M. Bones (2013). Genome scale transcriptional response diversity among ten ecotypes of Arabidopsis thaliana during heat stress. Frontiers in plant science. 4: 532.

Beguiristain, T., M.-A. Grandbastien, P. Puigdomènech, and J. M. Casacuberta (2001). Three Tnt1 subfamilies show different stress-associated patterns of expression in tobacco. Consequences for retrotransposon control and evolution in plants. Plant physiology. 127(1): 212-221.

Bourque, G., K. H. Burns, M. Gehring, V. Gorbunova, A. Seluanov, M. Hammell, M. Imbeault, Z. Izsvák, H. L. Levin, and T. S. Macfarlan (2018). Ten things you should know about transposable elements. Genome biology. 19(1): 1-12.

Bucher, E., J. Reinders, and M. Mirouze (2012). Epigenetic control of transposon transcription and mobility in Arabidopsis. Current opinion in plant biology. 15(5): 503-510.

Cakmak, I., and H. Marschner (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant physiology. 98(4): 1222-1227.

Casacuberta, E., and J. González (2013). The impact of transposable elements in environmental adaptation. Molecular ecology. 22(6): 1503-1517.

Cavrak, V. V., N. Lettner, S. Jamge, A. Kosarewicz, L. M. Bayer, and O. M. Scheid (2014). How a retrotransposon exploits the plant's heat stress response for its activation. PLoS genetics. 10(1): e1004115.

Chénais, B., A. Caruso, S. Hiard, and N. Casse (2012). The impact of transposable elements on eukaryotic genomes: from genome size increase to genetic adaptation to stressful environments. Gene. 509(1): 7-15.

Cheng, C., Y. Tarutani, A. Miyao, T. Ito, M. Yamazaki, H. Sakai, E. Fukai, and H. Hirochika (2015). Loss of function mutations in the rice chromomethylase Os CMT 3a cause a burst of transposition. The Plant J. 83(6): 1069-1081.

Chevin, L.-M., R. Lande, and G. M. Mace (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol. 8(4): e1000357.

Contour-Ansel, D., M. L. Torres-Franklin, M. H. Cruz De Carvalho, A. D'Arcy-Lameta, and Y. Zuily-Fodil (2006). Glutathione reductase in leaves of cowpea: cloning of two cDNAs, expression and enzymatic activity under progressive drought stress, desiccation and abscisic acid treatment. Annals of Botany. 98(6): 1279-1287.

Cui, Y.-Y., D. M. Pandey, E.-J. Hahn, and K.-Y. Paek (2004). Effect of drought on physiological aspects of Crassulacean acid metabolism in Doritaenopsis. Plant Science. 167(6): 1219-1226.

de Koning, A. J., W. Gu, T. A. Castoe, M. A. Batzer, and D. D. Pollock (2011). Repetitive elements may comprise over two-thirds of the human genome. PLoS genetics. 7(12): e1002384.

Elbarbary, R. A., B. A. Lucas, and L. E. Maquat (2016). Retrotransposons as regulators of gene expression. Science. 351(6274): aac7247.

Fox, H., A. Doron-Faigenboim, G. Kelly, R. Bourstein, Z. Attia, J. Zhou, Y. Moshe, M. Moshelion, and R. David-Schwartz (2018). Transcriptome analysis of Pinus halepensis under drought stress and during recovery. Tree physiology. 38(3): 423-441.

Gill, S. S., N. A. Khan, and N. Tuteja (2012). Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up

regulates sulfur assimilation and antioxidant machinery in garden cress (Lepidium sativum L.). Plant Science. 182: 112-120.

Gonzalez-Dugo, V., F. Orgaz, and E. Fereres (2007). Responses of pepper to deficit irrigation for paprika production. Scientia Horticulturae. 114(2): 77-82.

Grandbastien, M.-A. (1998). Activation of plant retrotransposons under stress conditions. Trends in plant science. 3(5): 181-187.

Grandbastien, M.-A., C. Audeon, E. Bonnivard, J. Casacuberta, B. Chalhoub, A.-P. Costa, Q. Le, D. Melayah, M. Petit, and C. Poncet (2005). Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. Cytogenetic and genome research. 110(1-4): 229-241.

Hernández-Pinzón, I., E. de Jesús, N. Santiago, and J. M. Casacuberta (2009). The frequent transcriptional readthrough of the tobacco Tnt1 retrotransposon and its possible implications for the control of resistance genes. J. Molecular Evolution. 68(3): 269-278.

Hirochika, H., K. Sugimoto, Y. Otsuki, H. Tsugawa, and M. Kanda (1996). Retrotransposons of rice involved in mutations induced by tissue culture. Proceedings of the National Academy of Sciences. 93(15): 7783-7788.

Huang, J., Y. Wang, W. Liu, X. Shen, Q. Fan, S. Jian, and T. Tang (2017). EARE-1, a transcriptionally active Ty1/copia-like retrotransposon has colonized the genome of Excoecaria agallocha through horizontal transfer. Frontiers in plant science. 8: 45.

Huang, Z., Z. Zou, C. He, Z. He, Z. Zhang, and J. Li (2011). Physiological and photosynthetic responses of melon (Cucumis melo L.) seedlings to three Glomus species under water deficit. Plant and soil. 339(1-2): 391-399.

Hummel, B., E. C. Hansen, A. Yoveva, F. Aprile-Garcia, R. Hussong, and R. Sawarkar (2017). The evolutionary capacitor HSP90 buffers the regulatory effects of mammalian endogenous retroviruses. Nature structural & molecular biology. 24(3): 234.

Inal, B., M. Türktaş, H. Eren, E. Ilhan, S. Okay, M. Atak, M. Erayman, and T. Unver (2014). Genome-wide fungal stress responsive miRNA expression in wheat. Planta. 240(6): 1287-1298.

Ismail, S. M. (2010). Influence of deficit irrigation on water use efficiency and bird pepper production (Capsicum annuum L.). J. King Abdulaziz University: Metrology, Environment and Arid Land Agricultural Sciences. 142(587): 1-29.

Ito, H., H. Gaubert, E. Bucher, M. Mirouze, I. Vaillant, and J. Paszkowski (2011). An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature. 472(7341): 115-119.

Ivancevic, A. M., R. D. Kortschak, T. Bertozzi, and D. L. Adelson (2018). Horizontal transfer of BovB and L1 retrotransposons in eukaryotes. Genome biology. 19(1): 85.

Kalendar, R., J. Tanskanen, S. Immonen, E. Nevo, and A. H. Schulman (2000). Genome evolution of wild barley (Hordeum spontaneum) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proceedings of the National Academy of Sciences. 97(12): 6603-6607.

Karakülah, G. (2018). Rtfadb: A database of computationally predicted associations between retrotransposons and transcription factors in the human and mouse genomes. Genomics. 110(5): 257-262.

Lichtenthaler, H. K., and A. R. Wellburn (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Portland Press Ltd.

Livak, K. J., and T. D. Schmittgen (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 25(4): 402-408.

Mhiri, C., J.-B. Morel, S. Vernhettes, J. M. Casacuberta, H. Lucas, and M.-A. Grandbastien (1997). The promoter of the tobacco Tnt1 retrotransposon is induced by wounding and by abiotic stress. Plant molecular biology. 33(2): 257-266.

Mirouze, M., J. Reinders, E. Bucher, T. Nishimura, K. Schneeberger, S. Ossowski, J. Cao, D. Weigel, J. Paszkowski, and O. Mathieu (2009). Selective epigenetic control of retrotransposition in Arabidopsis. Nature. 461(7262): 427-430.

Mita, P., and J. D. Boeke (2016). How retrotransposons shape genome regulation. Current opinion in genetics & development. 37: 90-100.

Negi, P., A. N. Rai, and P. Suprasanna (2016). Moving through the stressed genome: emerging regulatory roles for transposons in plant stress response. Frontiers in plant science. 7: 1448.

Noctor, G., and C. H. Foyer (1998). Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology. 49(1): 249-279.

Oliver, K. R., J. A. McComb, and W. K. Greene (2013). Transposable elements: powerful contributors to angiosperm evolution and diversity. Genome biology and evolution. 5(10): 1886-1901.

Pastenes, C., P. Pimentel, and J. Lillo (2005). Leaf movements and photoinhibition in relation to water stress in field-grown beans. J. Experimental Botany. 56(411): 425-433.

Pastori, G., C. H. Foyer, and P. Mullineaux (2000). Low temperature-induced changes in the distribution of H 2 O 2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. J. Experimental Botany. 51(342): 107-113.

Peccoud, J., V. Loiseau, R. Cordaux, and C. Gilbert (2017). Massive horizontal transfer of transposable elements in insects. Proceedings of the National Academy of Sciences. 114(18): 4721-4726.

Ramallo, E., R. Kalendar, A. H. Schulman, and J. A. Martínez-Izquierdo (2008). Reme1, a Copia retrotransposon in melon, is transcriptionally induced by UV light. Plant Molecular Biology. 66(1-2): 137.

Rozen, S., and H. Skaletsky (2000). Primer3 on the WWW for general users and for biologist programmers. *In* "Bioinformatics methods and protocols", pp. 365-386. Springer.

Sade, N., E. Galkin, and M. Moshelion (2015). Measuring arabidopsis, tomato and barley leaf relative water content (RWC). Bio-protocol. 5(8): 1451.

Sánchez, F., E. De Andres, J. Tenorio, and L. Ayerbe (2004). Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (Pisum sativum L.) subjected to water stress. Field Crops Research. 86(1): 81-90.

Sari, N., İ. Solmaz, İ. Biçer, and M. Namli (2018). Agronomic performance of some Kirkagac melon (Cucumis melo L. var. inodorus) hybrids developed by doubled haploidy technique. alatarim. 17(2): 118-127.

Schnable, P. S., D. Ware, R. S. Fulton, J. C. Stein, F. Wei, S. Pasternak, C. Liang, J. Zhang, L. Fulton, and T. A. Graves (2009). The B73 maize genome: complexity, diversity, and dynamics. science. 326(5956): 1112-1115.

Secco, D., C. Wang, H. Shou, M. D. Schultz, S. Chiarenza, L. Nussaume, J. R. Ecker, J. Whelan, and R. Lister (2015). Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. Elife. 4: e09343.

Slotkin, R. K., and R. Martienssen (2007). Transposable elements and the epigenetic regulation of the genome. Nature Reviews Genetics. 8(4): 272.

Suoniemi, A., A. Narvanto, and A. H. Schulman (1996). The BARE-1 retrotransposon is transcribed in barley from an LTR promoter active in transient assays. Plant molecular biology. 31(2): 295-306.

Takeda, S., K. Sugimoto, H. Otsuki, and H. Hirochika (1998). Transcriptional activation of the tobacco retrotransposon Tto1 by wounding and methyl jasmonate. Plant molecular biology. 36(3): 365-376.

Thiebaut, F., A. S. Hemerly, and P. C. G. Ferreira (2019). A role for epigenetic regulation in the adaptation and stress responses of non-model plants. Frontiers in Plant Science. 10: 246.

Todorovska, E. (2007). Retrotransposons and their role in plant—genome evolution. Biotechnology & Biotechnological Equipment. 21(3): 294-305.

Walsh, A. M., R. D. Kortschak, M. G. Gardner, T. Bertozzi, and D. L. Adelson (2013). Widespread horizontal transfer of retrotransposons. Proceedings of the National Academy of Sciences. 110(3): 1012-1016.

Woodrow, P., G. Pontecorvo, L. F. Ciarmiello, A. Fuggi, and P. Carillo (2011). Ttd1a promoter is involved in DNA–protein binding by salt and light stresses. Molecular biology reports. 38(6): 3787-3794.

Woodrow, P., G. Pontecorvo, S. Fantaccione, A. Fuggi, I. Kafantaris, D. Parisi, and P. Carillo (2010). Polymorphism of a new Ty1-copia retrotransposon in durum wheat under salt and light stresses. Theoretical and Applied genetics. 121(2): 311-322.

Yasuda, K., M. Ito, T. Sugita, T. Tsukiyama, H. Saito, K. Naito, M. Teraishi, T. Tanisaka, and Y. Okumoto (2013). Utilization of transposable element mPing as a novel genetic tool for modification of the stress response in rice. Molecular Breeding. 32(3): 505-516.