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Comparative Adaptation Responses of Melon (*Cucumis melo* L.) Genotypes to Salinity Stress

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ABSTRACT

The objective of this work was to understand the mechanisms of physiological, biochemical, and molecular responses to salinity stress of three Turkish melon genotypes (YYU 1, YYU 4 and CU 196) and cv. Ananas. The study used Randomized Complete Block Design (RCBD) and pots were irrigated with Hoagland nutrient solution after two-leaf stage until harvesting by 50 and 75 mM NaCl concentrations. For evaluation of responses, chlorophyll and carotenoid content, total phenolic and flavonoid amount, proline variations, and nutrient elements were determined. Moreover, qRT-PCR analyses were performed to identify the expression level of six TF (Transcription Factors) genes (WRKY24, TCP15, CmHD-Zip, mTERF2, Dof3 and CmADH2). Increase in salt application led to increase in chlorophyll content in the melon genotypes, but decrease (about 55%) in cv. Ananas. Phenolic, flavonoid, and proline contents varied based on the melon genotypes, but generally increased in Ananas. Expression levels of *TCP15* and *WRKY24* showed more fold change at 75 mM NaCl treatment. On the other hand, the expression of *CmADH2* and *Dof3* showed more fold change at 50 mM NaCl treatment. Finally, according to adaptation mechanisms of melon genotypes, the study might help in selection and detection of the salt tolerant ones.

Keywords: Biochemical responses, Salt stress, TF (Transcription Factors) genes.

INTRODUCTION

Soil salinity is one of the accumulative abiotic stress factors aggregating with high surface evaporation associated with inappropriate applications of excessive irrigation and fertilization in the arid and semi-arid regions with scarce precipitation (Solmaz *et al*., 2011; Yıldız and Balkaya, 2016). There is a salinity problem in 65% of the world's agricultural land (Yetişir, *et al*., 2016) and 20% in Turkey (Anonymous, 2014).

Melon is grown worldwide, with the production of about 31.2 million tons in 2016 (FAO, 2016). Also it is a salt sensitive crop and often faced with salinity problems. Several researchers who studied salinity stress on melon cultivars focused on the full crop cycle or at particular phenological phases and revealed that melon is sensitive, to moderately-sensitive, to salinity and demonstrated considerable genotypic variations in the response to salt stress (Carvajal, 1998;Tedeschi *et al*., 2011).

Generally, this research have found that the responses of crops vary according to

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severity of salinity stress and type of species and cultivars by highly complex differentiations and processes for making the organism able to survive. Salt stress is related with accumulation of certain compounds, synthesis of enzymatic or non-enzymatic antioxidants, variation in signal pathway and transduction networks, photosynthetic pathway, changes in gene expression, production of stress-related proteins, and metabolic profiles and ionic imbalance (Dhakarey *et al*., 2017). Each researcher tries to explain the mechanisms by working in different fields with various plants and cultivars.

The functions of stress related genes involved in signal transduction, transcriptional regulation, compartmentalization, osmolyte synthesis and detoxification have been characterized in Arabidopsis, which leads to the expression of stress-responsive genes regulated by a network of transcription factors (Mizoi *et al*. 2011). The most detrimental stresses, adversely affect growth and development by resulting in crop loss and yield reduction. Salt tolerance consists of various responses from cellular to whole plant coordination, depending on location, plant and variety, and the adaptation mechanisms are being clarified day by day. The salinity studies on tolerance of melon landraces could provide new directions for the selection of salt-tolerant plants and can help in revealing significant variation among melon landraces (Sarabi, 2017).

Ekincialp (2019) studied 13 melon genotypes and 4 commercial melon varieties collected from the Van Lake Basin based on morphological traits and found that YYU1 was medium-tolerant and YYU4 was susceptible to salinity stress. It was also reported that CU-196 genotype was tolerant to salt stress and cv. Ananas was susceptible (Kuşvuran *et al*., 2007; Kuşvuran, 2012). Here in the present study, we aimed to test the hypothesis that the mentioned genotypes (YYU-1 and Cu-196 as tolerant and YYU-4 and Ananas as susceptible) could have different physiological and molecular characteristics as well as different relationship with mineral uptake and ratios. The studied new traits could be effective in future breeding studies.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Stress Treatments

The study was carried out in the Van Yuzuncu Yil University, Faculty of Agriculture and Horticulture Department, Van-Turkey in 2015. Three melon (*Cucumis melo* L.) genotypes (YYU-1, YYU-4, CU-196; collected from Van Lake Basin) and Ananas cultivar were studied for 6 weeks in the present study with the aim of developing native gene resources and contributing to literature studies. Two of the studied genotypes (YYU1 and YYU4) were chosen based on their morphological aspects from previous studies (Ekincialp, 2019).

Melon seeds were sown in vials filled with peat:perlite at a ratio of 2:1 and were kept in protected cultivation conditions [30-19±2°C (day/night) and relative humidity of 50-72% (min/max)] until planting time and necessary maintenance procedures were carried out. After 45 days from seed sowing, the seedlings were planted in high plastic tunnel conditions, with 6 plants per repetition, into non-drainage 12 liter pots having sterile peat:perlite mixture at a ratio of 2:1. The study had three replications in the randomized complete block experimental design. When the seedlings reached two-true-leaf stage, about five days after planting seedlings, control (0 mM), 50 and 75 mM of NaCl doses were applied gradually for 3 days. For salt application acclimation, NaCl was dissolved in distilled water and NaCl doses were applied as 25 mM gradually until it reached the final concentrations of 50 and 75 mM. During the developmental period, the plants were irrigated with Hoagland nutrient solution (Aktas *et al*., 2009). Third and fourth leaves from the bottom were

sampled at the flowering time and were used for the analysis.

Chlorophyll and Carotenoid Contents

Chlorophyll and carotenoid contents in the samples were extracted with 80% acetone from the leaves of the treatments and the control plants. The absorbance values were measured at 450, 663 and 645 nm wavelengths in a UV-160 Shimadzu spectrophotometer. Chlorophyll a, total chlorophyll and carotenoids were calculated by using the formula according to Arnon (1949).

Proline Content

Proline contents were measured according to Bates (1973) method. One g of a fresh leaf was grounded and homogenized with 5 mL 40% methanol by using mortar and pestle. The mixture was centrifuged at 6,000 rpm for 15 minutes. One mL of supernatant was taken and mixed with 100 mg of ninhydrin, 1 mL of acetic acid and 1 mL ortho-phosphoric. The mixture was heated in water bath for an hour and then incubated on ice for 5 min. Two mL of the mixture was taken, extracted with 2 mL of toluene and quickly shaken with a vortex until phase differences were formed. The upper phase was taken and the absorbance was measured with spectrophotometer at 520 nm. A standard curve was prepared by using pure L-Proline to determine the proline content of watermelon cultivars. The content of proline was expressed in units of mg g^{-1} (FW).

Total Phenolic and Flavonoid Contents

Total phenolic contents of melon treatments were performed by the methods involving Folin–Ciocalteu reagent and gallic acid as standard. Extract solution (0.1 mL) containing 1,000 mg extract was taken in a tube, and 1 ml Folin–Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 minutes, 1 mL of a solution of 6% Na_2CO_3 was added and the mixture was allowed to stand for 1 hour with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for gallic acid solutions.

The total flavonoid content was estimated using aluminum chloride colorimetric assay. The 0.5 mL of test samples solution in methanol was mixed with 2 mL of distilled water and 150 µL of 5% $NaNO_2$. After 6 minutes, 150 µL of 10% $AlCl_3$ and 2 mL of 1M NaOH were added and shaken at room temperature for 15 minutes. The absorbance of the mixtures was measured at 510 nm. Quercetin was used as a standard to determine flavonoid contents of melon extracts.

RNA Isolation, cDNA Synthesis, and q-RT PCR Analysis

Total RNAs of leaf tissues of the four melon cultivars were extracted by Trizol reagent (Lot no. 135404, Invitrogen, USA) according to the manufacturer's protocol. Nano DropLite spectrophotometer was used to determine RNAs quality/quantity and the presence of RNAs were also confirmed by gel electrophoresis. cDNA synthesis was performed with 2 µg of RNA by using high fidelity cDNA synthesis kit which contained 2.5µM Anchored-oligo (dT)18, 1X transcriptor high fidelity reverse transcriptase reaction buffer, 20U protector RNAse inhibitor, 1mM deoxy-nucleotide mix, 5 mM DTT and 10U transcriptor high fidelity reverse transcriptase at final concentration. The following incubation conditions were applied: 10 minutes at 65^{o}C, 30 minutes at 55^{o}C and 5 minutes at 85^{o}C. To measure the expression level of *WRKY24*, *TCP15*, *CmHD-Zip*, *mTERF2*, *Dof3* and *CmADH2* genes, qRT-PCR was performed with Thermo Pico real system. All qRT PCR reactions were performed in three independent biological and technical

triplicates with a template free control to check any contaminations.

Amplifications of PCR product were monitored by using an intercalator based method including SYBR Green I dye. After pre-denaturation for 10 minutes at 95°C, 45 cycles of 15 seconds at 95°C, 20 seconds at 60°C and 20 seconds at 72°C were applied. Melting-Curve analysis was performed to confirm the presence of a single product and absence of primer-dimers. The abundance of target gene transcripts was normalized to18S rDNA and set relative to the control plants according to the 2-ΔΔCT method (Livak and Schmittgen, 2001). Changes in relative expression levels of the gene were checked for statistical significance according to one-way ANOVA. Fisher's least significant difference test at 0.05 significant levels was performed.

Nutrient Analysis

The samples were dried at room conditions (shadow and +27°C) and grounded by using a pestle and mortar. The pulverized and powdered samples were transferred into plastic bags. All these samples were treated in an identical manner. For acid digestion, approximately 0.50 g of the sample was weighed accurately into a PTFE digestion vessel. Ten milliliters of concentrated HNO_3 and 2 mL of concentrated H_2O_2 were added to the vessel and waited for about 25 minutes. The decomposition of the samples was carried out by Bergh of Speed wawe MWS-3 model microwave oven digestion system (Karcan and Cagran, 2009). Then, the residue was dissolved in Milli-Q water and filtered, and the filtrate was diluted to 25 mL. The metal analyses of the diluted solutions were also performed with the ICP-OES instrument. The certified reference material analysis (BCR670 Aquatic Plant Certified Reference Material) was made by using the dissolving method. Three replicate digestions were made for each sample type. Model Optima™ 7000 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Perkin Elmer) was used to determine the quantities of melon genotypes.

Statistical Analysis

In assessing the data from measurements and observations used in the study, with a view to determine how genotypes were affected by salt stress, change rates relative to the control (T0) were based on comparisons according to the following formula (Yılmaz *et al*., 2020):

$$Percent\ change = [(Salt\ treatment - Control)/Control] * 100$$

In addition, correlation analysis and Duncan's multiple comparison test ($P< 0.05$) based on two-way variance analysis (ANOVA) were performed by using SPSS software in order to determine the relationships between the variables.

RESULTS AND DISCUSSION

Responses on Biochemical Parameters

Chlorophyll contents and/or Chl/Carot ratios (degree of increase or decrease) are generally accepted as evidence of differential responses of melon cultivars to salinity stress. Leaf pigments including chlorophyll and carotenoid contents were significantly affected by salt stress treatment (Table 1).The results revealed that total chlorophyll contents increased in the genotypes compared to the control conditions, and average change made by salinity stress were 8.33% for YYU1, 26.67% for YYU4, and 50.96% for CU196. However, 44.06 % decrease was calculated for cv. Ananas. In case of carotenoid content, the genotypes of YYU1 and YYU4 significantly decreased by the average changes of 6.52 and 36.56%, respectively, while 31.25% increase was recorded in CU 196 landrace (Figure 1). The response of genotypes and cultivar were significantly varied according to their potential and

Table 1. Comparative results of chlorophyll, carotenoids, phenolic, flavonoid and proline content of melon exposed to salt stress. [a]

	Genotype	0 mM	50 mM	75 mM	Mean
Chlorophyll-a content (mg g^{-1})	YYU1	1.57±0.01 c-e	1.60±0.02 cd	1.71±0.03 bc	1.63±0.07 B
	YYU4	1.49±0.23 c-e	1.99±0.11 a	2.02±0.19 a	1.83±0.30 A
	CU196	1.42±0.07 de	1.89±0.07 ab	2.11±0.13 a	1.81±0.32 A
	Ananas	1.32±0.33 e	0.72±0.08 f	0.78±0.05 f	0.94±0.33 C
	Mean	1.45±0.19 B	1.55±0.53 AB	1.65±0.56 A	
Chlorophyll-b content (mg g^{-1})	YYU1	0.59±0.08 b	0.59±0.02 b	0.62±0.06 b	0.60±0.05 B
	YYU4	0.67±0.14 b	1.07±0.03 a	1.05±0.05 a	0.93±0.21 A
	CU196	0.70±0.16 b	0.99±0.18 a	1.04±0.18 a	0.91±0.22 A
	Ananas	0.76±0.08 b	0.38±0.02 c	0.35±0.03 c	0.49±0.20 B
	Mean	0.68±0.12	0.76±0.31	0.77±0.32	
Total chlorophyll content (mg g^{-1})	YYU1	2.16±0.09 b	2.18±0.03 b	2.34±0.04 b	2.23±0.10 B
	YYU4	2.25±0.41 b	3.00±0.15 a	2.85±0.56 a	2.70±0.49 A
	CU196	2.08±0.11 b	2.89±0.21 a	3.14±0.14 a	2.70±0.50 A
	Ananas	2.02±0.31 b	1.10±0.09 c	1.13±0.09 c	1.42±0.48 C
	Mean	2.13±0.24 B	2.29±0.79 ab	2.36±0.84 A	
Carotenoid content (mg g^{-1})	YYU1	0.92±0.37	0.86±0.38	0.86±0.34	0.88±0.32
	YYU4	0.93±0.49	0.84±0.39	0.59±0.08	0.79±0.35
	CU196	0.80±0.12	0.90±0.13	1.05±0.27	0.91±0.19
	Ananas	0.81±0.24	0.64±0.28	0.69±0.15	0.71±0.21
	Mean	0.86±0.29	0.81±0.29	0.80±0.27	
Total phenolic content (mg g^{-1})	YYU1	14.26±0.68 a	7.55±0.26 d	8.84±0.43 c	10.22±3.11 A
	YYU4	10.32±0.41 b	7.56±0.41 d	9.45±0.43 c	9.11±1.27 C
	CU196	7.59±0.31 d	10.64±0.62 b	10.83±0.41 b	9.69±1.63 B
	Ananas	9.19±0.47 c	8.83±0.41 c	6.68±0.35 e	8.24±1.23 D
	Mean	10.34±2.60 A	8.65±1.37 B	8.95± B	
Total flavonoid content (mg g^{-1})	YYU1	189.23±14.45 a	112.48±5.32 f	134.81±7.59 bc	145.51±35.25 A
	YYU4	147.93±8.64 b	116.04±8.66 ef	131.89±7.73 cd	131.95±15.59 B
	CU196	95.27±8.10 g	122.25±6.39 c-f	127.88±4.55 c-e	115.14±16.11 C
	Ananas	128.07±6.16 c-e	132.34±6.96 cd	119.73±5.03 d-f	126.71±7.67 B
	Mean	140.13±36.51 A	120.779.85 C	128.58±8.05 B	
Proline content (ug g^{-1})	YYU1	10.25±1.18 c-e	15.18±0.87 b	12.94±0.84 b-d	12.79±2.30 AB
	YYU4	9.52±1.27 de	16.02±5.06 b	16.64±1.71 b	14.06±4.38 AB
	CU196	13.71±2.43 bc	10.43±1.27 c-e	7.70±1.17 e	10.61±3.00 B
	Ananas	13.1±1.38 b-d	16.12±0.63 b	23.28±1.11 a	17.49±4.63 A
	Mean	11.64±2.33 B	11.44±3.34 A	15.14±6.02 A	

[a] (A-C) and (a-c): Capital letters in rows and columns are for the means of genotypes of salt applications. Small case letters are for Genotype×Salt interaction (P values ; Genotype: 0.001, Salt: 0.001; Genotype×Salt: 0.001).

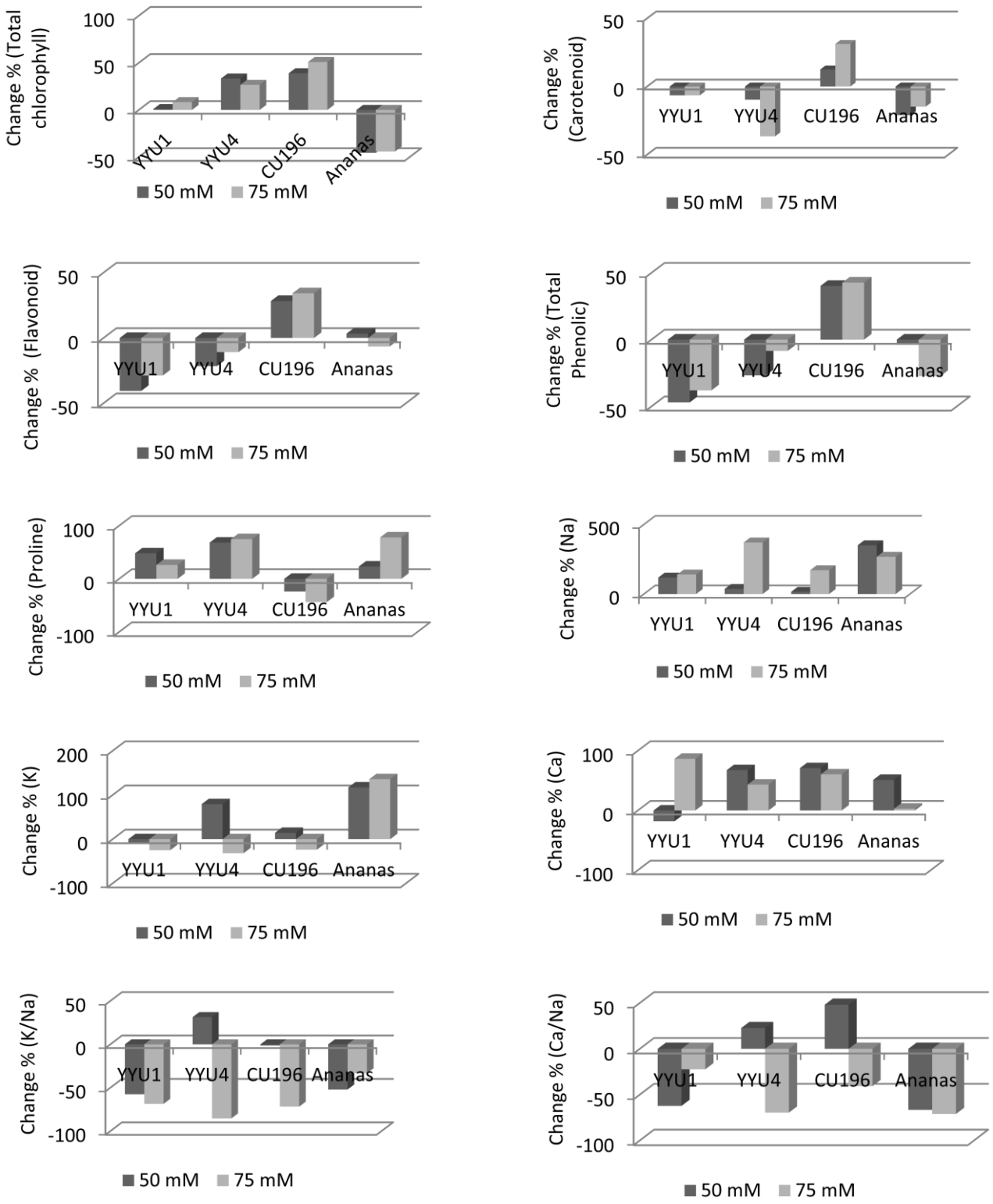

Figure 1. Changes relative to the control (%) of the tested parameters of melon genotypes (YYU 1, YYU 4, CU 196) and cultivar Ananas.

severity of salt treatment. Total chlorophyll and carotenoid results are related with Na content and *Mtef2* gene expression (Table 3). However, it is difficult to understand cause–effect-response relations between pigment content and growth reduction. The photosynthesis responses of the cultivars to salinity could be caused by alterations in the photosynthetic metabolism, or else by secondary effects caused by oxidative stress (Chaves *et al*., 2009). The chlorophyll fluorescence (CF) technique is fast and a powerful nondestructive method to detect changes in the photosynthetic activity in leaves influenced by environmental stress, Netondo *et al*. (2004) reported that maximum quantum yield of Photosystem II (PSII; Fv/Fm), Photochemical quenching coefficient (qP) and Electron Transport Rate (ETR) significantly decreased, but Non-photochemical quenching (qN) increased substantially under saline conditions. Each photochemical quenching coefficient and each non-photochemical quenching coefficient describe the same fluorescence signal in a different way, so, increased resistance of PSII to salt stress may help to improve the resistance of the photosynthetic metabolism and thus may increase the resistance of the whole plant.

Proline (osmo-compatible compound) content may protect plants from salt stress via detoxification of ROS. Proline content has an impressive role in osmotic adjustment process under abiotic stresses. While cv. Ananas had the highest proline content (23.28 mg g^{-1}), the CU196 cultivar had the lowest (7.70 mg g^{-1}) (Table 1). Increasing NaCl concentrations from 50 to 75 mM significantly increased proline accumulation by 26.24, 71.78, and 77.98% in YYU1, YYU4 and cv. Ananas, respectively; however, proline decreased by 43.83% in CU196 at 75 mM salt treatment compared to the control group (Table 3). The amounts of proline in YYU4 and CU196 genotypes were detected as stable by increment of salt application; this situation can be due to their tolerance potential. Moreover, similar situations were noticed for phenolic and flavonoid contents.

Phenolic and flavonoid contents of YYU4 and CU196 increased with salt applications (Table 1). Phenolic and flavonoids are the main groups of plant secondary metabolites possessing a wide range of biological functions such as protections of plants to adverse condition. Stressed-plants have also protection systems to overcome the oxidative damage by synthesis of secondary metabolites like phenolics and flavonoids. Furthermore, phenolics improve nutrient uptake through chelation of metallic ions, enhance active absorption sites, and soil porosity with accelerated mobilization of elements Sharma (2019). Their activities depend on biological factors and environmental conditions. In parallel with the increase in salt application, there was a significant increase in the phenolic and flavonoid amounts in the CU 196 genotype.

Nutrient Analysis

While the differences between genotypes and salt applications in the K content of melon genotypes were statistically significant, it was noteworthy that there was a positive increase compared to control in other genotypes, except YYU1 (-7.70%) at 50 mM NaCl concentration. In this context, it was determined that the highest rate of increase was 117.27% in cv. Ananas. In contrast, all of the genotypes, except cv. Ananas (135.88%) in 50 mM salt, had negative rates (Table 2; Figure 1). There is always a competition for Na^+ intake with K^+ (Zhu, 2003), which plays a significant role in physiological processes such as the maintenance of membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stoma movement and tropisms (Golldack *et al*., 2003; Shabala and Pottosin, 2014). Thus, K^+ and Na^+ homeostasis are vital for plants under salinity conditions. It has been reported by some researchers that the high K content of the plant increases tolerance against salt

Table 2. Potassium (K), calcium (Ca), and sodium (Na) contents in the leaves of melon genotypes.

	Genotype	0 mM	50 mM	75 mM	Mean
Na amount (ppm)	YYU1	4917.93±241.1 bc	10711.8±842 a-c	11801.34±609.5 a-c	9143.72±3248.38 A
	YYU4	3693.11±187.7 bc	5040.99±170.2 bc	17370.19±537.73 a	8701.43±6534.48 A
	CU196	5318.32±160.4 bc	6164.75±355.7 bc	14413.65±341.0 ab	8632.24±4359.23 A
	Ananas	1491.28±145.3 c	6717.9±238.3 a-c	5479.98±1444.90bc	4563.07±2477.19 B
	Mean	3855.16±2270.7 C	7158.89±4637.1 B	12266.3±4632.69 A	
K amount (ppm)	YYU1	20848.55±358.46 a	19242.8±251.29 b	15640.21±184.96 d	18577.21±2322.06 A
	YYU4	10593.30±25.44 e	18998.5±445.01 b	7217.46±297.57 f	12269.78±5260.87 C
	CU196	7164.05±188.42 f	8188.13±212.07 f	5454.69±885.50 g	6935.63±1205.14 D
	Ananas	7519.36±82.29 f	16337.6±342.6 cd	17736.40±494.2 bc	13864.48±4806.82 B
	Mean	11531.31±5791.2 B	15691.8±468.7 A	11512.19±5506.2 B	
Ca Amount (ppm)	YYU1	30438.18±922.75 i	24900.84±529.85 j	56755.24±137.46 c	37364.7±14764.80 BC
	YYU4	24369.18±332.52 j	40822.08±537.33 e	34924.27±1287.46 f	33371.84±7254.36 C
	CU196	41497.7±13840.3 e	70917.08±1759.59a	66611.27±296.95 b	59675.58±15428.22 A
	Ananas	31945.95±871.64 h	48306.72±1410.9 d	32874.23±895.64 g	37708.97±8014.08 B
	Mean	32062.77±8734.1 B	46236.8±17334.2A	47791.2±15005.2 A	
K/Na ratio	YYU1	4.24±0.16 b	1.80±0.15 e	1.33±0.06 e	2.46±1.36 B
	YYU4	2.87±0.15 d	3.77±0.13 bc	0.42±0.02 f	2.35±1.50 B
	CU196	1.35±0.06 e	1.33±0.10 e	0.38±0.01 f	1.02±0.48 C
	Ananas	5.07±0.47 a	2.43±0.07 d	3.38±0.82 c	3.63±1.25 A
	Mean	3.38±1.49 A	2.34±0.96 B	1.38±1.32 C	
Ca/K ratio	YYU1	6.20±0.46 cd	2.34±0.21 e	4.82±0.31 d	4.45±1.72 C
	YYU4	6.61±0.42 cd	8.11±0.34 c	2.01±0.08 e	5.58±2.76 C
	CU196	7.79±2.55 c	11.54±0.92 b	4.62±0.13 d	7.99±3.29 B
	Ananas	21.59±2.56 a	7.20±0.30 c	6.25±1.42 cd	11.68±7.58 A
	Mean	10.55±6.86 A	7.30±3.46 B	4.43±1.71 C	

(A-D) and (a-d): Capital letters in rows and columns are for the means of genotypes of salt applications. Small case letters are for Genotype×Salt interaction (Pvalues ; Genotype: 0.001, Salt: 0.001; Genotype×Salt: 0.001).

stress and potassium element decreases with increased NaCl (Hagin *et al*., 1990; Catalan *et al*., 1994; Naido, 1994; Erdinc *et al*., 2018; Erdinc, 2018).

As in the K content, it was observed that the differences between salt application and melon genotypes were important in Ca content (Table 2). In terms of calcium content, while the highest increase was observed in cv. Ananas at 25 mM, the genotype YYU1 showed an increase relative to the control in 50 mM salt concentration (70.90 and 86.46%, respectively). While the increase of sodium in the soil solution causes Ca^{+}, K^{+} and Mg^{+} deficiency in the plants, it has been reported that sufficient amount of Ca^{+} in the soil decreases the toxic effect of Na^{+} ion (Grattan 1993; Marschner 1995; Gomez *et al*., 1999).

It was determined that the salt doses increased the Na content in the genotypes, and CU196 was the genotype that had the

Table 3. Correlation analysis of parameters.

	Proline	Tot. Phen	Tot. flavo	Chlorol-*a*	Chlorl-*b*	Tot. Chl.	Caroten	TCP15	Dof 3	WRKY	Mterf2	CmADZP	CmADH2	Na	K	Ca	K/Na	Ca/Na
Proline	1	-0.714**	-0.372	-0.542	-0.421	-0.567	-0.790**	0.525	-0.060	0.506	-0.061	-0.004**	-0.127	-0.024	0.400	-0.357	0.178**	-0.125
Tot Phen		1	0.849**	0.311	0.180	0.298	0.466	-0.378	-0.134	-0.316	-0.012**	-0.021	0.166**	0.003	-0.065	0.137	0.084	0.029
Tot Flavo			1	0.016	-0.160	-0.028	0.219	-0.124	-0.174	-0.034	-0.287	-0.171**	0.055	-0.085	0.317	-0.194	0.351	-0.066
Chl-*a*				1	0.892**	0.987**	0.487	0.019	0.316	0.021	-0.107	0.689*	0.203	0.505	-0.328**	0.341**	-0.436	-0.207
Chl-*b*					1	0.939**	0.295	0.239	0.079	0.187	0.002	0.804**	-0.020	0.356**	-0.531	0.389**	-0.338	0.060
Tot Chl						1	0.508	0.047	0.262	0.032	-0.049	0.714**	0.154	0.425**	-0.398**	0.390	-0.401**	-0.110
Caroten							1	-0.593*	0.187	-0.542	0.122**	-0.064	0.299	-0.105	-0.102	0.293	-0.016	0.063*
TCP15								1	-0.200	0.948**	-0.416	0.557	-0.481	0.161	0.057	-0.252	0.092*	-0.103
Dof 3									1	-0.107	0.387	0.273	0.808**	0.426	0.231	0.457	-0.453	-0.324
WRKY										1	-0.333	0.570	-0.404	0.324	0.051	-0.135	0.006	-0.181**
Mter f2											1	0.092	0.579*	0.003	-0.211	0.772**	-0.267	0.233*
CmADZIP												1	0.135	0.580*	-0.212	0.420	-0.418	-0.237
CmADH2													1	0.209	0.394	0.453	-0.210	-0.197
Na														1	-0.212	0.301	-0.810**	-0.687*
K															1	-0.378	0.450	-0.295
Ca																1	-0.532	0.035
K/Na																	1	0.610*
Ca/Na																		1

* and ** significant at $P < 0.05$.

lowest rate of change (15.92%) in the amount of Na in the 50 mM salt. Moreover, depending on the amount of NaCl given in the 50 mM salt application, the plants contained high amounts of Na (Table 2). Compared to the control treatments, the lowest rate of change was found in the genotype YYU1 with 139.97%. There was a decrease in the genotypes, except for the genotype YYU4 (31.36%), for 50 mM in K/Na ratio, which is an effective parameter for determination of salt tolerance. However, in 50 mM salt, it was determined that there was a decrease in all melon genotypes and cv. Ananas had the lowest rate with -33.33%. While YYU1 was the genotype (-57.55%) which had the lowest K/Na ratio in 50 mM compared to the control treatment, the genotype YYU4 showed a negative ratio in 75mM salt with -85.37% (Table 2).

Na^+ exclusion mechanism is associated with Na^+/H^+ exchangers in the plasma membrane (Allen *et al*., 1995; Apse *et al*., 1999). There are chemical similarities between Na^+ and K^+, hence certain injury of Na^+ ion derives interference at some level with the transport and cytoplasmic functions of K^+ (Qi and Spalding, 2004). To implement the homeostasis of intracellular ion concentrations, it is fundamental for the physiology of cells, plant cells draw on primary active transport through H^+-ATPases, and secondary transport through channels and co-transporters. In this way, the plants keep high concentrations of K and low concentrations of Na in the cytosol (Zhu, 2003). Consequently, the interaction between Na^+/H^+ exchangers and H^+-ATPases is crucial for Na^+ exclusion. Heimler *et al*. (1995), Lopez and Satti (1996), Yu *et al*. (1998), Blumwald *et al*. (2000), Daşgan *et al*. (2002), Kıpçak and Erdinç (2016) reported that plants may have different amounts of Na^+ and K^+ absorption.

K/Na ratios play a role in tolerance against salinity. The uniformity of ion transport in plant cells is closely related to the equilibrium between monovalent (K^+ and Na^+) and divalent (Ca^{+2} and Mg^{+2}) cations. In particular, the competition between the monovalent Na and K results in favor of K causes increase in K/Na value, as a result, the plant can better protect itself against salt stress (Rubio *et al*., 2002; Yoshida, 2002). It is stated that salt tolerance is achieved by reducing the intake of Na^+ and Cl^- ions and increasing K^+ ion uptake in green parts (Gorham *et al*., 1985).

As in the K/Na ratio, there was a decrease in the Ca/Na ratio of genotypes with increasing salt doses. While the genotypes YYU4 and CU196 increased Ca/Na ratio compared to control in 50 mM salt application, there were decreases in the other genotypes. In 75 mM salt applications, all genotypes were negatively affected by salt and decreased Ca/Na ratio. The highest decrease was observed in cv. Ananas with -71.05% (Figure 1). It has been reported that the decrease of Ca/Na ratio in saline conditions leads to deterioration of membrane permeability and increases the toxicity intensity by taking more salts, mainly Na (Davenport *et al*., 1997; Kreij 1999; Villora *et al*., 2000). Grewal (2010) stated that high salt concentrations have a negative effect on the parameters such as shoot and root development, root/shoot ratio and water use efficiency; however, K/Na and Ca/Na ratios are higher in plant species resistant to salt. Volkmar *et al*. (1998) and Zeng *et al*. (2003) reported that K/Na ratio, in addition to Ca/Na ratio, is important in salt resistance and it is a crucial criterion to explain the response of plants to salt.

q-PCR Analysis in melon cultivars under Salt Stress

Totally, six different genes were used in this study and all these genes or transcription factors were known as molecular markers/indicator in plants under the stresses. These markers/indicator genes help researchers to provide inference and degree about the effects of stress on the plants.

The qRT-PCR analyses were performed to identify the expression level of six TF genes (WRKY24, TCP15, CmHD-Zip, mTERF2,

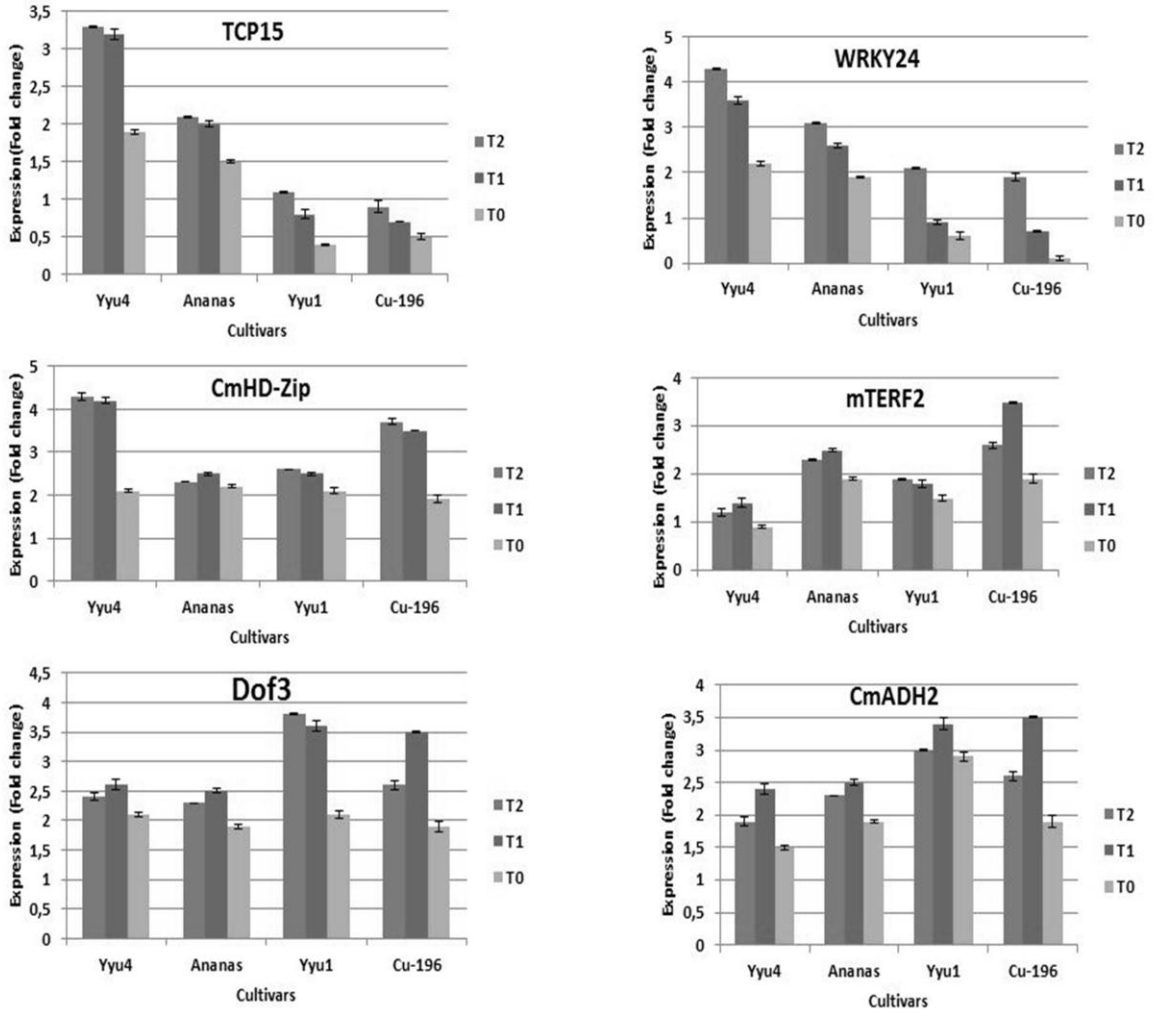

Figure 2. Expression patterns of genes in leaves of melon cultivars under salt stress (T0: Control, T1: 50 mM salt treatment, T2: 75 mM salt treatment).

Dof3, and CmADH2) in four melon cultivars exposed to salt stresses. Expression levels of six distinct TF genes were determined in the salt stressed melon leaves. Whole measurements were achieved with three independent biological triplicates per time point of analysis. As a result of analysis, it was observed that all six selected genes were up-regulated in all cultivars under the salt treatment. Similar expression patterns were observed between *TCP*, *WRKY24* and *CmHD-Zip* genes under salt stress conditions in all four cultivars. According to that result, expression level of the above genes observed at 75 mM was more than 50 mM salt treatment (Figure 2.) Over-expression of *AtWRKY57* in rice improves drought and salt tolerance (Jiang *et al*., 2016). In rice, OsWRKY24/45 negatively and OsWRKY72/77 positively regulates an ABA-inducible promoter which can be engineered to promote abiotic stress response (Xie *et al*., 2005). Genes consist of regulatory proteins like bZIP, which control the expression of many downstream salt stress tolerant genes (Shinozaki and Yamaguchi-Shinozaki, 2007). *TCP*, *TCP13* and TCP20 were found to be up-regulated in

common bean under the salt stress (Ilhan *et al*., 2018)

Expression levels of *mTERF2*, *Dof3* and *CmADH2* genes had almost the same profile and these genes showed a high gene expression profile response to salt stress in all genotypes. In this context, it was shown that expression levels of these genes were found to be up-regulated more in 50 mM salt treatment than 75 mM (Figure 2.). Expression of *mTERF* genes are regulated in maize seedling with light/dark, plant hormones, and salt application, showing the important roles in abiotic stress response (Zhao *et al*., 2014). Another previous study highlighted that the medium-chain ADHs in plant were involved in response to abiotic and biotic stress, which induced the specific expression of these ADHs in different tissues of soybean, wheat, and barley, implying that they may participate in different tissues development under stresses (Yamauchi *et al*., 2013). Most of the *DOF* genes were induced by the four abiotic stresses (cold, heat, salt and drought) treatments (Ma *et al*., 2015). Genotypic variation in response of the genes exposed to salt stress was also observed in the present study.

Identification of expression profiling of Transcription Factors (TFs) plays a crucial role in understanding the response of different crop cultivars against severe environmental changes. Diverse sets of genes related with biotic stress response have been identified (Waters *et al*., 2017). Among them, many families of TFs regulating the expression of many other downstream genes and gene clusters have been shown to have important role in drought and salinity tolerance mechanism. For example, *TCP* (TEOSINTE-BRANCHED1/CYCLOIDEA/PCF) (Wu *et al*., 2013; Zhuang *et al*., 2014; Ilhan *et al*., 2018), *bZIP* (Leucine Zipper Homeobox Protein Gene) (Saladié *et al*., 2015; Wang *et al*., 2003), DOF (Shaw *et al*., 2009; Singh *et al*., 2002), *ADH* (Alcohol dehydrogenases) (Jin *et al*., 2016), *mTERF* (Liang *et al*., 2015) and *WRKY* (Wei *et al*., 2013; Baloglu *et al*., 2014) families comprise a high proportion of abiotic stress responsive members. *DOF* (DNA binding with One Finger) family is an important example of such transcription factors which is a plant-specific transcription factor family containing a highly conserved DNA binding domain (DOF domain) (Yanagisawa, 2004).

Monitoring expression changes may provide important information for understanding the roles of these TFs under abiotic stresses in melon cultivars. Selected TFs might be potential targets for determination of salinity tolerant and susceptible cultivars for molecular breeding studies.

CONCLUSIONS

The reactions of plants to salt stress show a complex structure (as per our knowledge, breeding for abiotic stress, especially salt stress, face some difficulties due to multi-trait variations in crops). Therefore, it is difficult to breed salt tolerant cultivars. At the same time, tolerance varies according to plant species and even genotypes within species. In this study, it is noteworthy that there is variation between the genotypes studied in tolerance against salt stress in terms of the properties examined. There are very limited data in the scientific world about the studied melon genotypes. The genotype CU196 was found as comparatively tolerant because it showed better performance than the other genotypes with regard to photosynthetic pigments, total flavonoid and total phenolic amount, Na exception and some gene expressions. While expression levels of six different TF genes were up-regulated in all genotypes with salinity conditions, cv. Ananas and the genotype YYU4 decreased photosynthetic pigments, Na exception, and expression of mTERF2, Dof3 and CmADH2 genes. Moreover, the genotype YYU1 showed a decrease in total flavonoid and total phenolic amount and K content. It is known that the most effective method of coping with the

increasing salinity problem in the world is the development of varieties with high tolerance. For this reason, we believe that the importance of gene sources in terms of breeding will be better understood.

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پاسخ های سازگاری مقایسه ای ژنوتیپ های خربزه (*Cucumis melo* L.) به تنش شوری

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چکیده

هدف این پژوهش درک و شناسایی سازوکار پاسخ های فیزیولوژیکی، بیوشیمیایی، و مولکولی سه ژنوتیپ خربزه ترکی (YYU 1 و YYU 4و CU 196) و کولتیوار Ananas به تنش شوری بود. به این منظور، از طرح بلوک های کامل تصادفی (RCBD)استفاده شد و گلدانها بعد از دو برگه شدن بوته ها با محلول غذایی هوگلند با غلظت ۵۰mM یا ۷۵ mM کلرید سدیم آبیاری شد. برای ارزیابی پاسخ ها، مقدار کلروفیل، کاروتنوئید، فنول کل، فلا ونوئید، تغییرات پرولین، و عناصر غذایی تعیین شد. افزون بر این، برای شناسایی سطح بیان شش ژنTF(عامل رونویسی) شامل (WRKY24، TCP15، CmHD-Zip،mTERF2، Dof3، و CmADH2) از آزمون qRT-PCR استفاده شد. افزایش مقدار نمک منجر به افزایش محتوای کلروفیل در ژنوتیپ های خربزه شد ولی در کولتیوار Ananasکلروفیل کاهش یافت (تقریبا ۵۵٪). محتوای کاروتنوئید، فنول کل، و فلاونوئید بسته به نوع ژنوتیپ تغییر کرد ولی در کولتیوار Ananas به طور کلی افزایش یافت. سطح بیان TCP15و WRKY24تغییرات نسبی (fold change) در تیمار ۷۵ mM کلرید سدیم نشان داد. برعکس، سطح بیان CmADH2 و Dof3 تغییرات نسبی بیشتری در تیمار۵۰ mM کلرید سدیم داشت. بالاخره اینکه این مطالعه میتواند بر حسب سازوکارهای سازگاری ژنوتیپ های خربزه، درگزینش و شناسایی خربزه های مقاوم به شوری کمک نماید.