

THE DIFFERENCES AMONG MELON GENOTYPES AND VARIETIES UNDER SALT STRESS BASED ON CERTAIN MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES – MIXTURE MODELING AND PRINCIPAL COMPONENT ANALYSIS (PCA)

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Abstract. Present study aimed to determine the effects of salt stress in 13 melon genotypes and 4 commercial melon varieties collected from the Van Lake Basin. Two doses of salt applications were conducted at 0 mM and 50 mM NaCl concentrations and the plants were cultivated in 3 repeats under 25 ± 2 °C temperature and 16/8 light/dark periods based on the randomized block experimental design in climate chamber conditions. In order to determine the effect of stress, traits such as 0-5 scale, shoot and root lengths, shoot diameters, leaf number, fresh and dry weights of shoots and roots, leaf relative water content, membrane injury index, stoma widths and lengths, stomatal areas and stoma densities were evaluated. The reaction of the genotypes against stress was determined via mixture modeling and PCA analysis. In PCA analysis, three PCA components explained 71.48% of the total variation at 0 mM, while four components explained 69.53% of the total variation at 50 mM. As a result of the mixture modeling analysis, it was observed that 4 sub-populations for 0 mM and 3 sub-populations for 50 mM were formed and it was revealed that the salt-tolerant genotypes were in the sub-population 3 and salt-susceptible genotypes were in the sub-population 1.

Keywords: *Cucumis melo L.*, soil salinity, susceptibility, tolerance, Van Lake Basin

Introduction

Especially in arid and semi-arid regions with low precipitation, soil salinity, which occurs with the high surface evaporation due to the effect of incorrect applications such as excessive irrigation and fertilization, is among the increasingly significant abiotic stress factors (Maksimovic and Ilin, 2012; Yıldız and Balkaya, 2016). While salinity problem exists in 65% of global agricultural lands, this rate is around 20% in Turkey (Yetisir et al., 2016). Especially, 20% of the 230 million hectares of irrigated global farmlands face salinity problem (Peleg et al., 2011). Salinity causes stress in plants through different mechanisms. The plant initially undergoes water stress due to low osmotic pressure that occurs in the soil solution (Ashraf and Harris, 2004). Furthermore, ion imbalance and toxicity occur due to the accumulation of high amounts of Na⁺ and Cl⁻ ions (Lauchli and Grattan, 2007; Aktas et al., 2009). Generally, in the salt stress induced by the Na⁺ and Cl⁻ ions, the function and structure of the proteins deteriorate (Tuteja et al., 2012) and harmful effects occur in the function of metabolites at high concentrations of Na⁺ (Agarwal et al., 2013).

Since the tolerance mechanisms, including different genes under environmental conditions, have a complex structure (Ashraf and Harris, 2004), plants develop extremely diverse responses to salt stress due to their genotypic differences (Çulha and Çakırlar, 2011). While Levitt (1972) indicated that plants often cope with the negative effects of salt stress through escape, avoidance or tolerance strategies, Munns and Tester (2008) claimed that plant tolerance against soil salinity was ensured through

three components, namely, Na⁺ exclusion, tissue tolerance against Na⁺, and osmotic tolerance. It was reported that the most effective solution to salt stress is the development of varieties with tolerance (Dasgan and Koc, 2009).

Harlan (1951) indicated that Turkey, which is the second highest melon producer globally (FAO, 2013), was a micro-center for cucurbits, including melon (Sari et al., 2008). Furthermore, it was reported that Turkey is one of the gene centers for melon (Erdinc et al., 2013). Van province, which is located in the Eastern Anatolia region in Turkey, is known as one of the gene centers for cantaloupe melon (Turkmen et al., 2008). Thus, melon could be considered among the basic economic products of Turkey (Dasgan et al., 2012). It was noted that the threshold of Electrical Conductivity (EC) for the melon with moderate sensitivity against salt was 1 dS m⁻¹ (Maksimovic and Ilin, 2012). It was reported that although most cultivated plants such as beans, carrots and onions were known to be susceptible to salt (Petropoulos et al., 2017), melon exhibited a medium tolerance to salt stress, yet the condition was different among varieties due to the presence of tolerant and susceptible varieties (Damianos and Savvas, 2016).

Selection and breeding studies, conducted to develop high-yield commercial varieties, resulted in a reduced genetic diversity, and inevitably led to a reduction in genetic variations and a lower tolerance of cultivars to abiotic stress conditions. It was considered that genotypes with qualified breeding properties could provide gene resources for future breeding programs through determination of high tolerance genotypes via screening studies.

Mixture modeling intends to recognize previously unobserved homogenous sub-populations involving an apparently heterogeneous data set (Wang et al., 1996; Dalrymple et al., 2003; Martinez et al., 2009) utilizing Akaike's information criteria (AIC) and Bayesian information criteria (BIC) (Yeşilova et al., 2010) to characterize and distinct sub-populations. Mixture modeling is a novel methodology and has two significant benefits than the cluster and factor analysis (Muthén and Muthén, 2014). The first, for every observation, mixture modeling is settled by the likelihood of consideration inside the subgroup classes. The second, mixture model gives the parameter estimates for every subgroup (Mao et al., 2013). Present study aimed to determine the effects of salt stress in melon genotypes via mixture modeling and PCA analysis.

Materials and methods

Materials

Fourteen genotypes collected from different regions in Lake Van Basin (*Fig. 1*), 3 hybrid cultivars and 1 foreign standard cultivar were used in the present study where the reactions of the melon genotypes to salt stress were examined (*Table 1*).

NaCl treatment

In the study designed with three replications with 5 plants per repetition in randomized block experimental design, the seeds were sown into non-drainage 3-L pots having 2:1 ratio of sterile peat: perlite mixture. The seedlings were irrigated with Hoagland solution (Aktas et al., 2009). For salt application, based on previous studies (Yasar et al., 2006; Kusvuran et al., 2007b; Yarsi et al., 2017) the most suitable dose was chosen and when the seedlings reached into two-true-leaf stage, 50 mM NaCl

concentration was applied gradually for 2 days until a final concentration of 100 mM was obtained. The study was terminated after 18 days of salt application.



Figure 1. The locations where the genotypes used in the study were collected

Table 1. Passport information of melon accessions and cultivars used in the study

Accessions	Location	Accessions	Location
YYU-1	Van-Sihke-Kiratlı	YYU-21	Van-Unseli
YYU-4	Van-Sihke-Kiratlı	YYU-22	Van-Ercis
YYU-6	Van-Sihke-Kiratlı	YYU-23	Van-Ercek-Irgatlı
YYU-10	Van-Sihke	YYU-29	Van-Ercek-Irgatlı
YYU-11	Van-Sihke-Kiratlı	Cultivar	Location
YYU-13	Van-Sihke-Kiratlı	Kırkağaç F ₁ (Tolerant)	YükselTohum
YYU-14	Van-Sihke-Kiratlı	Lokum F ₁ (Tolerant)	YükselTohum
YYU-15	Van-Sihke-Kiratlı	Napolyon F ₁ (Susceptible)	YükselTohum
YYU-18	Van-Cakirbey	Galia (Susceptible)	Standard
YYU-20	Van-Unseli		

Seedling parameters

The morphological parameters such as shoot and root length, shoot diameter, leaf number, shoot-root fresh and dry weights were determined at the end of the experiment. The root: shoot ratio (Dry weight-DW%) (R: S) was determined after the dry weights were obtained.

Leaf relative water content (LRWC)

In order to determine the leaf relative water content in melon plants, primarily the fresh weights (FW) of the 3rd and 4th leaves of the three randomly selected plants in each repetition were determined and were stored in sterile distilled water for 4 h for the leaves to reach the maximum turgor weight (TW) and at the end of this duration their

turgor weights were measured. Leaf specimens, which were tested for turgor weight, were placed in an oven at 80 °C temperature and dry weights (DW) were determined. After measurements were completed, LRWC was calculated using *Equation 1* (Yamasaki and Dillenburg, 1999), consequent to the completion of measurements:

$$LRWC = [(FW - DW)/(TW - DW)] \times 100 \quad (\text{Eq.1})$$

Membrane injury index (MII)

The membrane injury index refers to the amount of electrolyte released from the cell. The amount of electrolyte released from the cell under stress conditions was determined with the methods developed by Dlugokecka and Kacperska-Palacz (1978) and Fan and Blake (1994). The discs retrieved from the third plant leaves were measured for their EC values after being kept in deionized water for 6 h at room temperature, then disc leaves were left in water at 100 °C for 10 min to retrieve the EC values again, and *Equation 2* was used for the calculations:

$$MII = \left[\frac{Lt - Lc}{1} - Lc \right] \times 100 \quad (\text{Eq.2})$$

Lt: EC value before autoclaving of stressed leaf/EC value after autoclaving of stressed leaf.

Lc: EC value before the control leaf is autoclaved / EC value after the control leaf is autoclaved.

Visual evaluation of salt stress (0-5 scale)

In the scale, used for visual evaluation of the salt injury in the seedlings, the scale values were as follows (Dasgan, 2002; Kuşvuran et al., 2007a):

- 0- No effect; 1-Local yellowing and curling of leaves, slow growth; 2- Necrosis and chlorosis in 25% of the leaf; 3- Necrotic spots on the leaves and defoliation by 25-50%; 4- Necrosis by 50-75% and death of several plants; 5- Formation of severe necrosis in leaves by 75-100% and predominant deaths in plants.

Stomatal traits

In order to determine the stoma density (StD) (unit mm²), stomatal area (StA) (µm²), stoma width (StW) and length (StL) (µm), the lower epidermis of the 4th plant leaf was stripped and spread on a slide with two drops of water (Kurtar et al., 2016). The stoma count was calculated with LAS EZ 3.0 software under three-field light microscope (LEICA DM500) with 40× magnification in three randomly selected 0.08 mm² tissue specimen sections spread on the slide.

Stomatal area was calculated with *Equation 3* (Orsini et al., 2013):

$$StA = \pi \times (SW \times 0.5) \times (SL \times 0.5) \quad (\text{Eq.3})$$

Statistical analysis

In the evaluation of the data obtained with the measurements and observations conducted in the study, the extent of the effects of salt stress on genotypes and varieties

were based on the variation rates when compared to the control group and the data were compared using *Equation 4*:

$$\text{Percent change} = \left[\frac{\text{Control} - \text{Salt treatment}}{\text{Control}} \right] \times 100 \quad (\text{Eq.4})$$

The mixture modeling statistical analyzes were performed with Mclust (mixture cluster) extension in R 5.2.3 statistical software (R Development Core Team, 2017). 0-5 scale, growth parameters, leaf relative water content, membrane injury index and stomatal features were classified with the Mclust Software. The principal component analysis (PCA) was conducted to identify the patterns of variation within the sets of melon accessions based on 14 properties using the PRINCOM procedure described by SAS (SAS Institute, 2015). Furthermore, Pearson correlation analysis was conducted with SPSS Software in order to determine the correlations among the variables.

Results and discussion

Seedling parameters

The leaf number, shoot diameter, shoot and root length values are presented in *Table 2*. It was observed that leaf number decreased in all genotypes and varieties due to salt stress, and the highest proportional decrease occurred in the genotype YYU29 (-54.24%) followed by cv. Lokum F₁ with a -52.61% decrease. YYU6 was the genotype that exhibited the lowest proportional decrease in leaf number due to salt stress (-7.46%). While the highest decrease in shoot diameter, -20.33%, was determined in genotype YYU10 when compared to the control group, the shoot diameter of 66.67% of the genotypes increased in saline conditions. It was established that salt stress affected the shoot length negatively in all melon genotypes and varieties. On the contrary, root length increased in half of the genotypes under salt stress. While YYU29 genotype and cv. Lokum F₁ were among the most adversely affected melons by salt stress in terms of shoot length (-62.93% and -62.59%, respectively), YYU15 genotype exhibited the lowest decrease in shoot length with a -21.42% decrease, when compared to the control group. Similar to the shoot diameter, YYU10 genotype was the most affected genotype by salt stress, similar to the decrease in root length (-25.31%). When compared to the control group, the highest increase in root length was observed in YYU6 genotype with 41.50%.

Although all genotypes and cultivars were adversely affected by salt stress, it was found that the shoot fresh weights of YYU18 and YYU6 genotypes were the least affected parameters (-13.94% and -18.55%, respectively). It was determined that the genotype with the highest decrease was YYU4, with -56.78%. In the presence of salt stress, increases in shoot dry weight were observed in only 3 genotypes, YYU6, YYU18 and YYU1 (20.00%, 6.76% and 1.11%, respectively). It was determined that about 67% of the genotypes exhibited an increase in root fresh weight in saline conditions and for root dry weight, the increase was 72%. The highest rate of increase was observed in YYU6 genotype in both cases (*Table 3*). The same trend was also reflected in the root to shoot ratio and 89% of the genotypes demonstrated an increase in root: shoot ratio due to salt stress. While, the highest increase was observed in the YYU6 genotype

(403.24%), only YYU11 and YYU10 genotypes exhibited a decrease of -7.65% and -1.19%, respectively (Table 4).

It was determined that salt application commonly affected plant growth negatively. Hence, the first response of the plants to salt was the reduction of growth rate and toxic effect symptoms on the leaves and shoot ends (Dasgan et al., 2002). While all varieties and genotypes were adversely affected in leaf number, shoot length and shoot fresh weight, it was established that there existed a variation between genotypes in shoot diameter, root length, shoot dry weight, root fresh and dry weight, and root:shoot rate. The reduction in leaf number ranged between 7.46 to 54.24%. Yetişir et al. (2016) also reported a reduction between 20 and 90% in Turkish gourd genotypes due to salt treatment. The soil salinity and low osmotic potential in the root zone reduce the water intake of plants (Lauchli and Grattan, 2007). These conditions create a negative effect especially on the fresh weight of plants that are salt intolerant. It was determined that once the amount of salt applied to the soil increased, the growth of plants, and thus the amount of dry matter decreased, hence in turn, the plant growth and the dry matter amount were affected more negatively (Romero et al., 2001). Although the root system is directly exposed to salinity, leaf growth is more susceptible to salt stress than root growth; therefore, root: shoot ratio increases under salt stress (Çulha and Çakırlar, 2011). Orsini et al. (2013) found that the root: shoot rate increased in plants under salt stress when compared to control conditions. Thus, it was determined that there was a significant correlation between R: S ratio and RFW ($r = 0.501$, $p < 0.01$) and RDW ($r = 0.620$, $p < 0.01$) (Table 5).

Table 2. Several growth parameters of melon accessions and cultivars with/without salt application

Accession	Leaf number per plant			Shoot diameter (mm)			Shoot length (cm)			Root length (cm)		
	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)
YYU1	6.11	4.38	-28.31	4.09	4.52	10.51	49.56	30.50	-38.46	16.41	22.38	36.38
YYU4	7.56	4.00	-47.09	4.31	3.90	-9.51	66.00	36.00	-45.45	19.80	16.27	-17.83
YYU6	4.56	4.22	-7.46	4.13	3.98	-3.63	43.11	31.67	-26.54	13.88	19.64	41.50
YYU10	5.11	3.11	-39.14	4.18	3.33	-20.33	45.44	27.22	-40.10	16.79	12.54	-25.31
YYU11	5.33	3.11	-41.65	5.38	4.84	-10.04	40.56	26.78	-33.97	22.50	19.90	-11.56
YYU13	4.86	3.22	-33.74	4.65	4.19	-9.89	39.80	30.00	-24.62	23.70	19.33	-18.44
YYU14	4.89	2.89	-40.90	4.53	4.62	1.99	42.56	26.56	-37.59	16.33	18.82	15.25
YYU15	5.00	4.50	-10.00	3.42	3.63	6.14	47.89	37.63	-21.42	13.44	16.65	23.88
YYU18	6.67	5.11	-23.39	3.45	3.98	15.36	63.56	43.33	-31.83	13.89	18.78	35.21
YYU20	8.22	4.44	-45.99	4.06	4.61	13.55	94.44	39.00	-58.70	18.83	19.22	2.07
YYU21	8.22	4.88	-40.63	4.27	5.19	21.55	84.86	44.25	-47.86	17.33	19.56	12.87
YYU22	6.89	4.78	-30.62	3.52	4.19	19.03	77.14	42.56	-44.83	18.63	15.22	-18.30
YYU23	8.00	5.11	-36.13	3.48	4.32	24.14	88.00	44.11	-49.88	14.66	16.03	9.35
YYU29	7.78	3.56	-54.24	4.05	4.48	10.54	81.22	30.11	-62.93	23.75	17.82	-24.97
Galia	5.56	4.75	-14.57	3.29	3.92	19.15	51.38	30.75	-40.15	14.19	13.90	-2.04
Kırkağaç F ₁	7.78	4.78	-38.56	3.69	4.02	8.94	104.71	43.22	-58.72	15.04	17.56	16.76
Lokum F ₁	8.44	4.00	-52.61	3.37	3.97	17.80	87.89	32.88	-62.59	15.63	15.38	-1.60
Napolyon F ₁	7.22	4.67	-35.32	3.44	3.44	0.00	68.38	32.78	-52.06	15.88	17.36	9.32

Table 3. Several growth parameters of melon accessions and cultivars with/without salt application

Accession	Shoot fresh weight (g)			Shoot dry weight (g)			Root fresh weight (g)			Root dryweight (g)		
	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)
YYU1	15.15	11.94	-21.19	0.90	0.91	1.11	0.56	1.10	96.43	0.04	0.07	75.00
YYU4	19.02	8.22	-56.78	1.34	0.77	-42.54	2.07	0.82	-60.39	0.08	0.06	-25.00
YYU6	9.65	7.86	-18.55	0.55	0.66	20.00	0.40	1.33	232.50	0.01	0.08	700.00
YYU10	12.50	5.45	-56.40	0.79	0.66	-16.46	0.61	0.39	-36.07	0.04	0.03	-25.00
YYU11	16.60	7.62	-54.10	1.29	0.87	-32.56	1.30	0.68	-47.69	0.10	0.06	-40.00
YYU13	18.26	9.73	-46.71	1.09	0.89	-18.35	1.54	1.58	2.60	0.10	0.08	-20.00
YYU14	16.94	8.78	-48.17	1.13	0.81	-28.32	0.98	1.42	44.90	0.05	0.09	80.00
YYU15	13.00	9.94	-23.54	0.61	0.52	-14.75	0.43	0.79	83.72	0.02	0.05	150.00
YYU18	11.41	9.82	-13.94	0.74	0.79	6.76	0.58	0.83	43.10	0.03	0.06	100.00
YYU20	15.74	11.29	-28.27	1.25	1.09	-12.80	0.85	1.71	101.18	0.05	0.12	140.00
YYU21	19.31	14.15	-26.72	1.61	1.27	-21.12	1.06	1.24	16.98	0.06	0.09	50.00
YYU22	14.87	9.29	-37.53	0.92	0.86	-6.52	0.77	1.20	55.84	0.03	0.06	100.00
YYU23	13.67	6.19	-54.72	0.88	0.70	-20.45	0.71	0.86	21.13	0.03	0.05	66.67
YYU29	19.74	9.41	-52.33	1.55	0.84	-45.81	1.71	1.21	-29.24	0.07	0.07	0.00
Galia	10.11	7.76	-23.24	0.53	0.50	-5.66	0.49	0.85	73.47	0.03	0.05	66.67
Kırkağaç F ₁	16.57	10.27	-38.02	1.23	1.00	-18.70	0.90	1.59	76.67	0.05	0.09	80.00
Lokum F ₁	22.25	11.24	-49.48	1.52	0.91	-40.13	1.27	1.26	-0.79	0.07	0.08	14.29
Napolyon F ₁	16.91	9.70	-42.64	1.15	0.68	-40.87	1.27	1.23	-3.15	0.06	0.07	16.67

Table 4. Several growth and physiological parameters of melon accessions and cultivars with/without salt application

Accession	Root:shoot ratio (DW %)			Leaf relative water content (%)			Membrane injury index (%)	0-5 scale
	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	50 mM	50 mM
YYU1	0.0433	0.0797	84.06	82.22	70.14	-14.69	21.49	1.78
YYU4	0.0570	0.0823	44.39	78.45	68.95	-12.11	33.96	2.22
YYU6	0.0247	0.1243	403.24	100.74	76.27	-24.29	15.59	2.00
YYU10	0.0503	0.0497	-1.19	63.80	72.44	13.54	23.35	2.22
YYU11	0.0693	0.0640	-7.65	76.39	75.34	-1.37	41.35	2.22
YYU13	0.0507	0.0820	61.74	82.78	71.72	-13.36	10.20	2.11
YYU14	0.0440	0.1010	129.55	76.36	72.15	-5.51	28.12	1.56
YYU15	0.0320	0.0850	165.63	79.92	78.52	-1.75	19.04	1.78
YYU18	0.0470	0.0763	62.34	71.39	74.59	4.48	18.01	1.33
YYU20	0.0410	0.1077	162.68	80.25	68.68	-14.42	26.51	1.78
YYU21	0.0400	0.0720	80.00	77.66	71.66	-7.73	11.60	1.67
YYU22	0.0367	0.0740	101.63	73.14	66.70	-8.81	24.30	1.89
YYU23	0.0340	0.0723	112.65	75.42	75.16	-0.34	40.04	1.67
YYU29	0.0400	0.0787	96.75	82.00	70.08	-14.54	-6.18	1.89
Galia	0.0500	0.0743	48.60	78.87	77.69	-1.50	6.00	1.89
Kırkağaç F ₁	0.0420	0.0863	105.48	82.15	71.28	-13.23	17.42	1.45
Lokum F ₁	0.0430	0.0917	113.26	79.10	72.06	-8.90	19.29	2.33
Napolyon F ₁	0.0503	0.0980	94.83	82.38	74.86	-9.13	-6.54	1.33

Table 5. Correlations among parameters evaluating salt stress

	SFW	SDW	RFW	RDW	RS	LRWC	LN	SD	SL	RL	StL	StW	StA	StD	MII	S
SFW	1	0.849**	0.433**	0.367**	-0.253**	0.231*	0.736**	0.164**	0.644*	0.127**	0.074	-0.084	0.017**	-0.149**	-0.402**	-0.553**
SDW		1	0.542**	0.529**	-0.134	0.064	0.648**	0.312	0.568	0.278	0.040	-0.142**	-0.048	0.048**	-0.183	-0.290**
RFW			1	0.750**	0.501**	-0.049	0.199**	0.342**	0.111	0.476	-0.026	0.070**	0.030**	0.152	-0.046	0.081
RDW				1	0.620**	-0.118	0.061	0.473**	-0.105	0.420	-0.047	0.079**	0.023**	0.275**	0.108	0.207*
RS					1	-0.256**	-0.379**	0.255	-0.476**	0.294**	-0.138	0.081**	-0.055	0.382**	0.322**	0.588**
LRWC						1	0.208	-0.155**	0.186	-0.106*	0.232*	0.002*	0.131	-0.174	-0.205	-0.340**
LN							1	-0.183**	0.898*	0.034**	-0.047	-0.286**	-0.186**	-0.109*	-0.369**	-0.585**
SD								1	-0.228	0.363	0.175	0.163	0.203**	0.116**	0.139	0.094
SL									1	0.022**	0.001	-0.254**	-0.143**	-0.083	-0.367**	-0.583**
RL										1	0.145	0.143	0.191**	0.055**	0.095	0.084
StL											1	0.363	0.826	-0.181	-0.210*	-0.158
StW												1	0.807	-0.188	0.034	-0.059
StA													1	-0.241	-0.130	-0.151
StD														1	0.252**	0.380**
MII															1	0.647**
S																1

*p < 0.05, **p < 0.01

SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, RS: R:S ratio (DW %), LRWC: Leaf relative water content, LN: Leaf number, SD: Shoot diameter, SL: Shoot length, RL: Root length, StL: Stoma length, StW: Stoma width, StA: Stomatal area, StD: Stoma density, MII: Membrane injury index, S: 0-5 Scale

Leaf relative water content (LRWC) and Membrane Injury Index (MII)

Among the genotypes and varieties examined in the present study, there was an increase in LRWC only in YYU10 and YYU18 (13.54 and 4.48%, respectively) in saline conditions, and it was determined that the LRWC decreased in all remaining genotypes and varieties due to salinity. While the injury rate varied between 6 and 41% in MII, which was evaluated 18 days after the salt treatment, it was determined that the YYU29 genotype and cv. Napolyon F₁ cultivar yielded negative values of -6.18% and -6.54%, respectively (Table 4). Salt stress decreased water uptake due to osmotic effect and furthermore led to membrane damage due to ion toxicity (Munns, 2002). The decrease in LRWC refers to low turgor pressure at limited water conditions (Katerji et al., 1997) and is, therefore, an important indicator of salt tolerance in cultivated plants (Sarabi et al., 2017). Membrane injury is caused by ion imbalance, which is caused by osmotic inconsistency inside and outside the cell under stress conditions such as salinity and drought (Ghoulam et al., 2002). It was found that membrane injury was lower in tolerant genotypes when compared to susceptible genotypes (Asha, 2007). Previous studies indicated that membrane injury increased due to salt stress and thus, MII could be used to determine the stress-effect rate in plants (Jamil et al., 2012).

Visual evaluation of salt stress (0-5 Scale)

In the 0-5 scale, which commonly assists the visual determination of leave damage, it was observed that cv. Lokum F₁ had the highest scale score (2.33) and YYU4, YYU10 and YYU11 genotypes were among the most affected genotypes with a scale score of 2.22. It was observed that YYU18 genotype had the lowest score with 1.33. Furthermore, it was found that 33.3% of the genotypes were more affected by salinity and their scores were above 2.0 (Table 4). Several studies reported that the scale findings could be used in estimating the reactions under salt stress by melon (Kusvuran

et al., 2007a), tomato (Daşgan et al., 2002), bean (Kıpçak and Erdinç, 2016) species. It was determined that there were negative correlation between the findings of the 0-5 scale utilized in present study on SFW ($r = -0.553$, $p < 0.01$), LN ($r = -0.585$, $p < 0.01$) and SL ($r = -0.583$, $p < 0.01$) and there were positive correlations between R:S ($r = 0.588$, $p < 0.01$), and MII ($r = 0.647$, $p < 0.01$) findings (Table 5).

Stomatal traits

It was observed that 78% of the melon cultivars and genotypes exhibited a decrease in stoma length, 56% exhibited a decrease in stomatal width and 61% exhibited a decrease in stomatal area under salt stress, and stoma density increased in 94%. The only negative ratio was observed in stoma density with 11.11% in YYU21 genotype and it was found that the highest increase in LRWC was observed in YYU18 genotype (216.67%), which had prominent scale scores. Thus, it could be observed that the stoma density (StD) increased as StL, StW and StA values decreased under salt stress (Table 6). It was reported that this mechanism is a functional plant response to suppress salt stress, regulate the respiration and preserve plant performance (Orsini et al., 2011) and it was also indicated that increased StD due to increase in salt stress led to an increase in photosynthesis (Chaves et al., 2009). Although several studies reported similar findings, indicating that salt stress led to a decrease in stoma dimensions and areas and an increase in stoma density (Orsini et al., 2013; Kurtar et al., 2016), Sarabi et al. (2017) reported that stoma density decreased due to the increase in salinity and interpreted this as a counter adaptation against salt stress.

Table 6. Stomatal characteristics of melon accessions and cultivars with/without salt application

Accession	Stoma length (μm)			Stoma width (μm)			Stomatal area (μm^2)			Stoma density (unit/ mm^2)		
	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)	0 mM	50 mM	Change (%)
YYU1	18.10	15.73	-13.09	14.33	10.53	-26.52	202.25	129.95	-35.75	104.17	108.33	3.99
YYU4	14.20	21.47	51.20	13.23	15.57	17.69	153.84	261.87	70.22	54.17	100.00	84.60
YYU6	21.77	17.73	-18.56	14.77	14.10	-4.54	250.70	196.56	-21.60	75.00	104.17	38.89
YYU10	17.93	13.47	-24.87	13.60	11.03	-18.90	193.76	117.48	-39.37	62.50	91.67	46.67
YYU11	20.43	20.77	1.66	14.77	14.83	0.41	236.58	242.78	2.62	145.83	166.67	14.29
YYU13	21.33	18.53	-13.13	19.13	14.20	-25.77	323.66	206.91	-36.07	62.50	95.83	53.33
YYU14	21.47	12.47	-41.92	14.13	22.43	58.74	239.40	219.67	-8.24	100.00	158.33	58.33
YYU15	19.30	18.63	-3.47	13.80	14.77	7.03	209.13	216.80	3.67	95.83	145.83	52.18
YYU18	15.47	15.13	-2.20	12.43	10.27	-17.38	156.30	122.26	-21.78	75.00	237.50	216.67
YYU20	15.77	11.67	-26.00	10.97	10.80	-1.55	135.07	99.28	-26.50	225.00	241.67	7.41
YYU21	15.73	23.70	50.67	12.47	12.87	3.21	153.37	246.64	60.81	187.50	166.67	-11.11
YYU22	15.63	15.50	-0.83	10.53	11.77	11.78	129.46	142.85	10.34	70.83	183.33	158.83
YYU23	16.40	16.27	-0.79	12.70	12.07	-4.96	163.54	154.94	-5.26	95.83	100.00	4.35
YYU29	23.30	19.53	-16.18	17.20	14.80	-13.95	314.50	227.72	-27.59	58.33	129.17	121.45
Galia	19.93	16.57	-16.86	17.30	13.17	-23.87	270.78	170.55	-37.02	83.33	100.00	20.00
Kırkağaç F ₁	22.07	14.53	-34.16	12.57	12.10	-3.74	218.41	139.27	-36.23	158.33	287.50	81.58
Lokum F ₁	14.80	19.20	29.73	10.77	12.83	19.13	132.51	195.74	47.72	95.83	229.17	139.14
Napolyon F ₁	20.23	19.10	-5.59	12.43	13.20	6.19	195.05	197.00	1.00	87.50	229.17	161.90

Principle component analysis (PCA)

Principal component analysis (PCA) method was used to determine the traits that led to the variation. Eigen value and variances obtained in the analysis and the properties that led to the differences among the genotypes were determined (Sönmez et al., 2015). 71.48% of the total variance in fourteen different traits was grouped in 3 PCA groups for the control application and the 69.53% in the 50 mM salt application were grouped in 4 PC groups, and it was found that the two applications were similar in total variance. In the control group, the variation ratios for three principal components were 35.42%, 25.26%, and 10.80%, respectively, and these ratios were observed as 28.16%, 18.56%, 11.62%, and 11.19% in the 4 principal components of in 50 mM salt application group (Table 7). In a study conducted by Sarabi et al. (2017) on salt stress using physiological and biochemical parameters, it was reported that the total variance was 97.17% in the PCA analysis and the first principal component explained 66.96 of the variance. Furthermore, Shelke et al. (2017) determined that 82.65% of the total variance of 95.07% was explained by the first principal component. It is considered that in both studies, the researchers were able to increase the variance by working with an excessive number of salt concentrations and the variances could stem from the reactions that plants exhibited indifferent concentrations.

Table 7. *Principal component analysis (PCA) of characters associated with melon accessions based on salt stress*

	PC axis						
	0 mM			50 mM			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Eigen values	4.96	3.54	1.51	3.94	2.60	1.63	1.57
Explained proportion of variation (%)	35.42	25.26	10.80	28.16	18.56	11.62	11.19
Cumulative proportion of variation (%)	35.42	60.68	71.48	28.16	46.72	58.34	69.53
Characters	Eigen vectors						
	0 mM			50 mM			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Shoot fresh weight	0.39	0.02	0.17	0.40	-0.02	-0.22	0.24
Shoot dry weight	0.42	0.01	0.11	0.34	0.06	0.13	0.49
Root fresh weight	0.37	0.17	0.02	0.40	0.08	0.24	-0.31
Root dry weight	0.40	0.18	-0.12	0.46	0.08	0.19	-0.08
R:S ratio (DW %)	0.18	0.27	-0.41	0.33	0.07	0.08	-0.51
Leaf relative water content	-0.02	0.06	0.61	0.04	-0.17	-0.55	0.03
Leaf number	0.36	-0.22	0.20	-0.03	0.59	-0.17	-0.02
Shoot diameter	0.15	0.30	-0.28	0.25	-0.08	0.14	-0.03
Shoot length	0.30	-0.26	0.26	0.21	-0.31	-0.18	-0.03
Root length	0.23	0.27	-0.12	0.24	0.21	0.09	0.45
Stoma length	-0.07	0.40	0.37	-0.15	-0.03	0.39	0.27
Stoma width	-0.12	0.42	0.09	-0.22	0.07	0.50	0.01
Stomatal area	-0.10	0.47	0.24	0.00	0.47	-0.23	0.14
Stoma density	0.13	-0.17	-0.06	-0.07	0.48	-0.04	-0.22

In the control group, it was found that 42% of the variance in PC1 was explained by SDW, StA explained 47% of the variance in PC2 and LRCW explained 61% of the variance in PC3, which were the highest ratios. The highest ratio in PC1, which is one of the principal components that occurred under salt stress, was observed with RDW (46%), in PC2, the highest ratio was observed with LN (59%), in PC3, the highest ratio was observed with LRWC (55%) and in PC4, the highest ratio was observed with SDW (49%). SDW and LRWC exhibited the highest variance in 0 and 50 mM, furthermore, SI (17%), which exhibited low variance in 0 mM, reached a higher value due to salt stress (48%) (Table 7). Genotypes and varieties were classified in two groups of 0- and 50-mM salt treatment (Fig. 2); YYU4, YYU20, YYU21 genotypes and cv. Lokum F1 in the control group and the YYU18, YYU20 genotypes and cv. Kırkağaç in the 50 mM salt treatment group were in the same group and the remaining genotypes and varieties were in the second group.

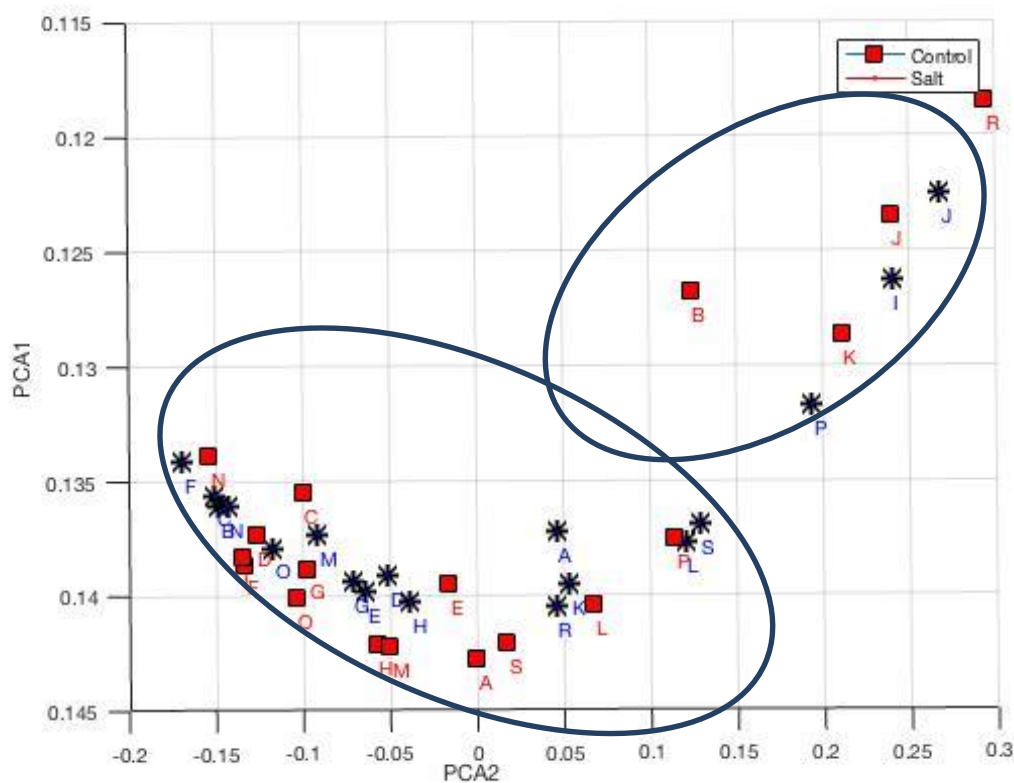


Figure 2. The first three principle components principle component analysis (PCA) plot conducted using 14 parameters (A: YYU1, B: YYU4, C: YYU6, D: YYU10, E: YYU11, F: YYU13, G: YYU14, H: YYU15, I: YYU18, J: YYU20, K: YYU21, L: YYU22, M: YYU23, N: YYU29, O: Galia, P: Kırkağaç F₁, R: Lokum F₁, S: Napolyon F₁)

Mixture modeling

The number of homogeneous sub-groups for the genotypes was determined with the Gaussian mixture model through the evaluation of inspected properties in order to determine the reactions of melon genotypes to salt stress. Based on all properties scrutinized in the present study and using the smallest AIC and BIC criteria (Yeşilova et al., 2016), it was determined that genotypes could have 4 homogenous sub-groups under

stress-free conditions and 3 sub-groups under conditions with salt stress (*Table 8*). Thus, it was found that the correct classification rate of entropy was 98% for 0 mM and 93% for 50 mM. In the control application, based on the sub-group distribution with respect to their traits, it was found that the highest mean values for all other traits except StD were observed in the second and fourth sub-groups, where no significant dispersion was observed in the lowest values and the majority of the lowest means were in the first group (*Table 8*). It was also determined that the high mean values were significantly observed in the first group based on the distribution of the variable means, and the low mean values were in the third group (*Table 8*). In other words, it is possible to state that the genotypes and varieties in the first group tend to increase their tolerance against salt stress. Thus, genotypes in the third group could be considered as more susceptible to salt.

Table 8. Estimated means of variables for model with four sub-population at 0 and 50 mM in melon genotypes

Characters	0 mM				50 mM		
	Sub-group 1	Sub-group 2	Sub-group 3	Sub-group 4	Sub-group 1	Sub-group 2	Sub-group 3
Shoot fresh weight	10.73	30.84	17.52	18.36	11.06	10.31	6.70
Shoot dry weight	0.63	2.11	1.26	1.35	0.93	0.76	0.72
Root fresh weight	0.53	1.99	0.99	1.67	1.55	0.98	0.70
Root dry weight	0.025	0.095	0.051	0.102	0.094	0.058	0.048
R:S ratio (DW %)	0.040	0.045	0.014	0.063	0.102	0.078	0.066
Leaf relative water content	77.95	86.33	79.35	78.55	71.16	76.75	71.52
Leaf number	5.18	10.58	7.79	5.72	4.14	5.28	3.50
Shoot diameter	3.68	3.83	3.84	4.96	4.41	4.07	3.96
Shoot length	50.60	106.25	83.50	44.58	35.22	41.69	30.12
Root length	15.01	17.34	17.49	21.33	19.12	16.26	16.88
Stoma length	19.16	19.00	16.69	20.26	17.96	16.14	17.15
Stoma width	14.34	12.08	11.70	16.71	14.20	11.84	13.61
Stomatal area	218.36	187.33	115.22	268.36	201.96	150.11	183.66
Stoma density	89.69	100.01	131.09	80.57	178.63	167.72	131.58
Membrane injury index	-	-	-	-	15.75	19.40	22.80
0-5 scale	-	-	-	-	1.81	1.57	2.20

It was found that the resulting homogeneous sub-groups exhibited variations in distribution of genotypes for 0 mM, and majority of genotypes were in the 1st and 3rd sub-groups, where the means were low. Furthermore, based on the mean values obtained with the variables, it was determined that the genotypes and varieties in the first group were tolerant to salt, those in the second group had moderate tolerance, and those in the third group were susceptible to salt under 50 mM salt treatment. Accordingly, it was determined that YYU4, YYU10 and YYU11 genotypes could be susceptible to salt, YYU15, YYU18, YYU23 genotypes and cv. Galia could be medium-tolerant, YYU6, YYU13, YYU14, YYU20, YYU21 and YYU29 genotypes and cv. Kırkağaç F₁ and cv. Lokum F₁ varieties could be tolerant to salt stress (*Table 9*).

Table 9. Mixture model results with correct classification values for four accession sub-populations

Accession	0 mM				50 mM		
	Sub-population1	Sub-population2	Sub-population3	Sub-population4	Sub-population1	Sub-population2	Sub-population3
YYU-1	0.987	0	0.013	0	0	0.996	0.004
YYU-1	1	0	0	0	1	0	0
YYU-1	0.001	0	0.999	0	0	0	1
YYU-4	0	0	0	1	0	0	1
YYU-4	0	0	0	1	0.99	0.001	0.009
YYU-4	0	0	1	0	0.004	0.023	0.973
YYU-6	1	0	0	0	0	0.018	0.982
YYU-6	1	0	0	0	1	0	0
YYU-6	1	0	0	0	1	0	0
YYU-10	1	0	0	0	0	0.004	0.996
YYU-10	1	0	0	0	0	0	1
YYU-10	0.932	0	0.065	0.003	0	0	1
YYU-11	0	0	0	1	0	0	1
YYU-11	0	0	0	1	0	0	1
YYU-11	1	0	0	0	0.954	0	0.046
YYU-13	0	0	0	1	0.992	0.01	0.002
YYU-13	0	0	0	1	1	0	0
YYU-13	0	0	0	1	0.04	0	0.96
YYU-14	0.019	0	0.972	0.009	1	0	0
YYU-14	0	0	0	1	1	0	0
YYU-14	1	0	0	0	0	0	1
YYU-15	1	0	0	0	0	0.999	0.001
YYU-15	1	0	0	0	0	1	0
YYU-15	1	0	0	0	0	0	1
YYU-18	0.988	0	0.012	0	0	0.537	0.463
YYU-18	0	0	1	0	0.006	0.994	0
YYU-18	1	0	0	0	0	1	0
YYU-20	0	0	1	0	1	0	0
YYU-20	0	0	1	0	1	0	0
YYU-20	0	0	1	0	0.997	0.003	0
YYU-21	0	0	1	0	1	0	0
YYU-21	0	0.001	0.999	0	1	0	0
YYU-21	0	0	1	0	0.013	0.990	0
YYU-22	0	0	1	0	0.913	0.090	0
YYU-22	0	0	1	0	0	1	0.004
YYU-22	0.998	0	0.002	0	0	0.14	0.865
YYU-23	0	0	1	0	0	1	0
YYU-23	0	0	1	0	0	0.936	0.064
YYU-23	1	0	0	0	0	0.035	0.964
YYU-29	0	0	0	1	0	0	1
YYU-29	0.926	0	0.073	0.001	0.982	0.001	0.017
YYU-29	0	1	0	0	1	0	0
Galia	1	0	0	0	0	0.763	0.237
Galia	1	0	0	0	0.02	0.98	0
Galia	1	0	0	0	0	0	1
Kırkağaç F ₁	0	0	1	0	0.999	0.001	0
Kırkağaç F ₁	1	0	0	0	0.001	0.994	0.004
Kırkağaç F ₁	0	1	0	0	1	0	0
LokumF ₁	0	0	0.999	0	0.001	0.027	0.972
LokumF ₁	0.981	0	0.019	0	1	0	0
LokumF ₁	0	1	0	0	1	0	0
NapolyonF ₁	0	0	1	0	0	1	0
NapolyonF ₁	1	0	0	0	0.001	0.001	0.998
NapolyonF ₁	0	1	0	0	0.998	0.002	0
Total Acc.	24	4	17	9	22	14	18

Conclusion

Salt stress is one of the most important stress factors for the majority of cultivated plants. Several studies were conducted on the subject. Tolerance to abiotic stress factors such as salinity displays a complex structure, and therefore, makes it difficult to develop tolerant varieties. Tolerance varies between plant varieties and even between variety genotypes. In the present study, it was determined that variations existed between the tolerances of studied genotypes against salt stress based on the examined traits. It was concluded that the examined traits could be used to determine the effect of salt stress, and the conducted correlation analysis demonstrated that there were correlations among various traits. It was found that traits such as leaf number, shoot length, shoot fresh weight and leaf relative water content of all genotypes were adversely affected under salt stress. While stoma length, stoma width and stomatal area decreased under salt stress, it was determined that the stoma intensity increased. It was observed that shoot dry weight, leaf relative water content and were among the traits that best explained the variance both salt stress and the control group in the PCA analysis. Mixture modeling analysis indicated that there was no significant difference between the 0 mM application sub-groups, however, especially in the 50 mM application, the distribution of the lowest and highest mean values of the variables was clearer; hence, the variance between the genotypes and varieties could be determined with mixture modeling analysis based on the reactions they exhibited against salt stress.

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