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Utilizing Some of Screening Methots in Order to Determine of Tolerance of Salt Stress in the Melon (*Cucumis melo* L.)

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Abstract: In order to determine the genotypical differences and basic selection parameters for salt tolerance 36 melon genotypes were grown in hydroponics culture. When seedlings reached to 4-5 leaf stages 150 mM NaCl was applied to them. 14 days after salt application, young plants were harvested from control and salt treated groups. The fresh weight of green parts of young plants and the amount of some ions (Na^+, K^+ and Cl^-) were determined. It was found that there were differences among melon genotypes in terms of salt tolerance and this tolerance had a relationship either with the accumulation Na^+ and Cl^- ions or not uptaking them at all. On the other hand 'plant stress index value (PSI) (%)' was found as an effective parameter for the determination of salt tolerance in melons.

Key words: *Cucumis melo,* Genotype, Ion uptake, Salt stress, Screening, Salt tolerance

INTRODUCTION

Salt damage reveals itself with different symptoms in plants. Salinity is a factor covering plant morphology and anatomy and affecting its entire metabolism[1]. When the salt concentration increases in soil solution and water potential decreases, osmotic potential of plant cells is reduced, with a sudden slowdown in cell division or elongation. Under such conditions of stress, generally stomata close, resulting in reduced photosynthesis. Plant growth may be completely hindered if stress conditions continue to exist[2]. Plants differ markedly within different species in salt tolerance. The first reaction plants show against salinity is reduction in shoot growth[3]. Saline stress may cause the death of plant, may inhibit growth depending on tolerance, may cause the formation of chlorosis, necrotic spots, and may result in reduced productivity and quality[4]. Plants growing under saline conditions have lower growth rates, with a dwarf structure, and their leaves are mostly small, with a dark green color[5].

Reduction in root, body and shoot lengths; reduction in plant fresh and dry weights; reduction in leaf area and numbers; reduction in chlorophyll amount; distortion in productivity, fruit taste and colors are listed among differences generally encountered in plants exposed to saline stress. When the plant is exposed to saline stress for extended periods, it has been noted that ion toxicity and water deficiency occurs in old leafs, with carbohydrate deficiency and associated symptoms in young leaves[6,7,8,9]. Günes *et al.* [10] have reported that salinity resulted in reduction in dry substance weight in pepper plants they have applied saline stress, with inhibited growth and development.

Sodium is known as an element that has the ability to move within both phloem and xylem in the plant[11]. Bohra and Döffling[12] have reported that ion balance was impaired in the body part of plant in saline stress; and that increased amount of sodium intake competed with the intake of other mineral substances and caused malnutrition. Levitt[1] suggests that where there is an abundance of sodium chloride in the growth media, plants took the Na^+ ion more than necessary and there occurred reductions in K^+ ion intake due to the resulting competition, thus resulting in K^+ deficiency. It is a known fact that potassium intake is reduced in the plant in a growth media with high sodium ion[2].

High saline concentrations reduce plant intake and transport of calcium, causing calcium deficiency and ion imbalance in the plant [13]. Calcium is an element with positive effects for the plant in saline stress. Unanimous opinions of researchers explaining calcium's playing a protective role against saline stress with various mechanisms is for ensuring calcium selectivity in cell membrane strengthening and ion intake and transport. Calcium and potassium display similar attitudes in the selective transport of ions from the cell membrane[14]. Ca/Na ratio in the plant cause the

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distortion of selectivity in root cell membranes just as in low K/Na ratio, resulting in passive intake of sodium into the cell and toxic level accumulation in the plant.

Salinity mostly causes early aging in leaves[15,16]. Leaf aging is generally expressed in terms of decrease in protein or chlorophyll concentrations[17] and increase in cell membrane conductivity[18]. Special effect of saline stress on leaf aging comes out in the form of accumulation of toxic ions (Na^+ and Cl^-) or their consumption of K^+ and Ca^{+2} ions[19,20].

Melon (*Cucumis melo* L.) is one of the firstly-remembered plants in the face of "salinity problem", one of the most important problems in front of plant growing in dry and semi-dry regions[21]. Although melon (*Cucumis melo* L.) has been stated to have medium tolerance to salinity by different researchers[22,23,24,25], it has also been reported that saline tolerance differs in melons by genotypes, with variables ranging from "sensitive" to "medium tolerant" with regards to this characteristic [26,27,28].

The purpose of this research is to study the genotypical variation in 36 melon genotypes with regards to saline tolerance, and to explain whether or not there are any relations between ion accumulation levels and saline tolerance ability in determining saline tolerance.

MATERIALS AND METHODS

Plant Material and Growth Conditions: In order to determine the genotypical differences and basic selection parameters for salt tolerance 36 melon genotypes were used (Table 1).

Plants were grown under controlled climatic conditions at 25/22^{0}C day/night temperatures, light/dark regimes of 16/8 h, light intensity of 280 µmol m^{-2} s^{-1} and 70% relative humidity. Seeds were germinated in vermiculite moistened with distilled water. After two week, seedlings were transferred to plastic vessels filled with 4lt half-strength Hoagland solution. Solution in vessels was replaced every week with fresh Hoagland solution. Two week later, salt treatment started and NaCI concentration was increased by increments of 50 mM per day until a final concentration of 150 mM was achieved. Non-salinized plants were kept as controls. Salt-stressed plants were subjected to 150 mM NaCI for 11 days after completing the salt addition and then all plants, including controls, were sampled. To determine the fresh weights of green partion of the plants, each five young plants from non-salt treatment and salt-treatment media were measured on digital scales. Plant stress index value (PSI) (%) was obtained as a result of division of each genotype with its respective control has bean impulse with 100.

Determination of Ion Content: For ion determinations, fresh leaf samples were extracted in concentrated 0.1N HNO_3. Na^+, K^+ and Ca^{+2} contents were determined by flame photometry in samples from leaves[29]. For chloride determination, fresh leaf materials were extracted with 0.1N HNO_3 in 10% (v:v) acetic acid and Cl^- was determined by the silver ion-titration method with a automatic chloridometer (Bucthler–Cotlove chloridometer) according to Bozcuk[30].

RESULTS AND DISCUSSIONS

Results: In the hydrophonic system, it has been seen that 14 days after addition of NaCl fresh weight of green parts in melon seedling generally decreased compared to control seedlings (Table 2).

Plant stress index value (PSI) obtained as a result of comparison of each genotype with its respective control has been found effective in that it demonstrates

Table 1: Number and locale name of the genotypes used in this study.

No	Local/name	No	Local/name
2	Ananas	35	Midyat - Mardin
5	Lüleburgaz - Kırklareli	36	Midyat – Mardin
6	Akbağ - Yalova	38	Bağpınar- Diyarbakır
8	Bursa	39	Erimli
12	Bucak - Burdur	41	Balıkesir
13	Van	42	Acurkavunu - Susurluk
14	Oltu - Erzincan	43	Sarıdilimli - Gönen
15	Gercüş - Mardin	44	Bayramiç
16	Oltu - Erzincan	45	Hasanbey - Ayvalık
18	Besni - Adıyaman	47	Erçek - Van- Şemame
21	Yuva	48	Erçek - Van- Şemame
22	Vedrantais (Fransa)	49	Erçek - Van- Şemame
24	Kış kavunu (Agromar)	50	Van - Merkez
26	Saf 4 - Adıyaman	52	HMK 208- US Eastern
28	Azerbeycan	53	Ogen
29	Azerbeycan	54	Yellow canary
30	Iğdır - Aralık	58	*Momordica charante*
34	Van	60	*Cucumis flexuosus*

Table 2: Fresh weight of green parts average of plants in the salt-treatment (NaCl) and control treatment mediums (g), plant stress index (PSI) values of plant (%), the amount of Na^+, K^+ and Cl^- ions (µg/mg F.W.)

Genotype No.	Plant fresh weight of green part			Na^+			K^+			Cl^-		
Control	NaCl	PSI (%)	Control	NaCl	Increase(%)		Control	NaCl	Losses(%)	Control	NaCl	Increase(%)
02	3.54c-g	0.76k	21.5	0.13c-e	13.75bc	10576.9	3.51a-h	6.78abc	-	0.24b	8.44ab	3516.7
05	2.97c-h	2.40c-f	80.8	0.19b-e	6.75bc	3552.6	3.90a-g	2.70ef	30.8	0.28ab	2.30cde	821.4
06	2.55d-h	2.20c-h	86.3	0.83b-d	9.00bc	1084.3	3.47a-h	2.06f	40.6	0.28ab	3.64cde	1300.0
08	1.28gh	1.28h-k	100.0	0.13b-e	7.75bc	5961.5	4.15a-d	1.93f	53.5	0.28ab	2.11cde	753.6
12	3.43c-h	1.44g-k	42.0	0.23b-e	7.00bc	3043.5	3.53a-h	3.50a-f	0.90	0.33ab	2.83cde	857.6
13	3.62c-g	1.93c-ı	53.3	0.30b-e	9.25bc	3080.0	3.08c-h	6.65a-d	-	0.19b	4.75b-e	2500.0
14	3.38c-h	1.70e-k	50.3	0.18b-e	6.88bc	3822.0	3.25a-h	4.20a-f	-	0.52a	2.59cde	498.1
15	3.38c-h	2.02c-ı	59.8	0.34abc	8.50bc	2500.0	4.23a-d	2.18f	48.5	0.23b	3.50cde	1521.7
16	2.12e-h	1.37f-k	64.2	0.53a	5.94bc	1120.7	3.40a-h	3.83a-f	-	0.28ab	1.97cde	703.6
18	3.69c-h	2.90cd	78.6	0.26b-e	5.13bc	1973.1	3.30b-h	4.10a-f	-	0.28ab	1.00e	357.1
21	2.84d-h	0.99ı-k	34.9	0.13cde	16.88abc	12984.6	4.33a-d	6.88ab	-	0.24b	10.21a	4254.2
22	1.93e-h	1.31f-k	67.9	0.30b-e	9.50bc	3166.7	4.63a-b	5.65a-f	-	0.19b	5.90bcd	3105.3
24	2.96c-h	1.24h-k	41.9	0.13cde	24.81a	19084.6	4.08a-e	3.70a-f	22.9	0.14b	4.55bcd	3250.0
26	4.06c-f	1.89d-j	46.5	0.18b-e	7.38bc	4100.0	3.59a-h	3.95a-f	-	0.28ab	3.07cde	1096.4
28	4.79cd	2.41c-f	50.3	0.19b-e	4.88bc	2568.4	3.95a-f	3.35b-f	15.2	0.28ab	1.06e	378.6
29	5.56bc	1.68e-k	30.2	0.23b-e	5.88bc	2556.5	3.23b-h	2.90c-f	10.2	0.24b	1.68cde	700.0
30	8.44a	2.62c-e	31.0	0.19b-e	4.63c	2436.8	3.51a-h	4.63a-f	-	0.19b	0.72e	378.9
34	7.31ab	2.33c-h	31.9	0.17b-e	4.56c	2682.4	2.82d-h	2.57e-f	8.9	0.27ab	1.59cde	588.9
35	4.34c-e	3.97b	91.5	0.22b-e	4.26c	1936.4	2.49f-h	2.06f	17.3	0.27ab	0.99e	366.7
36	1.45f-h	1.66e-k	114.4	0.11de	4.07c	3700.0	3.03a-d	3.03b-f	30.4	0.28ab	0.91e	325.0
38	1.99e-h	1.30g-k	65.3	0.14b-e	5.28bc	3771.4	2.32a-e	2.32f	43.5	0.28ab	1.39de	496.4
39	2.31d-h	1.06ı-k	45.9	0.13cde	4.00c	3076.9	2.51b-h	2.51f	20.1	0.33ab	0.97e	293.9
41	2.44d-h	1.37f-k	56.1	0.22b-e	5.71bc	2595.5	3.71b-h	3.71a-f	-	0.28ab	2.77cde	989.2
42	2.66d-h	1.50f-k	56.3	0.10e	4.94bc	4940.0	3.90e-h	3.90a-f	-	0.28ab	2.83cde	1010.7
43	2.86d-h	1.74e-k	60.8	0.12cde	5.81bc	4841.7	4.45f-h	4.45a-f	-	0.38ab	2.83cde	744.7
44	2.09e-h	0.85jk	35.6	0.14b-e	10.14bc	7242.9	7.37a-h	7.37a	-	0.32ab	6.17bc	1928.1
45	3.33c-h	1.64e-k	49.2	0.27b-e	5.33bc	1974.1	1.79a-h	1.79f	46.4	0.37ab	1.88cde	508.1
47	2.89d-h	2.97c	102.8	0.16b-e	4.91bc	3068.8	2.28c-h	2.28f	20.0	0.27ab	1.52de	562.9
48	3.20c-h	2.97c	92.8	0.35ab	4.60c	1314.3	2.16b-h	2.16f	32.9	0.36ab	1.95cde	541.7
49	7.91a	5.29a	66.9	0.10e	3.24c	3240.0	2.81c-h	2.81df	4.4	0.28ab	1.17e	417.9
50	4.37c-e	2.83cd	64.7	0.10e	4.57c	4570.0	4.96c-h	4.96a-f	-	0.37ab	1.99cde	537.8
52	0.86h	0.84jk	97.6	0.29b-e	4.00c	1379.3	5.62a	5.62a-f	-	0.22b	1.49de	677.2

Table 2: Continued.

53	2.02e-h	0.92ı-k	45.5	0.13b-e	4.32c	3323.1	3.73a-c	3.73a-f	15.1	0.23b	0.89e	386.9
54	3.46c-h	0.78jk	22.5	0.17b-e	6.43bc	3782.4	6.43g-h	6.43a-e	-	0.23b	3.90cde	1695.7
58	4.85cd	1.64e-k	33.8	0.10e	5.01bc	5010.0	2.20a-h	2.20f	35.1	0.28ab	1.21e	432.1
60	8.66a	2.83cd	32.7	0.12cde	8.04bc	6700.0	5.27h	5.27a-f	-	0.38ab	4.81b-e	1265.8

Mean values indicated by the same latter are not significant different ($p \leq 0.01$).

the abilities of genotypes to protect their growth performances under saline stress. It has been understood that, with regards to plant fresh weight, melon genotypes yielding the closest values to control plants under saline stress, in other words, least affected genotypes from salt were with the number of 36 (Midyat), 47 (Van – Şemame), 8 (Bursa), 52 (HMK 208), 48 (Van – Şemame), and 35 (Midyat). It has also been observed that in genotypes numbered as 36 and 47, salt application also had an effect stimulating plant growth instead of reducing plant growth. Average weights of the plants with saline application were found a bit higher than control plants of same genotypes.

When saline application was made, there has been a Na^+ ion increasing in the leaves of all melon genotypes in the experiment. Genotypes numbered as 24, 21, 2, 44, 60, 8, 58, 42, 43 and 50 have been the first 10 genotypes taking the most Na ion into their structures. In return, some genotypes have been selective in taking the Na ion into their structure and kept it away from themselves. Sequence, by increase rates, of the first 10 genotypes with least accumulation of Na ion in leaf tissue is as follows: 6, 16, 48, 52, 35, 45, 30, 15, 29, 28 (Table 2).

K^+ ion amount measured in leaf samples of melon plants with saline application did not yield lower values than control plants without saline application in all genotypes. A statistically significant difference has been found between the genotypes with regards to decrease in K^+ ion amount.

In some genotypes, there has never been a decrease in the amount of K^+ ions under saline stress, but increases compared to the control (2,13, 14, 16, 18, 21, 22, 26, 30, 41, 42, 43, 44, 50, 52, 54, 60); whereas in some genotypes saline stress cause reduction in the amounts of K^+ ions in plants (8, 15, 45, 38, 6, 58, 48, 5, 36, 24, 39, 35, 28, 53, 29, 34, 49, 12).

After saline application, there has been an increase in the Cl^- ion amount in all genotypes, however a significant increase has been observed with regards to 'increasing ratios' (%) compared to the control. Genotypes numbered as 21, 2, 24, 22, 13, 44, 54, 15, 6, 60 have been the first 10 genotypes taking the highest amount of Cl^- ion into their structures. In return, some genotypes proved to be more successful in keeping the Cl^- ion away from themselves. The first 10 genotypes accumulating least Cl^- ion have been (39, 36, 18, 35, 28, 30, 53, 49, 58, 38) at the 14th day of saline application of leaf tissue (Table 2).

Discussion: In the thirty six *Cucumis* sp. genotypes used in salt treatments, the first marked symptomatic effect of toxic-level NaCl salt applied in 150 mM dosage has been reduction on the plant fresh weight and inhibition on the plant growth. While some genotypes are affected less and grow equally with control plants and cause no inhibition effects on saline growth, there have been some genotypes that could grow only 50% and even 20% of control plants. Moreover, an opinion has formed to the effect that plant stress index value (PSI) use could be the easiest yet rather effective parameter that could give an opinion under the most restricted conditions in determining saline tolerance. This is completely in harmony with the opinions of Shannon *et al.*[26], Mangal *et al.*[27], Mendlinger and Pasternak[28] and Botia *et al.*[31] reporting the detection of variables ranging from "sensitive" to "medium tolerant" in melon indicated to be medium tolerant to salinity. Our study has also taken into consideration the plant stress index value listings in expressing tolerance to salinity. Comparisons were made with PSI for saline tolerance of genotypes in commenting about differences in ion accumulation.

It has been understood that one of the most important reasons of the reduction in growth in different melon genotypes is the sodium ion concentration accumulated more than necessary and at toxic level in plant body. It has been understood that the Na^+ ion increase measured in leaves after saline application was too much, that such increase in toxic levels significantly varied by genotypes; and except for some exceptions, it has been found that in general there was more saline tolerance in genotypes taking less Na^+ ion. As in some other plants[32,33,34], tolerance to salinity is has been related to Na^+ ion accumulation in plant green matter. Although it has been found that there were increases in Na ion intake in some of the melon genotypes, there has been decreases in K^+ ion intake, K^+ ion amounts in plants under plant stress has been found more compared to those in control plants in some including genotypes demonstrating high level of sensitivity to salt (2, 21,

44 and 60). Similarly, Heimler *et al.*[35], Lopez and Satti[36], Yu *et al.*[37], and Aktas[33], has reported that plant genotypes play role on tolerance to salinity for Na^+ and K^+ absorptions in different ratios, and types continuing to take potassium ion selectively have higher tolerance for salinity.

In our view, one of the most significant critical reasons of the reduction in the growth in different muskmelon genotypes is the more than necessary and at toxic level chloride ion concentration in plant body. In all melon genotypes, post-growing Cl^- ion has increased in the NaCl containing medium and there have been very big differences in such increase rates. Akıncı[38], has stated that Na^+ and Cl^- ion concentration very much over toxic dose limits (Na^+=0.01-10.0%, Cl^-=0.02-2.0%) were determined in the leaves of melon plants grown in saline media. In the present study, Cl^- ion accumulation in the leaves of plants subjected to salt in saline sensitive melon types Ananas (2) and Yuva (21) has been found rather higher compared to the control, but this increase was found low in Besni (18) and Mardin (35) melons that have high tolerance to salinity. And Carjaval *et al.* [39] and Navarro *et al.*[21] report that melon plant has a specific toxicity against Na^+ and Cl^- ions. Melon's tolerance mechanism for salinity is explained by Botia *et al.*[31] with the theory of accumulation of Na^+ and/or Cl^- in different plant sections; and Carjaval *et al.* [39] explains it by osmotic adjustment.

As a result, our study has concluded that chloride ion amount in plant leaves is the most enlightening feature in determining tolerance to salinity. A genotype has demonstrated tolerance to salinity as much as it takes chloride ion to leaves under saline stress conditions, and has given higher PSI value. We favor the opinion "Saline sensitivity comes out due to Na^+ and particularly Cl^- toxicity in muskmelons".

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