

Grafting for Sustainable Growth Performance of Melon (*Cucumis melo*) Under Salt Stressed Hydroponic Condition

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ABSTRACT

The aim of the present study was to find out whether grafting could improve salinity tolerance of melon plants, and whether possible induction of tolerance to salinity was linked with the defense of the photosynthetic apparatus. The climate chamber experiment was carried out to determine main stem length, leaf chlorophyll index (SPAD), fresh weight of shoot and root, and root length. Under climate chamber conditions, two melon cultivars [galia type Citirex F1) and standard type (Kirkagac Manisa Altinbas)] were grafted onto two different commercial *Cucurbita maxima* x *C. moschata* hybrid rootstocks (Kardosa and Nun 9075) and grown in 8 L pots filled continuously with aerated nutrient solution under two different salt levels (1 and 8 dS/m) with three replications. The results indicated that grafted and un-grafted plants were significantly ($P < 0.001$) affected by different salt levels. Grafted plants had better growth performance than un-grafted plants under both control and saline conditions. The SPAD value of the un-grafted plants significantly decreased as salinity stress increased, while root fresh weight of the grafted plants significantly increased under salinity stress. Under saline conditions the highest shoot fresh weight, and SPAD value were recorded in graft combination Nun 9075/ Citirex. These results suggest that the use of salt tolerant *Cucurbita maxima* x *C. moschata* hybrid rootstocks can improve crop performance in melon under salt stress, whereas the appropriate scion/ rootstock combinations under saline conditions will be investigated for fruit yield and quality parameters in further studies.

Keywords: Commercial rootstock, salinity tolerance, nutrient solution, grafting, melon

1. Introduction

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is an important horticultural crop, which is often cultivated in arid and semi-arid regions of the world, where salinity threats is to be, or already is, a serious problem [1], despite the advanced management techniques developed in recent decades [2]. Melon production was estimated to be 29.6 million tons in 2014 for a cultured area of 1.178,808 ha [3], the main producing countries being China (14.7 million tons) followed by Turkey (1.7 million tons), Iran (1.4 million tons), and Egypt (1.04 million tons). Melon is known to be moderately tolerant to salinity and salinity is a major environmental restriction limiting melon production worldwide. In the world's arable land up to 20%, and in all irrigated lands up to 50%, are adversely affected by salinity [4]. Saline irrigation water, low-level rainfall, high-level evaporation, excessive irrigation without proper drainage in inlands and underlying rocks rich in harmful salts can cause salinity problems in agricultural areas

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[5]. When crops are grown under saline conditions, a majority of crop plants are not able to survive; or are able to survive only with limited growth and yield [6, 7]. Saline irrigation causes several types of damage such as growth inhibition [8, 9], metabolic disturbances [10], and yield and quality losses [11]. In melon production, salt stress decreases biomass of the shoot and fruit production, and these changes are accompanied by enhanced Na^+ and Cl^- concentrations and a decreased K^+ concentration [12]. If a solution to problems that are caused by salt stress can be proposed, it will have a positive effect on crop yield and production. One possible way to reduce the detrimental effects of salt stress on high-yielding cultivars might be to graft them onto saline-tolerant rootstocks with the capability of inducing salt tolerance to the scion [13]. Grafting is known as the union of two or more properly selected pieces of living plant tissues or fractions that grow as a single plant.

A first grafted vegetable was performed in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks [13, 14]. It is an innovative technique for the suitable cultivation of fruit-bearing vegetables (tomatoes, bean, eggplant, cucumber, melon, and watermelon) in Japan, Korea, the Mediterranean basin, and several European countries [15], where land use is very intensive and continuous cropping is a common practice [16]. Grafting has contributed to sustainable agriculture by reducing the amount of chemicals used to disinfect the soil, as it has been used to confer tolerance to pests and diseases of the root system [17, 18, 19, 20], to improve fruit quality [21], to increase the absorption of nutrients and the mineral content in the aerial portion of the plant [22], to confer tolerance to high and low temperatures [23, 24], drought, salt, flooding, etc. [25, 26, 27, 28], and to improve plant vigour and the post-harvest lifetime of the fruits [29]. Up to now the effects of grafting on melon plants were carried out by many authors to achieve improved results such as resistance to pests and diseases in soil and the tolerance to low temperatures; increased plant growth, total and early yield and extending the plant's economic life by uptaking water and nutrients [30, 31, 32, 33, 34, 35, 36, 37]. Nevertheless, more comprehensive studies on the melon plants have to be conducted to better understand whether grafting could improve salinity tolerance and determine whether possible induction of tolerance to salinity was linked with the defense of the photosynthetic apparatus.

2. Materials and Methods

2.1 Plant material, treatments and experimental design

An experiment was conducted under climate chamber conditions at the Faculty of Agriculture, Erciyes University, Kayseri- Turkey. For the vegetation period, the average day/night temperatures was 25/22 °C, the relative humidity was 60-80% and a photoperiod of 16 h of light. Two melon cultivars [galia type (Citirex F1) and standard type (Kırkağaç Manisa Altınbas)] were used as scion and two different commercial *Cucurbita maxima* x *C. moschata* hybrid rootstocks (Kardosa and Nun 9075) were used as rootstock materials. The seeds were sown in multipots filled with mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). When the seedlings developed five or six true leaves, scions were grafted onto rootstocks. The un-grafted scion varieties were used as control plants. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week [38]. In order to

prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and growth room. This was done for the acclimatization of grafted plants to environmental conditions outside tunnel. After the end of healing and acclimatization, grafted plants were transplanted to plastic pots after roots were washed from growth media. The experiment was conducted with two different salt levels (1 dS/m and 8 dS/m). Each pot was filled with 8 L cultivation solution that was aerated by an air pump. The nutrient solution contained 1.5 mM calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), 250 μM monopotassium phosphate (KH_2PO_4), 500 μM potassium sulfate (K_2SO_4), 325 μM magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 50 μM sodium chloride (NaCl). Micronutrients were 80 μM iron (Fe) (III)- ethylenediaminetetraacetic acid (EDTA)- sodium (Na), 0.4 μM manganese sulfate (MnSO_4), 0.4 μM zinc sulfate (ZnSO_4), 0.4 μM copper sulfate (CuSO_4), 8 μM boric acid (H_3BO_3), 0.4 μM sodium molybdate (Na_2MoO_4). Solutions were changed completely every week in the first two weeks. The experiment was in a completely randomized block design with three replications and six plants in each replication. The experiment was terminated at the end of 35 days.

2.2. Shoot- root fresh weight and root length measurements

At the end of the experiment plants were harvested by separating them into shoot and roots. Main stem length (cm) was measured by using a ruler. For the fresh weight determination plant organs were fractioned into the leaf, stem and roots and then weighed. Each fully developed leaf was counted and recorded as leaf numbers (LN)/plant. The root length of the grafted and un-grafted plants were measured by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) after recording the root fresh weight.

2.3. Leaf chlorophyll content (SPAD) measurements

For each experimental treatment, SPAD readings were taken with the Minolta SPAD-502 chlorophyll meter. During the growth period, two series of SPAD 502 chlorophyll meter readings were obtained at the centre of the leaves on the fully expanded youngest leaf for each treatment.

2.4 Statistical Analysis

Analysis of variance (ANOVA) was performed using the SAS program (SAS Institute, Cary NC, USA). If ANOVA determined that the effects of the treatments were significant ($P < 0.05$ for F -test), then the treatment means were separated by Duncan's Multiple Range Test.

3. Results and Discussion

3.1 The leaf number and leaf chlorophyll index (SPAD)

At the end of the experiment generally plant growth was affected by increasing the salt level of the nutrient solutions. Because the NaCl concentration in the nutrient

solution was increased, plant growth significantly decreased. Growth performance of the plants was dependent on genotype and salinity. The results of the leaf number and leaf chlorophyll determined as SPAD readings at the end of the growing cycle of graft combination and control plants in different salt levels (1 dS/m and 8 dS/m) are shown in Table 1. Leaf number and leaf chlorophyll content were significantly ($P < 0.01$) affected by grafting, different salt levels and grafting x salt interaction. Under control conditions, the graft combination of Kardosa/ Citirex has significantly higher leaf number, whereas the un-grafted plant of Altinbas has the minimum leaf number. On the other hand, the graft combination of Nun 9075/ Citirex has significantly higher leaf number as compare to the un-grafted plants under salt conditions. Compared to the unstressed plants, 8 dS/m NaCl decreased the leaf number of the Altinbas and Kardosa/ Citirex plants by 34.3 and 60.5 %, respectively. Under salt stress conditions decline in number of leaves has been studied previously on grafted watermelon plants. Crimson Tide F1 watermelon variety was grafted on to some bottle gourd (*Lagenaria siceraria*) genotypes (Commercial rootstocks: Argentario and RS841; Bottle gourd genotypes: 70-07 and 35-02) and tested under two different saline conditions (1 and 6 dS/m). The graft combinations of Crimson tide/70-07 and Crimson tide/35-02 had significantly higher leaf number than un-grafted watermelon plants of Crimson tide under saline conditions [39]. Moreover, another study reported that using different rootstock genotypes had significantly positive effects on the total chlorophyll quantity and thus on the photosynthesis metabolism of grafted tomato plants grown under saline conditions [40].

Regarding leaf chlorophyll index, under control conditions the graft combination of Kardosa/Altinbas has significantly higher SPAD values, while the un-grafted plants of Citirex has significantly lower SPAD values. On the contrary, under salt condition significantly higher SPAD value was produced when Kardosa and Nun 9075 were used as a rootstock as compared to the un-grafted plants of Altinbas and Citirex. Under saline conditions, in many plants species growth inhibition is often linked with a reduction in their photosynthetic capacity [41, 42, 43, 44]. As the NaCl concentration increased, net photosynthesis level decreased in watermelon plants [45]. Leaf chlorophyll content decreased under saline conditions in melon [46], tomato [47] and Turkish tobacco varieties (*Nicotiana tabacum*) [48]. Under saline conditions, photosynthesis rate increased in grafted cucumber plants compared to the rate in non-grafted plants [49]. The study of [50] suggested that the grafted melons were more tolerant to salinity than non-grafted ones; they suggested that grafted plants developed various mechanisms to avoid the physiological damage caused by excessive accumulation of Cl^- and Na^+ in the leaves, including exclusion of Cl^- and/or reduction of Cl^- absorption by the roots, and the replacement or substitution of total K^+ by total Na^+ in the foliage.

Table 1. The effects of graft combination and different salt levels (1 dS/m and 8 dS/m) on leaf number and leaf chlorophyll index of melon plants. Values denoted by different letters (upper and lower case letters for control and salt treatment, respectively) are significantly different between genotypes within columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Graft combination	Leaf number (LN/plant)			Leaf chlorophyll index (SPAD)		
	Control	Salt	%	Control	Salt	%

			Reduction			Reduction
Altınbas	25.6 D	16.8 c	34.3	38.83 C	33.72 b	13.2
Citirex	30.9 C	12.9 d	58.1	36.65 D	33.46 b	8.7
Nun 9075/ Citirex	45.9 B	27.4 a	40.2	41.02 AB	40.78 a	0.6
Nun 9075/ Altınbas	46.2 B	22.4 b	51.4	40.53 BC	38.59 a	4.8
Kardosa/ Citirex	48.3 A	19.1 b	60.5	39.22 C	38.33 a	2.3
Kardosa/ Altınbas	47.4 B	20.2 b	57.4	42.0 A	37.08 a	11.7
F Test	Genotype	Salt	Gen.xSalt	Genotype	Salt	Gen.xSalt
	***	***	***	***	***	***

3.2 Biomass production and partitioning

Results of shoot and root fresh weight of the grafted and un-grafted plants grown in different salt levels (1 dS/m and 8 dS/m) at the end of the growing cycle are shown in Table 2. Fresh weight of shoot and root were significantly affected ($P < 0.01$) by grafting, different salt levels and grafting x salt interaction. Fresh matter of the shoot decreased by different rates in each genotype with salt application. The grafted plants had significantly higher shoot fresh weight as compared to un-grafted plants under both control and saline conditions. Under control conditions, the graft combinations of Nun 9075/Citirex had significantly higher shoot fresh matter, though the un-grafted plants of Altınbas had lower shoot fresh matter. In grafted and un-grafted melon plants shoot fresh matter decreased in response to an increase of salinity in the nutrient solution. Conversely, the graft combination of Nun 9075/Citirex had significantly higher shoot fresh matter as compared to the un-grafted plants under salt stress conditions. On the other hand, the un-grafted plants of Citirex and Altınbas had lower shoot fresh matter under saline conditions. As compared to the unstressed plants, salinity decreased the shoot fresh matter of Kardosa/ Altınbas and Citirex plants by 28.8 and 68.5 %, respectively. Likewise, in a study by [51], as the NaCl concentration increased, reduction in shoot and leaf dry matter was observed. The shoot growth inhibitions are related with a decline in number of leaves, fresh and dry biomass accumulation, and shoot elongation [52, 53, 54]. Under saline conditions, shoot dry matter and leaf area decreased progressively in cucumber plants. Though, plants grafted onto salt-tolerant pumpkin rootstock of Figleaf Gourd (*Cucurbita ficifolia* Bouche) and especially the ones grafted onto salt-tolerant pumpkin rootstock of Chaojiquanwang (*Cucurbita moschata* Duch) showed smaller reductions compared to the self-grafted cucumber plants [55]. In another study, shoot dry matter was significantly higher in grafted melon plants than un-grafted melon plants under salt stress conditions [56].

Concerning the root fresh weight, the results in our study show that grafted and un-grafted melon plants varied differentially in response to the salinity of their root environments. Under both control and salt stress conditions the un-grafted plants of Altınbas had significantly higher root fresh matter than all graft combinations. The reason might be that the high salinity stress, which has restricted mineral nutrients in the root zone, induces expanded root growth to produce more root surface. Calcium (Ca) plays an important role in processes that preserve the structural and functional integrity

of the plant membrane, stabilizes cell wall structures, regulates ion transport and selectivity, and controls ion exchange behavior as well as cell wall enzymes [57]. To provide more Ca elements in plant tissues, the more root mass was occurred in grafted plants that is formed by un-grafted plants. These findings are contrary to those of [39] who found that the grafted watermelon plants have significantly higher root fresh mass than un-grafted watermelon plants under salt stress conditions. The differences between our and Yamac's (2017) studies could be the results of using different types of graft materials (e.g. *Lagenaria*) as the rootstocks.

The reduction in the root fresh mass was ranked between -70.2 and 65.5 %. The minimum reduction was observed in the graft combination of Kardosa/ Citirex (-70.2 %), while the maximum reduction was observed in Citirex (65.5 %). Decline in plant shoot and root fresh- dry weights in response to salt stress has been reported for other crops, such as melon [58], cucumber [59], strawberry [60], tomato [61], eggplant [62], pepper [63], and broad bean [51]. Up to now in many published studies it was reported that, because of the strong and intense root structure of the grafted plants, the improved crop growth and performance of grafted plants was dependant on the increment of root hairs, and root length; thus they acquire more water and mineral elements from the soil and send them to aerial parts of the plant [64, 65, 66, 67,68, 69,30].

Table 2. The effects of graft combination and different salt levels (1 dS/m and 8 dS/m) on shoot and root fresh weight of melon plants. Values denoted by different letters (upper and lower case letters for control and salt treatment, respectively) are significantly different between genotypes within columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Graft combination	Shoot fresh weight (g/plant)			Root fresh weight (g/plant)		
	Control	Salt	% Reduction	Control	Salt	% Reduction
Altinbas	198.8 F	80.0 d	59.8	123.9 A	72.4 a	41.6
Citirex	241.5 E	76.0 d	68.5	72.4 B	25.5 c	65.5
Nun 9075/ Citirex	357.6 A	225.5 a	37.0	30.8 E	52.5 b	-70.2
Nun 9075/ Altinbas	301.0 C	185.6 c	38.3	43.1 D	53.7 b	-24.7
Kardosa/ Citirex	332.3 B	212.1 b	36.2	32.2 E	52.8 b	-64.0
Kardosa/ Altinbas	271.0 D	192.8 c	28.8	50.9 C	56.2 b	-10.3
F Test	Genotype	Salt	Gen.xSalt	Genotype	Salt	Gen.xSalt
	***	***	***	***	***	***

The results of the main stem length and root length at the end of the growing cycle are shown in Table 3. The main stem length and root length of the grafted and un-grafted melon plants at the end of the growing cycle varied under different salt levels. The grafted and un-grafted plants grown in the hydroponic, salt conditions were noticeably smaller and less vigorous than the plants grown in the non-saline conditions. Main stem length was significantly ($P < 0.01$) affected by grafting, different salt levels and grafting x salt interaction. The grafted plants had significantly longer stem length as

compared to un-grafted plants under saline conditions. Under control conditions, the longest plant main stem length was measured in Altınbas, while the shortest was measured in Nun 9075/ Citirex and the control plant of Citirex. Salinity decreased the main stem length of Kardosa/ Altınbas and Citirex plants by 31.3 and 57.9 %, respectively. Conversely, the graft combination of Kardosa/ Altınbas has significantly longer stem length as compared to the un-grafted plants of Citirex under salt conditions. As the salinity level increased, plant main stem length decreased [70]. It has been reported that salinity causes reduction at stem length parameter in okra plants [71]. In our study, similar results were also detected (Table 3). Our results are in line with those of [39] who reported that the grafted watermelon plants have significantly longer stem lengths than un-grafted watermelon plants under salt stress conditions.

Regarding the root length, root length was significantly affected ($P < 0.01$) by grafting and grafting x salt interaction, but not in different salt levels. Under control conditions the graft combination of Nun 9075/ Citirex has significantly higher root length; while the un-grafted plant of Altınbas produced lower root length. Under saline conditions, the graft combination of Kardosa/ Citirex has significantly higher root length; while the un-grafted plant of Citirex produced lower root length. Salinity causes a decline in root length between -44.6% and 24.7% depending on the rootstocks. The root length was ranked between 28.3 m/plant and 69.8 m/plant under salinity. The graft combination of Kardosa/Citirex (69.8 m/plant) has significantly higher root length, while the un-grafted plant Citirex (28.3 m/plant) produced lower root length. Salt stress reduces transpiration and respiration as well as water uptake and root development [72]. Likewise in our study, [39] observed that salt stress caused a reduction in root length depending on genotypes as compare to control plants in watermelon plants.

Table 3. The effects of graft combination and different salt levels (1 dS/m and 8 dS/m) on main stem length and root length of melon plants. Values denoted by different letters (upper and lower case letters for control and salt treatment, respectively) are significantly different between genotypes within columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Graft combination	Main stem length (cm/plant)			Root length (m/plant)		
	Control	Salt	% Reduction	Control	Salt	% Reduction
Altınbas	89.2 A	40.3 c	54.8	32.7 D	41.2 c	-26.0
Citirex	70.0 D	29.4 d	57.9	37.0 CD	28.3 d	23.5
Nun 9075/ Citirex	70.1 D	41.4 c	40.9	75.9 A	57.1 b	24.7
Nun 9075/ Altınbas	74.1 CD	47.0 b	36.6	52.6 B	45.4 c	13.6
Kardosa/ Citirex	76.3 BC	49.6 b	34.9	48.2 B	69.8 a	-44.6
Kardosa/ Altınbas	80.2 B	55.1 a	31.3	41.5 C	55.8 b	-34.5
F Test	Genotype ***	Salt ***	Gen.xSalt ***	Genotype ***	Salt ns	Gen.xSalt ***

Conclusions

Our study provides evidence that melon plants grafted onto different commercial *Cucurbita maxima* x *C. moschata* hybrid rootstocks and ungrafted plants performed differently under stress caused by saline conditions. The graft combination of Nun 9075/ Citirex was found more tolerant to salinity than ungrafted melon plants, and the other graft combinations used in regard to investigated parameters. Grafting onto salt tolerant *Cucurbita maxima* x *C. moschata* hybrid rootstocks can improve the growth performance of melon plants under salt stress. Future research is planned to study how suitable scion/ rootstock combinations under saline conditions affect fruit yield and quality parameters.

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