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Rootstock effects on alkali stressed melon plants

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ABSTRACT

The aim of the present study was to determine alkalinity tolerance of gourd rootstocks grafted with melon and to examine physiological and morphological response of the grafted plants under different pH levels. The climate chamber experiment was carried out to determine main stem length, leaf chlorophyll content (SPAD), branching number, shoot and root fresh mass, as well as root length. Under climate chamber conditions, two melon cultivars [Galia type (Çıtırex F_1) and Kırkağaç Manisa Altınbaş type (PI-169303 open pollinated)] were grafted onto two different commercial *Cucurbita maxima* × *C. moschata* hybrid rootstocks (Kardosa and Nun 9075) and grown in 8L pots filled continuously with aerated nutrient solution under two different pH levels (7 and 9) with three replications. The results indicated that grafted and ungrafted plants were significantly (P<0.001) affected by pH levels regarding leaf chlorophyll content (SPAD), branching number, shoot and root fresh mass, as well as root length, whereas there were no significant differences observed regarding main stem length. Grafted plants had better growth performance than ungrafted plants under both control and high pH conditions. Shoot fresh mass of the ungrafted and grafted plants significantly decreased as pH level increased, whereas root fresh mass of the ungrafted and grafted plants significantly increased under high pH level. Branching number was significantly (P<0.001) affected by grafting combinations and pH levels. Leaf chlorophyll content (SPAD) of the ungrafted and grafted plants significantly decreased as pH level increased, whereas branching number of the grafted plants significantly increased under high pH level. These results suggested that use of interspecific *Cucurbita* hybrid rootstocks could improve crop performance in melon plants under alkaline conditions.

Key words: *Cucurbita maxima*, *cucurbita moschata*, *Citrullus lanatus*, alkalinity, grafting.

INTRODUCTION

At the agricultural production one of the main factors that determining physicochemical state of the soil and soil fertility is soil pH. When soil pH is between 4.0 and 7.0, it characterized as acidic soil conditions. When soil pH is between 6.5 and 7.0, it characterized as neutral soil conditions. And when soil pH is between 7.0 and 10.0, it characterized as alkaline soil conditions. When crop cultivated on alkaline soils, crop yields are often poor or changeable. The main ions that cause alkalinity are bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. Because alkaline soils, especially those with free lime, can contain high concentrations of bicarbonate that detrimentally affects plant growth either directly or indirectly by decreasing the solubility and availability of nutrients (Valdez-Aguilar and Reed, 24; Roosta, 20). Bicarbonate ions interfere among the uptake of macro elements, in particular phosphorus (P), potassium (K), and magnesium (Mg) (Pissaloux *et al*., 15). Due to the formation of metal complexes [e.g. calcium (Ca)–P and Mg–P], P is largely unavailable to plants in alkaline soils. Additionally, HCO_3^- concentration interacts strongly with the availability of various micro ions, especially Fe^{2+}, leading to serious yield and quality losses (Colla *et al*., 4). One way to avoid or reduce losses in production caused by alkalinity in high-yielding genotypes would be to graft them onto rootstocks capable of reducing the detrimental effect of external pH on the shoot (Colla *et al*., 4). 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.

Grafting on vegetables was first performed in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks (Lee, 9). Vegetable grafting is an inventive technique for the appropriate cultivation of fruit-bearing vegetables (tomatoes, bean, eggplant, cucumber, melon, and watermelon) in Japan, Korea, the Mediterranean basin, and several European countries (Pogonyi *et al*., 16), where continuous cropping is a common practice and land use is very intensive (Khah *et al*., 7). 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 (Bletsos *et al*., 2; Oka *et al*., 13), to improve fruit quality (Davis et al., 5), to

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increase the absorption of nutrients and the mineral content in the aerial portion of the plant, to confer tolerance to high and low temperatures (Rivero *et al*., 19), drought, salt, flooding, etc. (Yetisir and Uygur, 26; Colla *et al*., 4; Schwarz *et al*., 21), and to improve plant vigour and the post-harvest lifetime of the fruits (Lee and Oda, 10). Up to now several studies have been conducted in alkaline conditions in many crop species, whereas limited information is available about effects of the grafting on melon plants under alkaline conditions (Colla *et al*., 4). Therefore the aim of the present study was to determine whether alkalinity tolerance could be improved by grafting of melon onto gourd rootstocks and to examine the grafted plants based on the physiological and morphological response mechanisms under different pH levels.

MATERIALS AND METHODS

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 (Cıtırex F_1) and Kırkağaç Manisa Altınbaş type (PI-169303 open pollinated)] were used as scion and two different commercial *Cucurbita maxima* × *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 one or two true leaves, scions were grafted onto the rootstocks. The ungrafted 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 (Lee *et al*., 11). 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 pH levels (7 and 9). 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 ($Ca(NO_3)_2$,) 250 µM monopotassium phosphate (KH_2PO_4), 500 µM potassium sulfate (K_2SO_4), 325 µM magnesium sulfate ($MgSO_4.7H_2O$), 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 replaced 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.

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 weighted (g/plant). Branching number was counted and recorded as BN/plant. The root length of the grafted and ungrafted 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 mass.

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 performed at the centre of the leaves on the fully expanded youngest leaf for each treatment.

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.

RESULTS AND DISCUSSION

Branching number and leaf chlorophyll index (SPAD): At the end of the experiment generally plant growth was negatively affected by increasing the pH level of the nutrient solutions. Branching number of the grafted and non grafted plants differed in response to solution pH. Growth performance of the plants was dependent on genotype of scion and rootstock and pH level. The results of the branching number and leaf chlorophyll determined as SPAD readings at the end of the growing cycle of graft combinations and control plants in different pH levels (7 and 9) was shown in Table 1. The results indicated that grafted and ungrafted plants were significantly affected by different pH levels regarding branch number ($P<0.001$) and leaf chlorophyll index (SPAD) ($P<0.01$). In both grafted and ungrafted plants of branch number decreased consistently with increased solution pH. Under control conditions, the graft combinations of Kardosa/ Cıtırex and

Table 1. Effects of graft combinations and pH levels (7 and 9) on branching number and leaf chlorophyll index of melon plants.

Graft combination	Branching number (BN/plant)			Leaf chlorophyll index (SPAD)		
	pH 7	pH 9	% Reduction	pH 7	pH 9	% Reduction
Altınbas	1.2 d	1.0 c	16.0	37.8 bc	36.5 b	3.7
Cıtırex	2.2 c	1.7 bc	23.5	37.1 c	36.1 b	2.7
Nun 9075/ Cıtırex	4.1 a	2.7 a	35.1	42.11 a	40.11 a	4.9
Nun 9075/ Altınbas	3. 1 b	2.8 a	10.7	40. 71 a	39.9 a	1.9
Kardosa/ Cıtırex	4.0 a	3.0 a	25.0	40.5 ab	39.8 a	1.9
Kardosa/ Altınbas	3.0 b	2.5 ab	16.3	40.1 ab	40.0 a	0.3
LSD (0.05)	***	**		**	**	

Statistics: t test, means with the same letter, denote **and ***significant differences at p< 0.01 and 0.001.

Nun 9075/ Cıtırex have significantly higher branch number, whereas ungrafted plants of Cıtırex and Altınbas have the minimum branch number. On the other hand, the graft combination of Kardosa/ Cıtırex, Nun 9075/ Altınbas and Nun 9075/ Cıtırex have significantly higher branch number as compare to the ungrafted plants under alkaline conditions. Compared to the unstressed plants, alkaline conditions decreased the branch number of Nun 9075/ Altınbas and Nun 9075/ Cıtırex plants by 10.7 and 35.1 %, respectively (Table 1). These phenomena might consequence from ion imbalance, nutritional damage, and metabolic disorders caused by alkali stress (Li *et al*., 12; Yang *et al*., 25).

Regarding leaf chlorophyll index, under control conditions the graft combination of Nun 9075/ Cıtırex and Nun 9075/ Altınbas have significantly higher SPAD value, while the ungrafted plants of Cıtırex has significantly lower SPAD value. On the contrary, under alkaline conditions significantly higher SPAD value was produced when Kardosa and Nun 9075 were used as rootstock as compare to the ungrafted plants of Altınbas and Cıtırex (Table 1). Likewise in the current study, the decline in the values of leaf chlorophyll content in lettuce plants at high solution pH was also observed by Roosta, 20.

Biomass production and partitioning: Results of shoot and root fresh mass of the grafted and ungrafted plants grown in different pH levels (7 and 9) at the end of the growing cycle were shown in Table 2. Since the pH level in nutrient solution increased plant growth significantly decreased. The results indicated that grafted and ungrafted plants were significantly (P<0.001) affected by different pH levels regarding shoot and root fresh mass. Fresh matter of the shoot decreased by different rates in each genotype under high pH level conditions. The grafted plants had significantly higher shoot fresh mass as compare to ungrafted plants under both neutral and high pH conditions. In the grafted and ungrafted melon plants shoot fresh matter decreased in response to an increase of pH level in the nutrient solution. Under control conditions, the grafted plants of Nun 9075/ Cıtırex and Kardosa/ Cıtırex have significantly higher shoot fresh matter, though the ungrafted plants of Cıtırex and Altınbas have lower shoot fresh matter. Conversely, the grafted plants onto the Kardosa and Nun 9075 rootstocks have significantly higher

Table 2. Effects of graft combinations and pH levels (7 and 9) on shoot and root fresh mass of melon plants.

Graft combination	Shoot fresh mass (g/plant)			Root fresh mass (g/plant)		
	pH 7	pH 9	% Reduction	pH 7	pH 9	% Reduction
Altınbas	71.1 d	57.3 c	19.3	127.0 a	201.2 a	-57.8
Cıtırex	80.5 c	73.3 b	8.9	72.1 b	106.6 b	-47.7
Nun 9075/ Cıtırex	106.5 a	91.0 a	14.2	30.9 d	54.7 d	-77.3
Nun 9075/ Altınbas	95.8 b	83.7 a	12.6	43.1 cd	63.9 c	-48.3
Kardosa/ Cıtırex	110.0 a	89.1 a	19.3	32.2 d	54.9 d	-70.4
Kardosa/ Altınbas	92.7 b	86.1 a	7.1	50.6 c	71.6 c	-41.3
LSD (0.05)	***	***		***	***	

Statistics: t test, means with the same letter, denote ***significant differences at p< 0.001

shoot fresh matter as compare to the ungrafted plants under high pH level conditions. As compared to the unstressed plants, alkalinity decreased the shoot fresh matter of Kardosa/ Altınbas and Altınbas plants by 7.1 and 19.3%, respectively (Table 2). Up to now in many studies are observed that crop plants react to elevated concentrations of $NaHCO_3$ in growing medium solution or in soil with decreased shoot growth (Campbell and Nishio, 5). This might be due to either HCO_3^- or Na^+ (Pearce *et al*., 14). Inhibition of shoot growth at high solution pH is related with a decline in number of leaves, fresh and dry biomass, and shoot elongation (Valdez-Aguilar, 23). The decline in shoot fresh mass was observed in grafted watermelon plants (Colla *et al*., 4). Likewise, in the current study, considerable depressions in shoot biomass production in grafted melon plants were observed under alkaline conditions and that effect differed as a function of rootstock (Table 2). Decreased shoot growth might be attributed to a low photosynthetic rate occurring in the HCO_3^- induced leaves. A lower photosynthetic rate results from impaired chlorophyll synthesis due to low translocation of Fe (Bavaresco *et al*., 1).

Concerning the root fresh mass, the results show that grafted and ungrafted melon plants varied differentially to the pH level of their root environments. Root fresh mass increased with increasing solution pH in both ungrafted and grafted plants. The ungrafted plants have greater root mass as compare to grafted plants under both neutral and alkaline conditions. The ungrafted Altınbas has significantly higher root biomass under alkaline conditions than all the graft combinations. The reason might be explained the high alkalinity stress which is restricted mineral nutrients in the root zone is exceeded to produce more root surface. 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 (Rengel, 18). To provide more Ca elements in plant tissues, the more root mass was occurred in grafted plants that is formed by ungrafted control plants.

The increase in the root fresh mass ranged between 77.3 and 41.3% (Table 2). The root dry mass also increased in ungrafted cucumber plants as compared to grafted ones under alkaline conditions (Tachibana, 22). Conversely, higher root dry mass in grafted watermelon plants than ungrafted plants was observed under alkaline conditions (Pulgar *et al*., 17). Up to now in many articles 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 depending on the increment of root hairs, and root length and to acquire more water and mineral elements from soil and transfer them to aerial parts of the plant (Kovalev and Lisovskaya, 8).

The results of the main stem length and root length at the end of the growing cycle are presented in Table 3. The main stem length and root length of the grafted and ungrafted melon plants at the end of the growing cycle varied under different pH levels. The grafted and ungrafted plants grown in alkaline conditions were noticeably smaller than the plants grown in the neutral conditions, however there were no significant differences found within graft combinations regarding main stem length. The grafted plants had longer stem lengths as compare to ungrafted plants under alkaline conditions, whereas we have observed that the alkalinity had no significant effect on main stem length. Stem length was ranged in between 70 and 86 cm/plant under neutral pH conditions, whereas it was ranged in between 59 and 79 cm/plant under alkaline conditions (Table 3).

Regarding the root length, the results indicated that grafted and ungrafted plants were significantly ($P<0.001$) affected by different pH levels. The results show that the root lengths followed a similar pattern to root fresh mass in response to solution alkalinity. Root length of grafted and ungrafted plants increased under alkaline conditions as compare to neutral conditions. The increase in the root length was ranged between 34.3 and 143.6%. The ungrafted Altınbas has significantly higher root length under both neutral and alkaline conditions. The root length was ranged between 10047 cm/plant and 15345 cm/plant under alkalinity (Table 3). Similar to the present study, sugar beet plants also increased root thickness, and a higher production of lateral roots was observed a few days after Fe deficiency and HCO_3^- treatments started (Campbell and Nishio, 3).

In conclusion, our study suggests that, in response to an increase of alkalinity in the nutrient solution shoot biomass, leaf chlorophyll index (SPAD), main stem length and branching number were affected negatively in both grafted and ungrafted plants, whereas root biomass increased positively under alkaline condition. Different melon cultivars grafted onto different commercial interspesific *Cucurbita* hybrid (*Cucurbita maxima* x *C. moschata)* rootstocks confirmed better performance than ungrafted melon plants under alkaline conditions. Furthermore, in response to alkalinity, the current study exposed to capable of maintaining better vegetative growth, considerable differences in the agronomical and physiological responses among graft combinations. These results suggest that the use of interspecific

Table 3. Effects of graft combinations and pH levels (7 and 9) on main stem length and root length of melon plants.

Graft combination	Main stem length (cm/plant)			Root length (cm/plant)		
	pH 7	pH 9	% Reduction	pH 7	pH 9	% Reduction
Altınbas	86.0	59.1	31.3	11424 a	15345 a	-34.3
Cıtırex	79.3	64.2	19.0	8385 b	14341 ab	-71.0
Nun 9075/ Cıtırex	70.1	74.2	-5.9	7088 bc	10047 d	-41.7
Nun 9075/ Altınbas	75.0	74.3	0.9	7558 b	11026 d	-45.9
Kardosa/ Cıtırex	71.6	64.7	9.6	5146 d	12101 c	-135.1
Kardosa/ Altınbas	83.3	78.6	5.7	5626 cd	13706 b	-143.6
LSD (0.05)	ns	ns		***	***	

Statistics: t test, means with the same letter and ns are non significant, denote ***significant differences at p< 0.001

Cucurbita hybrid rootstocks can improve crop performance in melon plants under alkaline conditions.

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