

EFFECTS OF SALINITY STRESS ON CHLOROPHYLL AND CAROTENOID CONTENTS AND STOMATA SIZE OF GRAFTED AND UNGRAFTED GALIA C8 MELON CULTIVAR

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Abstract

Salinity is known as the most important abiotic stress that decreases crop production and plant growth, and changes the anatomy and morphology of plants. In this study, the growth rate of grafted and ungrafted melon plants were studied under salinity stress. Maximus F₁, Shintoza F-90 F₁ and Nun 9075 F₁ (*Cucurbita maxima* x *Cucurbita moschata*) were used as a rootstock and Galia C8 melon cultivar was used as a scion. In this study, the stomata size and chlorophyll and carotenoid contents were investigated. According to the results, chlorophyll and carotenoid contents and stomata length and width of upper and lower surface of leaf were generally reduced under salinity stress.

Key words: Grafting melon, Salinity stress, Stomata size, Pigments.

Introduction

The melon cultivation is intensive in Turkey. But the melon cultivation is restricted by monoculture agriculture and soil-borne pests and diseases such as *Fusarium* spp. and nematodes in the melon cultivation area. In recent years, the applications of pesticides have been reduced in view of the importance of organic farming. One of ways to reduce the applications of pesticides is to use the soil-borne pest and disease resistant rootstocks. For this purpose, especially hybrids of *Cucurbita maxima* and *Cucurbita moschata* are used as rootstocks in melon cultivation. Because the rootstocks are resistant to biotic and abiotic stress, the production with grafted seedlings has been increased. The melon production was about 1.7 million tons in Turkey in 2015 (Anon., 2015). Some characteristics as plant height, leaf damage, yield, survival, vigor have been used to identify salinity tolerance criteria (Sahannon, 1984; Gama *et al.*, 2007).

The problem of soil salinity which is likely to increase in the future as a result of global warming has attracted the researchers to focus on this important issue. The use of grafted plants will be important in resistance to salinity stress.

In Turkey, biotic and abiotic factors are limiting crop production as well as in other producing countries. Therefore, in recent years, the studies have been concentrated on finding out of genotypes which are resistant to drought and salinity stress, and ensuring better growing in these circumstances (Yasar, 2003; Saruhan *et al.*, 2008; Dasgan & Koc, 2009; Kusvuran, 2004; Kusvuran *et al.*, 2007; Yagmur, 2008; Aktas, 2002). Sonmez (1990) reported that 4 million hectares of land was affected by salt in Turkey. Çevik (1986) reported that the total area of land in Turkey is 78 million hectares; 36% of this land is cultivated, and 3.2% of 36% has salinity problems.

There are reports that grafted melons increased plant growth, total and early yield; the tolerance to low temperatures and resistance to pests and diseases in soil; and prolonged the plant's economic life by uptaking water and nutrients. (Yarsi & Sari, 2006; Yarsi *et al.*, 2012; Yetisir & Sari 2003; Zijlstra *et al.*, 1994; Blanco & Folegatti, 2005; Edelstein *et al.*, 2005; Colla *et al.*, 2006; Dasgan & Akhoundnejad, 2012).

It has been reported that salinity reduces the plant's water uptake and root development. As a result of this, the hormonal balance is affected negatively; photosynthesis and protein synthesis and plant height are decreased as a result of the decrease in nitrate uptake; fresh and dry weight, consequently the number of flowers and yield is decreased. (Shalhevet & Bernstein, 1968; Robinson *et al.*, 1983; Çakırlar & Topcuoglu, 1985; Drew *et al.*, 1990; Aktas *et al.*, 2006; Kusvuran, 2010; Topaloglu, 2010; Kulak, 2011; Ozturk *et al.*, 2004).

Some researchers also reported that the salt stress causes a reduction in the amount of chlorophyll. (Akca & Samsunlu, 2012; Dhanapackiam & Muhammad İlyas, 2010; Molazem *et al.*, 2010; Sevengör *et al.*, 2011; Zhani *et al.*, 2012; Turan *et al.*, 2007; Roupael *et al.*, 2008). It is also reported that stomata size and the number of the plants are affected by stress factors such as drought, salt and heavy metal (Özyigit & Akıncı, 2009; Jafri & Ahmad, 1995; Çavuşoğlu *et al.*, 2007). Melons have stomata which are smaller size and more amount of stomata under the salt stress (Solmaz *et al.*, 2011).

The genotypes resistant to salinity which are used as rootstock will be important in future to combat from adverse effects of global warming. In this study, the effects of salt stress on stomata size and amount of chlorophyll and carotenoids of grafted and ungrafted melon plants were investigated.

Materials and Methods

Plant materials: To determine the growth performance under salinity stress, 3 squash rootstock (Maximus F₁, Shintoza F-90 F₁ and Nun 9075 F₁) were used as rootstock and Galia C8 melon varieties was used as a scion. The grafting procedures were performed in Antalya Seedling Company and Grow Seedlings Company. Grafted seedlings were planted in plastic pods which were filled with peat: perlite by 2: 1 ratio. The pods size were 72cmx19.5cm x 15.5 cm (14 liter; No:4). The grafted and ungrafted plants were exposed to a salt concentration of 50 mM NaCl for 5 days and 100 mM NaCl for 5 days then the final concentration of 150 mM NaCl was applied for 10 days. The salt treatments were started when the plants had 3-4 true leaves and the treatment was given every day (Table 1).

The study was carried out at about 26-30°C during the day and 16-20°C night temperature in plastic greenhouse. The humidity inside the greenhouse was measured as an average of 55-65% in the same period. For each replicate, 4 plants in one pot were used as one replication and for salt and control treatments 3 replicates were used separately. Plants were watered with nutrient solution having 177.2 ppm N; 52.70 ppm P, 240.44 ppm K, 53.46 ppm Mg, 120.30 ppm Ca; 3.36 ppm Fe; 0.85 ppm Mn; 0.45 ppm B; 0.50 ppm Zn; 0,10 ppm Cu and 0.05 ppm Mo (Suyum *et al.*, 2012).

Table 1. The source of rootstocks and scion

| Rootstocks | Company | |
|------------------------------|-----------------|---------------------------------------|
| Maximus F ₁ | Agromar | <i>C. maxima</i> X <i>C. moschata</i> |
| Shintoza F-90 F ₁ | Fito tohumculuk | <i>C. maxima</i> X <i>C. moschata</i> |
| Nun 9075 F ₁ | Nunhems | <i>C. maxima</i> X <i>C. moschata</i> |
| Scion | | |
| Galia C8 | Hazera | Melon (<i>Cucumis melo</i> L.) |

Grafting: Tube grafting method was used. The grafting was done when rootstocks and scions created the first true leaves.

Planted: Seedlings were planted in equally sized plastic pots which contained a perlite-peat (2:1) mixture. After planting, the leaves of the rootstock were taken from time to time and it was permitted only the growth of the scion.

Stomata size: Leaves from the plants (in the same period and were taken from the same location from each plant) were put in plastic bottle filled by 70% ethyl alcohol. Then, the cross-sections of the upper and lower of the leaves were placed in lamellar. Stomatal length and width of upper and lower parts of the leaves was measured with a light microscope. Photographs were also taken.

Determination of total chlorophyll a, chlorophyll b and carotenoids: One gram of leaf samples taken from melon varieties extracted in acetone and filtered. Then, samples were centrifuged at 3000 rpm (De Kok & Graham 1989). 662 nm and 645 nm absorbance for total chlorophylls, and 470 nm for carotenoids were read on spectrophotometer (Schimadzu UV-1800) and the amounts were calculated according to Lichtenthaler & Wellburn (1983). The analyses were repeated three times.

Table 2. Stomata size of upper and lower leaf surfaces and % changing compare to the control of grafted and non-grafted melon plants grown under salinity stress (150 mM NaCl)

| lower leaf surfaces | Rootstocks | | Control Plants | | Salt application | | |
|--|------------|------------|----------------|------------|------------------------------|-------------|------------------------------|
| | Width (µm) | length(µm) | Width (µm) | length(µm) | % change relative to control | length (µm) | % change relative to control |
| Ungrafted | 15.00±0.6 | 19.35±0.8 | 12.44±0.9 | 19.22±1.0 | -17.06 | 19.22±1.0 | -0.67 |
| Maximus F ₁ /Galia C8 | 15.76±0.9 | 19.61±1.0 | 15.01±1.3 | 17.79±1.2 | -4.76 | 17.79±1.2 | -9.28 |
| Shintoza F-90 F ₁ /Galia C8 | 15.27±1.2 | 20.21±1.0 | 15.02±0.9 | 19.12±1.5 | -1.64 | 19.12±1.5 | -5.39 |
| Nun 9075 F ₁ /Galia C8 | 14.87±1.4 | 19.26±1.0 | 14.17±1.4 | 17.87±1.1 | -4.71 | 17.87±1.1 | -7.21 |
| Upper leaf surfaces | Width (µm) | length(µm) | Width (µm) | length(µm) | % change relative to control | length (µm) | % change relative to control |
| Ungrafted | 15.57±1.1 | 20.15±1.3 | 15.08±0.9 | 21.57±0.6 | -3.15 | 21.57±0.6 | +7.05 |
| Maximus F ₁ /Galia C8 | 15.41±1.3 | 20.95±1.2 | 14.68±1.2 | 19.70±1.1 | -4.74 | 19.70±1.1 | -5.97 |
| Shintoza F-90 F ₁ /Galia C8 | 15.47±0.9 | 20.65±1.2 | 14.76±0.8 | 17.54±1.4 | -4.59 | 17.54±1.4 | -15.06 |
| Nun 9075 F ₁ /Galia C8 | 14.53±0.8 | 20.18±1.2 | 14.84±1.4 | 20.26±1.4 | +2.13 | 20.26±1.4 | +0.39 |

Data collection and statistical analysis: All of the analyses were repeated thrice. To determine the differences between means were used *t*-test (with significance at $p < 0.05$).

Results and Discussion

Stomata Size: The lower leaf surface's length and width were reduced in varying proportions under salt stress (Table 2). This decrease was 17.06% of stomatal width in ungrafted plants while stomatal length was 0.67%. This decrease in grafted plants for stomata width were *Maximus F₁/Galia C8* (4.76%), *Shintoza F-90 F₁/Galia C8* (1.64%) and *Nun 9075 F₁/Galia C8* (4.71%), respectively. Stomata's length was decreased, too. The data in Table 2 shows that the stomatal length of lower leaf surface was a contraction of between 0.67% and 9.28%.

The data (Table 2) show that the stomata size of upper leaf surface were generally affected by salt stress on both grafted and non-grafted plant; but the salt stress has increased the stomata width and length by 2.13% to 0.39% of *Nun 9075 F₁/Galia C8*. While the reduction in width was 3.15% in un-grafted plants, the increase was 7.05% in the stomata length.

The decrease in stomatal size of plants can be explained as an adaptation mechanism to stress conditions. In a study carried out by Solmaz *et al.* (2011), it was reported that the stomata size was smaller and the number of stomata was increased in melon under salt stress. It was stressed that plants created a protection mechanism against salt stress (Fig. 1).

Chlorophylls and carotenoids: Data (Table 3) shows that chlorophyll *a*, chlorophyll *b* and carotenoid contents are decreased due to salt stress. Salt stress decreased the amount of chlorophyll *a* and *b* in both grafted and ungrafted plants. While chlorophyll *a* content of *Maximus F₁/Galia C8* was 13.95 mg g⁻¹ FW ($p < 0.05$), as receiving the highest value, the lowest value was with *Nun 9075 F₁/Galia C8* (12.00 mg g⁻¹) and ungrafted (12.02 mg g⁻¹) under salinity ($p < 0.05$). Chlorophyll *b* contents were the highest in *Maximus F₁/Galia C8* (3.61 mg g⁻¹ FW) and in *Shintoza F-90 F₁ C8* (3.64 mg g⁻¹ FW) under salinity. When the value of carotenoids

was observed, the salt applications did a difference, statistically. *Nun 9075 F₁/Galia C8* grafted combination was the highest value of carotenoids with 4.55 mg g⁻¹ FW ($P > 0.05$). In similar studies, it is reported that salt stress reduced chlorophyll content (Sevengör *et al.*, 2011) and Zhani *et al.*, 2012 in pepper (*Capsicum frutescens* L.) Akca & Samsunlu (2012) in walnut. Yasar *et al.* (2008) in green bean, Malik *et al.* (2010) in cucumber.

The changing percentage of chlorophyll *a* was -31.33% for ungrafted, -22.02% for *Maximus F₁/Galia C8*, -27.31% for *Shintoza F-90F₁/Galia C8* and -34.78% for *Nun 9075 F₁/Galia C8*, whereas changing percentage of chlorophyll *b* were -35.56% for ungrafted, -15.20% for *Maximus F₁/Galia C8*, -27.63% for *Shintoza F-90F₁/Galia C8* and -22.96% for *Nun 9075 F₁/Galia C8* and the change percentage of carotenoids content were -23.10% for ungrafted, -18.51% for *Maximus F₁/Galia C8*, -29.35% for *Shintoza F-90F₁/Galia C8* and -15.22% for *Nun 9075 F₁/Galia C8*.

Table 3 shows that chlorophyll *a* and chlorophyll *b* of *Maximus F₁/Galia C8* grafted plants combination were less affected and carotenoids in *Nun 9075 F₁/Galia C8* combination was less affected by salt stress

Conclusion

It is concluded that salinity had adverse effect not only on the amount of chlorophyll and carotenoids, but also on stomatal size of lower and upper leaf surfaces. It was found that the salinity stress decreased chlorophyll *a*, chlorophyll *b* and carotenoid contents of both grafted and un-grafted plants. But the un-grafted plants generally were more effected than grafted plants (except *Nun 9075 F₁/Galia C8* for chlorophyll *a* and *Shintoza F-90F₁/Galia C8* for carotenoids). The regional genotypes which are resistant to salinity should be investigated and the studies must be done on rootstocks characteristics in melons.

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Table 3. Chlorophyll *a*, chlorophyll *b* and carotenoids contents of grafted and non-grafted melon plants grown under salinity stress (150 mM NaCl).

| | chlorophyll <i>a</i> | | chlorophyll <i>b</i> | | carotenoids | |
|--|------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | no salt | salt | no salt | salt | no salt | Salt |
| Ungrafted | 17.81±0.2 ^a | 12.02±0.9 ^b | 4.96±0.5 ^a | 3.33±0.5 ^b | 5.02±0.4 ^a | 3.76±0.2 ^b |
| <i>Maximus F₁/Galia C8</i> | 17.83±0.8 ^a | 13.95±0.4 ^b | 5.02±0.4 ^a | 3.61±0.6 ^a | 5.51±0.5 ^a | 4.26±0.2 ^a |
| <i>Shintoza F-90F₁/Galia C8</i> | 17.65±0.5 ^a | 12.83±0.9 ^b | 5.03±0.2 ^a | 3.64±0.3 ^a | 5.87±0.1 ^a | 4.08±0.1 ^a |
| <i>Nun 9075 F₁/Galia C8</i> | 18.40±0.8 ^a | 12.00±1 ^b | 4.52±0.4 ^a | 3.40±0.7 ^a | 5.61±0.4 ^a | 4.55±0.3 ^a |

Means followed by the same letter are not significant according to *t*-test (confidence limit 95 %).

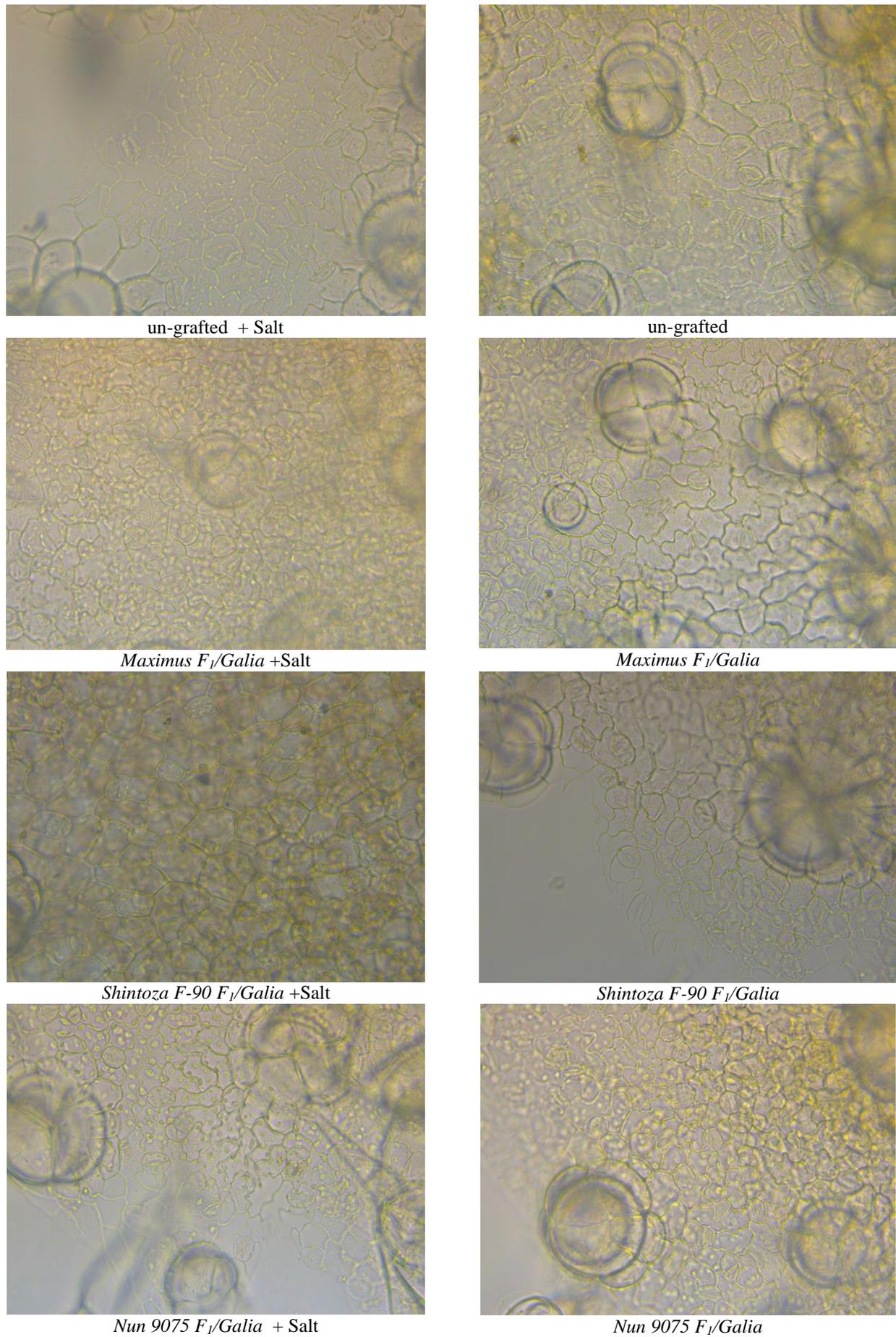


Fig. 1. Stomata photos of lower surface grafted and ungrafted Galia C8 melon under salt stress and control plants.

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