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Research Article

Shading Alleviates Damage of Short Term Salinity and Protects Leaf Pigments in Sweet Cherry

Gölgelemenin Kirazda Kısa Süreli Tuzluluğun Zararını Azaltması ve Yaprak Pigmentlerini Koruması

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Abstract: Salt stress negatively influences fruit yield and quality in trees. In addition to environmental stresses, excess energy from sunlight possesses harmful effects on plant metabolisms. Here we reveal the effects of shading on short term salinity stress in cherry plants. Salinity and shading treatments were introduced approximately two months after planting. Three shading levels (40%, 60%, and 80%) were applied. To induce moderate salinity stress, 35 mM NaCl (sodium chloride) was utilized for one month. Many morphological and physiological aspects and chlorophyll metabolism were evaluated. We found that the plant growth, stomatal conductance and chlorophyll biosynthesis were significantly retarded under salinity during a month. However, shading treatments alleviated the salinity damage. Moreover, salt stress hindered the biosynthesis of chlorophyll at Mg-Proto IX step. Shading treatments mitigated salinity damage associated with decreasing air and leaf temperature and preventing the loss of chlorophyll and the precursors and increasing anthocyanins. The results of the study showed that shading treatments possessed a protective effect on short term salinity in cherry plants.

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1. Introduction

Salt stress represents a detrimental environmental factor that constrains plant growth and productivity. Numerous plants are adversely affected by salinity, often exacerbated by excessive fertilization and inadequate drainage (Aras and Eşitken, 2018). Sweet cherry is known as salt-sensitive (Aras and Eşitken, 2019a; Aras et al., 2019). Salinity causes ion toxicity, decline in mineral uptake, and growth depression in sensitive plants (Ghaffari et al., 2014). Malignant influences of salt stress depend on the period of salt exposure (Vigo et al., 2005). In previous experiments, we stated that short term salinity led decline in leaf chlorophyll, water content and stomatal conductance in cherry (Aras and Eşitken 2018; 2019b) and apple plants (Aras and Eşitken, 2019a). Salt stress also hampers chlorophyll biosynthesis (Santos, 2004) and nitrogen metabolism (Yang et al., 2013).

Leaves cannot use photosynthetically active radiation (PAR) when exposed to stress factors. This excess energy must be dissipated, otherwise production of reactive oxygen species (ROS) increases which harms photosynthetic machinery (Pompelli et al., 2010). Plants reveal some mechanisms to dissipate excess energy that decrease photosynthetic capacity (Hichem et al., 2009; Wilhelm and Selmar, 2011). Neto et al. (2014) reported that salt-tolerant plants can dissipate excess photosynthetic energy that contributes to salinity tolerance. One defense mechanism to dissipate excess energy under stress conditions could be a decrease in environmental light conditions. Shezi et al. (2020) studied the photoprotection of avocado leaves between within and outside the canopy. They stated that optimum photoprotection was maintained under low light intensity. Da Silva et al. (2019) applied calcium particle films to promote the shading effect in grapevine and suggested that shading promoted photoprotection and alleviated thermal stress.

Several studies demonstrated that shading promotes plant growth and fruit quality (Dussi et al., 2005). Furthermore, shading treatments were used against water stress in many studies (Barradas et al., 2005; Nicolás et al., 2005). The impact of shading practices on salinity stress has been less investigated. Within the above-mentioned context, the current paper aims to reveal the effects of artificial shadings on salinity in sweet cherry plants. Many morphological and physiological aspects and chlorophyll metabolism were evaluated.

2. Material and Method

The experiment was conducted in a greenhouse in March of 2020. 1-year-old 0900 Ziraat/MaxMa 14 sweet cherry was utilized. About 2 months after planting in pots, salinity stress and shading treatments were initiated. Three levels of black colored net shadings (40, 60 and 80%) were used. All plants were watered with tap water until the beginning of the study and then during one month the treated plants were watered with a fertilizer solution (Hoagland's nutrient solution (Hoagland and Arnon, 1950) containing 35 mM NaCl and the untreated control plants were watered without NaCl (Aras and Eşitken, 2018; 2019b). The treatments were: no shading and no salinity as control (A), no shading + NaCl (B), shading with 40% + NaCl (C), shading with 60% + NaCl (D) and shading with 80% + NaCl (E). The plants were stressed for one month. The experiment was carried out following a randomized complete plot design with three replications and 5 plants per replication.

2.1. Morphological measurements

The assessment included the examination of relative growth rates for shoot diameters and shoot length. A digital caliper (Mitutoyo) was employed for measuring shoot diameter, while a ruler was used for determining shoot length. The calculation of relative growth rates (RGR) followed the formula established by Del Amor and Marcelis (2003).

2.2. Physiological measurements

Stomatal conductivity and leaf temperature were assessed using a leaf porometer (SC-1 porometer, Decagon Devices). Leaf relative water content (LRWC) was calculated according to the formula proposed by Smart and Bingham (1974).

Total phenolic content was determined according to the study of Singleton and Rossi (1965).

Chlorophyll a, b, and a+b, as well as carotenoid concentrations, were determined using the equations provided by Corte Real et al. (2017). The total anthocyanin concentration was computed utilizing the formula outlined by Mita et al. (1997).

2.3. Chlorophyll precursors

The assessment of chlorophyll precursor concentrations followed the methods outlined by Hodgins and Van Huystee (1986) and Liu et al. (2015). Chlorophyll yield was approximated using the ratio of chlorophyll a + b to Proto IX, as suggested by Aras et al. (2021).

2.4. In-situ detection of cell death

Cell death was detected through leaf staining with 0.025%. Evans blue, employing the methodology outlined by Ray et al. (2016) and Riaz et al. (2019) with certain adaptations.

2.5. Microclimate of experimental areas and soil EC

Light intensity was gauged using a luxmeter (CEM, DT-1308). The air temperature of the phyllosphere (on the leaf surface) beneath the nets and above the unshaded control plants was documented using a digital thermometer. Soil electrical conductivity (EC) was assessed following the methodology outlined by Richards (1954).

2.6. Statistical analyses

Statistical analyses were performed with the statistical software package SPSS, version 20.0. The data were compared by the Duncan multiple range test at the 5% level of significance.

3. Results

The investigation illustrated that shading enhanced salinity tolerance in cherry plants. Various plant parameters were assessed to unveil the protective effects of shading against salt stress.

3.1. Morphological responses

Salt stress caused a remarkable decrease in plant growth (Table 1). RGR of shoot diameter decreased by 69% in salinized plants compared to control. Salt treatment declined RGR of shoot length by 54% compared to the control and shading treatments increased RGR of shoot length compared to salinized plants.

Table 1. Effects of shading on plant growth of cherry under salinity condition

Treatments	RGR of shoot diameter	RGR of shoot length
Control	0.5713 ab	0.6360 a
Salt	0.1747 c	0.2923 b
%40 + Salt	0.5447 ab	0.4075 b
%60 + Salt	0.5044 b	0.4050 b
%80 + Salt	0.7695 a	0.3855 b

3.2. Physiological responses

Salinity and shading treatments had a significant impact on the physiological responses of cherry, as indicated in Table 2. The highest decline in stomatal conductance was found in salinized plants and shading treatments had higher stomatal conductance than salt treated plants. Leaf temperature decreased with increasing shading levels (60 and 80% shadings). LRWC value decreased by salinity and increased by shading with 60 and 80% treatments. The highest carotenoid content was found in salinized plants (Table 3). Shading treatments decreased carotenoid content.

Table 2. Effects of shading on stomatal conductance, leaf temperature and LRWC of cherry under salinity condition

Treatments	Stomatal conductance (mmol m^{-2} s^{-1})	Leaf temperature (°C)	LRWC (%)
Control	77.8 a	29.63 a	75.9 bc
Salt	63.4 c	29.40 ab	72.4 c
%40 + Salt	64.3 c	29.83 a	73.8 bc
%60 + Salt	72.0 ab	29.00 ab	76.8 b
%80 + Salt	69.0 bc	28.60 b	82.0 a

Table 3. Effects of shading on total carotenoid, anthocyanin and phenolics contents of cherry under salinity condition

Treatments	Carotenoid (mg L^{-1})	Anthocyanin (mg L^{-1})	Total phenolics (µg GAE 100 g^{-1} fw)
Control	10.92 a	9.06 c	0.0762 b
Salt	10.98 a	5.87 e	0.1049 a
%40 + Salt	9.06 b	9.66 b	0.0730 b
%60 + Salt	7.57 d	6.77 d	0.0549 c
%80 + Salt	7.92 c	10.15 a	0.0426 d

3.3. Chlorophyll and precursors in chlorophyll pathway

Salinity decreased chlorophyll content and shading treatments significantly increased chlorophyll compared to control (Table 4). In the presence of salinity, chlorophyll a, chlorophyll b, and chlorophyll a+b exhibited reductions of 26%, 25%, and 26%, respectively, as compared to the control. Shading treatments led to remarkable increases in chlorophyll a, b and a+b contents compared to salinized plants. Moreover, the contents of chlorophyll precursors were significantly influenced by treatments (Table 5). Salinized plants had higher Proto IX content than the control, however salinity caused decreased Mg-Proto IX and Pchlide contents. Shading with 80% possessed the highest Proto IX, Mg-Proto IX and Pchlide contents among treatments.

Table 4. Effects of shading on chlorophyll a, b, a+b contents of cherry under salinity condition

Treatments	Chlorophyll a (µg g^{-1} fw)	Chlorophyll b (µg g^{-1} fw)	Chlorophyll a+b (µg g^{-1} fw)
Control	16.07 a	5.29 a	21.39 a
Salt	11.83 e	3.99 b	15.84 d
%40 + Salt	13.08 d	4.04 b	17.13 c
%60 + Salt	13.76 c	4.13 b	17.89 bc
%80 + Salt	14.86 b	4.10 b	18.90 b

Table 5. Effects of shading on chlorophyll precursors of cherry under salinity condition

Treatments	Proto IX (µg g^{-1} fw)	Mg-Proto IX (µg g^{-1} fw)	Pchlide (µg g^{-1} fw)	Chl a+b/Proto IX
Control	0.0917 d	0.0574 c	0.0417 c	233.6 a
Salt	0.0999 c	0.0554 d	0.0363 e	155.3 c
%40 + Salt	0.1059 b	0.0476 e	0.0404 d	161.7 bc
%60 + Salt	0.1069 b	0.0643 b	0.0462 b	167.4 b

%80 + Salt	0.1157 a	0.0722 a	0.0536 a	163.2 bc

3.4. In-situ detection of cell death

The assessment of leaf cell death was conducted using the Evans blue staining method (Figure 1). No necrotic lesions were observed in the leaves of the control group, as well as in plants subjected to 60% and 80% shading. In plants treated with salt, necrotic spots appeared at the leaf edge and tip. Shading at 40% demonstrated fewer necrotic lesions in comparison to the plants exposed to salinity.

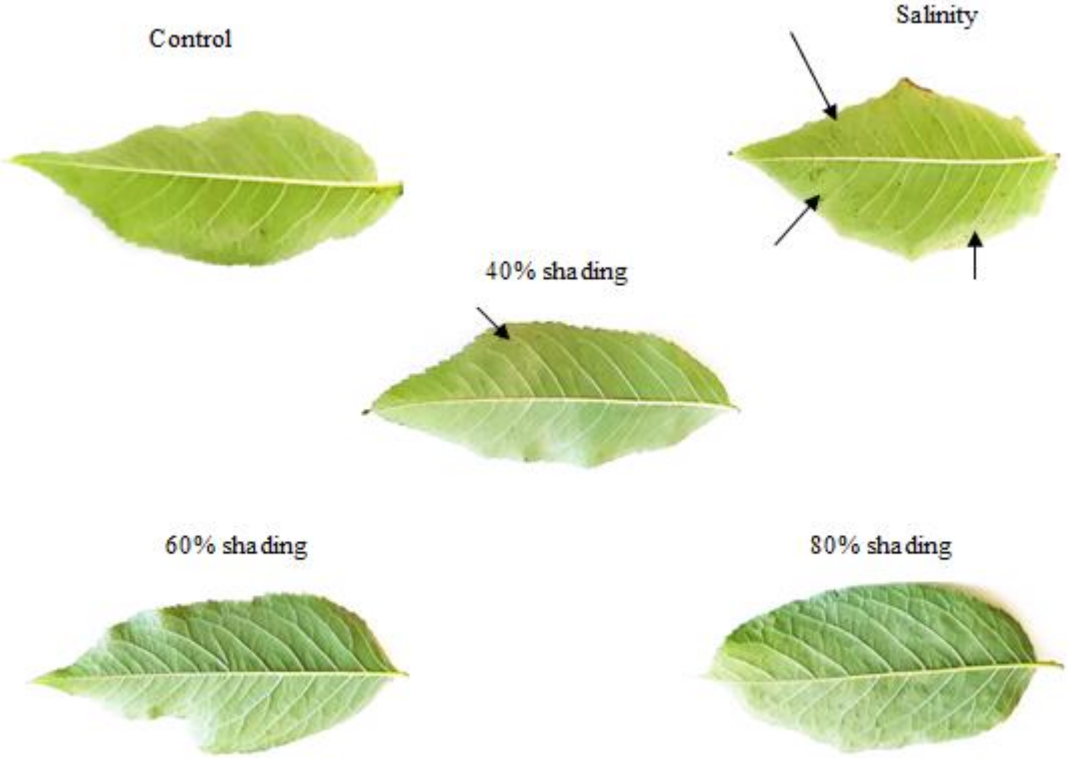

Figure 1. Leaf salinity damage observation by Evans blue, *arrows show necrotic spots on the leaves.

3.5. Microclimate of experimental areas and soil EC

Maximum mean light intensities were 70 klux in non-shaded area and 50, 30, and 15 klux in areas of shading with 40, 60 and 80%, respectively. Maximum mean temperatures were 29.0^{o}C in non-shaded area and 27.0, 26.7 and 25.9^{o}C in areas of shading with 40, 60 and 80%, respectively. Soil EC values were found as 441, 561, 508, 484 and 464 µS in control, salinized plants, shading with 40, 60 and 80% plants, respectively.

4. Discussion

Salt stress critically causes impaired plant growth and dysfunctions in metabolisms. Our study presented that shading treatment remarkably mitigated damage of NaCl stress in cherry plants.

4.1. Morphological responses

Salt stress depresses plant growth and the response to salinity depends on rootstocks and the period of salt exposure. In previous experiments, we defined short term salinity for cherry and apple trees as treatment of 35 mM NaCl during one month (Aras and Eşitken, 2019a; b). In the current experiment, cherry growth was depressed by short term salinity and promoted by different shading levels. RGR of shoot diameter and length was suppressed by salt stress, however shading treatments improved the parameters compared to salinized plants. Shading with 50% enhanced height and canopy of lemon trees in a study by García-Sánchez et al. (2015). They stated that improvement in plant growth could be due to better CO_2 assimilation and soil water status. Moreover, improved growth was attributed to a decrease in leaf temperature. Under 80% shading condition, leaf temperature decreased from 29.63 to 28.60^{o}C (control and 80% shading values, respectively). Furthermore, mean air temperature decreased from 29.0 to 25.9^{o}C (control and 80% shading values, respectively). A remarkable increment in RGR of shoot diameter by 80% shading could be a result of remarkable decreases in leaf and air temperatures. Shading conditions, as demonstrated by Aras and Eşitken (2019c), lead to a reduction in soil temperature, thereby inhibiting water loss. Therefore, we consider that evaporation was higher in salinized plant due to high soil and air temperatures that increased salinity concentration in the soil and higher salinity damage was found in salinized plants. The soil EC decreased by shading treatments compared to salinized plants as shown in the results.

4.2. Physiological responses

In the current experiment, stomatal conductance decreased by salinity. This result was consistent with previous experiments demonstrating that salinity reduced stomatal conductance in cherry (Aras and Eşitken, 2018) and apple (Aras and Eşitken, 2019a). Shading treatments led to an increment in stomatal conductance compared to salinized plants. Furthermore, shading treatments decreased leaf temperature and increased LRWC. We suggest that higher stomatal conductance and LRWC and less leaf temperature are due to a decline in air temperature by shading treatments. Moreover, improvement in leaf water status by shading may have favored stomatal conductance and leaf temperature values.

Carotenoids belonging to plant pigments play important roles in photosynthesis and dissipation of excess energy (Muzzopappa et al., 2020). Under stress conditions, plants accumulate carotenoids as a photoprotective mechanism (Nisar et al., 2015). Moreover, carotenoids act as signals against stress factors (Shumbe et al., 2014). In the present experiment, salinized plants increased carotenoid content for defense mechanism, however shaded plants did not require higher contents of carotenoids may be due to lowering excess light by shading. In addition to carotenoids, phenolics play a critical role in the regulation of stress tolerance (Parida et al., 2004). Similar to the carotenoid behavior of plants in our study, salinized plants increased and shading treatments decreased total phenolic content.

Anthocyanins are also plant pigments that play an important role in plant tolerance against stress conditions (Gu et al., 2019). Anthocyanin content increased in water spinach under 25 mM NaCl condition in a study by Kitayama et al. (2019). Zhu et al. (2013) stated that increased anthocyanin content led to an increment in tolerance against chilling stress. In the present study, salt stress remarkably decreased total anthocyanin content and shading increased accumulation of total anthocyanin. Increment in anthocyanin content may be also a result of capturing more light under shading conditions.

4.3. Chlorophyll and precursors in chlorophyll pathway

Chlorophyll, a tetrapyrrole incorporating Mg, plays a crucial role in light absorption for photosynthesis (Tanaka and Tanaka, 2007). Within plants, two primary types of chlorophyll exist—chlorophyll a and b. Numerous studies have indicated that salinity induces a reduction in chlorophyll content (Zhu et al., 2019). Consistent with these findings, chlorophyll a, b, and the combined a+b exhibited a notable decrease under conditions of salinity stress. However, shading treatments maintained a protective effect on chlorophyll. Prevention of chlorophyll loss was greater in shading with 80% among treatments. Plants increase chlorophyll content to capture more light under low irradiance conditions (Brouwer et al., 2014). In our study, shading promoted chlorophyll content compared to salinized plants and alleviated salinity damage.

The synthesis of chlorophyll necessitates the involvement of specific precursors, with Proto IX, Mg-Proto IX, and Pchl serving as crucial elements in chlorophyll biosynthesis (Shen et al., 2007). Proto IX, among these precursors, acts as the final common intermediate in both chlorophyll and heme synthesis. The initial step in the chlorophyll branch involves the insertion of Mg^{2+} ions into Proto IX (Tanaka and Tanaka, 2007). In our study, Proto IX increased in salinized plants compared to the control, however, Mg-Proto IX and Pchlide decreased by salinity treatment. We consider that salinity blocked the biosynthesis of chlorophyll at Mg-Proto IX step in our study. In a previous experiment, we reported that biosynthesis of chlorophyll was hampered at Mg-Proto IX step in peach under calcium deficiency conditions (Aras et al., 2021). Xiong et al. (2018) demonstrated that salt stress caused a decline in Proto IX, Mg-Proto IX, and Pchlide. Chlorophyll yield was also assessed in the experiment. The lowest value was found in salinized plants which may be a consequence of the participation of Proto IX to other tetrapyrrole, heme (not determined in the study). Therefore, the reason for chlorophyll loss in salinized plants may be caused by the failure of Proto IX to Mg-Proto IX synthesis. Shading 60 and 80% mitigated the detrimental effects of salinity through the accumulation of Proto IX, Mg-Proto IX and Pchlide.

Conclusion

The study provides insights into physiological responses and chlorophyll metabolism in cherry in response to short term salinity stress and shading treatments. The findings obtained in the current experiment demonstrated that short term salt stress remarkably decreased plant growth, stomatal conductance, LRWC and contents of chlorophyll and chlorophyll precursors in cherry plants. Salt stress hampered the biosynthesis of chlorophyll at Mg-Proto IX step. Shading treatments alleviated salinity damage associated with decreasing air and leaf temperature and preventing the loss of chlorophyll and the precursors. Anthocyanins may be involved in the protective mechanism of shading treatments. The results of the study showed that shading with 80% had a higher protective effect on short term salinity in cherry plants.

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