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RESEARCH ARTICLE

Shading Treatments Improved Plant Growth and Physiological Responses of Sweet Cherry Plants Subjected to Salt Stress

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ABSTRACT

The responses of sweet cherry plant to salt stress and shading treatments were investigated in the study. The study was performed in a greenhouse with 1-year-old 0900 Ziraat sweet cherry cultivar grafted onto MaxMa 14 grown in 10 L pots. About 2 months after planting, salinity and shading treatments were initiated. Three levels of shading (40, 60 and 80%) were employed. 35 mM NaCl (sodium chloride) was used to expose plants to moderate salt stress. The plants were stressed during four months. Many morphological and physiological responses were assessed. Salt stress remarkably decreased plant growth and chlorophyll, anthocyanin contents and stomatal conductance. Shading treatments protected cell membranes, leaf water status and photosynthetic pigments and leaded increment in gas exchange under salinity conditions. Cell death of leaves was observed by Evans blue staining. Necrotic lesion was not observed in the leaves of control, shading with 60 and 80% plants. The results of the study showed that shading with 80% had higher protective effect on salt stress in sweet cherry.

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Introduction

Salinity is a common stress in plants caused by excessive fertilization, drought and poor drainage in soils (Aras and Eşitken, 2018; Pehlivan and Güler, 2019). Many fruit trees including sweet cherry plants are known to sensitive to salt stress (Aras et al., 2019; Aras and Eşitken, 2019a). Salinity damages of salt stress have been previously explained in temperate fruit trees. Decrease in leaf chlorophyll and water status and increment in cell membrane permeability were reported in apple (Yin et al., 2010; Aras and Eşitken, 2019a) and sweet cherry (Aras and Eşitken, 2019b). García-Legaz et al. (2008) stated that salinity depressed loquat plant growth that could be attributed to decreases in CO_2 assimilation, photosynthesis and leaf gas exchange.

Plants cannot utilize photosynthetically active radiation (PAR) when subjected to environmental stresses.

Shading treatments can be used to dissipate excess energy. Shading has been studied for many purposes such as fruit quality (De Freitas et al., 2013), plant tolerance against drought stress (Nicolás et al., 2008), dormancy breaking (Campoy et al., 2010). Morandi et al. (2011) used shading on apple trees to control fruit size. Barradas et al. (2005) studied the effects of shading on water stress in apricot trees and they found that shading nets improved plant tolerance against drought by decreasing water loss. Furthermore, it has been reported that shade plants use lower light irradiance by chloroplast movement to protect photosystem II (Park et al., 1996).

As far as we know shading treatments with different shading levels against salinity has not been studied so far. Therefore, the aim of the current experiment was to evaluate the utilize of shading nets on improving salinity tolerance in sweet cherry and which shading level is the best. Many plant growth and leaf physiological parameters were measured in the study.

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Materials and Methods

The experiment was conducted in a semi-controlled greenhouse of Yozgat Bozok University in March of 2020. 1-year-old 0900 Ziraat sweet cherry cultivar grafted onto MaxMa 14 was grown in 10 L pots filled with peat and perlite (4:1). About 2 months after planting, salinity and shading treatments were initiated. Nets (black colored) placed over a metal tunnel were 2 m high, and each group was separated from the other group. Three levels of shading (40, 60 and 80%) were employed. 35 mM NaCl (sodium chloride) was used to expose plants to moderate salt stress (Aras and Eşitken 2018; 2019b). The plants were stressed during four months. During the experiment plants were watered regularly and excess solution was allowed to drain from the pot. Plants were fertilized fortnightly with Hoagland's nutrient solution (Hoagland and Arnon, 1950). The experiment was carried out following a randomized complete plot designs with three replications and 5 plants per replication.

Morphological Measurements

Relative growth rates of shoot diameters and shoot length were evaluated. Shoot diameter was measured with a digital caliper (Mitutoyo). Shoot length was measured with a ruler. The relative growth rates (RGR) were calculated using the equation given below (Del Amor and Marcelis, 2003):

$$RGR = 100 \times [(\ln Xt_2 - \ln Xt_1) / (t_2 - t_1)]$$

with $t_2 - t_1$= 120 days (during the experiment), Xt_2 = final shoot diameter and shoot length, Xt_1 = initial shoot diameter and shoot length.

Physiological Measurements

Relative chlorophyll (SPAD) content was determined by a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd, Osaka, Japan). Relative anthocyanin content of the leaves was measured with an Anthocyanin Content Meter (ACM-200 plus). Stomatal conductivity and leaf temperature were conducted on the youngest fully expanded leaves on upper branches of the plants with leaf porometer (Li-COR).

For the measurement of membrane permeability (electrolyte leakage), the procedure of electrolyte leakage based on Lutts et al. (1996) was used to assess membrane permeability. Electrolyte leakage was measured using an electrical conductivity (EC) meter. Mature leaves per plant were taken and cut into 1 cm segments. Leaf samples were then placed in individual stoppered vials containing 10 mL of distilled water after three washes with distilled water for removing surface contamination. These samples were incubated at room temperature (25◦C) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC_1) was measured after incubation. The same samples were then placed in an autoclave at 120◦C for 20 min and the second measurement (EC_2) was taken after cooling solution to room temperature. The electrolyte leakage was calculated as EC_1/EC_2 and expressed as percent.

Leaf relative water content (LRWC) was determined by the formula of Smart and Bingham (1974): LRWC(%) = [(FW-DW)/(TW-DW)] × 100

with FW= fresh weight, DW= dry weight, TW= turgor weight

In-situ Detection of cell Death

Cell death was observed by staining of the leaves with Evan's Blue according to Ray et al. (2016) and Riaz et al. (2019) with some modifications. The leaves of each treatment were excised and imbibed in 0.025% of Evan's Blue staining solution for 15 min at room temperature. We examined different concentrations of Evan's Blue solution and 0.025% was the best to stain the leaves as shown for roots in a previous experiment (Zhang et al. 2011). The necrotic lesions were observed as spots on the leaves.

Microclimate of Experimental Areas

Light intensity was measured with a luxmeter (CEM, DT-1308). Air temperature and relative humidity of phyllosphere (on the leaf surface) under the nets and above the un-shaded control plants was recorded with a digital thermometer.

Statistical Analyses

Statistical analyses were performed with the statistical software package SPSS, version 20.0. The means were compared by the Duncan's test at 5%.

Results

The study revealed that shading treatments improved salinity tolerance in cherry plant. Many plant morphological and physiological responses were assessed in order to reveal shading effects against salinity.

Morphological Responses

Plant growth was significantly influenced by salinity and shading treatments (Table 1). Salt stress decreased RGR of shoot diameter and length by 6.8 and 29.9%, respectively compared to control. However, shading treatments improved morphological status. Shading with 80% possessed the highest RGR of shoot diameter and length values among the treatments including control.

Table 1. Effects of shading and salt stress treatments on morphology of sweet cherry plants

Treatments	RGR of shoot diameter	RGR of shoot length
Control	0.4509 b	0.3093 bc
Salt	0.4200 b	0.2183 c
%40 + Salt	0.6136 a	0.4412 bc
%60 + Salt	0.6300 a	0.5547 ab
%80 + Salt	0.6378 a	0.8499 a

Means separation within column by Duncan's multiple range test. $P<0.05$

Physiological Responses

Salt stress and shading treatments statistically affected leaf physiological responses of sweet cherry plants (Table 2 and 3). SPAD and anthocyanin values decreased by 18 and

29%, respectively under salt stress conditions. Shading with 80% and control had the highest SPAD and anthocyanin values among the treatments. Salinity caused decline in stomatal conductance from 124.83 mmol m^{-2} s^{-1} to 65.30 mmol m^{-2} s^{-1}. Shading treatments improved gas exchange under salinity conditions. Shading with 80% had the lowest leaf temperature and LRWC values under salinity conditions. Membrane permeability as shown damage at cell level increased by 38% in salinized plants compared to control. Shading treatments protected cell membrane against salt stress.

Table 2. Effects of shading and salt stress treatments on SPAD, anthocyanin and stomatal conductance of sweet cherry plants

Treatments	SPAD	Anthocyanin	Stomatal conductance (mmol m^{-2} s^{-1})
Control	43.56 a	8.33 a	124.83 a
Salt	35.76 c	5.90 c	65.30 b
%40 + Salt	38.46 b	6.76 b	75.76 b
%60 + Salt	42.06 a	7.20 b	79.43 b
%80 + Salt	43.8 a	8.43 a	110.16 a

Means separation within column by Duncan's multiple range test. P<0.05, NS: Non Significant

Table 3. Effects of shading and salt stress treatments on leaf temperature, LRWC and membrane permeability of sweet cherry plants

Treatments	Leaf temperature (°C)	LRWC (%)	Membrane permeability (%)
Control	33.26 a	78.18 a	18.57 c
Salt	32.73 b	73.17 b	25.63 a
%40 + Salt	31.80 c	78.91 a	22.44 b
%60 + Salt	29.66 d	76.23 ab	21.50 bc
%80 + Salt	28.13 e	78.91 a	20.73 bc

Means separation within column by Duncan's multiple range test. P<0.05, NS: Non Significant

In-situ Detection of Cell Death

Cell death of leaves was observed by Evans blue staining method (Figure 1). Necrotic lesion was not observed in the leaves of control, shading with 60 and 80% plants. In salt treated plants, necrotic spots were in leaf edge and leaf tip. Shading with 40% had less necrotic lesions compared to salinized plants.

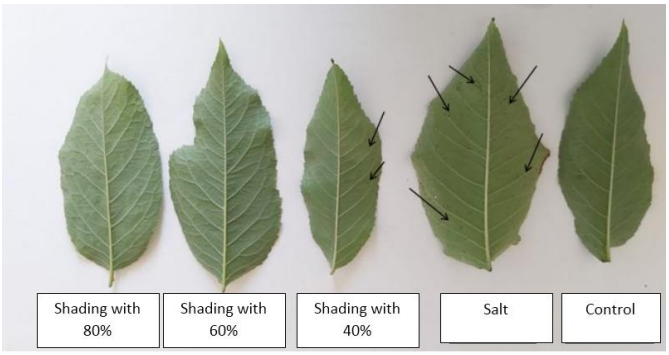

Figure 1. Leaf salinity damage observation by Evans blue
*Arrows show necrotic spots on the leaves.

Microclimate of Experimental Areas

Maximum mean light intensities were 75 klux in non-shaded area and 54, 32, and 17 klux in areas of shading with 40, 60 and 80%, respectively. Maximum mean temperatures were 35.6°C in non-shaded area and 35.0, 34.3 and 29.8°C in areas of shading with 40, 60 and 80%, respectively. Moreover, maximum mean relative humidity values were found as 15% in non-shaded area and 20, 22 and 23% in areas of shading with 40, 60 and 80%, respectively.

Discussion

Morphological Responses

The influence of salt stress on trees is a reduction in plant growth (Chartzoulakis et al., 2002; Zrig et al., 2011). Our study showed reductions in RGR of shoot diameter and length. In a previous experiment, we stated that 35 mM NaCl is appropriate to subject cherry plants to moderate salinity and cherry plant growth remarkably decreased by salinity (Aras et al., 2019). In the current study, shading treatments stimulated growth under salt stress conditions. Moreover, shading treatments increased plant growth when compared to control. In a previous experiment, height and canopy of lemon trees increased by shading with 50% thanks to better CO_2 assimilation and soil water status (García-Sánchez et al., 2015). Shading treatments, especially 80% level, leaded reductions in leaf and air temperature compared to control. In addition that, it has been reported that soil temperature also declines under shading (Aras and Eşitken 2019c) that prevents water loss from soil (Chen et al., 2007) and consequently prevents increment in soil EC. Therefore, shading treatments improved plant growth against salinity. Furthermore, it has been stated that plants enable to outgrow shade to capture more light known as shade-avoidance response (Park and Runkle, 2017). We consider that shaded plants increased plant growth to capture more light.

Physiological Responses

Salinity decreased SPAD value that represents relative chlorophyll content. Reduction in chlorophyll content by salinity was reported in many fruit trees (Zrig et al., 2011; Papadakis et al., 2018). Shading treatments prevented chlorophyll loss and shading with 80% possessed similar content of chlorophyll pigment. Under shading conditions, plants increase chlorophyll content to capture more light (Brouwer et al. 2014). Therefore, loss in chlorophyll content was not observed in shading treatments. Similar with SPAD value, anthocyanin content decreased by salinity and loss in anthocyanin content was suppressed by shading treatments. Anthocyanins also absorb light playing role in photosynthesis (Kyparissis et al., 2007). Depressed in anthocyanin content by salinity was well reported (Zrig et al., 2016). Increase in anthocyanin content could be a consequence of capturing more light under shading conditions.

Stomatal conductance decreased by salinity as reported in previous studies (García-Legaz et al., 2008; Aras and Eşitken 2018). Shading treatments increased stomatal aperture compared to salinized plants. Moreover, shading

declined leaf temperature and increased LRWC in the study. Shading with 80% protected plant growth by increasing gas exchange and conserving leaf water compared to salinized plants.

We evaluated cell membrane damage of salinity by measuring membrane permeability and observing Evans blue staining. Membrane permeability increased by salt stress and the damage decreased through shading treatments. Increment in membrane damage caused by salinity was found in many studies (Yin et al., 2010; Liu et al., 2012). Shading with 60 and 80% promoted cell membranes shown in less membrane permeability and as no visual necrosis observed by Evans blue dye.

Conclusion

The overall findings of the current experiment showed that salinity damaged sweet cherry plants and shading treatments improved the salinity tolerance. Salt stress remarkably decreased plant growth and chlorophyll, anthocyanin contents and stomatal conductance. Shading with 80% protected cell membranes, leaf water status, photosynthetic pigments and leaded increment in gas exchange under salinity conditions. The results of the study showed that shading with 80% had higher protective effect on salt stress in sweet cherry.

Compliance with Ethical Standards

a) Authors' Contributions

Author SA: Designed the study, interpreted data, performed the laboratory work and drafted the paper.

Author HK: Performed the laboratory work.

Author EB: Performed the laboratory work.

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Statement of Human Rights

This study does not involve human participants.

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