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Morphological, Physiological and Biochemical Responses of Safflower (*Carthamus tinctorius* L.) Exposed to Salinity Stress

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ABSTRACT

The morphological, physiological (biomass, water content-WC) and biochemical (proline, membrane damage-malondialdehyde-MDA, H_2O_2 content) responses of safflower to NaCI salt stress in different concentrations (0, 50, 75, 150, and 300 mM) were investigated for the first time in *in vitro* conditions in this study. At the end of the 3-week period, it was determined that NaCl had a negative effect on germination percentages. The percentage of germination was 100% in the control group, while it decreased to 30% in 150 mM NaCl and 5% in 300 mM. In general, morphological development of seedlings was significantly slowed down and seedling growth was not observed at 300 mM concentration. It was determined that the WC, fresh weights, shoot and root length decreased in all NaCl concentrations but there was no significant decrease in dry weights. MDA, proline and H_2O_2 contents increased in safflower seedlings in parallel with the intensity of salt treatments. While the highest MDA and proline content was found in 150 mM NaCl treatment, the highest H_2O_2 content was found in 75 mM NaCl treatment. In the light of these data, it has been proven that the Balcı safflower variety is sensitive by showing a negative effect on the applied salt concentrations.

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Tuz Stresine Maruz Bırakılan Aspir (*Carthamus tinctorius* L.) Bitkisinin Morfolojik, Fizyolojik ve Biyokimyasal Tepkileri

ÖZET

Aspir tohumlarının farklı konsantrasyonlarda (0, 50, 75, 150, 300 mM) NaCI stresine karşı morfolojik, fizyolojik (biyokütle, su içeriği-WC) ve biyokimyasal (proline, membran hasarı-malondialdehit-MDA, H_2O_2 içeriği) tepkileri *in vitro* koşullarda ilk defa incelenmiştir. Üç haftalık kültür sürenin sonunda NaCl tuz stresinin çimlenme yüzdelerini olumsuz etkilediği belirlenmiştir. Çimlenme yüzdesi kontrol grubunda % 100 iken 150 mM NaCl'de % 30'a, 300 mM'de % 5'e düşmüştür. Genel olarak, fidelerin morfolojik gelişimi önemli ölçüde yavaşlamış ve 300 mM konsantrasyonda ise fide büyümesi gözlenmemiştir. Tüm NaCl konsantrasyonlarında su içeriği (WC), taze ağırlık, sürgün ve kök uzunluğunun azaldığı ancak kuru ağırlıklarda önemli bir azalma olmadığı belirlenmiştir. Aspir fidelerinde tuz uygulamalarının yoğunluğuna paralel olarak MDA, proline ve H_2O_2 içerikleri de artmıştır. En yüksek MDA ve proline içeriği 150 mM NaCl uygulamasında ve en yüksek H_2O_2 içeriği 75 mM NaCl uygulamasında bulunmuştur. Bu veriler ışığında, Balcı aspir çeşidinin uygulanan tuz konsantrasyonlarında negatif etki göstererek hassas olduğu kanıtlanmıştır.

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an annual bushy dicotyledonous plant with its yellow, orange, red, cream-colored and white flowers. Firstly used as an ornamental and dyeing plant purposes, safflower has today become an invaluable plant with a strategic importance in the production of two main products, oil and biodiesel, and it will be even more valuable in the future. Also, it has been reported that safflower seed oil is healthier than canola and olive oils (Dajue & Mündel, 1996). In addition to its use as an alternative oil plant, other areas of use of safflower are animal feed, fuel, paint industries and medical (cardiovascular diseases, analgesic, antipyretic, osteoporosis) (İşler, 2014; Birecikli Hamidi & Akbaş, 2018; Nadas et al., 2023).

In ever-changing world, the environmental conditions in which plants grow may change as a result of various abiotic and biotic stress factors. Salinity, one of the abiotic factors, has a significant impact on every stage of the life cycle of plants (Çulha, 2011). Increasing salinity in the soil as a result of higher NaCl negatively affects plant growth because this situation not only restricts the water uptake from the soil but also inhibits nutrient intake (Pessarakli & Szabolcs, 1999).

Revealing the stress behaviors of plants provides great convenience in breeding varieties with high adaptability to stress conditions. Therefore, plant breeding programs are becoming increasingly important (Özen & Onay, 2013). Safflower is a plant that is not very selective in climatic and soil demands and has high adaptability (Kaya, 2017). It is highly important to conduct breeding activities to produce new varieties with high productivity, quality and resistance to stress in order to improve the cultivation of safflower plants which can easily adapt to different climatic conditions.

Biotechnological methods are increasingly used to overcome the problems that cannot be solved using traditional methods in breeding activities and is an alternative method used in salt stress factor research, tolerance determination and variety selection (Hamidi Birecikli & Akbaş, 2018; Kaya, 2017). However, there are a few studies about the effect of salinity stress factor on the safflower plant (*Carthamus tinctorius* L.) in *in vitro* (Hosseini et al., 2010; Çulha, 2011). This study is the first that was conducted in vitro on the effect of salt stress on the Balcı safflower cultivar. From this point of view, the morphological, physiological and biochemical responses given against NaCl stress of safflower (*Carthamus tinctorius* L.) under *in vitro* culture conditions investigates in this study.

MATERIAL and METHOD

In this study, the seeds of the registered Balcı safflower (*Carthamus tinctorius* L.) were used as plant materials. After the seeds were soaked in 70% ethanol for 30 seconds, optimum surface sterilization was performed by soaking them in %5 NaOCl for 60 minutes. Aseptic safflower seeds were cultured in Magenda GA-7 containing 1/4 MS medium (Murashige & Skoog, 1962) supplemented with 0, 50, 75, 150, and 300 mM NaCl and were left to germinate in the growth chamber. All nutrient media were supported with 30 g sucrose and 5.458 g agar. For each application group, 4 seeds were planted in 20 Magenda GA-7 and a total of 80 seeds were used. All salt stress applications on the germination of safflower seeds were carried out in the growth room where optimum conditions were provided. The growth chamber was provided with mercury fluorescent lamps (400 W, MBFR/U, Thorn) with a light intensity of 30-60 $\mu m/m^2s^1$ and a temperature control system that keeps the ambient temperature constant at 25±2ºC. In addition, the light period of the growth room was adjusted to be 16 hours of light and 8 hours of dark (3000-5000 lux).

After a period of 3 weeks, the germinated ones were determined among the seeds (n: 20) incubated with NaCl applications. Seeds with a radicle outflow of about 2.0 mm were considered germinated. At the end of the 3-week culture period, the aerial/root parts of the safflower seedlings were harvested separately and then kept in deep- freeze (-42 °C) until the analysis was performed. Shoot/root length and fresh/dry weight were measured immediately after harvesting to determine salinity effects on the growth of seedlings. Water contents (WC) of every sample were calculated in % according to the formula below:

$$WC\ \% = (FW - DW) / FW \times 100$$ (DW=Dry weight, FW=Fresh weight). (1)

Malondialdehyde (MDA) content was determined according to Ohkawa et al. (1979). Proline content was calculated spectrophotometrically by using acidic ninhydrin method (Bates et al., 1973; Ghoulam et al., 2002). H_2O_2 content was determined according to Velikova et al. (2000).

Statistical Analysis

A completely randomized design was used to analyze the data. Means were compared by DMRT (Duncan Multiple Range Test) using SPSS 20.0 for Windows (SPSS Inc., USA) to evaluate the presence of any significant difference between the means. Significance refers to the statistics at 0.05 probability level.

RESULTS and DISCUSSION

Salinity stress is known to lead insufficient water uptake, ion toxicity, restrains in metabolic activity, enzymatic inhibition and imbalances in plant growth and thus prevents seed germination to a great extent (Leblebici & Işık, 2018). It has been also reported by a

number of researchers that increasing salt concentrations decrease the percentage of germination in the safflower plant (Çulha & Çakırlar, 2011; Çulha, 2011; Bina & Bostani, 2017). As compatible with the literature, this study conducted on the Balcı safflower variety revealed that NaCl treatments had a negative effect on the percentages of germination when compared with the control group ($p \leq 0.05$). The seeds were germinated 100% in the control group while they were germinated 75% in 50 mM and 75 mM NaCl treatment groups. It was detected that the germination percentage decreased to 30% in 150 mM NaCl treatment and decreased to 5% in 300 mM which is the highest salt treatment (Figure 1). Supporting the findings of this study, Echi et al. (2013) reported that the salinity conditions led to a significant reduction in the germination of safflower seeds and the germination decreased significantly at high salt concentrations (210 mg L^{-1} NaCl) compared to the control group.

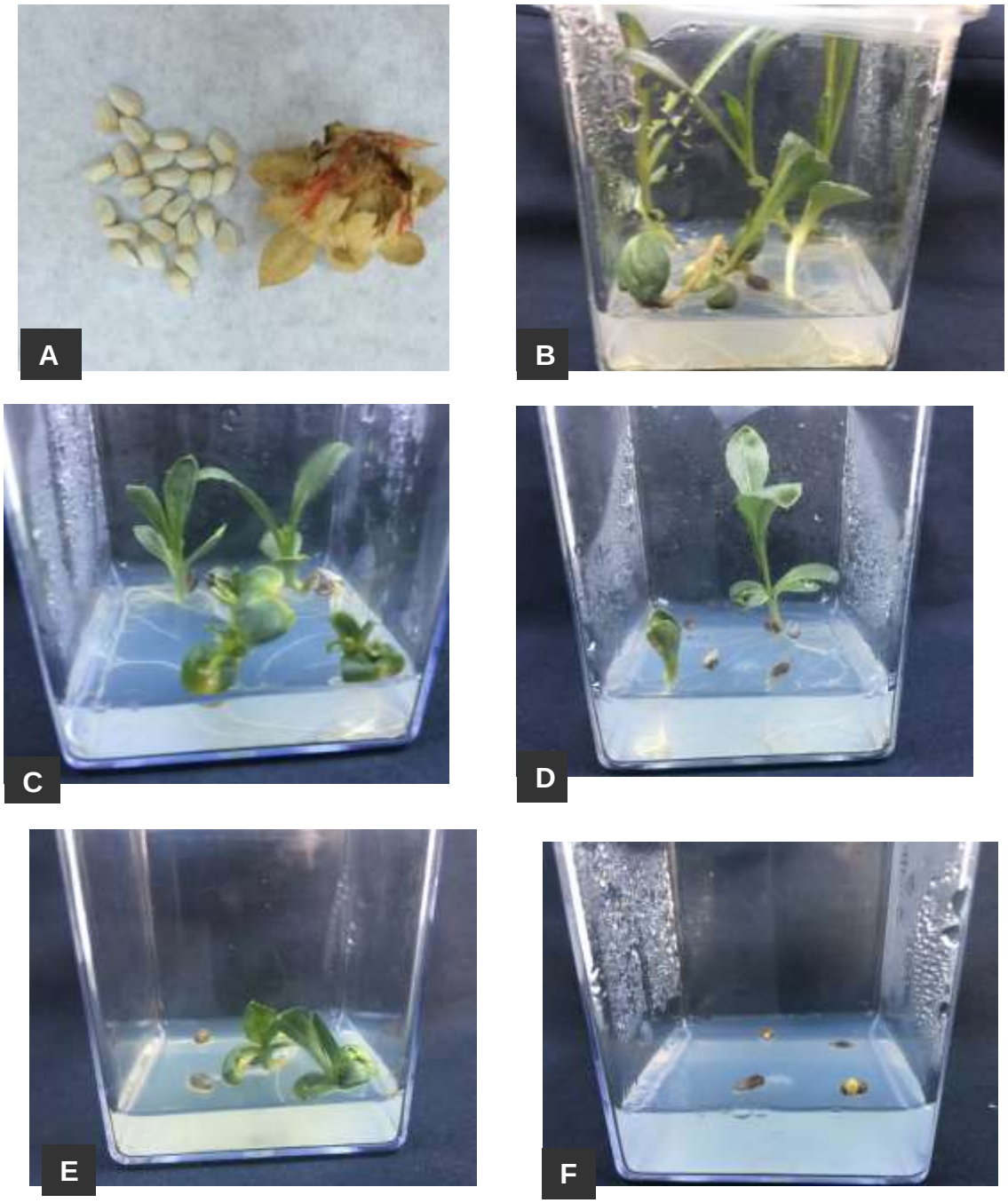

Figure 1. A) A general view of mature seeds of Balcı safflower variety **B)** Control, General views of safflower seeds cultured in **C)** 50 mM NaCl **D)** 75 mM NaCl **E)** 150 mM NaCl **F)** 300 mM NaCl groups for 3 weeks

Şekil 1. A) *Balcı aspir çeşidinin olgun tohumlarının genel görünüşü* ***B)*** *Kontrol grubundaki* ***C)*** *50 mM NaCl* ***D)*** *75 mM NaCl* ***E)*** *150 mM NaCl* ***F)*** *300 mM NaCl grubundaki aspir tohumlarının 3 haftalık genel görünümleri*

The impacts of salt stress on plants may impair the metabolism of plants and result in a reduction in growth. In the present study, a significant decrease was observed in the shoots length of Balcı safflower seedlings compared to the control group depending on increasing NaCl concentrations (Table 1).

Table 1 Effect of salinity stress on shoot and root length (mean ± sd)
Çizelge 1. Tuz stresinin sürgün ve kök boyu üzerindeki etkisi

Treatments	Germination Percentage (%)	Shoot length (cm)	Root length (cm)
Control	100	5.22 ± 2.05^{a}	1.95 ± 1.97^{a*}
50 mM NaCI	75	2.38 ± 1.89^{b}	0.51 ± 0.71^{b}
75 mM NaCI	75	1.79± 0.95^{cb}	0.54 ± 1.11^{b}
150 mM NaCI	30	1.20 ± 0.31^{c}	0.49 ± 0.63^{b}
300 mM NaCI	5	-	-

Numbers given are the mean of 20 materials. Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test.
Rakamlar 20 materyalin ortalamasını göstermektedir. Duncan's multiple range testine dayalı olarak, her sütunda farklı harfi alan ortalamalar arasındaki farklılık $p \leq 0,05$ seviyesinde önemlidir.

It was also found that all NaCl salt treatments had a negative effect on the roots length of safflower seedlings and caused a significant reduction compared to control group (approximately 74%). The longest root was obtained from the control group with 1.95 cm and the shortest root was obtained from the 150 mM NaCl treatment with 0.49 cm. Parallel to this study, there are numerous studies reporting that salinity stress causes a reduction in plant length, root length and all growth and productivity parameters (Çulha & Çakırlar, 2011; Echi et al., 2013; Erdal & Çakırlar, 2014; Zhang et al., 2015; Toprak & Tunçtürk, 2018). It is thought that the decrease in plant length and root length may be a result of osmotic pressure differences, Na^{+} accumulation in leaves and inhibition in cell growth (Alasvandyari & Mahdavi, 2017).

In this study, a significant decrease was detected in fresh weights of the green parts of growing seedlings in all NaCl treatments compared to the control group and such decrease was statistically significant ($p \leq 0.05$). As shown in Table 2, when the effects of salt treatments on the dry weights of green parts were analyzed, it was detected that there was no significant decrease when compared to the control group and to each other and the differences were statistically insignificant. In some studies on safflower, decreased fresh and dry weights of shoots in association with the increasing NaCl content were reported (Çulha & Çakırlar, 2011; Zhang et al., 2015; Toprak & Tunçtürk, 2018). It is considered that salinity stress causes a decrease in water content, chlorophyll and carotenoid content in tissues, inhibition of photosynthesis activity and, as a result, weight loss in the plant (Sairam et al., 2002).

Table 2: Effect of salinity stress on water content and fresh/dry weights (mean ± sd)
Çizelge 2. Tuz stresinin taze/kuru ağırlık ve su içeriği üzerindeki etkisi

Treatments	Fresh Weight (g/plant)	Dry Weight (g/plant)	WC (%)
Control	0.22±0.02^{a}	0.02±0.004^{a}	89.31±1.005^{a}
50 mM NaCl	0.16±0.01^{b}	0.02±0.003^{a}	87.23±1.038^{a}
75 mM NaCl	0.15±0.03^{b}	0.01±0.003^{a}	87.32±0.902^{a}
150 mM NaCl	0.16±0.01^{b}	0.02±0.004^{a}	83.95±1.916^{b}
300 mM NaCl	-	-	-

Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test.
Duncan's multiple range testine dayalı olarak, her sütunda farklı harfi alan ortalamalar arasındaki farklılık $p \leq 0,05$ seviyesinde önemlidir.

It is reported that the first damage caused by salinity stress in plants indicated itself with water deficiency. The increase in salt content in culture medium reduces the osmotic potential of water and makes it difficult for the root to take up water, causing water deficiency in the plant (Sairam & Srivastava, 2002). As a result of this event which is described as physiological drought, various metabolic irregularities and reduced growth rate occur (Levitt, 1980). In this study, a decrease was detected in water content (WC) compared to the control group as a result of increased in all NaCl concentrations. However, it was found that the decreases observed in 50 and 75 mM NaCl treatment groups were statistically insignificant ($p \leq 0.05$) while the decrease in 150 mM NaCl group was statistically significant compared to the control group (Table 2). Among all the tested applications, the lowest WC ratio was determined in the cultivated safflower seedlings in the 150 mM NaCl application group with 83.95%. In the light of all data, it was determined that high NaCl concentrations caused a decrease in WC ratio in Balcı safflower cultivars and low salt concentrations were not very effective (Table 2). Similarly, Siddiqi and Ashraf (2008) as well as Alasvandyari & Mahdavi

(2017) reported in their studies conducted with safflower varieties that salinity stress decreased water-related parameters (such as relative water content, water potential, and osmotic potential). Çulha (2011) stated in a study conducted with 3 different safflower varieties that water content (relative and real) decreased as a result of increased salinity stress.

Under salt stress, the active oxygen species react with polyunsaturated fatty acids and cause lipid peroxyl radicals to form. These radicals cause the disruption of membrane organization and integrity (Radić et al., 2006). As a result of the peroxidation of lipids in the cell membrane, MDA is formed as a reaction product (Ohkawa et al., 1979). The damage caused by salt stress on the cell membranes of Balcı safflower variety was determined by analyzing the MDA content calculated with thiobarbituric acid test (Table 3). It was observed that NaCl applications led to an increase in MDA content and that it continued rising in parallel with the increased concentration compared to the control group. In the 150 mM NaCl application group, it was detected that MDA content was maximum (4.58 µmol g^{-1}) and this value was statistically significant difference from the values in both NaCl applications and control group. In studies conducted with different safflower varieties, it was reported that salinity stress caused an increase in MDA content as compatible with this findings (Çulha, 2011; Erdal & Çakırlar, 2014; Alasvandyari & Mahdavi, 2017; Kazemeini et al., 2017). In support of the findings of this study, it has been reported that high salt concentrations in different plant species cause an increase in the amount of MDA [rice (Orcan et al., 2019), and wheat (Zhang et al., 2013).

Table 3: Changes in MDA, Proline and H_2O_2 Contents Following Salinity Stress (mean ± sd)
Çizelge 3. Tuz stresinde MDA, prolin ve H_2O_2 içeriğindeki değişimler

Treatments	MDA (µmol g^{-1} TA)	Proline (mmol g^{-1} TA)	H_2O_2 (µmol g^{-1} TA)
Control	2.76±0.04^{d}	0.69±0.01^{d}	14.55±0.69^{d}
50 mM NaCl	3.02±0.02^{c}	0.94±0.04^{c}	17.81±1.04^{c}
75 mM NaCl	3.68±0.05^{b}	1.33±0.03^{b}	34.03±0.87^{a}
150 mM NaCl	4.58±0.05^{a}	2.18±0.03^{a}	25.66±1.10^{b}
300 mM NaCl	-	-	-

Differences between means marked with different letters are significant ($p \leq 0.05$)
Her sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir ($p \leq 0.05$)

Plants accumulate large quantities of low molecular weight osmolytes in order to resist the salinity stress factor. There are a number of studies reporting that the content of proline, which is one of these osmolytes, increases in plants under stress (Ashraf & Orooj, 2006; Eyidogan & Öz, 2007). Similarly, in the present study conducted with the Balcı safflower variety, it was determined that the osmotic stress resulting from salinity stress caused an increase in proline content (Table 3). It was observed that proline content increased in proportionally to the NaCl concentration and such increase was statistically significant. The increase in proline content (0.94 mmol g^{-1}) at 50 mM concentration, which was the lowest NaCl application, indicated a significant difference ($p \leq 0.05$) compared to the control. 2.18 mmol g^{-1} proline content which was quite high compared to the control group was detected in the 150 mM NaCl treatment group. Supporting these findings, a number of studies were reported in different safflower varieties that proline content increased as the severity of stress increased (Erdal & Çakırlar, 2014; Alasvandyari & Mahdavi, 2017; Kazemeini et al., 2017).

It is expressed that increased H_2O_2 in plants under salinity stress causes lipid peroxidation and disrupts the membrane structure (Mandhania et al., 2006). In this study, the changes in H_2O_2 contents in all of NaCl salinity stress treatments were analyzed and a significant ($p \leq 0.05$) increase in H_2O_2 content was detected generally in all groups (50, 75, 150 and 300 mM NaCI) compared to the control group (Table 3). Among the salt applications, the highest H_2O_2 content was obtained in safflower seedlings developed at 75 mM NaCI concentration with 34.03 µmol g^{-1}, while the lowest H_2O_2 content was found at 50 mM NaCI application with 17.81 µmol g^{-1}, and these values were found to be much higher than the control group. Çulha (2011) reported in his study conducted with 3 different safflower varieties that H_2O_2 content increased as a result of the induction of oxidative stress as the NaCI content increased. The researcher reported that increased NaCI stress escalated not only the lipid peroxidation but also the ion leakage and, in this case, H_2O_2 acted as a toxic molecule, not as a signal molecule. Chaparzadeh et al. (2004) detected an increase in the H_2O_2 content of the leaves of *Calendula officinalis* plant under salinity stress compared to the control group. The researchers reported that H_2O_2 content had a significant role in the regulation of growth in plants and their development of tolerance to salinity stress as a result of interaction with different enzyme activities.

CONCLUSION

Salinity which is one of the most important environmental stress factors affects the growth and development of plants negatively. However, responses

to salinity stress may vary depending on the growth stages of plants. During seed germination that known the first stage of growth in plants, analyzing the stress effects and responses is regarded as an important step in determining the tolerance degree of plants to salinity. Therefore, we analyzed and assessed in this study the effect of various NaCl concentrations in the germination stage of the Balcı safflower variety. As a result, it was detected that NaCl salt had a negative impact on germination percentage, shoot/root length, fresh weight of green parts but had no effect on dry weight. In addition, significant increases were found in MDA content, proline and H_2O_2 content of seedlings, particularly in high concentrations.

In order for plants to adapt to changing adverse environmental conditions, it is important to determine tolerant plant species and varieties that can cope with abiotic stress factors such as salinity. In this study, for the first time, a scientific data that can be a reference in the selection of possible agricultural areas by determining the sensitivity level of the Balcı safflower variety to salinity stress is presented. Our results showed that Balcı safflower cultivar is sensitive to salinity. Planning future studies to increase the resistance of the plant against salt stress through elicitors will be beneficial in the cultivation of safflower, which is an alternative oil plant.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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