

Morphological and biochemical changes with hormone and hydro-priming applications in safflower (*Carthamus tinctorius* L.) seedlings under salinity stress conditions

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Abstract

In this study, the ameliorative effects of hydro- and hormoprimer applications against salt stress of safflower seeds during germination and seedling development stage were investigated. Primed (hydropriming (0, 1, 2 and 3 days) with distilled water and hormoprimer for 24 hours (0, 50, 100 and 150 mg L⁻¹ with kinetin) and nonprimed seeds were sown under control (no salt) and salt stress (0, 50, 100 and 150 mM NaCl) conditions. When priming applications under salt stress are compared with the control, germination percentage (GP), seedling length (SL), root length (RL), seedling fresh weight (SFW), root fresh weight (RFW), leaf relative water content (RWC), electrolyte leakage (EL) parameters reducing the effects of stress, however, it was determined that caused an increase in carotenoid (Car), superoxide dismutase (SOD), total phenolic compounds (TPC) parameters. Besides this, it has an inhibitory effect on the increase in proline (Pro) and malondialdehyde (MDA) content. According to the correlation analysis, significant positive correlation was determined in all parameters. A significant positive correlation was determined for hydropriming GP with SL, RL, SFW, RFW, and for hormoprimer with SL, RL, SFW, RFW, total Chl, RWC and GP. According to the PCA analysis, the parameters examined in both applications it is seen that they are divided into four different groups. In conclusion, this study priming applications are compared with each other, it has been determined that hormoprimer is more effective in reducing the effects of stress than hydropriming.

Keywords: priming applications; plant growth regulators; seed germination

Introduction

As a result of global climate change, plants are exposed to more stress. Salt stress, which is one of the most important of stress factors, negatively affects germination and seedling growth. Soil salinity is one of the most important factors limiting crop production all over the world. (Genc *et al.*, 2019; Gürsoy, 2020; Granaz *et al.*, 2022). As a result, it becomes difficult to reach safe food for the increasing world population and malnutrition problems arise. Saline conditions result in a severe decline in yield due to poor germination and seedling establishment (Narejo *et al.*, 2022). In addition, salt stress impairs plant processes such as growth and nutrient uptake and production of secondary (membrane lipids and reactive oxygen species) metabolites (Gill

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and Tuteja, 2010; Song *et al.*, 2017; Zhang *et al.*, 2019). In addition, salt stress negatively affects the development and morpho-physiological properties of plants (Granaz *et al.*, 2022).

Since salt stress negatively effects especially germination and seedling growth, it causes a decrease in yield, as well as osmotic stress, ion toxicity and increased production of ROS species, along with a decrease in the uptake of some ions, etc. causes such situations (Ali *et al.*, 2021; Akhter *et al.*, 2022). Excessive ROS production damages plants by disrupting cellular processes (Zahra *et al.*, 2018). Plants produce secondary metabolites as a response mechanism to these stresses. In addition, plants have the ability to synthesize phenolic compounds under stress conditions (Zamljen *et al.*, 2022).

Growth and development in plants begins with germination and plants need to adapt to environmental conditions in order to survive (Gürsoy, 2022a). Plants are affected by salt stress throughout their lives, but the germination and first seedling period are much more sensitive periods (Ali *et al.*, 2021). Besides this, the germination stage is one of the most sensitive phases related to drought, temperature and salinity stresses (Moori and Lahijani, 2020). It is tried to reduce the effects of salt stress by applying various methods in sustainable agricultural systems. Seed priming is a technique to improve the uniform emergence and rapid growth to achieve seedling vigor and good establishment of the seeds (Kumar and Rajalekshmi, 2021). Seed priming for salt stress reduction is an inexpensive, easy to apply and alternative method compared to other techniques (Elsiddig *et al.*, 2022). Therefore, seed priming leads to rapid and synonym germination and subsequently contributes to the plant resilience for the next growth period of the plant through certain regulations regarding metabolic profile (Sheteiwy *et al.*, 2018). Today, various priming applications are made some of which are hydropriming, hormoprining, bioprining, and osmoprining (Neto *et al.*, 2020).

Hydropriming is a simple technique of low cost that may be efficient to enhance the germination percentage and early seedling growth in plants grown under stressful conditions (Yan, 2016; Kumar and Rajalekshmi, 2021). Hydropriming is the simplest method to hydrate seeds and minimize the use of chemicals (Neamatollahi *et al.*, 2009).

Hormoprining application is one of the applications that affect seed germination with plant hormones (Moori and Ahmadi-Lahijani, 2020). Hormoprining is one of the priming techniques that seed imbibition occurs in the presence of plant hormones. Besides this hormoprining can directly affect seed metabolisms (Moori and Ahmadi-Lahijani, 2020). Cytokinins, which are plant hormones, affect plant growth and development by acting in various ways under abiotic and biotic stress conditions (Bozsó and Barna, 2021). Cytokinins are a group of plant hormones that increase plant growth and development, increase cell division, as well as support the response of plants to abiotic and biotic stresses. The cytokinin group is the first identified kinetin hormone and promotes cell division (Bozsó and Barna, 2021). Cytokinin is crucial hormone involved in plant growth and development, as well as plant responses to stresses (Cortleven *et al.*, 2019).

Safflower is an annual oilseed crop and it is an oil plant with flowers in yellow, red, orange, colors, with and without thorns, with an average oil rate of 30-50% (Gürsoy, 2019, 2020; Jam *et al.*, 2023). In addition to these, various parts of the safflower plant are used, some of which are stem, leaf, seed and flower parts (Beyyavaş and Dogan, 2022). Although the safflower plant withstands many adverse conditions, its yield decreases under stress conditions (Jam *et al.*, 2023; Culpan, 2023).

The objective of the present study is to determine the effects of hydro- and hormo-priming applications on the germination, seedling growth, total chlorophyll, carotenoid, proline, relative water content, total phenolic compounds, electrolyte leakage, malondialdehyde (MDA) and superoxide dismutase (SOD) parameters of safflower seeds under salt stress.

Materials and Methods

In this study, safflower seeds were obtained from Central Field Crops Research Institute, Ankara, Türkiye. The research was carried out at the Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). Before commencing the experiment, all seeds were pretreated with a 5% sodium hypochlorite solution for 5 min and then subsequently rinsed with distilled water for sterilization. After this process, the seeds were dried until they reached their initial weight. The study of two independent experiments.

Experiment I

Safflower seeds were kept in distilled water for different times (0, 1, 2 and 3 days) for hydropriming (Hy) process which are represented as Hy1, Hy2, Hy3, Hy4. After priming, they were dried until they reached their initial weight. After drying, the seeds were germinated at 24 ± 1 °C above filter papers in petri dishes in different salt compositions (0, 50, 100, 150 mM NaCl, designed as S0, S1, S2, S3 respectively). Only distilled water was added to the control petri dish. In order to prevent evaporation the petri dishes are wrapped with parafilm. Filter papers in petri dishes were changed every two days and salt solutions were added. Germination was monitored daily and the seeds were considered germinated when the radicle length reached about 2 mm out of seed surface. The research was carried out according to the randomized plot design with three replications for each treatment.

Experiment II

Safflower seeds were kept in solutions containing kinetin (0, 50, 100 and 150 mg L⁻¹) for 24 hours for the hormoprimering (Hr) process, which are represented as Hr1, Hr2, Hr3, Hr4 respectively. After priming, they were dried until they reached their initial weight. After drying, the seeds were germinated at 24 ± 1 °C above filter papers in petri dishes in different salt compositions (0, 50, 100, 150 mM NaCl, designed as S0, S1, S2, S3 respectively). Only distilled water was added to the control petri dish. In order to prevent evaporation the petri dishes are wrapped with parafilm. Filter papers were changed every two days and salt solutions were added. Germination was monitored daily and the seeds were considered germinated when the radicle length reached about 2 mm out of seed surface. The research was carried out according to the randomized plot design with three replications for each treatment.

Measurements

Germination percentage (%)

Given formula was applied to calculate germination percentage.

$$\text{Germination\%} = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100 \text{ (Siddiqi et al., 2007)}$$

Seedling Growth (cm)

After 14 days of applications, the seedling and root lengths of the plants were measured in meters. In the same seedlings, seedling and root fresh weights were also determined by weighing on precision scales.

Relative Water Content (%)

In order to determine the relative water content in the leaf tissues, leaf samples taken from the plants of the safflower in the control and stress groups were weighed and their fresh weight was determined, then put in glass tubes containing 5 mL of distilled water and kept in the light for 24 hours at room temperature. At the end of this period, the hydrated leaf samples were weighed again and their turgor weights were determined. Then, these leaf samples were dried in an oven at 80 °C for 48 hours, weighed again and their dry weights were determined. Finally, the relative water contents were found according to the formulas below. (Ritchie *et al.*, 1990).

$$\text{RWC(\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

FW: fresh weight, TW: turgor weight, DW: dry weight

Total Chlorophyll Content (mg g⁻¹ FW)

In order to determine the chlorophyll content, 0.25 g of the green leaves of the seedlings were weighed with the help of a precision scale and homogenized in acetone (80%). The extract was made up to 25mL with acetone. The absorbance of the extract was measured at 663 nm and 645 nm using a spectrophotometer. Total chlorophyll amounts were determined according to the formula determined by the values found (Lichtenthaler and Welburn, 1983).

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = (12.7 \times 663 \text{ nm}) - (2.69 \times 645 \text{ nm}) \times V / W \times 10000$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = (22.91 \times 645 \text{ nm}) - (4.68 \times 663 \text{ nm}) \times V / W \times 10000$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

V = volume leaf extract in 80% Acetone

W = fresh weight of leaf material

Carotenoid Content (mg g⁻¹ FW)

A sample of 0.25 g was taken from the leaves of the safflower seedlings, homogenized with 80% acetone and filtered. Then, to determine the amount of carotenoids, the homogenate was made up to 25 mL with acetone and the samples were measured in a spectrophotometer at a wavelength of 450 nm. The amount of carotenoid was calculated with the help of the following formula (Lichtenthaler and Welburn, 1983).

$$\text{Carotenoid (mg g}^{-1}\text{)} = (4.07 \times 450 \text{ nm}) - (0.0435 \times \text{Chlorophyll a} + 0.367 \times \text{Chlorophyll b})$$

Lipid peroxidation (MDA) (nmol mL⁻¹)

After the 0.5 g leaf sample taken from the plants was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA), the homogenate was centrifuged at 15000 rpm for 10 minutes. TBA (thiobarbituric acid) 0.5 ml was dissolved in 4 ml 20% TCA that was taken from the upper phase or supernatant of the centrifuged samples. It was cooled and centrifuged for 10 minutes at 10000 rpm and its absorbance was determined at 532 nm and 600 nm wavelength spectrum by taking its clear part. The content of malondialdehyde (MDA) was calculated from the obtained values with the help of the formula (Heath and Packer, 1968).

$$\text{MDA (nmol mL}^{-1}\text{)} = [(A532 - A600) / 155000] \times 10^6$$

Superoxide dismutase (SOD) activity (U g FW⁻¹)

Fresh leaf sample (0.5 g) was homogenized with 10 mL sodium phosphate buffer and centrifuged at 15000rpm for 15 minutes to obtain the plant extract. Na-phosphate buffer (50 mM) (Na₂HPO₄ × H₂O₂), Na-EDTA (0.1 mM), NBT (33 μM), riboflavin (75 μM), methionine (13 mM) was used as the reaction solution (pH: 7.0). Then, reaction solution (2.5 ml) and plant extract (0.1 ml) solution were mixed. The control solution and reaction solution readings were taken at 560 nm (Rahnama and Ebrahimzadeh 2005).

Electrolyte leakage (%)

Electrolyte leakage was determined according to Bajji *et al.* (2002). 0.1 g of the fresh leaves of the seedlings were placed in test tubes and after adding 10 mL of deionized water, they were kept at room temperature for 24 h. At the end of this period, the samples were measured with an EC meter (EC1). The test tubes were then heated to 100 °C for 15 minutes. After this process, they were cooled to 25 °C and measured again with an EC meter (EC2). Electrolyte leakage was calculated with the following formula.

$$\text{EL(\%)} = (\text{EC}_1 / \text{EC}_2) \times 100$$

Proline ($\mu\text{mol g}^{-1}$ FW)

To calculate the proline content (Bates, 1973), 0.5 g of fresh leaf sample was homogenized with 10 mL of sulfosalicylic acid. After the obtained extract was filtered with filter paper, 2 mL of acetic acid and 2 mL of ninhydrin were added. The mixture was heated to 100 °C for 1 hour. The reaction was terminated by immersion in an ice bath and 4 mL of toluene was added and mixed. The absorbance was read in a spectrophotometer at 520 nm using toluene as a blank. The proline content was determined as $\mu\text{mol g}^{-1}$ FW by plotting the calibration curve.

Total phenolic content (mg GAE g^{-1} FW)

To determine the total phenolic content, the fresh leaf sample was homogenized in 80% acetone and centrifuged at 10000 rpm for 10 minutes. 1 mL of folin-ciocalteu, 2 mL of distilled water and 5 mL of Na_2CO_3 are added to the obtained extract. It is then made up to a total of 10 mL with distilled water. It was read in a spectrophotometer at 750 nm. The results were expressed as mg GAE g^{-1} FW by plotting the standard calibration curve (Julkanen-Titto, 1985).

Data analysis

The experimental data obtained at the end of the research, was subjected to analysis of variance using MSTAT-C computer software. Duncan test was applied to determine the significance levels of the differences between means of applications. SPSS was used for the correlation and PCA analysis.

Results*Germination percentage (GP)*

Germination percentage (GP) was significantly affected ($p < 0.01$) by all applied factors, and the interaction (Priming applications \times Salt doses) ($p < 0.01$) is also important. When the results of the averages (Figure 1) were examined and compared with the control, it was determined that Hy and Hr applications were effective in increasing the germination percentage. It was determined that hydropriming applications were more effective in increasing the germination percentage compared to hormoprimering as the salt doses increased. However, it was determined that Hy3 and Hr4 applications were effective in increasing germination in S4, which is the highest salt dose.

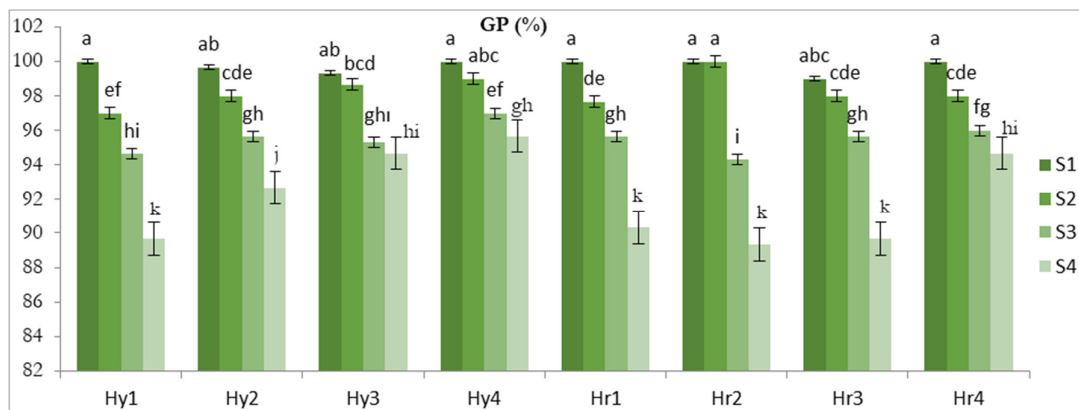


Figure 1. Interaction effects of salt treatments and hydro- and hormo- priming effects on germination percentage (GP)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Seedling length (SL)

The bilateral interaction of priming application, salt doses and (Priming applications \times Salt doses) was found to be significant at $p < 0.01$ level in safflower seedlings where hydro- and hormo- priming applications were made under salt stress (Figure 2). For example, when the 4th dose applications of hydro- and hormo-priming applications (Hy4, Hr4) were compared within themselves, it was determined that the seedling height increased by 0.35% and 0.20% respectively. It has been determined that hormopriming application is more effective in elongation of seedlings than hydropriming.

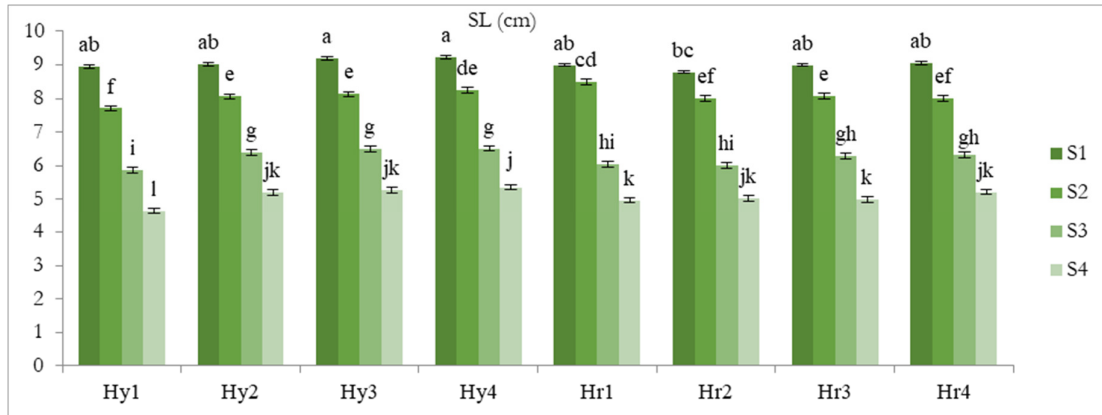


Figure 2. Interaction effects of salt treatments and hydro- and hormo- priming effects on seedling length (SL)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Root length (RL)

When the results in terms of root length are examined, priming applications, salt doses and priming applications \times Salt doses were found to be significant at the $p < 0.01$ level (Figure 3). These results show that as the doses of salt application increased, the root length was shortened. However, the root length was prolonged at the 4th dose (Hy4) in hydropriming application, and in Hr3 and Hr4 in hormopriming application despite the increase in salt doses. In addition, the longest root length was obtained from Hr4 and S1 applications.

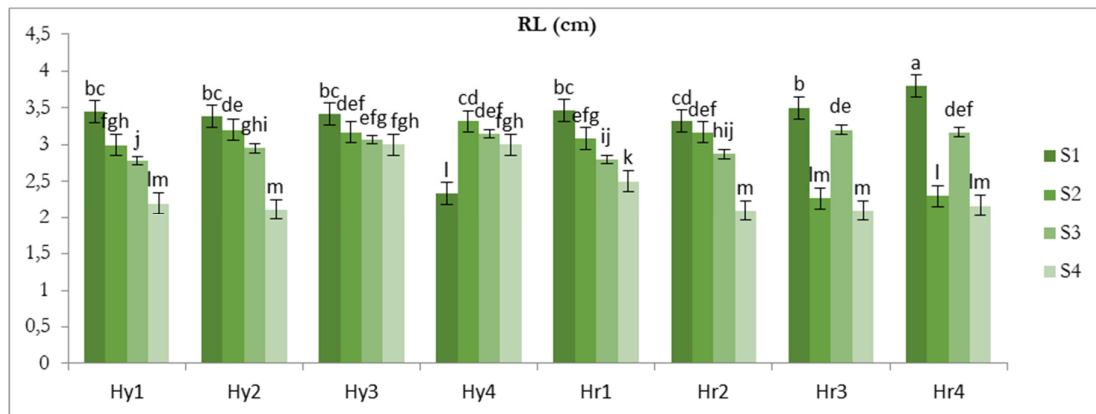


Figure 3. Interaction effects of salt treatments and hydro- and hormo- priming effects on root length (RL)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Seedling fresh weight (SFW)

When the fresh weight of the plant was examined, the bilateral interaction of priming application, (Priming applications × Salt doses) in safflower seedlings was found to be significant at $p < 0.01$ level (Figure 4). When all treatments are compared with the control, it is seen that there is a decrease in plant fresh weight compared to the control. However, it is seen that both applications cause a little decrease in wet weight compared to the control with the increase in salt doses in the 2nd, 3rd and 4th doses (Hy2, Hy3, Hy4, Hr2, Hr3, Hr4, respectively). This reduction was much greater in Hy1 and Hr1. However, when both applications in this study were evaluated, it was determined that hormoprimer application was more effective against salt stress than hydropriming application in terms of seedling fresh weight.

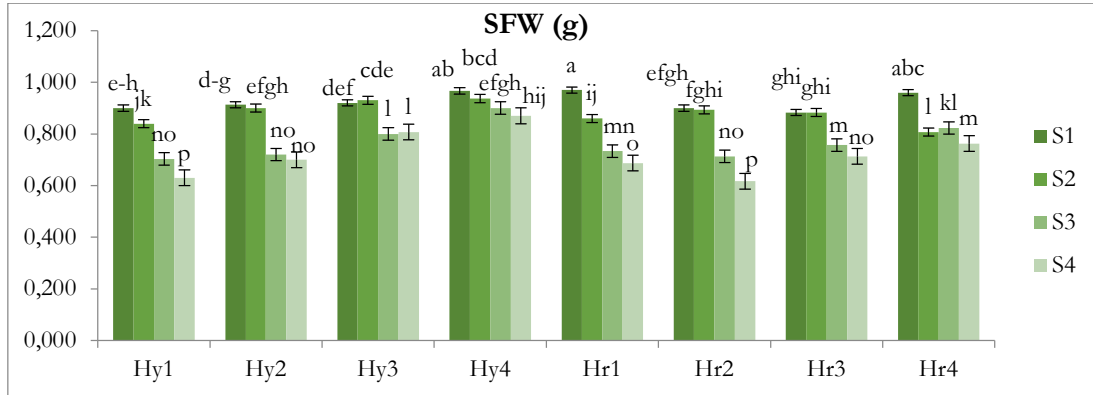


Figure 4. Interaction effects of salt treatments and hydro- and hormo- priming effects on seedling fresh weight (SFW)
Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests

Root fresh weight (RFW)

Root fresh weight was significantly affected ($p < 0.01$) by all treatments and their combinations. Priming applications × Salt doses interaction caused an increase in SFW in Hy3 and S2 compared to S1. In Hy4 S4 application, SFW caused an increase of 38% compared to Hy1 S4, and similarly, in Hr4 S4 application, it increased by 10% compared to Hr1 application and S4. Although SFW decreased as the doses of salt stress increased, the effects of stress were reduced with Hy and Hr applications (Figure 5).

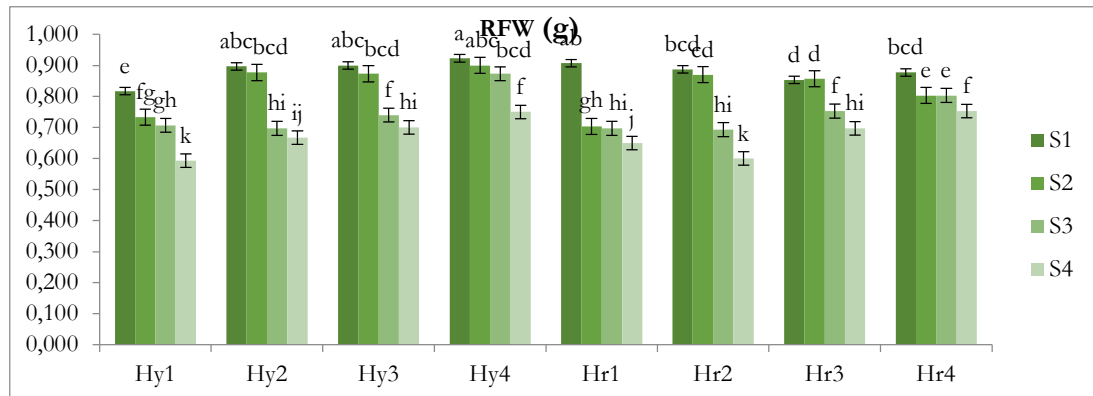


Figure 5. Interaction effects of salt treatments and hydro- and hormo- priming effects on root fresh weight (RFW)
Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Total chlorophyll (Total Chl)

Total chlorophyll was significantly ($p < 0.01$) affected by all treatments and their combinations. When the interaction of Priming applications \times Salt doses is examined (Figure 6) it is seen that the amount of chlorophyll in all applications is the highest in the S1 application. It was determined that the chlorophyll content decreased as the salt content increased. Hy4 application, in which the most chlorophyll was determined in hydropriming application, decreased 83%, 72% and 74% in S2, S3 and S4, respectively, when compared to the control. In the application of hormoprimering, the highest chlorophyll was determined in Hr1 S1 without any application. After that, the highest chlorophyll was determined in Hr2 and Hr4, and it caused reductions in Hr2 by 98%, 87%, 84%, and in Hr4 by 79%, 73%, and 71%, respectively, when compared to the control. However, Hr application was more effective in reducing the negative effects of salt stress and the chlorophyll content was less affected (Figure 6).

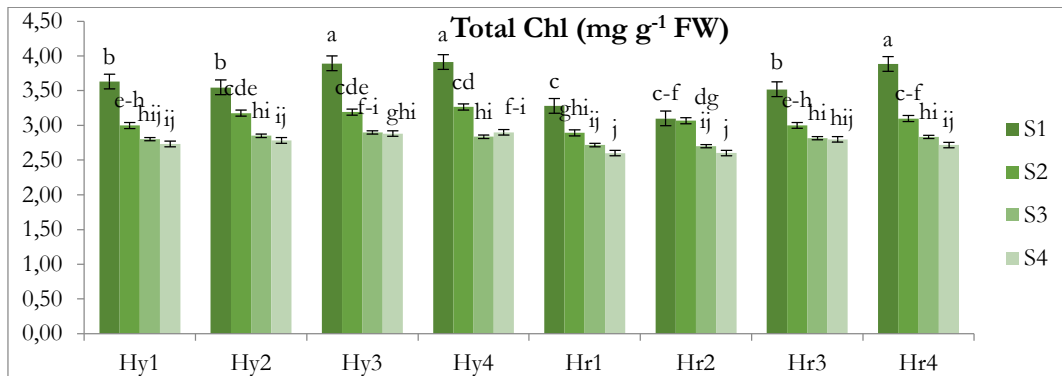


Figure 6. Interaction effects of salt treatments and hydro- and hormo- priming effects on total chlorophyll (Total Chl)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Carotenoid (Car)

Carotenoid content was significantly affected ($p < 0.01$) by priming applications, salt doses and priming applications \times salt doses interaction (Figure 7). The highest carotenoid was obtained in S4 application in Hy1, Hr1, Hr2 and Hr3, respectively. Compared with the control, the treatments caused 73% increases in Hy1, 82% in Hr1, 92% in Hr2 and 82% in Hr3, respectively. However, in all applications, it is seen that the carotenoid increases as the salt stress increases. The increase in S3 only in Hr4 application was more than S4. With the effect of all applications (except Hr4), the increase in S3 and S4 was higher than S2.

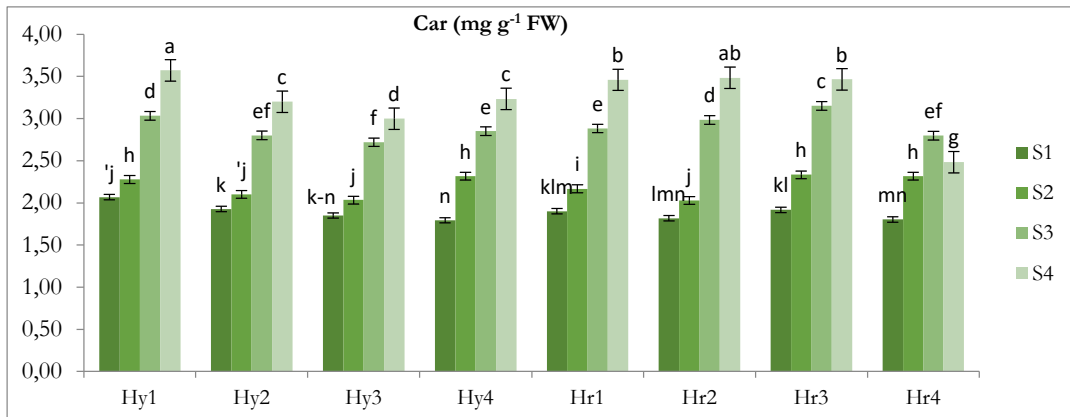


Figure 7. Interaction effects of salt treatments and hydro- and hormo- priming effects on carotenoid (Car) Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Electrolyte leakage (EL)

Electrolyte leakage content was significantly affected by priming applications, salt doses and priming applications \times salt doses interaction ($p < 0.01$) (Figure 8). In all applications, the most electrolyte leakage was in S4. In addition, Hy and Hr priming applications were effective in reducing EL. When both priming applications were compared, it was determined that Hy3 application was more effective in reducing EL. When the Hy3 application is compared to the control, it is seen that EL occurs at a lower rate in S2, S3, and S4 compared to other applications (Figure 8).

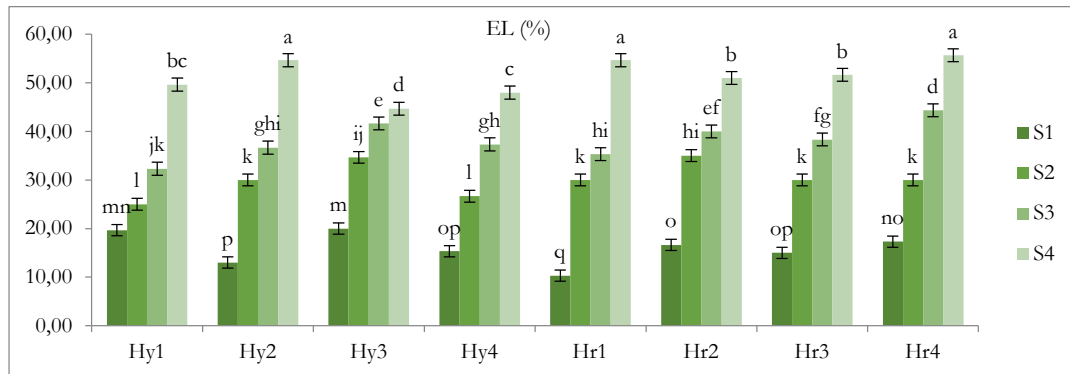


Figure 8. Interaction effects of salt treatments and hydro- and hormo- priming effects on electrolyte leakage (EL) Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Lipid peroxidation (MDA)

MDA content of all plants grown under salt stress was significantly affected by all treatments ($p < 0.01$) (Figure 9). The highest MDA content was obtained from Hr1S4 application. However, it was determined that MDA tended to decrease despite the increase in salt doses in Hy2, Hy3, Hy4, Hr2, Hr3 and Hr4 applications with the effect of the applications. It is observed that MDA increased approximately 4 fold in S4 compared to control (S1) in Hr1 without priming application. On the other hand, there is a 55% increase in Hr4 application. It has been determined that priming applications are effective in reducing the negative effects of MDA.

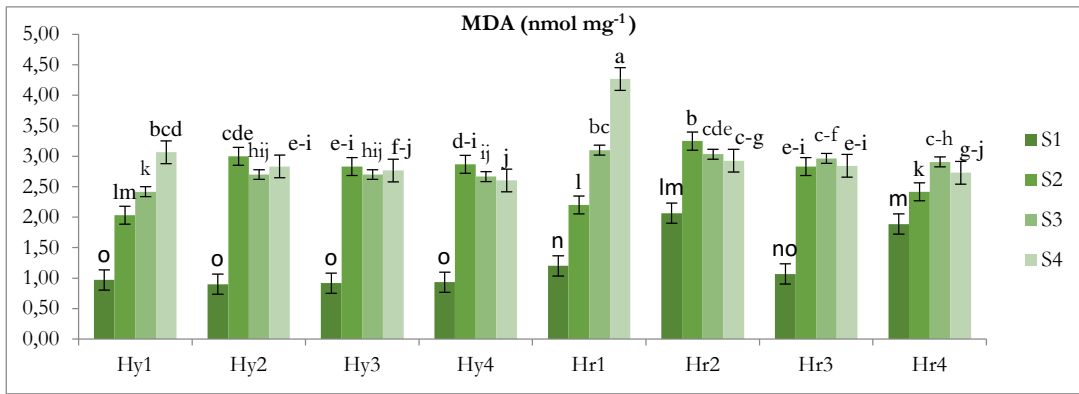


Figure 9. Interaction effects of salt treatments and hydro- and hormo- priming effects on lipid peroxidation (MDA)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Superoxide dismutase (SOD) activity

When the applications are examined in terms of SOD activity (Figure 10), the SOD content of all plants grown under salt stress was significantly affected by all applications (salt doses, priming applications and priming applications \times salt doses interaction) ($p < 0.01$). When the mean values were examined, the lowest SOD content was determined in the Hy1 S1 application. The highest was determined in Hy4 S3 and S4. It was determined that the SOD content increased as the salt stress increased in all applications. In parallel with this, as the doses of Hy and Hr applications increased, the SOD content increased.

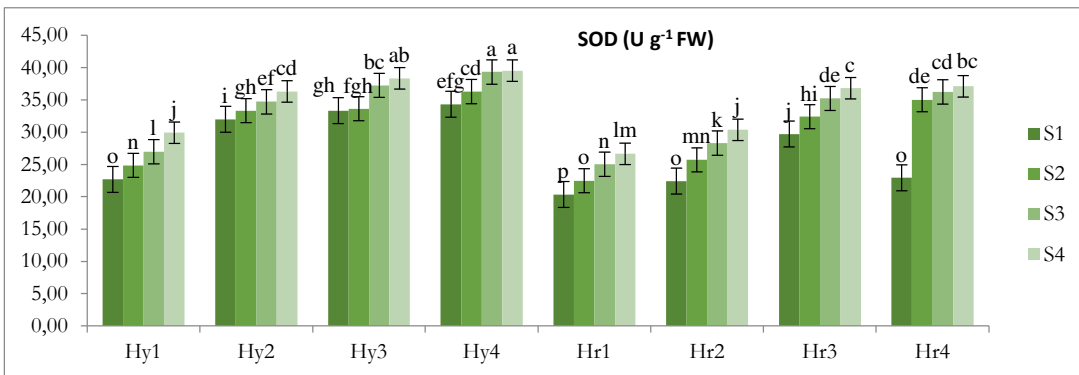


Figure 10. Interaction effects of salt treatments and hydro- and hormo- priming effects on superoxide dismutase (SOD)

Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Total phenolic content (TPC)

TPC in the leaves of plants grown under salt stress was also significantly affected by the treatments ($p < 0.01$). TPC increased in all applications. However, the highest TPC was at the highest salt (S4) dose in both treatments (Hy, Hr) (Figure 11). Other applications that followed were Hy3 S4, Hy4 S3, Hy3 S3, Hr4 S3 and Hy2 S4, respectively.

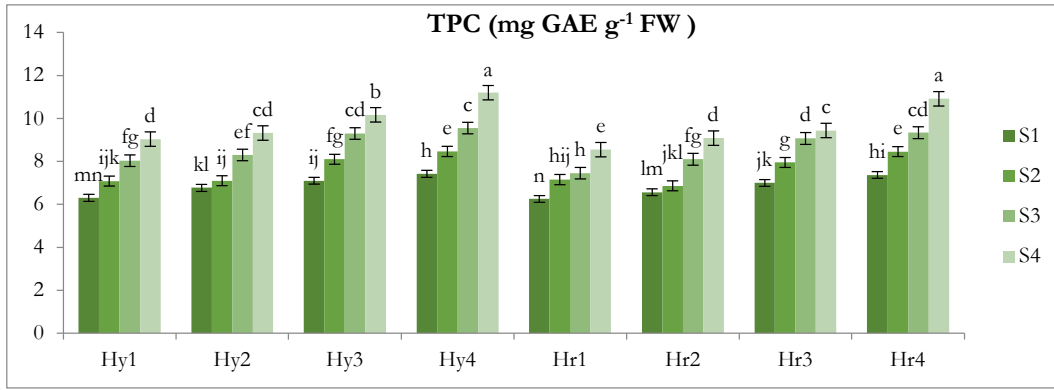


Figure 11. Interaction effects of salt treatments and hydro- and hormo- priming effects on total phenolic content (TPC)
 Different letters indicated significant differences among priming applications and salt doses to Duncan’s multiple range tests at $p < 0.01$

Relative water content (RWC)

The water content in the leaves was affected by salt stress. In addition, priming practices were also effective in RWC content. With the increase in salt stress, the water content in the leaves decreased, and the opposite is the case in Hr2 application. It is seen that the RWC is higher in the S2 treatment compared to the control in Hr2 treatment (Figure 12). It was determined that both applications were effective in reducing RWC with the negative effect of salt stress. For example, while there was an 80% decrease in S4 in the Hr4 application compared to the control, there was a 97% decrease in S4 in the Hr1 application compared to the control.

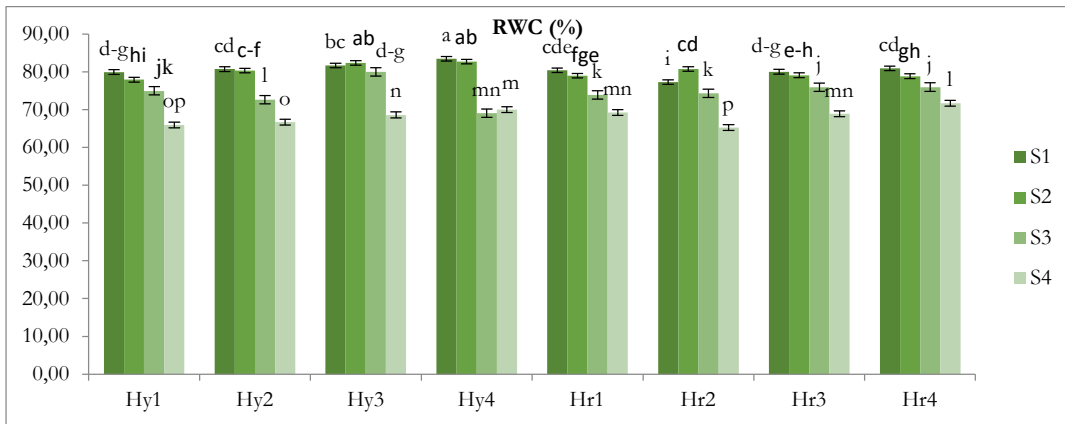


Figure 12. Interaction effects of salt treatments and hydro- and hormo- priming effects on relative water content (RWC)
 Different letters indicated significant differences among priming applications and salt doses to Duncan’s multiple range tests at $p < 0.01$

Proline (Pro)

Proline production in leaves under salt stress increased in all treatments compared to control. However, it is seen that this increase is mostly observed in Hr1 and S4 applications (15%). Similarly, it was determined that the proline content was high in Hy1 S4, which was not treated. In Hy4 application, it was realized at the same level in S3 compared to S1 (control). It has been determined that Hy4 application has an inhibitory effect on the increase of proline content. A similar situation has been determined in Hy2 and Hy3 applications.

However, in Hr applications, the lowest proline content was determined in Hr4. In Hr2, Hr3, and Hr4, the proline content was decreased at the S3 dose compared to S2 (Figure 13).

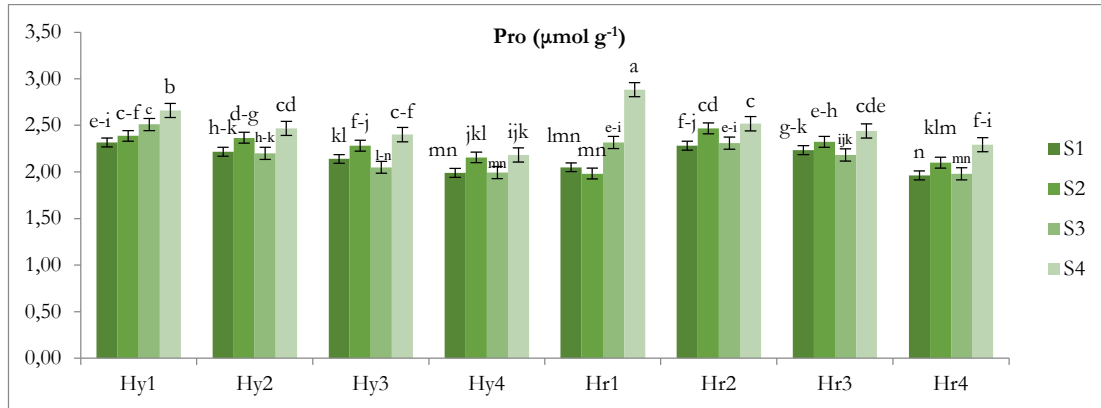


Figure 13. Interaction effects of salt treatments and hydro- and hormo- priming effects on proline (Pro) Different letters indicated significant differences among priming applications and salt doses to Duncan's multiple range tests at $p < 0.01$

Correlation analysis

Correlation coefficients showing the effects of hydro- and hormoprimer applications on safflower seeds on germination, seedling characteristics and biochemical properties under salt stress are given in Table 1 (hydropriming) and Table 2 (hormoprimer). When both tables are examined, Table 1 shows positive correlation were determined between GP with SL, RL, SFW, RFW and Total Chl, SL with RL, SFW, RFW, RWC, Total Chl, RL with SFW, RFW, Total Chl, RWC, SFW with RFW, Total Chl, SOD, RWC, RFW with Total Chl, SOD, RWC, Total Chl with RWC, Crt with EL, MDA, SOD, TPC, Pro, EL with MDA, SOD, TPC, Pro, MDA with SOD, TPC, Pro SOD with TPC. Other correlations are negative or insignificant.

In the application of hormoprimer (Table 2), strong positive correlation of GP with SL, RL, SFW, RFW, total Chl, RWC, strong positive correlation of SL with RL, SFW, RFW, total Chl, RWC, RL with SFW, RFW, Total Chl, RWC, SFW positive correlation with RFW, total Chl, RWC, RFW positive correlation between total Chl and RWC, Total chl with RWC, Crt with EL, MDA, SOD, TPC and there was a positive correlation between EL with MDA, SOD, TPC, Pro, MDA with SOD, TPC, Pro SOD with TPC, Pro, TPC with Pro. Other correlations are negative. According to the correlation coefficients, it is seen that the application of hormoprimer creates a significant positive correlation in more parameters.

Table 1. Correlation coefficients of hydropriming applications on physiological and biochemical parameters of safflower seedlings under salt stress

Correlated parameters	GP	SL	RL	SFW	RFW	Total Chl	CRT	EL	MDA	SOD	TPC	RWC
SL	0.916**											
RL	0.661**	0.545**										
SFW	0.902**	0.803**	0.597**									
RFW	0.882**	0.843**	0.562**	0.910**								
Total Chl	0.778**	0.870**	0.360*	0.704**	0.738**							
CRT	-0.909**	-0.974**	-0.536**	-0.805**	-0.836**	-0.837**						
EL	-0.843**	-0.917**	-0.506**	-0.671**	-0.734**	-0.809**	0.883**					
MDA	-0.640**	-0.719**	-0.275	-0.487**	-0.494**	-0.828**	0.671**	0.800**				
SOD	-0.126	-0.312*	-0.070	0.117	0.109	-0.221	0.267	0.452**	0.410**			
TPC	-0.594**	-0.793**	-0.319*	-0.340*	-0.452**	-0.640**	0.775**	0.828**	0.638**	0.725**		
RWC	0.820**	0.895**	0.526**	0.711**	0.753**	0.734**	-0.902**	-0.810**	-0.519**	-0.269	-0.692**	
PRO	-0.626**	-0.472**	-0.368*	-0.671**	-0.651**	-0.423**	0.462**	0.386**	0.336*	-0.459**	-0.005	-0.436**

** : significance level at $p < 0.01$, * : significance level at $p < 0.05$. GP: Germination Percentage, SL: Seedling Length, RL: Root Length, SFW: Seedling Fresh Weight, RFW: Root Fresh Weight, Total Chl: Total Chlorophyll, CRT: Carotenoid, EL: Electrolyte Leakage, MDA: Molandialdehyde, SOD: Superoxide Dismutase, TPC: Total Phenolic Content, RWC: Relative Water Content

Table 2. Correlation coefficients of the physiological and biochemical parameters of safflower seedlings of hormoprimer applications under salt stress

Correlated parameters	GP	SL	RL	SFW	RFW	Total Chl	CRT	EL	MDA	SOD	TPC	RWC
SL	0.912**											
RL	0.702**	0.687**										
SFW	0.908**	0.908**	0.701**									
RFW	0.881**	0.819**	0.604**	0.919**								
Total Chl	0.725**	0.823**	0.686**	0.807**	0.765**							
CRT	-0.937**	-0.921**	-0.627**	-0.917**	-0.856**	-0.779**						
EL	-0.848**	-0.940**	-0.732**	-0.831**	-0.771**	-0.806**	0.833**					
MDA	-0.621**	-0.744**	-0.539**	-0.680**	-0.612**	-0.732**	0.718**	0.803**				
SOD	-0.450**	-0.550**	-0.586**	-0.466**	-0.260**	-0.377**	0.490**	0.608**	0.301*			
TPC	-0.672**	-0.789**	-0.662**	-0.650**	-0.530**	-0.551**	0.646**	0.838**	0.482**	0.846**		
RWC	0.948**	0.904**	0.696**	0.896**	0.837**	0.735**	-0.867**	-0.817**	-0.577**	-0.383**	-0.657**	
PRO	-0.620**	-0.579**	-0.516**	-0.590**	-0.485**	-0.522**	0.569**	0.561**	0.666**	0.078	0.206	-0.628**

** : significance level at $p < 0.01$, * : significance level at $p < 0.05$. GP: Germination Percentage, SL: Seedling Length, RL: Root Length, SFW: Seedling Fresh Weight, RFW: Root Fresh Weight, Total Chl: Total Chlorophyll, Crt: Carotenoid, EL: Electrolyte Leakage, MDA: Molandialdehyde, SOD: Superoxide Dismutase, TPC: Total Phenolic Content, RWC: Relative Water Content

PCA analysis

The results of PCA analysis showing the effects of hydropriming (a) and hormoprimer (b) applications on safflower seeds on germination, seedling characteristics and biochemical properties under salt stress are shown in Figure 14. When the graph (a) of hydropriming is examined, the 1st principal component is 65.89%, and the 2nd principal component is 16.92%, in total 82.81% of the variation. When hormoprimer (b) was examined, the 1st principal component was 72.05%, and the 2nd principal component was 10.73%, accounting for 82.78% of the total variation. Yan *et al.* (2000) reported that the coefficients of the investigated properties were high when the total value of the two principal components approached 100%. It is seen that the properties examined in both applications form 4 different groups. In hydropriming application, GP, RL, SFW, RFW 1st Group, SL, Total Chl, RWC 2nd Group, SOD, TPC, MDA, EL and Crt 3rd Group, and Pro 4th Group. In hormoprimer application, GP, SFW, RFW, RWC, Total Chl 1st Group, RL, SL 2nd Group, SOD, TPC and EL 3rd Group and Crt, MDA, Pro 4th Group. Since the angle between the vectors is less than 90°, the investigated features are in 4 different groups. Since the angle between the parameters in separate groups is more than 90°, it was determined that the relationship between them was weak (Kızılgeçi *et al.*, 2019).

Discussion

Germination percentage (GP)

Salt stress causes excessive production of reactive oxygen species (ROS), which prevents germination and plant growth, leading to an increase in antioxidant potential, proline and phenolic compounds. Seed germination is the initial stage of a plants life. It is a three-phase process. The first phase is imbibition. The second phase is the regulation of germination and the third phase represents the completion of germination (Sghaier *et al.*, 2022). However, plants cannot achieve sufficient germination due to adverse environmental conditions and stress factors. In this study, germination and seedling growth were increased with hydro- and hormoprimer applications. Similarly, Alom *et al.* (2016) reported the same results in wheat, three bean and two sorghum cultivars, and Moori and Ahmadi-Lahijani (2020) thyme, Kka *et al.* (2022) faba bean crops.

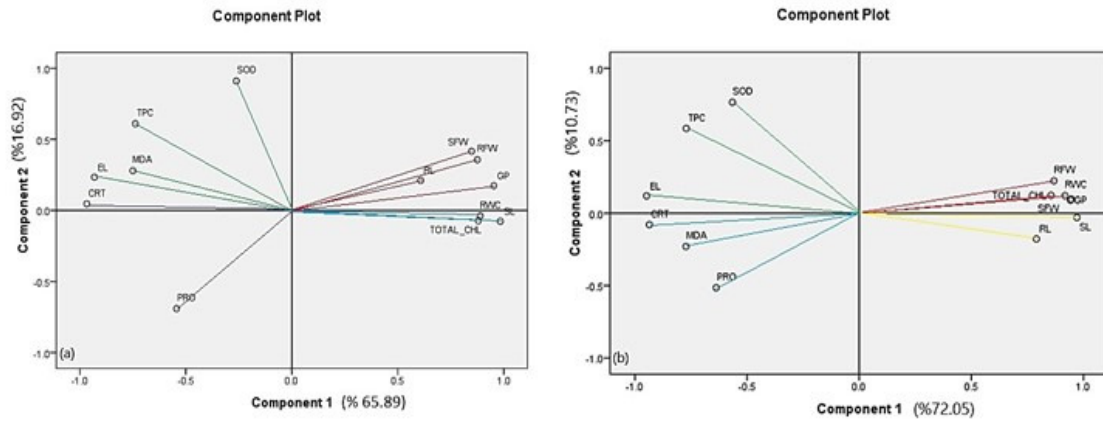


Figure 14. Principal component analysis (PCA) (a) hydropriming, (b) hormoprimer for different physiological and biochemical parameters of safflower under salinity stress conditions
GP: Germination Percentage, SL: Seedling Length, RL: Root Length, SFW: Seedling Fresh Weight, RFW: Root Fresh Weight, Total Chl: Total Chlorophyll, CRT: Carotenoid, EL: Electrolyte Leakage, MDA: Molandialdehyde, SOD: Superoxide Dismutase, TPC: Total Phenolic Content, RWC: Relative Water Content, Pro: Proline

Seedling growth parameters (SL, RL, SFW, RFW)

Salinity reduces plant growth through osmotic and toxic effects. Due to these effects, high Na ion uptake occurs and root growth slows down (Acosta-Motos *et al.*, 2017). In this study, it was determined that although SL, RL, SFW and RFW decreased as salt stress increased besides this priming applications were effective in reducing negative effects. When the applications were compared with each other, it was determined that the application of hormoprimer was more effective than hydropriming. Kaur *et al.* (2002) reported that the root and seedling length of the hydropriming application was longer than the non-priming seeds under drought conditions. Gürsoy (2022b) investigated the germination and seedling properties under salt stress by applying hormoprimer (salicylic acid) to safflower and linseed seeds. As a result of the study, it was reported that SA applications in both plants had a positive effect on the root and seedling growth of the plants. Álvarez-Méndez *et al.* (2022) applied gibberellic acid and proline in order to reduce the effects of salt stress on *Carica papaya* L.. As a result of the study, they reported that GA₃ or proline application as foliar induced a better performance of plants under salt stress by increasing stem height (142% or 144%) and applications can be recommended as plant growth and osmo regulator. Kayaçetin (2022), in study to determine the effects of drought and salt stress on two safflower varieties and four safflower lines, reported that as the severity of salt and drought stress increased, seedling growth parameters decreased.

Total chlorophyll (Total Chl)

In long term, salt accumulation can decrease photosynthetic pigments chlorophyll a, chlorophyll b and carotenoid content in plants, and this situation overall photosynthetic efficiency can also be decreased. Besides this in the short term salinity can affect photosynthesis by stomatal limitations (Parida and Das, 2005; Munns and Tester, 2008; Acosta-Motos *et al.*, 2017). According to Arora *et al.* (2012), photosynthetic rate has a positive relationship with relative chlorophyll content. Therefore, change in chlorophyll content is a good indicator of plant health Kumar and Rajalekshmi (2021). Because this situation causes a decrease in chlorophyll due to the toxicity of Na⁺ ions and the increase of Cl⁻ ions (Huang *et al.*, 2021). With various priming applications, it is tried to increase the chlorophyll content under salt stress conditions. In this study, salt stress caused a significant decrease in total chlorophyll. However, the ameliorative effect of priming applications had an effect on the increase in chlorophyll content. Similarly, the findings obtained in other studies are consistent with the findings of Kumar and Rajalekshmi (2021) *Psophocarpus tetragonolobus* (L.) DC. they applied hydro-, halo- and osmoprimer to their seeds. As a result of the study, they reported that they obtained the highest

chlorophyll from osmopriming application. Huang *et al.* (2021) reported that the chlorophyll content increased (60-92%) compared to the control in their study in which they investigated the effects of sorghum water extract by priming the seeds under salt stress. Granaz *et al.* (2022) in their study in which they applied thiourea (TU; 10 mM), salicylic acid (SA; 250 μ M), and kinetin (KIN; 3 μ M) to corn plant under salt stress, and it was found that photosynthetic pigments (Chl a, b and carotenoids) reported that they determined that it increased especially with thiourea and kinetin applications.

Carotenoid (Car)

Carotenoids have a very important role as antioxidants, and they are also related to the response of plants to environmental stresses (Havaux, 2013). Among the antioxidants present in the chloroplasts, carotenoids (Car) play an important role in the mechanisms protecting the photosynthetic parts against dangerous environmental factors (Ramel *et al.*, 2012). Shaki *et al.* (2017) in their study in which they applied hormopriming to safflower plant under salt stress conditions and carotenoid content increased under salinity while exogenous hormopriming (SA) significantly increased this compound in plants. Khosrowshahi *et al.* (2020) reported that the carotenoid in the leaves decreased due to oxidative stress in their study investigating the effects of spermine on the antioxidant enzymes and pigment contents of the safflower plant under water stress conditions. Álvarez-Méndez *et al.* (2022) applied gibberellic acid and proline priming to reduce the effects of salt stress on *Carica papaya* L. plant. As a result of the study, they reported that the content of carotenoids decreased in plants exposed to salt stress, whereas the pigment contents of plants treated with gibberellic acid and proline were at the same level as control plants. Gürsoy (2022b), in her study in which he applied salicylic acid to sunflower and linseed plants under salt stress, reported that pretreatment applications in both plants caused an increase in carotenoid content compared to the control.

Electrolyte leakage (EL)

The EL is a good indicator as it reflects the degree of plant injury by salt stress. EL values indicate membrane damage and are expressed by the electrical conductivity value of cell fluid leaking from leaf tissues (Dongsansuk *et al.*, 2021). In this study, priming applications caused a decrease in EL. However, the application of Hy3 is important in reducing the most effective EL. In their study in which they examined the physiological properties of soybean varieties under salt stress, they reported that EL increased significantly with the effect of stress in the leaf tissues of plants (El Sabagh *et al.*, 2015). Kumar and Rajalekshmi (2021) reported that electrolyte leakage is high in unprimed seeds in their study in which they applied hydro-, halo-, and osmopriming, whereas the leakage decreases as the application time increases in hydopriming seeds. They determined that electrolyte leakage was reduced in hydroprimed seeds compared to control and other applications. This situation, also known as the stability of cell membranes, occurs in the form of reduced leakage by restructuring the membranes (Pandey *et al.*, 2014).

Lipid peroxidation (MDA)

Lipid peroxidation measured as MDA content is a good indicator of oxidative damage from stress (Nimir *et al.*, 2015). Ella *et al.* (2011) applied presoaking and priming to rice seeds with KCl and hydropriming. As a result of the study, they reported that the MDA ratio decreased in seeds with priming application. They reported that the lowest MDA content was determined in 48 h with the most concentrated KCl solution (22.25 MPa). Similar results were reported by Ebrahimian and Bybordi (2012), who noticed that an increase in salinity was accompanied by an increase in MDA content in sunflower. Under salinity and temperature stresses, ROS accumulation cause membrane lipid peroxidation, reducing membrane fluidity and selectivity. Nimir *et al.* (2015) applied gibberellic acid, kinetin and salicylic acid priming to the seeds of sweet sorghum plant under salt and heat stress conditions. In the study, they determined that the MDA content increased with salt and heat stress. However, they stated that hormone applications had a positive effect. They reported that

MDA decreased by 17.6% with salicylic acid application at 37 °C compared to the control. Samea-Andabjadid *et al.* (2018) reported that cytokinin and salicylic acid application to faba bean plant under salt stress in greenhouse conditions increased lipid peroxidation under stress conditions, but lipid peroxidation decreased with the effect of foliar application and cytokinin and salicylic acid application. Farooq *et al.* (2022) It also reduced MDA contents (65-75%) and regulated ROS production resulting in improved membrane stability.

Superoxide dismutase (SOD)

Plants respond to stress by increasing their antioxidant enzymes. SOD plays a key role in the cellular defense system against an oxidative effect by ROS (Fujii *et al.*, 2022). In this study, the increase in SOD played an important role in reducing the effects of salt stress. Similarly, Ella *et al.* (2011) applied presoaking and priming to rice seeds with KCl and hydropriming. As a result of the study, they reported that by increasing the scavenging property of reactive oxygen species in the seeds with priming application, it caused an increase in SOD and CAT activities. Wani *et al.* (2017) reported that the application of 24-epibrassinolide under salt or cadmium stress in chickpea plant caused an increase in antioxidant enzymes (CAT, POX and SOD). Hussain *et al.* (2018) applied hormoprimering (aspirin) to wheat seeds under salt stress. In their study, they reported that priming caused a decrease in oxidative stress, as well as an increase in antioxidant enzymes (catalase, peroxidase, and superoxide dismutase). Shah *et al.* (2020) applied hormoprimering (3-epibrassinolide) to the cucumber plant under cadmium stress. As a result of the study, they determined that the content of SOD, CAT and POD increased in plants.

Total phenolic content (TPC)

The phenolic compounds alter the chlorophyll content, photosynthesis and phytohormone activity (Huang *et al.*, 2021). Likewise, phenolic compounds are effective in regulating auxin homeostasis in tissues by also inhibiting the oxidation of auxins induced by peroxidases and oxidases (Huang *et al.*, 2021). Therefore, the increase in phenolic compounds is of vital importance for the response mechanism of plants against stress. In this study, the increase of phenolic compounds with priming applications is extremely important in resisting salt stress. Shaki *et al.* (2017) reported that an increase in phenolic compounds occurred under salt stress conditions of the safflower plant. They reported that the highest increase was obtained from 200 mM salt and foliar spray (hormone application, SA) application. Farooq *et al.* (2020) reported that phenolic compounds increased under water stress conditions in safflower. They reported that the application of ascorbic acid played an important role in the increase of phenolic compounds. Golkar *et al.* (2021) reported that the phenolic content increased under stress conditions. Farooq *et al.* (2022) reported that in nanoprimering, hydropriming and control applications of wheat seeds, TPC decreased as the priming doses increased. He *et al.* (2022) *Mesembryanthemum crystallinum* L. primed with sea water under salt stress. As a result of the study, they reported that phenolic compounds increased as the percentage of priming application increased.

Relative water content (RWC)

Similar results were obtained by Salwa *et al.* (2010) with peanut cultivars. Hniličková *et al.* (2017) reported that in the salt-tolerant 'Astro', the osmotic potential decreased with increasing NaCl concentrations, while RWC decrease didn't take place until 200 mM NaCl. Gürsoy (2022a) decreased RWC in sunflower cultivars under salt stress.

Proline (Pro)

Proline is a very important osmoprotectant in reducing the negative effects of salt stress in plants (ElSayed *et al.* 2022). Proline is known as a multifunctional molecule that accumulates at high concentrations in various abiotic stress situations (Kishor and Sreenivasulu, 2014). Proline has a very important role in

protecting plants against osmotic stress (Razi and Khadhir, 2021). Matias *et al.* (2018) applied hydropriming to increase the salt tolerance of sunflower plants. They reported that there was no accumulation of proline in hydroprimed seeds and that proline showed protective properties. Golkar *et al.* (2021), in their study to determine the effects of drought stress on safflower genotypes, reported an increase in proline accumulation due to the effect of stress. The significant increase in proline content observed in the present study is consistent with those previously reported on safflower (Farooq *et al.*, 2020; Yeloojeh *et al.*, 2020)

Conclusions

As a result of this study, it was determined that hydro- and hormoprimer applications play an important role in reducing the negative effects of salt stress. Both priming applications had a positive effect on the germination, seedling growth and biochemical properties of safflower seeds. However, priming applications have played an important role in reducing the electrolyte leakage and MDA content of safflower seeds. In this study, when both applications were compared, it was determined that the hormoprimer process was more effective in reducing the effects of stress.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The author declares that there are no conflicts of interest related to this article.

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