Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1183604

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 2: 164-173 / March 2023

DETERMINATION OF TOLERANCE AND SENSITIVITY OF SAFFLOWER GENOTYPES BASED ON GERMINATION INDICES AND COMPARISON OF BIOCHEMICAL CONTENTS UNDER SALT STRESS

Sercan ÖNDER[1]*, Nagihan YAVUZ[1], Muhammet TONGUÇ[1]

[1]*Isparta University of Applied Sciences, Faculty of Agriculture, Department of Agricultural Biotechnology, 32200, Isparta, Türkiye*

Abstract: Safflower germination and seedling growth stages are extremely sensitive to salinity. The study aimed to identify safflower genotypes' germination, seedling growth responses, and biochemical changes in tolerant and susceptible genotypes in response to salt stress. Total of 28 genotypes were subjected to salt (NaCl) treatments (0, 180, 240 mM), and germination percentage, mean germination time, seedling and root lengths, and vigor index of the genotypes were determined. The genotype, treatments, and interaction effects were significant for germination, seedling, and biochemical parameters. The genotypes' germination percentage, seedling length, root length, and vigor index decreased under salt stress. While the reduction in germination percentage of salt-tolerant genotypes was between 6-21%, it was between 46-65% in sensitive genotypes at 240 mM salt treatment. Five tolerant (Shufu, Sidwill, Finch, Yuyao, Oleic Leed) and sensitive (Huaxian, Linas, 4022, Oker, Rehbein) genotypes were chosen based on reductions in germination percentage and vigor index, and the proline, hydrogen peroxide, and malondialdehyde (MDA) contents of these genotypes were investigated. The proline content of the genotypes increased by 26 to 56 fold at 180 mM salt concentration. The hydrogen peroxide content of sensitive and tolerant genotypes increased at 180 mM salt treatment, but at 240 mM salt treatment, the hydrogen peroxide content of the sensitive genotypes continued to increase by 6-50%, hydrogen peroxide content decreased in tolerant genotypes by 10-30%. MDA contents increased in the sensitive and tolerant genotypes, but the level of increase was higher in sensitive genotypes (307-631%) than the tolerant genotypes (103-323%) at 240 mM salt treatment. The heatmap generated by means of sensitive and tolerant genotypes showed 28 coefficients and 5 of which were significant. These results show that changes in hydrogen peroxide and MDA contents are different between tolerant and sensitive genotypes. They could be useful selection criteria along with germination percentages for determining tolerant and susceptible safflower genotypes at the seedling stage.

Keywords: *Carthamus tinctorius*, Hydrogen peroxide, Lipid peroxidation, Proline, Salinity

***Corresponding author:** Isparta University of Applied Sciences, Faculty of Agriculture, Department of Agricultural Biotechnology, 32200, Isparta, Türkiye
E mail: sercanonder@isparta.edu.tr (S. ÖNDER)
Sercan ÖNDER https://orcid.org/0000-0002-8065-288X
Nagihan YAVUZ https://orcid.org/0000-0001-5791-8486
Muhammet TONGUÇ https://orcid.org/0000-0003-1292-2910

Received: October 03, 2022
Accepted: February 03, 2023
Published: March 01, 2023

Cite as: Önder S, Yavuz N, Tonguç M. 2023. Determination of tolerance and sensitivity of safflower genotypes based on germination indices and comparison of biochemical contents under salt stress. BSJ Agri, 6(2): 164-173.

1. Introduction

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae family and the only cultivated species in the *Carthamus* genus. Safflower is an alternative crop grown for its flowers and seeds. Safflower seeds contain 20-40% oil and are mainly utilized in the industry for edible oil and dying purposes.

Soil salinity has become a problem for agricultural production, particularly in arid and semi-arid regions, affecting approximately 800 and 1.5 million hectares worldwide and in Türkiye, respectively (Türkan and Demiral, 2009). Soil salinity reduces the amount of water utilized and causes water stress in the plants. Ion accumulation due to soil salinity reduces nutrient uptake, chlorophyll synthesis, and the rate of photosynthesis, increases water loss and causes toxicity, and thus reduces plant growth and yield. It also causes the formation of reactive oxygen species, which causes oxidative stress in the cells, and further impedes the metabolic process (Munns and Tester, 2008; Hussain et al., 2016).

Salinity has detrimental effects on germination, seedling establishment, growth, and yield of crops. Different approaches could be employed to alleviate the effects of salinity, and biotic approaches, such as cultivating salt-tolerant genotypes to grow saline soils, should be preferred because they are sustainable, efficient, and economical (Ashraf and McNeilly, 2004). Plants could be classified based on their performance under saline and non-saline conditions. Therefore, screening genotypes for identification and selection for salt tolerance within the available germplasm resources is necessary. Safflower is classified as tolerant to saline conditions, showing variation in salt tolerance within safflower germplasm (Dajue, 1993; Siddiqi et al., 2007; Kaya et al., 2019). Salt stress affects all developmental stages of safflower

(Irving et al., 1988; Kaya et al., 2003), but germination and seedling development phases are more susceptible to salinity stress (Hussain et al., 2016).

Plants produce or accumulate different solutes and ions under salinity stress, such as proline, hydrogen peroxide, and malondialdehyde (MDA) to maintain the osmotic balance of cells or as a result of oxidative damage. Proline accumulation effectively prevents membrane damage and maintains osmotic balance (Hasegawa et al., 2000; Hosseini et al., 2010). Safflower genotypes under salt stress increase proline content to protect themselves from the effects of salt stress (Hosseini et al., 2010; Karimi et al., 2014). Hydrogen peroxide and MDA accumulation are associated with the production of reactive oxygen species leading to oxidative damage and causing lipid peroxidation. Therefore, the accumulation of these molecules is considered an indicator of cellular damage and seed deterioration in plants (Priestley, 1986; Bailly, 2004).

It is necessary to conduct salt tolerance tests under extreme conditions for identification and differentiation of safflower genotypes' tolerance to salinity (Siddiqi et al., 2007). Determination of biochemical changes occurring under stress conditions could be useful to identify tolerance mechanisms associated with tolerant and sensitive varieties. Therefore, the study aimed to test the response of many safflower genotypes under high salt concentrations during germination and early seedling growth to determine tolerant and sensitive genotypes. Then to study the effects of salt stress on proline, MDA and hydrogen peroxide contents in sensitive and tolerant genotypes to deduce stress responses of these genotypes.

2. Materials and Methods

2.1. Plant Material and Germination Tests

The study was carried out in the Agricultural Biotechnology Department laboratories at Isparta University of Applied Sciences (ISUBU) in 2021. Seeds from 28 safflower genotypes were used as plant material in the study. Enana, Rinconado and 4022 were breeding lines and the rest was registered safflower genotypes. A previous publication provides detailed information about origins, registration status, oil contents and other agronomic characteristics (Erbaş et al., 2016).

Seeds were surface sterilized with 2% sodium hypochlorite solution for 10 min, rinsed under running tap water for 5 min, and dried at room temperature. Seeds (50x4) were placed into two layers of filter papers, moistened with three different (0, 180, 240 mM) NaCl solutions, and put into sealed plastic containers to prevent evaporation. High salt concentrations were chosen because salt-tolerant and sensitive genotypes were distinguished better at stringent conditions (Siddiqi et al., 2007; Tonguç et al., 2021). Germination tests were conducted in a growth cabinet at 25±1 °C under dark conditions for 14 days. Seeds with radicle growth of 2 mm were counted as germinated and allowed to grow further for seedling measurements. Germination percentage and mean germination time were calculated as described (ISTA, 2009). After 14 days of germination, seedling and root lengths (cm) were measured using five seedlings from each replication. The vigor index of genotypes under different salt stress conditions was calculated by multiplying germination percentage and seedling length (Abdul-Baki and Anderson, 1973).

2.2. Biochemical Measurements

Percent reduction in germination percentage along with vigor index of the genotypes at 240 mM salt concentration was used to discriminate salt tolerant and sensitive genotypes. Seedlings were washed with distilled water, dried with paper towels, and used to measure biochemical parameters. Five seedlings (5x4) from each replication were powdered in liquid nitrogen and used to determine proline, MDA, and hydrogen peroxide contents of selected genotypes.

Proline was determined following the protocol of Zhang and Huang (2013). Ground seedlings (0.5 g) was homogenized with 2 mL 3% sulphosalicylic acid to determine the proline content of the samples. The slurry was centrifuged at 5.000 x g for 5 min, the 500 µL supernatant was taken to a new tube, added 500 µL acetic acid and 500 µL ninhydrin solution prepared with glacial acetic acid and orthophosphate. Tubes containing samples were boiled for 45 min and cooled on ice. An equal volume of toluene was added to each sample and vortexed for 1 min, then centrifuged at 1.000 × g for 5 min. The absorbance of the samples was measured at 520 nm by spectrophotometer. The standart curve was generated by known concentrations of proline prepared in 3% sulphosalicylic acid.

The hydrogen peroxide and MDA content of the samples was determined following Velikova et al. (2000), and Zhang and Huang (2013); respectively, and the analyses were performed as described by Önder et al. (2022).

2.3. Statistical Analysis

The experiment was a complete randomized block design with four replications. Each replication contained 50 seeds for germination parameters, and contained five biological replications for biochemical analysis. Germination percentage, mean germination time, and seedling and root lengths were measured. Germination data were transformed for normalization. The vigor index of the genotypes was also calculated using germination percentage and seedling length. Germination, seedling, and biochemical indices were subjected to ANOVA with SPSS 22.0 software (SPSS Inc, USA), and significant effects for genotypes, treatments, and their interactions on all examined germination and seedling indices were detected at $P \leq 0.001$ level of significance. Duncan's multiple range test was used to discriminate the differences between the means. To reveal the relationships between germination, seedling, and biochemical indices, Pearson's linear correlation analysis was carried out using OriginPro software's trial version, and results were presented as a heatmap.

3. Results

Safflower genotypes were tested for their responses at 0, 180, and 240 mM NaCl concentrations (Table 1). Germination percentages varied between 52-93% in the control group. While the genotypes with the lowest germination were Enana, Huaxian, and Olas; the genotypes with the highest germination were Linas, S-517, and 55633 (Table 2). Germination decreased at 180 mM, and 240 mM NaCl treatments, and germination percentages varied between 41-85% among the genotypes at 180 mM NaCl concentration. The lowest germination percentage in this treatment was observed in Olas and Enana, and the highest germination percentage was obtained from Frio, 55633, and S-517. At 240 mM NaCl treatment, germination percentage varied between 24-69%, and Rehbein, Girard, Huaxian, and 4022 had less than 30% germination. Twelve genotypes had higher than 50% germination, and the highest germination percentages were obtained from Frio, Finch, and S-517 at 240 mM salt treatment.

Mean germination times were shorter than two days for all genotypes except Montola 2000, Enana, and Oleic Leed in control. Germination time prolonged under 180 mM NaCl concentration, but germination times of Montola 2000, Enana, and Oleic Leed, contrary to the general trend, decreased. Hartman had over three days, and 13 genotypes had over two days of mean germination time at 180 mM NaCl concentration. At 240 mM NaCl treatment, ten genotypes had over two days, and Rehbein had over three days of mean germination time. With the increased NaCl concentration, the changes observed in the germination times of the genotypes differed, showing a response depending on genotype (Table 2). For example, Shufu, Yuyao, and Sidwill shortened, Linas, 55633, and Rehbein prolonged, and Yenice and Huaxian had the same mean germination times at 240 mM NaCl compared to 180 mM NaCl concentration.

Table 1. Mean squares of ANOVA results for germination and seedling parameters of safflower genotypes

Variance sources	DF	GP	MGT	VI	SL	RL
Genotypes	27	403.95***	0.56***	325285 ***	19.31***	9.27***
Treatments	2	6764.02***	11.67***	33356311***	4528.03***	2310.36***
Interaction	54	80.64***	0.58***	120691***	9.85***	5.95***
Error	252	13.76	0.14	10324	1.51	0.97

GP= germination percentage, MGT= mean germination time, VI= vigor index, SL= seedling length, RL= root length, ***= significant at $P \leq 0.001$ level of significance according to Duncan's multiple range test.

Table 2. Effects of salt treatments on germination percentage, mean germination time and vigor index of safflower genotypes. Values within columns are the means ± standard deviations

	Germination percentage (%)			Mean germination time (day)			Vigor index		
	Control	180 mM	240 mM	Control	180 mM	240 mM	Control	180 mM	240 mM
Shufu	59±6.3^{h-j}	52±6.1^{f-i}	51±2.4^{a-e}	1.4±0.1^{e-h}	1.9±0.2^{b-e}	1.5±0.1^{f}	1079±79^{hi}	381±38^{i-l}	377±31^{f-i}
Saffire	82±6.4^{b-e}	63±5.3^{a-d}	46±1.8^{c-h}	1.8±0.3^{c-e}	2.3±0.4^{b-d}	1.8±0.4^{d-f}	1636±125^{c-e}	630±67^{a-e}	449±61^{d-g}
Sidwill	56±4.0^{j}	51±5.5^{f-i}	50±1.5^{b-f}	1.7±0.3^{c-e}	2.0±0.3^{b-e}	1.6±0.2^{ef}	1177±125^{gh}	484±140^{f-j}	519±75^{a-d}
PCA	63±8.9^{g-j}	47±3.6^{h-j}	45±3.4^{c-h}	1.7±0.2^{c-e}	2.2±0.2^{b-e}	2.0±0.1^{c-f}	1377±127^{fg}	341±34^{kl}	315±20^{h-j}
Frio	76±10.1^{d-f}	69±4.9^{a}	59±4.9^{a}	1.2±0.1^{gh}	2.0±0.2^{b-e}	2.2±0.1^{b-f}	1546±132^{d-f}	700±71^{ab}	555±49^{a-c}
Montola 2000	54±4.0^{j}	44±3.0^{i-j}	38±7.0^{ij}	2.8±0.7^{a}	2.1±0.3^{b-e}	1.9±0.5^{d-f}	901±121^{ij}	291±33^{l}	231±21^{j-l}
Rinconada	82±8.1^{b-f}	58±3.0^{c-f}	52±3.8^{a-d}	1.1±0.1^{gh}	1.9±0.2^{b-e}	1.8±0.2^{d-f}	1603±88^{d-f}	401±43^{h-l}	344±42^{g-i}
Enana	52±2.6^{j}	42±1.5^{j}	37±8.1^{ij}	2.3±0.4^{b}	1.8±0.2^{c-e}	1.7±0.5^{d-f}	981±65^{h-j}	377±32^{j-l}	328±72^{h-j}
Huaxian	52±1.5^{j}	46±2.3^{h-j}	25±3.6^{kl}	1.9±0.1^{b-d}	2.2±0.5^{b-e}	2.2±0.5^{b-e}	795±77^{j}	374±68^{j-l}	207±31^{kl}
Linas	93±5.5^{a}	61±8.2^{b-e}	46±1.8^{c-h}	1.1±0.1^{gh}	1.7±0.3^{c-e}	2.4±1.3^{a-d}	1538±44^{d-f}	674±110^{a-c}	487±54^{a-e}
Dinçer	80±8.6^{b-f}	60±7.6^{b-e}	43±3.5^{f-i}	1.2±0.1^{f-h}	2.0±0.3^{b-e}	1.8±0.4^{d-f}	1610±121^{d-f}	562±144^{c-g}	402±89^{e-h}
4022	78±10.9^{b-f}	59±5.5^{c-f}	27±1.9^{kl}	1.3±0.1^{f-h}	2.5±1.2^{b}	2.7±0.8^{ab}	1405±173^{ef}	560±56^{c-g}	202±12^{kl}
Finch	72±3.7^{e-h}	58±3.5^{c-f}	57±3.6^{ab}	1.3±0.2^{f-h}	1.9±0.2^{b-e}	2.0±0.2^{c-f}	1389±111^{fg}	391±51^{h-l}	391±28^{e-h}
Sahuaripa 88	79±6.3^{b-f}	64±0.9^{a-d}	53±4.1^{a-d}	1.2±0.1^{gh}	2.0±0.3^{b-e}	2.2±0.3^{b-f}	1620±135^{c-f}	624±68^{a-e}	488±59^{a-e}
Oker	87±6.7^{a-d}	65±3.2^{a-c}	42±1.5^{g-i}	1.1±0.1^{gh}	1.9±0.1^{b-e}	1.6±0.2^{ef}	1706±220^{cd}	739±130^{a}	462±75^{c-f}
55633	88±1.5^{a-c}	67±3.2^{ab}	54±2.6^{a-c}	1.1±0.1^{h}	2.0±0.3^{b-e}	2.7±0.5^{a-c}	1943±36^{b}	611±52^{b-f}	588±38^{a}
Yenice	69±7.2^{f-i}	55±6.9^{d-g}	43±1.1^{e-i}	1.3±0.2^{f-h}	1.9±0.3^{b-e}	1.9±0.3^{d-f}	1478±135^{d-f}	515±87^{d-h}	390±26^{e-h}
Leed	70±8.2^{e-i}	50±0.9^{g-i}	45±0.9^{d-h}	1.1±0.1^{gh}	1.7±0.2^{de}	2.0±0.1^{c-f}	1533±109^{d-f}	561±49^{c-g}	480±52^{b-f}
Ole	78±10.8^{b-f}	61±1.8^{b-e}	52±1.8^{a-d}	1.5±0.1^{d-h}	2.4±0.5^{bc}	2.7±0.1^{ab}	1497±332^{d-f}	507±50^{e-i}	490±2^{a-e}
Hartman	73±8.4^{e-g}	61±6.9^{b-e}	54±0.9^{a-d}	1.1±0.1^{gh}	3.2±0.4^{a}	1.6±0.2^{ef}	1548±154^{d-f}	640±92^{a-d}	569±65^{ab}
Ziyang	78±5.5^{c-f}	62±1.5^{a-d}	47±8.6^{c-h}	1.2±0.1^{gh}	1.9±0.3^{b-e}	2.1±0.4^{b-f}	1842±137^{bc}	407±43^{h-l}	332±84^{h-j}
S-517	89±4.9^{ab}	67±5.8^{ab}	59±3.6^{a}	1.1±0.1^{h}	1.5±0.3^{e}	1.9±0.4^{d-f}	2167±212^{a}	447±56^{g-k}	398±66^{e-h}
Yuyao	62±7.6^{g-j}	54±3.2^{e-h}	54±2.8^{a-d}	1.2±0.1^{gh}	2.2±0.4^{b-e}	2.0±0.2^{c-f}	1017±150^{h-j}	353±25^{kl}	354±38^{g-i}
Girard	56±1.8^{j}	51±1.8^{f-i}	29±6.1^{kl}	1.6±0.3^{d-g}	2.0±0.2^{b-e}	1.9±0.3^{d-f}	1042±60^{hi}	505±67^{e-i}	210±104^{kl}
FO-2	58±1.8^{ij}	46±1.8^{h-j}	31±4.4^{jk}	1.4±0.1^{e-h}	2.1±0.2^{b-e}	1.7±0.3^{d-f}	1104±70^{hi}	512±29^{e-h}	346±79^{g-i}
Olas	52±1.8^{j}	41±0.9^{j}	40±3.7^{hi}	1.7±0.4^{c-f}	2.1±0.3^{b-e}	1.6±0.2^{ef}	1050±48^{hi}	421±44^{h-k}	320±58^{h-j}
Oleic Leed	54±3.2^{j}	51±1.8^{f-i}	50±0.9^{b-g}	2.0±0.5^{bc}	1.9±0.1^{b-e}	2.1±0.3^{b-f}	1092±63^{hi}	549±41^{c-g}	378±71^{f-i}
Rehbein	54±2.3^{j}	44±1.5^{ij}	29±3.2^{kl}	1.9±0.4^{cd}	1.8±0.1^{b-e}	3.0±0.4^{a}	1057±113^{hi}	412±332^{h-l}	276±77^{i-k}

Different letters within each column indicate significant differences between the genotypes within treatments.

Table 3. Seedling length and root length of safflower genotypes under different salt treatments. Values within columns are the means ± standard deviations

	Seedling length (cm)			Root length (cm)		
	Control	180 mM	240 mM	Control	180 mM	240 mM
Shufu	18.6±3.1^{h-j}	7.4±0.2^{h-j}	7.4±0.3^{g-j}	11.5±2.5^{f-h}	3.7±0.4^{h-j}	4.2±0.6^{c-h}
Saffire	20.0±0.8^{c-i}	10.1±1.3^{a-f}	9.9±1.2^{a-d}	11.7±0.5^{f-h}	4.7±0.67^{d-h}	4.9±0.6^{a-g}
Sidwill	21.0±1.0^{c-h}	9.5±1.8^{c-g}	10.4±1.6^{a-c}	13.3±1.0^{c-f}	4.6±1.2^{d-i}	5.5±1.1^{a-c}
PCA	22.0±1.1^{b-e}	7.3±0.2^{ij}	7.0±0.2^{h-j}	13.3±1.0^{c-f}	3.7±0.2^{h-j}	3.7±0.1^{e-h}
Frio	20.5±1.3^{c-i}	10.1±0.5^{a-e}	9.5±0.9^{a-e}	13.4±1.7^{c-f}	5.0±0.4^{c-f}	5.0±0.6^{a-f}
Montola 2000	16.7±1.5^{jk}	6.6±0.4^{j}	6.3±0.6^{j}	9.4±1.2^{ij}	3.3±0.3^{j-k}	3.3±0.3^{h}
Rinconada	19.8±1.4^{d-i}	6.9±0.5^{ij}	6.7±0.6^{ij}	12.4±1.3^{d-g}	3.6±0.5^{h-j}	3.5±0.5^{gh}
Enana	18.7±0.7^{g-j}	9.0±0.5^{e-h}	8.9±0.4^{b-h}	11.7±0.8^{f-h}	4.2±0.3^{e-j}	4.4±0.4^{c-h}
Huaxian	15.3±1.5^{k}	8.2±1.4^{g-j}	8.5±2.0^{c-i}	8.3±1.3^{j}	3.8±0.7^{g-j}	4.3±0.9^{c-h}
Linas	16.7±1.1^{jk}	11.1±1.0^{a-c}	10.7±1.2^{ab}	9.8±0.8^{h-j}	6.0±1.0^{a-c}	6.0±0.8^{ab}
Dinçer	20.3±1.0^{c-i}	9.4±1.5^{c-g}	9.4±1.4^{a-f}	12.9±0.9^{c-g}	4.7±0.8^{d-h}	4.6±0.8^{c-h}
4022	18.1±1.0^{ij}	9.6±0.7^{b-g}	7.5±0.2^{f-j}	11.7±0.5^{e-h}	4.7±0.3^{d-h}	3.3±0.2^{h}
Finch	19.3±0.7^{f-i}	6.8±0.7^{ij}	6.8±0.3^{ij}	11.6±1.2^{f-h}	3.2±0.1^{k}	4.6±0.5^{b-h}
Sahuaripa 88	20.5±0.5^{c-h}	9.8±1.1^{a-g}	9.3±1.0^{a-g}	13.4±0.6^{c-f}	5.0±0.7^{c-e}	4.9±0.6^{a-g}
Oker	19.7±1.1^{e-i}	11.3±1.8^{a}	11.0±1.8^{a}	12.5±0.5^{d-g}	5.5±1.1^{a-d}	5.5±1.2^{a-c}
55633	22.1±0.8^{b-d}	9.1±0.4^{e-g}	10.9±0.6^{ab}	14.8±1.0^{a-c}	3.9±0.3^{f-j}	5.3±0.4^{a-c}
Yenice	21.5±0.8^{b-f}	9.3±0.6^{d-g}	9.1±0.4^{a-g}	14.3±0.6^{a-d}	4.8±0.2^{d-g}	4.9±0.1^{a-f}
Leed	22.2±1.8^{bc}	11.2±1.0^{ab}	10.8±1.0^{ab}	13.9±1.6^{b-e}	6.2±0.8^{ab}	6.1±0.8^{a}
Ole	19.0±2.0^{f-i}	8.4±0.9^{f-i}	9.5±0.4^{a-e}	11.0±1.7^{g-i}	3.5±0.2^{j-k}	6.0±0.5^{ab}
Hartman	21.3±0.6^{c-g}	10.5±1.0^{a-e}	10.6±1.1^{ab}	14.3±0.8^{a-d}	5.1±0.8^{c-e}	5.3±0.5^{a-c}
Ziyang	23.6±0.7^{ab}	6.6±0.7^{j}	7.0±0.6^{h-j}	15.6±0.8^{ab}	3.4±0.5^{j-k}	3.8±0.6^{d-h}
S-517	24.5±1.4^{a}	6.7±0.6^{j}	6.7±0.7^{ij}	16.4±1.2^{a}	3.5±0.4^{j-k}	3.6±0.5^{f-h}
Yuyao	16.6±1.9^{jk}	6.7±0.6^{j}	6.6±0.4^{ij}	9.1±1.5^{ij}	3.6±0.4^{i-k}	3.6±0.2^{f-h}
Girard	18.8±1.6^{g-j}	10.0±1.0^{a-f}	7.1±1.7^{h-j}	12.0±1.6^{e-g}	5.2±0.7^{b-e}	3.6±1.3^{f-h}
FO-2	19.2±1.0^{f-i}	11.3±0.3^{ab}	11.1±1.0^{a}	11.6±1.0^{f-h}	6.3±0.4^{a}	6.1±0.8^{a}
Olas	20.4±1.6^{c-i}	10.4±1.0^{a-e}	8.0±1.1^{d-j}	13.4±1.9^{c-f}	5.4±0.8^{a-d}	5.1±0.8^{a-d}
Oleic Leed	20.4±0.6^{c-i}	10.9±1.0^{a-d}	7.6±1.4^{e-j}	13.3±0.8^{c-g}	5.4±0.70^{a-d}	5.1±1.2^{a-e}
Rehbein	19.7±1.6^{d-i}	9.4±0.9^{c-g}	9.7±2.7^{a-d}	12.3±1.4^{d-g}	4.7±0.6^{d-h}	5.4±2.0^{a-c}

Different letters within each column indicate significant differences between the genotypes within treatments.

Seedling and root lengths were measured to observe the effects of salt concentrations on the growth and development of germinated seeds under saline conditions (Table 3). Seedling lengths in the control group varied between 15.3-24.5 cm. Huaxian and S-517 had the control group' lowest and highest seedling lengths, respectively. Seedling lengths decreased with the increased salt concentrations, and as a result, seedling lengths reduced to 6.6-11.3 cm at 180 mM NaCl and to 6.3-11.1 cm at 240 mM NaCl treatments. Seedling lengths of six genotypes (Montola 2000, Rinconado, Finch, Ziyang, S-517, Yuyao) remained below 7 cm at 180 mM NaCl concentration. However, the length of these seedlings varied from 16.7-24.2 cm in the control group. Oker and Linas had the highest seedling length at 180 mM salt concentration. Oker (11.0 cm) still had the highest seedling length at 240 mM NaCl treatment. Montola 2000, Rinconado, Finch, S-517, and Yuyao had the shortest seedling lengths at the same treatment.

Root length measurements of the genotypes also showed that salt concentrations decreased seedling root lengths. While the root length of the seedlings varied from 8.3-14.8 cm in the control group, the root lengths of the seedlings varied from 3.2-6.3 cm and from 3.3-6.1 cm grown at 180 and 240 mM salt concentrations, respectively. Genotypes with the longest root lengths were Ziyang and S-517, and genotypes with the shortest root lengths were Huaxian and Yuyao in the control group. At 240 mM NaCl treatment, 4022 and Montola 2000 had the shortest root lengths with 3.3 cm, while Leed and FO-2 had the longest root lengths.

The vigor index values of the genotypes were between 795-2167. S-517 and Huaxian had the control group's highest and lowest vigor index values, respectively. Montola 2000 and Enana also had low vigor index values. Significant decreases in vigor index values were observed, and vigor indices of the genotypes were reduced to 291-1653 at 180 mM NaCl treatment. The vigor index values decreased further at 240 mM NaCl treatment, and Girard, Montola 2000, Huaxian, 4022 and Rehbein had the lowest, whereas Sidwill, Frio, 55633, and Hartman had the highest vigor index values (Table 2).

After measuring germination and seedling indices, the percent reduction in germination percentage and vigor index values were compared at 0 and 240 mM salt

concentrations. The results were used to select salt-tolerant and sensitive genotypes at the germination stage to investigate biochemical changes within these two groups of plants. Initial germination percentages of Oleic Leed, Shufu, Sidwill, Yuyao, and Finch were between 54-72%, and germination percentages were reduced by 6, 13, 11, 13, and 21% at 240 mM NaCl treatment, respectively. Germination percentages of sensitive genotypes (Huaxian, Linas, 4022, Oker, and Rehbein) were between 52-93% and dropped to 25-46% at 240 mM NaCl treatment, corresponding to 46-65% reduction in germination percentage between 0 and 240 mM NaCl treatments. Girard and Rehbein had the same germination percentages at 0 and 240 mM NaCl treatments, but their vigor index values were significantly different at 240 mM NaCl treatment; therefore, Rehbein was more vigorous and selected for biochemical evaluations.

The proline, MDA, and hydrogen peroxide contents were analyzed using sensitive and tolerant genotypes to determine the biochemical changes caused by salt stress in safflower seedlings grown at three different salt concentrations. Variance analysis revealed that genotypes, treatment, and genotypes x treatment interactions for proline, hydrogen peroxide, and MDA contents were significant at $P \leq 0.001$ levels of significance (Table 4).

1.00 µg g^{-1}among the sensitive genotypes. Shufu and Yuyao among the tolerant and Linas and 4022 among the sensitive genotypes significantly differed for proline content at the control (Figure 1). Salt stress increased proline contents of sensitive and tolerant safflower genotypes, but the increase from 0 to 180 mM treatment was far more dramatic than the increase from 180 mM to 240 mM NaCl treatment. Proline content increased by 26 to 56 fold to reach 25.04 µg g^{-1} and 28.24 µg g^{-1} in Rehbein and Shufu at 180 mM NaCl treatment, respectively. The highest increases in proline contents were observed in Shufu, 4022, and Oleic Leed, and the lowest increase was observed in Rehbein. At 240 mM NaCl treatment, the highest increases in proline contents were observed in 4022 and Rehbein by 1.6 and 1.2 times, respectively. The proline content of Shufu, Sidwill, Yuyao, Linas, and Rehbein was significantly higher at 240 mM salt concentration.

The hydrogen peroxide content of the genotypes was between 73.7-169.3 µmol g^{-1} in the control treatment. Huaxian and Oker had the highest hydrogen peroxide contents, whereas 4022 had the lowest hydrogen peroxide content in the control treatment (Figure 2). Exposure to 180 mM salt concentration increased hydrogen peroxide content among both tolerant and sensitive genotypes. The lowest increase was observed in Huaxian (7%) from 168.1 to 179.6 µmol g^{-1}, and the highest increase was observed in Finch (115%) from 92.9 to 200.1 µmol g^{-1} at 180 mM NaCl treatment. Oker had the highest hydrogen peroxide content (327.8 µmol g^{-1}) among the genotypes at 180 mM NaCl treatment. At 240 mM NaCl treatment, sensitive and tolerant genotypes exhibited different responses to salt stress. While hydrogen peroxide content increased in sensitive genotypes by 6-50%, it decreased in tolerant genotypes by 10-30%. The lowest and the highest increase among the sensitive genotypes was observed in Rehbein from 265.5 to 279.8 µmol g^{-1} and in Linas from 167.8 to 251.4 µmol g^{-1}. Shufu showed the lowest decrease in hydrogen peroxide content from 281.4 to 254.7 µmol g^{-1}. Finch had the highest reduction from 200.1 to 141.4 µmol g^{-1} for hydrogen peroxide content among the tolerant genotypes at 240 mM NaCl concentration.

The MDA content of the genotypes was between 0.89 to 2.10 nmol g^{-1} in the control group. Shufu had significantly higher MDA content than the rest of the genotypes within this group (Figure 3). MDA contents of the genotypes increased with increased salt concentrations, but the increase was higher in sensitive genotypes. MDA content increased by 27-94% in tolerant genotypes, whereas it increased by 132-349% in sensitive genotypes exposed to 180 mM salt concentration. The lowest and the highest MDA contents of tolerant genotypes were between 1.23-3.77 nmol g^{-1} in Sidwill and Shufu, respectively. Sidwill and Yuyao had higher MDA content than the other tolerant genotypes at 180 mM NaCl treatment. On the other hand, the MDA contents of the sensitive genotypes were significantly higher than the MDA contents of the tolerant genotypes, except for Shufu. The highest MDA content was observed in Linas (4.05 nmol g^{-1}), and the lowest MDA content was found in Huaxian (3.15 nmol g^{-1}). MDA contents of the genotypes continued to increase at 240 mM NaCl treatment.

Table 4. Mean squares of analysis of variance for biochemical measurements among the tolerant and sensitive safflower genotypes

Variables	DF	Mean squares		
		Prolin	Hydrogen peroxide	MDA
Genotypes	9	14.02***	23452.17***	8.59***
Treatments	2	7077.73***	82877.53***	134.99***
Interaction	18	11.99***	4324.03***	4.67***
Error	60	0.16	108.35	0.44

MDA= malondialdehyde, ***= significant at P≤0.001 levels of significance according to Duncan's multiple range test.

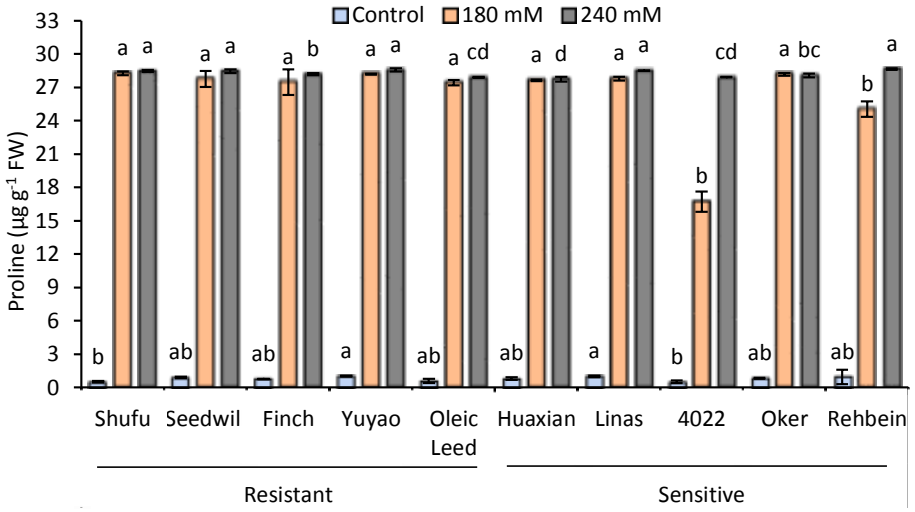

Figure 1. Proline contents of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

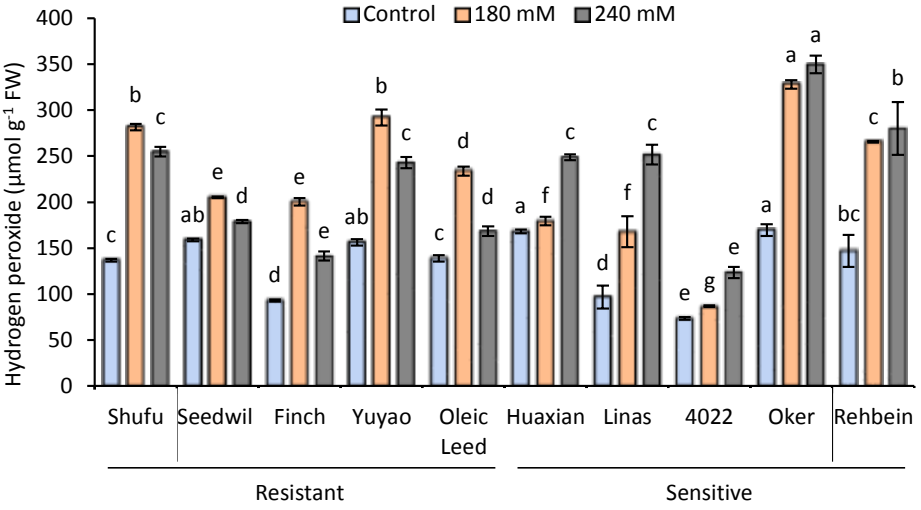

Figure 2. Hydrogen peroxide content of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

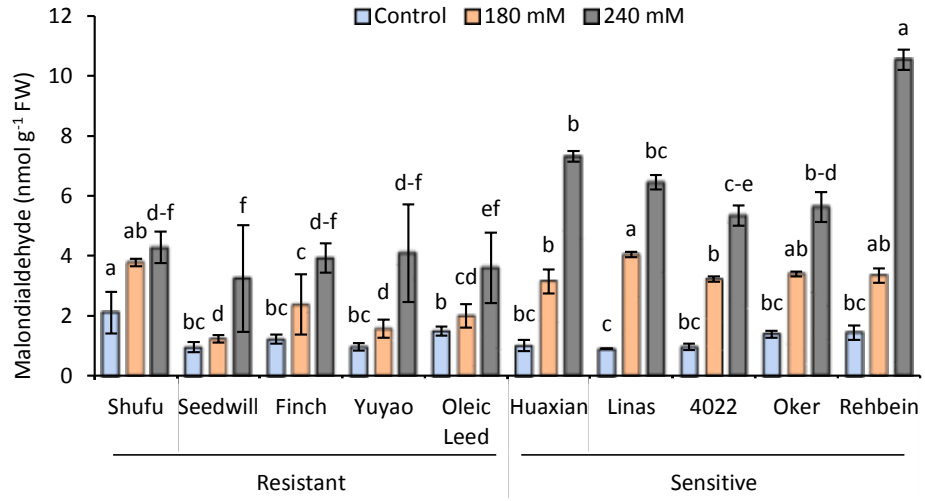

Figure 3. MDA content of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

The increase in the tolerant genotypes was between 14-166% and was between 61-215% in the susceptible genotypes. Even though Sidwill's MDA content increased by 166% to reach 3.25 nmol g^{-1}, but it still had the lowest MDA content among the genotypes, and Rehbein, the highest MDA content, increased by 215% to reach 10.54 nmol g^{-1} at 240 mM NaCl treatment (Figure 3).

Pearson's correlation analysis was carried out among the tolerant and sensitive genotypes to show the relationships between germination, seedling, and biochemical parameters. The results are given in Figure 4. A total of 28 correlation coefficients were calculated, two were negative, and three were positive and significantly correlated with each other. Germination percentage had a significant negative correlation with mean germination time (-0.83) but had a significant positive correlation with vigor index (0.91). Mean germination time negatively correlated with the vigor index (-0.72). Root length showed positive correlations with seedling length (0.98). Proline content positively correlated with hydrogen peroxide content (0.73).

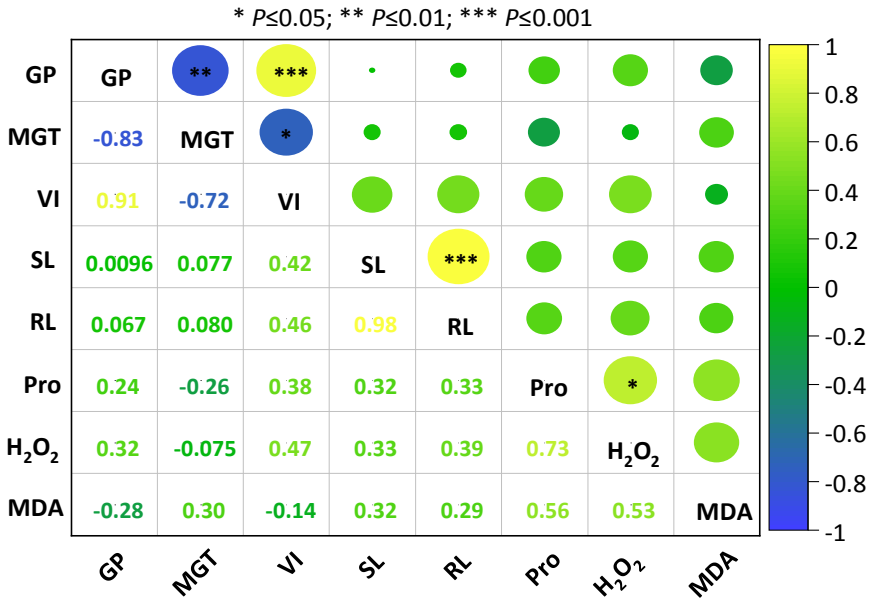

Figure 4. Relationships and correlation between 8 germination, seedling and biochemical parameters generated by a heat map. Color and scale display the intensity of mean values. (GP, germination percentage; MGT, mean germination time; VI, vigor index; SL, seedling length; RL, root length; Pro, proline; H2O2, hydrogen peroxide; MDA, malondialdehyde).

4. Discussion

Salinity, along with drought, is becoming a major constraint for agricultural production in arid and semi-arid regions of the world. Different biological treatments could be employed to mitigate stress conditions exerted by environmental factors, such as different seed treatments and foliar applications (Jabeen and Ahmad, 2012; Ashrafi and Razmjoo, 2015; Turan et al., 2022). Screening and identifying plant germplasm resources for salinity tolerance and cultivation is also another means to maintain reasonable yields under saline conditions (Irving et al., 1988; Siddiqi et al., 2007).

It is recommended that high salt concentrations should be used to differentiate genotypes for salt tolerance (Siddiqi et al., 2007), and we also observed that higher salt concentrations were better for differentiating safflower genotypes (Tonguç et al., 2021); therefore, we have screened safflower genotypes at 0, 180 and 240 mM salt concentrations in the present study. Safflower genotypes exhibited significant variations in germination and seedling indices. Salt stress negatively affected all germination and seedling parameters, especially at 240 mM salt concentration. The germination percentage of the genotypes varied between 52-93% at the control and varied between 27-59% at 240 mM NaCl treatment. The reduction in germination percentages was between 9-65% at 240 mM NaCl treatment (Table 2). Adverse effects of high salt concentrations on the germination of plant species are well known and have been documented for safflower (Kaya et al., 2003; Siddiqi et al., 2007; Çulha and Çakırlar, 2011; Kaya et al., 2019; Tonguç et al., 2021; Kurtuluş and Boydak, 2022). Mean germination time, measuring germination speed, was higher at higher salt concentrations compared to the control, which shows the germination speed was slower under salt stress conditions. In the present study, Montola 2000, Enana, and Oleic Leed had germination times over two days at the control, but 14 genotypes had over two days of germination times at 240 mM salt concentration. Similarly, increased mean germination time with the increased salt concentrations was reported for safflower (Kaya et al., 2019; Tonguç et al., 2021; Kurtuluş and Boydak, 2022). The results presented in the paper confirm that high salt concentrations reduce germination percentage and increase the time necessary for germination.

Germinated seedlings were allowed to grow within filter papers during the experiment to study the effects of salt concentrations on seedling growth. Seedling and root lengths were measured to calculate safflower genotypes' growth performance under stress and non-stress conditions. Plants in the control group had the highest values for these parameters. As the salt concentrations increased, measured values for seedling and root lengths decreased for all genotypes (Table 3). While seedling lengths decreased from 180 mM NaCl treatment to 240 mM NaCl treatment, root lengths of some genotypes, such as Shufu, Sidwill, Ole, and Rehbein, increased at 240 mM NaCl treatment compared to 180 mM NaCl treatment. Changes in seedling and root lengths have also been reported for safflower genotypes exposed to salt stress. In all reported experiments, shoot and root lengths reduced with increased salt concentrations (Kaya et al., 2003; Çulha and Çakırlar, 2011; Erdal and Çakırlar, 2014; Toprak and Tunçtürk, 2018; Kaya et al., 2019; Kurtuluş and Boydak, 2022). However, shoot growth was affected more severely and found more sensitive to salt stress than the root growth in safflower (Kaya et al., 2019). Increased root length under increased salt concentrations was reported for some safflower genotypes (Toprak and Tunçtürk, 2018; Kaya et al., 2019), which was also observed at 240 mM salt concentration in this study.

The vigor index is calculated using germination percentage and seedling length and shows the relative vigor of the genotypes under stress conditions (Abdul-Baki and Anderson, 1973), and genotypes with higher vigor index are considered to be more vigorous. Increased salt concentrations reduced the vigor index values of the genotypes, and the decline was more severe at the highest salt concentration. The vigor index of some safflower genotypes at low salt concentrations increased, but it decreased under higher salt concentrations (Kaya et al., 2019). We have not observed such an increase in vigor index because salt treatments were higher from the beginning. Still, our results for vigor index were similar at higher salt concentrations reported for safflower.

Different selection criteria, such as germination percentage, ion accumulation, ion balance, principal coordinate analysis, and gas exchange rates, are used to discriminate between tolerant and sensitive safflower genotypes to salinity (Siddiqi et al., 2007; Siddiqi et al., 2009; Kaya et al., 2019). We have used reduction in germination percentage as the main selection criteria along with vigor index values for selecting salt sensitive and tolerant genotypes. Based on these results, five tolerant (Shufu, Sidwill, Finch, Yuyao, and Oleic Leed) and five susceptible (Huaxian, Linas, 4022, Oker, and Rehbein) were selected for studying biochemical changes in the sensitive and tolerant genotypes exposed to salt stress.

Proline is accumulated as an osmoregulator to maintain the osmotic balance of cells and prevent the deterioration of proteins. Proline levels of plants increase in response to salt and drought stresses (Hussain et al., 2016). Proline contents of safflower genotypes were very low at the control, but exposure to salt stress increased proline content by 26-56 folds at 180 mM salt concentrations. Only Rehbein and 4022 had lower proline content at 180 mM salt concentration. Further increasing salt concentration caused little change in tolerant genotypes, but it further increased proline contents of Rehbein and 4022 to comparable levels to the other genotypes (Figure 1). Proline accumulation in response to salt stress is a common mechanism and has been reported for safflower (Hosseini et al., 2010; Erdal and Çakırlar, 2014). Dramatic increases in proline levels in response to salt stress, regardless of salt tolerance levels, were reported for safflower (Karimi et al., 2014). Even though the level of increase was very rapid in response to salt stress, proline accumulation did not differ between sensitive and tolerant genotypes and continued to increase in both groups suggesting that proline accumulation may be a common mechanism under stress conditions and may not be a particular part of salinity tolerance in safflower.

Reactive oxygen species are produced under stress conditions, and hydrogen peroxide and other reactive oxygen molecules cause lipid peroxidation (Priestley, 1986). Lipid peroxidation is the main cause of membrane damage, and the level of lipid peroxidation could be measured by monitoring MDA levels (Sharma et al., 2012). Superoxide dismutase scavenges superoxide radicals and converts them to hydrogen peroxide, which is scavenged by catalase and peroxidases. Therefore, antioxidant defense systems play important roles in mitigating oxygen species' effects under stress conditions. Hydrogen peroxide content was lower among the genotypes in the control treatment. Salt stress caused sharp increases in hydrogen peroxide content at 180 mM salt concentration across the genotypes (Figure 2). However, unlike proline contents of sensitive and tolerant genotypes, raising salt concentration to 240 mM did not increase hydrogen peroxide content across the safflower genotypes. Hydrogen peroxide accumulation showed a distinct difference between the sensitive and tolerant genotypes. In sensitive genotypes, hydrogen peroxide accumulation continued. As a result, the hydrogen peroxide content of sensitive genotypes increased by 6-50% at 240 mM salt concentration, whereas the hydrogen peroxide content of tolerant genotypes decreased by 10-30% at the same treatment. Studies showed that salt-tolerant safflower genotypes increase or maintain antioxidant enzyme activity under salt stress in safflower (Hosseini et al., 2010; Erdal and Çakırlar, 2014; Önder et al., 2022). Catalase and peroxidase activity between the salt-tolerant and sensitive safflower genotypes showed that the activity of these enzymes remained high in the tolerant genotype, but their activity ceased at a high salt concentration in the sensitive genotype (Hosseini et al., 2010). Similarly, the activity of different antioxidant enzymes followed the increased salt concentrations (Erdal and Çakırlar, 2014;

Önder et al., 2022). Though we did not determine the antioxidant enzyme activities in the study, these results show that genotypes differed in their abilities to detoxify hydrogen peroxide at 240 mM salt concentration, and the action of enzymes in the antioxidant defense mechanism regulated the level of hydrogen peroxide in tolerant genotypes. We have monitored MDA levels in tolerant and sensitive genotypes to measure lipid peroxidation damage. MDA contents of the genotypes in control were lower. Salt treatment increased the MDA contents of both sensitive and tolerant genotypes, and the increase in the sensitive genotypes at 180 mM NaCl treatment was higher than the increase in the tolerant genotypes at the same treatment (Figure 3). MDA levels of the sensitive genotypes continued to increase, and Rehbein, Huaxian, and Linas had significantly higher MDA levels than the tolerant genotypes at 240 mM NaCl treatment. MDA levels were between 3.25-4.28 nmol g^{-1} and 5.34-10.54 nmol g^{-1} in tolerant and sensitive genotypes at 240 mM NaCl treatment, respectively. MDA levels increase in response to salt stress in safflower (Erdal and Çakırlar, 2014; Önder et al., 2022). Our results show a link between hydrogen peroxide and MDA contents; as hydrogen peroxide content was reduced in tolerant genotypes, MDA levels in tolerant genotypes were also lower than in the sensitive genotypes. Önder et al. (2022) reported that hydrogen peroxide content was significantly associated with MDA, catalase, and antioxidant enzyme activities in two safflower genotypes under salt stress.

Correlation analysis revealed some insights between germination and biochemical parameters. Germination percentage and mean germination times were negatively correlated, as previously reported (Tonguç et al., 2021; Önder et al., 2022). However, our study found that germination percentage did not significantly correlate with MDA, proline, and hydrogen peroxide contents; these parameters were significantly and negatively associated with germination (Önder et al., 2022).

5. Conclusion

According to the results, Germination decreased under salt stress, but decline in the germination of seeds at 240 mM salt concentration was higher. Mean germination times also differed from the control depending on salt concentrations. The vigor index of the genotypes in the control group was higher, and significant decreases in vigor indices occurred with the increased salt concentrations. The highest seedling and root lengths were obtained from the control group, and seedling and root lengths decreased under salt stress. Proline content increased at very high rates in sensitive and tolerant genotypes and remained high under salt stress. MDA levels also increased, but the increase in the tolerant genotypes was lower than the increase in the sensitive genotypes grown under higher salt concentrations. Hydrogen peroxide levels also increased in response to salt stress. However, the hydrogen peroxide levels in the tolerant genotypes decreased, while the hydrogen peroxide levels continued to increase in the sensitive genotypes when the salt concentration increased to 240 mM. The amount of hydrogen peroxide could be a feature that can be used to distinguish between tolerant and sensitive genotypes for their germination ability under high salt concentrations.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	S.Ö.	N.Y.	M.T.
C	50	50	
D	100		
S			100
DCP		100	
DAI		50	50
L	40	30	30
W	50		50
CR	50		50
SR	100		
PM			100
FA		100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

Scientific and Technological Research Council of Türkiye (TUBITAK) financially supported this work under 2209-A program. Sercan Önder is a recipient of YÖK 100/2000 fellowship program for graduate students financed by Council of Higher Education.

References

Abdul-Baki AA, Anderson JD. 1973. Vigor determination in soybean seed by multiple criteria. Crop Sci, 13: 630-633.

Ashraf M, McNeilly T. 2004. Salinity tolerance in Brassica oilseeds. Crit Rev Plant Sci, 23: 157-174.

Ashrafi E, Razmjoo J. 2015. Seed treatment to overcome salt and drought stresses during germination in safflower (*Carthamus tinctorius* L.). J Plant Nutr, 38: 2151-2158.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. Seed Sci Res, 14: 93-107.

Çulha Ş, Çakırlar H. 2011. Effects of salt stress induced by NaCl on safflower (*Carthamus tinctorius* L.) cultivars at early seedling stages. Hacettepe J Biol Chem, 39: 61-64.

Dajue L. 1993. Progress of safflower (*Carthamus tinctorius* L.)

research and production in China. In: Proceedings of the Third Interactional Safflower Conference, June 14-18, Beijing, China, pp: 35-46.

Erbaş S, Tonguç M, Şanlı A. 2016. Variations in the agronomic and quality characteristics of domestic and foreign safflower (*Carthamus tinctorius* L.) genotypes. Turk J Field Crop, 21: 110-119.

Erdal ŞC, Cakirlar H. 2014. Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars. Turk J Biol, 38: 549-560.

Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol, 51: 463-499.

Hosseini T, Shekari F, Ghorbanli M. 2010. Effect of salt stress on ion content, proline and antioxidative enzymes of two safflower cultivars (*Carthamus tinctorius* L.). J Food Agric Environ, 8:1080–1086.

Hussain MI, Lyra DA, Farooq M, Nikoloudakis N, Khalid N. 2016. Salt and drought stress in safflower: A review. Agron Sustain Dev, 36: 1-31.

Irving DW, Shannon MC, Breda VA, Mackey BE. 1988. Salinity effects on yield and oil quality of high-linoleate and high-oleate cultivars of safflower (*Carthamus tinctorius* L.). J Agric Food Chem, 36: 37-42.

ISTA, 2009. International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland, pp: 48.

Jabeen N, Ahmad R. 2012. Improving tolerance of sunflower and safflower during growth stages to salinity through foliar spray of nutrient solutions. Pak J Bot, 44: 563-572.

Karimi S, Arzani A, Saeidi G. 2014. Differential response of ion and osmolyte accumulation to salinity stress in salt-tolerant and salt-sensitive seedlings of safflower (*Carthamus tinctorius* L.). Res Crops, 15: 802-809.

Kaya MD, İpek A, Öztürk A. 2003. Different soil salinity levels on germination and seedling growth of safflower (*Carthamus tinctorius* L.). Turk J Agric For, 27: 221-227.

Kaya MD, Akdoğan G, Kulan EG, Dağhan H, Sarı A. 2019. Salinity tolerance classification of sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) by cluster and principal component analysis. Appl Ecol Environ Res, 17: 3849-3857.

Kurtuluş M, Boydak E. 2022. Bazı aspir (*Carthamus tinctorius* L.) çeşitlerinde farklı tuz konsantrayonlarının çimlenme ve çıkış üzerine etkisi. Türk Tarım ve Doğa Bilimleri Dergisi, 9: 696-704.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Ann Rev Plant Biol, 59: 651-681.

Önder S, Dayan E, Karakurt Y, Tonguç M. 2022. Changes in germination, antioxidant enzyme activities and biochemical contents of safflower (*Carthamus tinctorius* L.) under different salinity levels. Süleyman Demirel Üniv Fen Edeb Faki Fen Derg 17: 186-196.

Priestley DA. 1986. Seed aging. Cornell University Press, Ithaca, USA, pp: 304.

Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot, 2012: 1-26.

Siddiqi EH, Ashraf M, Hussain M, Jamil A. 2009. Assessment of intercultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange characteristics as selection criteria. Pak J Bot, 41: 2251-2259.

Siddiqi EH, Ashraf M, Akram NA. 2007. Variation in seed germination and seedling growth in some diverse lines of safflower (*Carthamus tinctorius* L.) under salt stress. Pak J Bot, 39:1937-1944.

Tonguç M, Önder S, Mutlucan M. 2021. Determination of germination parameters of safflower (*Carthamus tinctorius* L.) cultivars under salt stress. Süleyman Demirel Üniv Fen Bil Enst Derg, 25: 155-161.

Toprak T, Tunçtürk R. 2018. Farklı aspir (*Carthamus tinctorius* L.) çeşitlerinin gelişim performansları üzerine tuz stresinin etkileri. Doğu Fen Bil Derg, 1: 44-50.

Turan M, Ekinci M, Kul R, Boynueyri FG, Yıldırım E. 2022. Mitigation of salinity stress in cucumber seedlings by exogenous hydrogen sulfide. J Plant Res, 135: 517-529.

Türkan I, Demiral T. 2009. Recent developments in understanding salinity tolerance. Environ Exp Bot, 67: 2-9.

Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci, 151: 59-66.

Zhang Z, Huang R. 2013 Analysis of malondialdehyde, chlorophyll proline, soluble sugar, and glutathione content in Arabidopsis seedlings. Bio-protocol 3 (14) URL: http://www.bio-protocol.org/e817. (access date: August 10, 2022).