doi:10.14720/aas.2025.121.1.16550 Original research article / izvirni znanstveni članek

Evaluation of salinity tolerance in seedlings of *Iris* × *germanica* L. hybrids

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Received November 08, 2023; accepted December 14, 2024
Delo je prispelo 8. november 2023, sprejeto 14. december 2024

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Abstract: Salinity is an abiotic stress that primarily impacts plant development and agricultural productivity worldwide and typically occurs in arid and semi-arid areas. Less research has been done on the impact of salt irrigation on the growth and development of ornamental plants, particularly-plants with bulb . In order to identify salt-tolerant in *Iris* × *germanica* L. genotypes (four genotypes including OPRC 14,18, 23 and 54), an experiment was carried out with four NaCl levels (4 as control, 6, 8, and 12 dS m^{-1}). The variation among genotypes caused different responses to salinity conditions. The results showed that the morphological, physiological, and biochemical characteristics of OPRC23 genotype were superior to those of other genotypes. The highest peroxidase enzyme activity was observed at 8 dS m^{-1} salinity level. The highest content of chlorophyll a, b, and carotenoid was obtained at a salinity level of 6 dS m^{-1} (NaCl). The OPRC54 genotype had the highest levels of chlorophyll a, b, and proline content at 12 dS m^{-1} salinity. In conclusion, different levels of salinity can expose to different genotypes, which leads to the selection of specific salt tolerant genotypes. The genotypes examined in this experiment of Iris germanica were resistant to salinity stress and this trait is of interest to landscape designers.

Key words: kperoxidase enzyme, carotenoid, proline content, chlorophyll content

Ovrednotenje tolerance na slanost pri sejankah križancev nemške perunike (*Iris* × *germanica* L.)

Izvleček: Slanost je abiotski stres, ki prvenstveno prizadane razvoj rastlin in ogroža kmetijsko pridelavo širom po svetu in se značilno pojavlja v sušnih in polsušnih območjih. Relativno manj raziskav je bilo opravljenih o vplivu namakanja s slano vodo, še posebno tistih na rastlinah z gomolji, korenikami in čebulicami. Z namenom ugotoviti toleranco na slanost pri genotipih nemške perunike (*Iris* × *germanica* L.) (štiri genotipi ko so obsegali OPRC 14,18, 23 in 54) je bil izveden poskus s štirimi ravnmi NaCl (4 kot kontrola, 6, 8, in 12 dS m^{-1}). Variabilnost med genotipi je povzročila različen odziv na slanostne razmere. Rezultati so pokazali, da je bil genotip OPRC23 glede na mofološke, fiziološke in biokemijske lastnosti najbojši. Največja aktivnost peroksidaze je bila ugotovljena pri slanosti 8 dS m^{-1}. Največja vsebnost klorofilov a, b in karotenoidov je bila pri slanosti 6 dS m^{-1}(NaCl). Genotip OPRC54 je imel največjo vsebnost klorofilov a, b, in prolina pri slanost 12 dS m^{-1}. Zaključimo lahko, da ispostavitev različnih genotipov različnim nivojem slanosti vodi k selekciji genotipov s specifično odpornostjo na slanost. V tem poskusu preučevani genotipi nemške perunike so bili odporni na slanostni stres in bi lahko bili zanimivi v tem pogledu za krajinske arhitekte.

Ključne besede: peroksidaza, karotenoidi, vsebnost prolina, vsebnost klorofila

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1 INTRODUCTION

Iris × *germanica* L. is one of the most important varieties of the bearded iris (Azimi et al., 2017), which is cultivated as a perennial ornamental plant (Azimi et al., 2017). In previous studies, new genotypes of *Iris* × *germanica* with a broad color range and different shapes have been recognized in Iran (Azimi et al., 2017) and the genetic difference between 43 F1 new hybrids and introduced superior hybrids has been evaluated (Azimi et al., 2018). Colorful flowers, rhizome propagation, resistance to calcareous soils, tolerance to undesirable environmental conditions, and low water requirements of that species are the causes for their selection for landscape design (Azimi, 2015).

In arid and semi-arid areas, salinity is a common abiotic stressor that affects plant development and agricultural productivity (Evelin and Kapoor, 2009; Evelin et al., 2012; Porcel et al., 2012). Around the world, it has been estimated that salinity affects roughly 7 % of agricultural crops; it has also been anticipated that the increase in salinity will cause the loss of 36 % of arable land in the ensuing 27 years (Evelin and Kapoor, 2009; Procel et al., 2012; Ruiz-Lozano et al., 2012; Kapoor et al., 2013). When the plants are exposed to high salt concentrations, plants' growth is distrurbed because of toxicity of Na^+ and Cl^- ions (Fatma et al., 2016). The main cause of the declines in growth and yield under salinity is the significant accumulation of Na^+ ions in plant's tissues (Munns & Tester, 2008). Salt stress affects levels of growth regulators and metabolic enzyme activity, damages membranes, impairs enzyme performance, and disturbs the nutritional balance of minerals (Munns & Tester, 2008; Hasanuzzaman et al., 2013). Therefore, these physiological changes result in reduced cell division, expansion, or promotion of cell death and induce a decrease in growth rate and yield; they also destroy chlorophyll in leaves, which leads to leaf senescence (Rahemi et al., 2017). Physiological and biochemical processes like ion balance, seed germination, osmotic management, photosynthesis, respiration, and nitrogen metabolism are disrupted as soil salinity rises (Kaya et al., 2009; Porcel et al., 2012). The first effect of salinity is osmotic stress; further, oxidative stress occurs due to the accumulation of reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and peroxide (Noctor et al., 2014) that have detrimental effects on normal cell growth and metabolism (Aroca et al., 2013). Increased Na^+ and Cl^- absorption leads to disruption in the potassium, phosphorus, calcium, and nitrogen ions' absorption (Ulczycka-Walorska et al., 2020).

Regarding the cultivation of ornamental plants in landscaping, it is necessary to use salinity-resistant ornamental species or induce resistance traits to these stresses through plant breeding and physiological methods (Bayat et al., 2013). Considering the effects of salinity stress on vegetative traits in ornamental plants including petunia, tagetes, scarlet sage, snapdragon, madagascar periwinkle, marigold, and cockscomb, it was shown that marigold is more tolerant to salinity and the sodium accumulation is less than the others (Saki, 2014). Bayat et al. (2013) reported that the flower number and diameter of *Gerbera aurantiaca* Sch. Bip. exposed to salinity decreased compared to control plants. In another study by Wen Yuan et al. (2012), root and leaf relative water content, stomatal conductivity, and the rates of net photosynthesis and evapotranspiration of *Iris lactea* Pall. var. chinensis seedlings declined under salt stress.

According to above references, *I.* x *germanica* as a perennial plant with a beautiful appearance and a high compatiblity with salinity, is an appropriate selection for cultivation in saline conditions. Less research has been done on the impact of salt irrigation on the growth and development of ornamental plants, particularly bulbous plants. Therefore, due to the drought and salinity crisis, which are considered limiting factors for landscape development, the physiological and morphological study of that species is important. The specific objective of the present study was to the effect of saline irrigation water or soil sanity on the performance of iris.

2 MATERIALS AND METHODS

This experiment was carried out at the municipality research farm of Mahallat, Iran; with latitude = 33°54'N, longitude = 50°27'E and 1600 m above sea level. The seeds of *Iris* × *germanica* L. genotypes were obtained from the "Ornamental Plants Research Center (OPRC) of Mahallat, Iran". The genotypes of *Iris germanica* were encoded as OPRC14, OPRC18, OPRC23, and OPRC54. For rapid and uniform germination, seeds were stratified in wet cocopeat at 4-6 °C for 45 days (Azimi et al., 2016). The seeds were cultivated in a cultivation tray and kept in a greenhouse with 70 ± 5 % relative humidity and 25 ± 5 °C conditions. The seedlings were transplanted at the three-leaf stage into the pots (in late April, 2017). Then, the uniform seedling genotypes were selected and transplanted into the pots with 20 cm in diameter and 40 g in mass filled with loamy soil, rotten animal manure, and compost with the ratio of 1:1:1, then transferred to open space. Amount of soil per pot was 2 kg. The NPK fertilizer was used with the ratio of 18:18:18.The single salt (NaCl) was

Table. 1: The chemical characteristics of water used in the salinity experiment.

	EC	Nitrate	Calcium	Magnesium	Bicarbonate	Carbonate	Sodium	Chlorophyllorine
pH	(dS m^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(meq l^{-1})
7.5	363	20	32	12	70.15	0	16	24.85

Con. Table 1: The physical and biochemical characteristics of soil used in the experiment.

Depth	EC	Saturation	Organic carbon	Total N	Phosphorous	Potassium	Texture	Clay	Silt	Sand
(cm)	(Ec × 10 dS/cm)	(pH)	(%)	(%)	(ppm)	(ppm)		(%)	(%)	(%)
	7.44	7.5	4	0.4	300	3000	Loam	28.5	20	51.5

applied. The amount of water/ irrigation/pot was 400 ml.The experiment consisted of four salinity concentrations [EC = 4 dS m^{-1}, and electrical conductivity (EC) at 6, 8, and 12 dS m^{-1}] with three replicas (Table 1).

The salt for the salinity treatments was added gradually to the soil with irrigation water at 7-day intervals. To prevent salt accumulation, the pots were leached twice a week. The fresh water and soil characteristics are given in tables 1 and 2. Three months after the salinity application, the plants were harvested. Leaf freshness, leaf length and width, leaf area, plant height, water relative content, leaf cell membrane permeability (electrolyte leakage), proline, chlorophyll, and carotenoids content, and peroxidase enzyme activity were measured.

2.1 MEASUREMENTS OF PARAMETERS

The leaf length, width, area, and number, plant height and plant diameter of the *Iris* × *germanica* genotypes seedlings were measured at the beginning and end of the experiment. The rate of these changes was determined using the information gathered at the beginning and end of salt stress treatments. After the leaves were detached from the roots and thoroughly rinsed with deionized water, the mass of the freshly harvested leaves was ascertained. The plant samples were oven-dried for 24 hours at 60 °C, and the dry mass was recorded.

2.2 MEASUREMENT OF CHLOROPHYLL A, B, TOTAL CHLOROPHYLL AND CARTENOIDS CONTENTS

The chlorophyll content was determined by rinsing the leaves in an 80 % acetone solution and measuring absorbance at 645 and 663 nm with a spectrophotometer (Nazemi Rafi et al., 2019). The carotenoids content of petals and leaves was measured at 480 and 510 nm, and estimation values were calculated using the following formula:

Chlorophyll a mg g^{-1} = 12.7(A663) - 2.69 (A645) × V/1000 × 10

Chlorophyll b mg g^{-1} = 22.9(A645) - 4.68 (A663) × V/1000 × 10

Total Chlorophyll mg g^{-1} = 20.2(A645) - 8.02 (A663) × V/1000 × 10

Carotenoids mg g^{-1} = 7.6(OPRC5480) - 1.49 (A510) × V/1000 × 10

2.3 PEROXIDASE ACTIVITY

The Guaiacol technique was used to measure the peroxidase (POD) activity (Guan et al., 2015). For three minutes, the variations in 470 nm absorbance were used to follow how well guaiacol was being oxidised. 50 ml of 100 mM PBS (pH 6.0), 19 ml of 30 % H_2O_2, and 28 l of guaiacol comprised the reaction mixture solution. The enzyme extract was added to the reaction mixture solution to initiate the reaction.

POD activity was calculated using the following equation:

POD activity ($\Delta OPRC54_{70}$ min·g FM^{-1}) = $\Delta OPRC54_{70} \times V_T M^{-1} \times V_S \times t$. $\Delta OPRC54_{70}$: the changes of absorption; were: V_T: total volume of the extracted solution; V_S: volume of enzyme solution for testing; M: the mass of samples."

2.4 PROLINE CONTENT

The method developed by Bates et al. (1973) was used to measure the proline content in the leaves.

Each of the four replications' fresh leaves (1.0 g) were homogenised in 10 ml of 30 ml l^{-1} sulfosalicylic acid. Proline was measured spectrophotometrically using the extract.

2.5 STATISTICAL ANALYSIS

The experiment was conducted as a factorial design based on complete randomized block design with three replications, and in each replicate, 8 seedling genotypes were planted.

Using the "SAS statistical programme", data were examined by variance mean comparison and the "Duncan multiple range test".

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE

Variance analysis of interaction salinity and genotypes had a significant impact ($p \leq 0.01$) on leaf length

Table 2: Mean comparison of genotypes and salinity levels on *Iris* × *germanica* characteristics.

Genotypes	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	PH (cm)	Leaf number	Plant diameter (cm)	LDM (g)
OPRC14	14.58d	0.91d	13.43d	14.57d	5.14c	2.79d	0.83b
OPRC18	19.43b	1.77b	34.53b	19.43b	6.54a	3.55b	0.91ba
OPRC23	21.84a	1.87a	40.32a	21.90a	6.61a	3.85a	1.11a
OPRC54	16.09c	1.32c	21.43c	16.09c	6.18b	2.87c	1.10a
Salinity							
*EC= 4 (control)	17.58b	1.35d	24.69c	17.61b	5.54c	3.23b	0.90a
*EC = 6	17.89ab	1.43c	26.92b	17.89ab	5.97b	3.22b	0.94a
*EC = 8	18.27a	1.49b	28.39a	18.24a	6.43a	3.3a	1.128a
*EC = 12	18.22a	1.56a	29.71a	18.22a	6.41a	3.3a	0.99a

Con. Table 2: Mean comparison of genotypes and salinity levels on *Iris* × *germanica* characteristics.

Genotypes	leaf fresh mass (g)	Proline content (µmol g^{-1} DM)	Chlorophyll a (µmol g^{-1} DM)	Chlorophyll b (µmol g^{-1} DM)	Total Chlorophyll (µmol g^{-1} DM)	cartenoids contents (µmol g^{-1} DM)	Peroxidase activity (µmol min^{-1} g^{-1} FM)
OPRC14	8.52c	0.89b	573.17c	695.67c	1793.24b	403.88c	0.43c
OPRC18	7.94c	0.91b	735.5b	878.91b	1812.86b	513.49b	0.67b
OPRC23	12.01a	0.85b	664.84bc	851.05bc	1515.99c	493.68bc	0.78a
OPRC54	10.38b	1.58a	923.42a	1142.19a	2065.62a	647.47a	0.5c
Salinity							
*EC= 4 (control)	9.07b	0.86c	723.35ab	876.29ab	1799.51a	521.40a	0.61ab
*EC = 6	10.12a	0.93bc	782.86a	971.35a	1754.30b	567.07a	0.54b
*EC = 8	10.71a	1.15ab	769.95a	969.66a	1739.61b	555.68a	0.64a
*EC = 12	8.95b	1.28a	620.78b	750.54b	1894.30a	414.37b	0.59ab

Mean value followed by the same letters in each column are not significantly different (Duncan multiple range test).

Table. 3: Mean comparison of interaction between genotype and salinity levels on *Iris* × *germanica* characteristics.

Genotype × salinity	leaf length (cm)	leaf width (cm)	leaf area (cm2)	PH (cm)	leaf number	plant diameter (cm)	Relative water content (%)	Electrolyte leakage (%)
OPRC14*EC = 4	14.07f	0.94h	13.37f	14.07f	4.5g	2.86c	74.33c	9.07c
OPRC14*EC = 6	13.81f	0.74i	10.25g	13.81f	4.91g	2.60d	94.85ab	9.79bc
OPRC14*EC = 8	15.39de	0.91h	14.12f	15.39de	5.58f	2.85c	0.99a	11.37ab
OPRC14*EC = 12	15.05e	1.05g	15.97f	15.05e	5.58f	2.85c	93.79ab	9.97bc
OPRC18*EC = 4	18.75c	1.7d	32.07d	18.75c	6.25bdec	3.50b	83.38bc	9.32c
OPRC18*EC = 6	19.58b	1.76dc	34.7dc	19.58b	6.5abc	3.50b	91.89ab	9.41c
OPRC18*EC = 8	19.41bc	1.80dc	34.96dc	19.41bc	6.75ab	3.58b	84.22bc	9.94bc
OPRC18*EC = 12	20.00b	1.81dcb	36.39c	20.00b	6.66abc	3.61b	86.92abc	9.86bc
OPRC23*EC = 4	21.33a	1.52e	32.54d	21.33a	6.00fdec	3.76a	85.26abc	10.42abc
OPRC23*EC = 6	22.00a	1.87bc	41.29b	22.00a	6.58abc	3.88a	97.87ab	12.00a
OPRC23*EC = 8	22.12a	1.92ab	42.65a	22.12a	6.83ab	3.58a	87.87abc	10.21bc
OPRC23*EC = 12	21.91a	2.03a	44.82a	21.91a	7.00a	3.88a	94.49ab	11.26ab
OPRC54*EC = 4	16.16d	1.28f	20.79e	16.16d	5.75fe	2.80c	92.60ab	9.82bc
OPRC54*EC = 6	16.16d	1.32f	21.45e	16.16d	5.83fde	2.90c	94.66ab	8.83c
OPRC54*EC = 8	16.16d	1.35f	21.83e	16.16d	6.58abc	2.92c	95.62ab	9.10c
OPRC54*EC = 12	15.93d	1.35f	21.66e	15.93d	6.41abcd	2.85c	89.40ab	8.92c

Con. Table 3: Mean comparison of interaction between genotype and salinity levels on *Iris* × *germanica* characteristics.

Genotype × salinity	leaf fresh mass (g)	Proline content (μmol g^{-1} DM)	Chlorophyll a (μmol g^{-1} DM)	Chlorophyll b (μmol g^{-1} DM)	Total chlorophyll (μmol g^{-1} DM)	cartenoids contents (μmol g^{-1} DM)	peroxidase (μmol min^{-1} g^{-1} FM)
OPRC14*EC = 4	7.15f	0.79dc	837.4abc	1014.1abcd	1857.2dce	538.11abc	0.45fg
OPRC14*EC = 6	8.20fe	0.91dc	734.8c	837.5dc	1529.6hg	481.59bc	0.42fg
OPRC14*EC = 8	9.61dec	0.93dc	732.9abc	899.2abcd	1632.1e-h	517.54abc	0.51fe
OPRC14*EC = 12	9.12fde	0.91dc	950.00ab	1204.00ab	2154.00ab	336.28d	0.33g
OPRC18*EC = 4	7.80fe	0.80dc	669.80c	809.70dc	2273.00a	469.42c	0.62dec
OPRC18*EC = 6	7.78fe	0.76dc	710.60bc	810.80dc	1521.40h	494.81bc	0.69abcd
OPRC18*EC = 8	8.36fe	0.86dc	849.60abc	1032.90abcd	1882.40dc	593.89abc	0.73abcd
OPRC18*EC = 12	7.81fe	1.22c	712.10bc	862.30bdc	1574.4 f-h	495.85bc	0.64bdec
OPRC23*EC = 4	10.69bdc	1.12dc	577.30c	699.10c	1276.3i	468.39c	0.79ab
OPRC23*EC = 6	13.28a	1.12dc	731.60abc	1033.10abcd	1765.00d-g	565.73abc	0.74a-d
OPRC23*EC = 8	13.67ab	0.65d	626.00c	1033.10abcd	1420.6h	565.73abc	0.84a
OPRC23*EC = 12	11.41abc	0.66d	724.60abc	877.50abcd	1602.00hgf	436/05c	0.77abc
OPRC54*EC = 4	10.64bdc	1.43a	808.90abc	982.30abcd	1791.2dfe	564.68abc	0.58fde
OPRC54*EC = 6	11.23abcd	1.07dc	977.20ab	1204.00ab	2201.2ab	726.15a	0.30g
OPRC54*EC = 8	12.21ab	1.001dc	871.44abc	1151.90abc	2023.34bc	606.75abc	0.49fe
OPRC54*EC = 12	7.45fe	1.82b	1016.20a	1230.60a	2246.8ab	692.28ab	0.63bdec

Mean value followed by the same letters in each column are not significantly different (Duncan multiple range test).

and width, plant height, plant diameter, chlorophyll a, b, total chlorophyll and carotenoids contents, proline content and peroxidase enzyme activity (Table. 3).

3.2 COMPARISON OF SALINITY LEVELS AND GENOTYPES

As shown in Table 2, the highest leaf length and width, leaf area, plant height, leaf number, plant diameter, electrolyte leakage, leaf fresh and dry mass, soluble protein, and peroxidase enzyme activity were observed in OPRC23 genotype seedlings. OPRC54 genotype seedlings had the highest relative water content, proline content, chlorophyll a, chlorophyll b, total and carotenoids contents. In morphological and physiological characteristics, OPRC23 was superior to other genotypes. The lowest morphological characteristics were observed in OPRC14 genotype seedlings. OPRC54 genotype seedlings were superior to other genotypes in biochemical characteristics, especially proline content; also, the lowest biochemical characteristics were observed in OPRC14 genotype seedlings. The highest leaf length, plant height, leaf number, plant diameter, electrolyte leakage, fresh leaf mass, and peroxidase enzyme activity were obtained at 8 dS m^{-1} salinity level. At 6 dS m^{-1} salinity, the highest relative water content, chlorophyll a, b, and carotenoid content were observed. The greatest leaf width, leaf area, total chlorophyll, and proline contents corresponded to a salinity level of 12 dS m^{-1}. There was no significant difference between salinity levels with soluble protein, electrolyte leakage, and leaf dry mass between *Iris* × *germanica* genotypes**.**

3.3 INTERACTION OF SALINITY AND GENOTYPES ON *IRIS* X *GERMANICA* CHARACTERISTICS

3.3.1 Growth characteristics

Reduced growth traits are one of the earliest impacts of salt stress on plants. Plant responses to salt stress can be separated into two phases (Munns, 2005). Osmotic stress causes the first stage of growth reduction to proceed more quickly. The accumulation of salt in leaves, which results in salt toxicity in the plants, initiates the second phase, which is a much slower process. During the study period, the NaCl treatments had a considerable impact on plant development (Table 1). Based on morphological characteristics, the highest leaf length, plant height, and leaf number at all salinity levels, especially at 8 dS m^{-1}, corresponded to OPRC23 genotype seedlings (Table 3). Increasing salinity levels reduced these characteristics in the OPRC54 genotype. The highest leaf width (2.03 cm) and leaf area (44.82 cm) were observed in the OPRC23 genotype at 12 d dS m^{-1}salinity level. OPRC23 and OPRC18 genotypes showed higher morphological characteristics in comparison to others under salinity conditions. Increasing salinity levels reduced the leaf width and leaf area of the OPRC54 genotype, so that increasing salinity up to 12 dS m^{-1} decreased the leaf length. Leaf area change is an important process that controls water consumption under stress conditions. Most of the plants tolerate salinity up to a certain threshold; at higher salinity levels, plant growth decreases linearly with increasing salinity (Amir-Jani, 2010). Plants' reduced ability to absorb water as a result of osmotic stress brought on by salt is the reason of the decrease in leaf surface area (Sarvandi et al., 2020).

Chlorophyllide and sodium ion absorption has an impact on hormone synthesis and transport between roots and shoots, which lowers leaf area, plant dry biomass, and specific leaf area (SLA) In addition to lowering LA, salinity inhibits the growth of the root system, delays the development of apical buds, and results in chlorophyllorosis with subsequent necrosis on the leaf edge (Oliveira et al., 2017).

The highest plant diameter (3.88 cm) was related to the OPRC23 genotype at 6 dS m^{-1} salinity level (Table 3). The results indicated that, while increasing salinity, the diameter of the plant was reduced; in this condition, the plants of the OPRC23 genotype were more able to absorb nutrients than other genotypes.

The fresh and dry mass of leaves decreased as salinity increased. These findings were consistent with previous research on different crop species of Chinese iris (Wang et al., 2012). OPRC23 genotypes had the highest fresh and dry mass of leaf. The highest leaf dry mass was obtained in OPRC23 genotypes (1.38 g) at 8 dS m^{-1} salinity level. The leaf dry mass of OPRC18, OPRC13, and OPRC54 seedlings decreased at salinity level of 12 dS m^{-1}. The highest fresh leaf mass belonged to OPRC23 genotype seedlings (13.28 g). At 12 dS m^{-1}, the leaf fresh mass of OPRC23 and OPRC54 decreased with increasing salinity (Table 3). As a general rule, the lowest morphological characteristics (plant height, leaf length, plant diameter, leaf number, and leaf fresh mass) were observed in OPRC14 and OPRC18, respectively. Dry matter reduction under stress conditions has been reported due to decreased leaf area index, photosynthesis rate, growth of aerial organs, and the relative growth rate of the plant (Soheili-Movahed et al., 2017). Fresh and dry mass of roots and shoots in response to high salt concentrations in Poa pratensis L. declined (Esmaeili & Salehi, 2016).

Vegetative growth, including leaf area, number of

leaves, number of shoots, plant height and chlorophyll content, decreased in tuberose as the concentration of sodium chlorophylloride increased. Furthermore, the decrease in root biomass caused by salinity has been attributed to either direct Na or Cl toxicity or high Na content, which results in an inability to maintain cell turgor. Root elongation may have been reduced due to an inhibition of cell expansion as cell turgor decreased under salinity stress (Dlamini et al., 2019). Salinity stress decreased length and width of leaves, relative water content, and chlorophyll content (Naseri Moghadam et al., 2020), and salinity stress was more damaging to the growth, ornamental, and physiological traits of the *N. tazetta* L. flower than drought stress (Naseri Moghadam et al., 2020). A restriction in leaf expansion followed by a reduction in leaf area is one of the first signs of plants exposed to excessive salinity (Navarro et al., 2007). Changes in the cells and a decline in leaf turgor can both be used to explain it. Reduced cell elongation and cell division cause leaves to appear more slowly and to grow to a smaller size under salt stress. Leaves become smaller and thicker as a result of a shift in cell size that reduces area more than depth (Go´mez-Bellot et al., 2013).

3.3.2 Proline content

An organic osmolyte called proline is primarily found in protoplasm. Proline increases in response to osmotic stress caused by salt. It might be connected to their excellent salinity resistance (Guan et al., 2015). The maximum proline content at 12 dS m^{-1} salinity level was found in OPRC23 (1.82 µmol gr^{-1} DM) and OPRC18 (1.22 µmol gr^{-1} DM) genotype seedlings. The results indicate that a higher proline content was observed in OPRC54 genotype seedlings at all salinity levels. The proline content increased significantly with the increase in saline stress (Table 3). Proline content increases when the water potential of the leaf decreases, which leads to the maintenance of cellular turgor and reduces the damage to the membrane in the plant; therefore, with osmotic adjustment, tolerance to water stress increases (Rahdari and Hosseini, 2012). The rate of proline synthesis depends on the development of stress, the age of the plant organ, and genetic variation (Bajji et al., 2001). In this experiment, the proline content was different among the genotypes. The changes in proline concentration at different levels of salinity showed that with increasing salinity, the proline content of genotypes increased. However, the difference in proline content between sensitive and tolerant genotypes was statistically significant. Saneoka et al. (2004) reported that proline accumulation has a positive and direct relationship with increasing salinity and drought resistance in plants. Under salt stress, proline's primary functions include osmotic adjustment, protecting membranes and enzymes, and serving as a store of energy and nitrogen that can be used (Amini et al., 2015). A well-known defence mechanism against salt stress in plants is proline accumulation. Proline accumulation has also been proposed as a selection factor for salt tolerance due to the possibility that an increase in proline content will boost salt tolerance (García-Caparrós & Lao, 2018). Proline oxidase and proline dehydrogenase have higher inhibitory rates, which helps to explain why this accumulation occurs when exposed to salt stress. However, because proline breaks down quickly when stress is removed, it is also possible to discover a decrease in proline accumulation in plants. The breakdown products provide reducing agents that aid mitochondrial oxidative phosphorylation and ATP production for stress recovery and repair (García-Caparrós & Lao, 2018). The increase in proline concentration in the roots and leaves of all species exposed to salinity could be due to a decrease in proline degradation or an increase in proline biosynthesis (García-Caparrós et al., 2016).

3.3.3 Chlorophyll content

The chlorophyll a and b content was the highest in OPRC54 genotype seedlings at a salinity level of 12 dS m^{-1}. At a salinity level of 4 dS m^{-1}, the highest content of chlorophyll a and b belonged to OPRC14. Also, at 6 and 8 dS m^{-1} salinity levels, the highest content of chlorophyll a was observed in genotype OPRC54 (Table 3). The study of salinity stress in Crocus sativus L. revealed that at a salinity level of 6 dS m^{-1}, the chlorophyll a and b content decreased. A significant decrease in total chlorophyll content in the leaves as a result of increased salinity reduces photosynthesis and exacerbates stress-induced damage (Tuna et al., 2013). Changes in the content of photosynthetic pigments, according to some reports (Noreen & Ashraf, 2009), are also affected by plants' tolerance to soil salinity, i.e., their genotype. Most studies on plant responses to salinity conditions have found a decrease in total chlorophyll content in plant leaves as a result of salt stress (Silva et al., 2010). Under stress conditions, degradation of the pigment protein complex and the oxidative complex causes damage to the chloropplast lipids, pigments, and proteins (Tambussi et al., 2005). The stimulatory effect of salinity on leaf pigment content could be attributed to an increase in the number of chloroplasts per mesophyll cell. Under salinity stress, however, both epidermal and mesophyll thickness and intercellular spaces decrease in Bruguiera parviflora (Roxb.) Wight & Arn. ex Griff. (Salachna et al., 2016). In salt-treated hyacinth, the leaf content of chlorophyll a, chlorophyll b, and carotenoids decreased as NaCl concentration increased. In

Ornithogalum saundersiae Baker, the intensity of photosynthesis and transpiration decreased as NaCl concentration increased. The chlorophyll and carotenoids content of NaCl-treated Ornithogalum saundersiae plants was significantly higher than that of control plants (Salachna et al., 2016). The leaf greenness index and proline content in Hyacinthus orientalis L. were also higher when sodium chlorophylloride was applied to the soil on the day of bulb planting, and the length of the forcing period was longer (in the second season) (Ulczycka-Walorska et al., 2020; Salachna et al., 2016) and stage Calla lilies (Zantedeschia K. Koch) (Ayad et al., 2019).

The main photosynthetic pigments that play an important role in photosynthesis are chlorophyll a, chlorophyll b, and carotenoids. The main electron donor is chlorophyll a, while the primary accessory pigment for light harvesting and energy transfer is chlorophyll b.

Plant chlorophyll content is well known to be directly related to plant health (Barry, 2009). Chlorophyll concentration decreases in response to salt stress, which can be used as a sensitive indicator of cellular metabolic state. This drop could be due to membrane deterioration. Nonetheless, under salt stress, plants may exhibit an increase in chlorophyll concentration, which may be due to an increase in the number of chloroplasts per unit leaf area in the stressed plant leaves. This decrease in photosynthetic rate in plants under salt stress could be due to a number of factors, including: a) Dehydration of cell membranes reduces their permeability to CO_2, b) salt toxicity, c) reduction of CO_2 supply due to hydroactive closure of stomata, d) increased senescence caused by salinity, and e) changes in enzyme activity caused by changes in cytoplasmic structure. Under salt stress, stomatal conductance decreases, limiting the availability of CO_2 for carboxylation reactions. Furthermore, this stomatal closure reduces water loss through transpiration, which has an impact on light-harvesting and energy-conversion systems, resulting in a change in chlorophylloroplast activity (García-Caparrós and Lao, 2018).

Plants accumulatelow-molecular-mass compounds as a compatible solutes to adjust the osmotic potential of the cytoplasm during salt stress because they do not interfere with normal biochemical reactions. Nonetheless, producing enough osmotica is metabolically costly, potentially limiting plant growth by consuming large amounts of carbon that could otherwise be used for growth. Compatible solutes include proline, sugars, glycine-betaine, and other related quaternary ammonium compounds; however, due to a lack of data on the effects of salt stress on solute accumulation in ornamental plants, we will concentrate on soluble sugars and proline (García-Caparrós and Lao, 2018).

Another study found that stabilisation of chloroplasts is a well-known mechanism for increasing stress tolerance (Veatch-Blohm et al., 2019). Calcium is required for cell division, cell wall formation, cell signalling, and the permeability of the cell membrane to ions. It may be able to mitigate some of the negative effects of salinity by reducing the impact of Na on Ca homoeostasis (Veatch-Blohm et al., 2019). Dry mass of roots is regarded as an important parameter in the response to salt stress because the greater the root growth, the greater the water and nutrient uptake that can occur, favouring the accumulation of toxic ions in roots, particularly Na^+, and thus minimising its negative effects on shoot growth. In the presence of reduced stomatal aperture, these NaCl-induced anatomical changes may facilitate CO_2 reaching the chloroplast more efficiently. These modifications appeared to be an adaptive response to protect the photosynthetic process (Acosta-Motos et al., 2015).

3.3.4 Carotenoid content

The highest carotenoid content was present in OPRC54 genotype seedlings at 6 dS m^{-1} salinity level, and the lowest carotenoid content belonged to the OPRC14 genotype seedlings at 14 dS m^{-1} salinity level. The maximum carotenoid content was observed in OPRC54 genotype seedlings at different salinity levels (Table 3). One of the plant salt resistances under salinity conditions is carotenoid content (Juon et al., 2005). Muller et al. (2010) found that salinity stress increased carotenoid content while decreasing chlorophylls a and b. This was consistent with the findings of this study on salt stress. These findings could be attributed to stressed leaves increased thickness and compacted mesophyll cells, resulting in more chloroplasts per unit area, as is often the case under stress conditions (Sarvandi et al., 2020). Controlling the salt concentration of plant aerial parts, limiting entry through the roots, and limiting transport to the shoots (by retaining these ions in the root and lower stem) is an important mechanism that allows plant survival and growth in a saline environment (Álvarez and Sánchez-Blanco, 2015).

K^+ and Na+ are important in plant growth and development, but they are also important in maintaining osmotic adjustment and cell turgor. Furthermore, plants

irrigated with saline water had lower K^+/Na^+ and $K^+/Na+$ ratios, which correlated with lower biomass production (Álvarez and Sánchez-Blanco 2015). A large number of enzymes were inhibited, and several aspects of plant physiology were disrupted due to high NaCl, including photosynthesis and pigment synthesis (Munns & Tester, 2008; Cirillo et al., 2016).

3.3.5 Peroxidase activity

At 8 dS m^{-1} salinity level, OPRC23 genotype seedlings had the highest peroxidase activity (0.84 mol min^{-1}.mg $protein^{-1}$). At 6 dS m^{-1} salinity, OPRC54 genotype seedlings had the lowest peroxidase activity (0.30 mol min^{-1}.mg $protein^{-1}$). The lowest carcinoid content was observed in OPRC14 genotype seedlings at different salinity levels (Table 3). Plants tend to counteract reactive oxygen species produced by stress (Ahmad et al., 2012, 2011; Bano et al., 2013; Kaya et al., 2013). Plants subjected to salt stress exhibited up-regulation of the antioxidant defence system (Hussain et al., 2016). According to these studies, salinity increased the activity of peroxidase enzymes in salt-sensitive cultivars. Salinity inhibits cell division and also reduces cell size, resulting in a reduction in plant length. These stresses generate ROS compounds, which cause protein, lipid, carbohydrate, and nucleic acid damage. Plants use enzymatic (catalase, superoxide dismutase, and so on) and non-enzymatic (phenolic compounds and carotenoids) defence systems to scavenge and detoxify these compounds from the cell surface (Naseri Moghadam et al., 2019).

Plants commonly respond to saline conditions by accelerating the generation of reactive oxygen species (ROS), which include the cytotoxic superoxide radical (O_2), singlet oxygen ($^1O_2$), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). The mitochondria, chloroplasts, and peroxisomes are the primary sources of ROS generation in the cell. These reactive oxygen species participate in a variety of processes, including DNA damage, lipid peroxidation, and protein oxidation.

Plants have an antioxidative machinery composed of enzymatic and non-enzymatic components such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), and catalase (CAT) to mitigate the harmful effects of ROS at the cellular level (Garca-Caparrós and Lao, 2018). In contrast to what happened with CAT activity, myrtle plants experienced a sharp decline in APX activity during the experimental period. APX and CAT appear to compensate each other to some extent, as evidenced by increased CAT expression in knock-out cytAPX *Arabidopsis* plants in response to light stress. At the end of the recovery period, APX, despite showing low activity data, is partially recovered in S4 plants compared to control plants but not in S8 plants. NaCl has been shown to reduce APX activity in other plant species, including grapevine plantlets in vitro. A link has been established between CAT activity and photosynthesis because an increase in CAT reduces CO_2 photorespiratory loss (Acosta-Motos et al., 2015).

Increased CAT and SOD levels were observed in salt-stressed *Eugenia* plants at 44 and 88 mM, particularly in recovered plants. The response of CAT activity suggested that the photorespiratory pathway could be induced under salinity conditions, whereas SOD is thought to be the first line of defence against oxidative stress in plants. From the decarboxylation of glycine in the mitochondria, photorespiration can provide electron acceptors to PSI and CO_2 for the chloroplast. Furthermore, a close relationship between CAT activity and photosynthetic rate has been described. Increased CAT activity has been found to limit the H_2O_2-dependent decarboxylation of the keto-acids glyoxylate and hydroxypyruvate in the peroxisome, thereby reducing CO_2 photorespiratory loss (Acosta-Motos et al., 2015).

4 CONCLUSION

The results showed that the morphological, physiological, and biochemical characteristics of OPRC23 genotypes were superior OPRC54 genotype. The OPRC54 genotype had the highest levels of chlorophyll a, b, and proline content at 12 dS m^{-1} salinity. In conclusion, different levels of salinity can expose to different genotypes, which leads to the selection of specific salt tolerant genotypes. The genotypes examined in this experiment of *Iris* × *germanica* were resistant to salinity stress and this trait is of interest to landscape designers.

5 STATEMENTS & DECLARATIONS

5.1 FUNDING

There is no funding with this work. The original data were obtained from Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran

5.2 COMPLIANCE WITH ETHICAL STANDARDS (CONFLICT INTEREST)

The authors declare that they have no conflict of interest.

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