Morpho-physiological and biochemical responses of sugar beet cultivars (*Beta vulgaris* L.) to pretreatment and irrigation salty soil

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Abstract

Osmotic stress can elicit a complex response in plants, leading to a range of abnormal symptoms that can limit growth and development, and even result in yield loss. The current study aimed to assess the morpho-physiological responses of various sugar beet cultivars, namely 'Shokofa', 'Sina', 'Paya', 'Turbata', and 'Aria', to different seed priming methods (non-priming, osmo-priming, and hydro-priming) and varying levels of salinity stress through irrigation (>2, 8, and 16 dS/m). The experiment was carried out using a randomized complete block design (RCBD) with a factorial split-plot arrangement. The study was conducted with three replications at a research farm in Saveh, Iran during the 2019-2020 growing season. Results showed that non-priming of 'Shokofa' genotype, hydro-priming of 'Aria' genotype, and osmo-priming of 'Paya' genotype under non-salinity conditions had the highest total chlorophyll content (31.04, 32.80, and 28.50 µg/g FW, respectively). The highest proline content was related to the hydro-and osmo-priming of seeds under high salinity stress (1.91 and 1.23 µmol/g FW, respectively). The hydro-priming treatment of 'Shokofa' seeds resulted in the highest LAI under high salt stress conditions. Additionally, the highest root yield (62.9 tons/ha) was observed in the hydro-priming treatment of 'Aria' genotype under non-saline condition. Among the sugar beet cultivars, 'Sina', 'Torbata', and 'Aria' had the highest root yield under non-stress and hydro-priming conditions. Therefore, these cultivars and the hydro-priming technique are recommended for planting in areas with saline soil or irrigation water.

Keywords: chlorophyll, hydro-priming, leaf area index, proline, root yield

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Introduction

Sugar beet (*Beta vulgaris* L.), one of the newly domesticated crops, belongs to the Caryophyllales (Skorupa et al., 2019). Sugar beet is a hardy and resistant plant with strong root and lateral roots widely adaptable to various climatic conditions. This plant is not sensitive to salty soil and grows optimally at a temperature of 20 to 24 ℃ (Abbasi et al., 2019). The global sugar production from sugar beet is approximately 42 million tons, accounting for about 30% of the worldwide sugar supply (Lv et al., 2019). Russia has the highest

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cultivated area and production rate while Belgium obtains the highest sugar beet yield (FAO, 2019).

Among the abiotic stresses, high salt concentration in the soil can lead to severe harmful effects such as poor germination, low yield, changes in many physiological processes, including photosynthetic pigments, amino acid, proline, and the activity of antioxidant enzymes (Shahverdi et al., 2019 and 2020).

Sugar beet has the ability to withstand high levels of salt, and this is due to its salt-tolerant nature. According to Bouras et al. (2021), the maximum salinity threshold for sugar beet is 7.0 $dS \cdot m^{-1}$. Sugar beet utilizes two mechanisms to tolerate salinity: firstly, it stores Na^+ ions in vacuoles to avoid toxicity, as high concentrations of Na^+ ions are generally detrimental to plants. Secondly, it adjusts osmotically by accumulating compatible osmolytes in high cytoplasmic concentrations (Katerji et al., 1997).

Salty soil affects the physiological activities of plants in various ways. The damage caused by the salinity usually manifests itself in the plant when the soil solute concentration is very high (Shahverdi et al., 2018; Farzami Sepehr et al., 2022). Under salt stress, compounds with low molecular weight, soluble and compatible (e.g., proline and sugars) in plants accumulate for ionic balance in vacuoles and cytoplasm (Choudhary et al., 2022). In plant cells, proline serves as a substance to maintain the osmotic balance between the cytoplasm and vacuole (Shahverdi et al., 2019). Bouras et al. (2021) found that sugar beet yield decreased by 21% under saline irrigation with an EC value of 4 $dS \cdot m^{-1}$, and by 26% with an EC value of 8 $dS \cdot m^{-1}$, compared to the control. In addition, the total sugar content increased significantly by 5% and 7% under saline irrigation with an EC value of 4 and 8 $dS \cdot m^{-1}$, respectively.

Seed priming efficiently increases seed germination rate and synchronizes crop germination and seedling growth under environmental stress conditions such as drought and salinity (Aghighi Shahverdi et al., 2017; Nejati et al., 2020). An interesting approach in this process regarding compounds' effect on improving tolerance to environmental stress is that priming the seeds with nutrients or hormonal compounds can reduce oxidative stress (Subramanyam et al., 2019). Nutri-priming is a recently developed method for pre-sowing seed treatment that involves using micro- or macronutrients. This method was introduced in studies by Mirshekari (2012) and Aghighi Shahverdi et al. (2017). Compared to other treatments, hydro-priming resulted in the highest number of early flowerings, pods per plant, ratio of filled pods, grain weight, yield, and harvest index for lentils (Azarnia et al., 2020). According to Farooq et al. (2011), one of the significant advantages of seed planting is using nutri-priming with commercial fertilizers, which can enhance crop yield.

An essential micronutrient required by plants and humans, zinc plays a vital role in numerous enzymes, including DNA, RNA polymerases, and marker proteins (Rehman et al., 2018). Its deficiency is common in agricultural soils in developed and developing countries, especially in areas with drought and salinity stress (Mahmood et al., 2019). In addition, it has been shown that the seeds primed in solutions containing zinc increase the amount of zinc in them or help the growth and performance of seedlings (Tounekti et al., 2020). In various studies, the positive effect of seed priming with zinc has been reported, for example, in quinoa under drought stress (Pakbaz et al., 2022), soybean under drought stress (Alijani et al., 2022) and sunflower under salt stress (Nejati et al., 2020).

Seed priming is a widely used and cost-effective method to enhance plant tolerance to environmental stresses, such as salinity. However, given the significance of sugar beet as an industrial crop within the country, further investigation and research are needed to increase both the quantitative and qualitative yield and identify the most suitable genotype for cultivation in specific regions. Hence, the current study aimed to evaluate the morpho-physiological characteristics of various sugar beet cultivars under salt stress conditions following seed priming treatment.

Table 1
Analysis of the physicochemical characteristics of the farm soil

Depth (cm)	Texture	pH	EC (dS/m)	SAR	K (ppm)	Mg (mq/lit)	Ca (mq/lit)	Na (mq/lit)
0-30	Sandy loam	7.96	2.32	3.78	165.6	5.9	8.1	10

Table 2
Information on the sugar beet cultivars tested in the current research

Cultivars	Name of breeder(s)	The owner of the genotype	Introduce year	Genotype code	Resistance type
Ariya	SBSI	SBSI	1394	1.3.3.108	resistant to rhizomania and nematodes
Paya	SBSI	SBSI	1394	1.3.3.110	drought tolerant
Shokofa	SBSI	SBSI	1394	1.3.3.137	resistant to rhizomania and nematodes
Torbata	SBSI	SBSI	1399	1.3.3.197	resistant to rhizomania and cyst nematode
Sina	SBSI	SBSI	1397	1.3.3.168	resistant to rhizomania and rhizoctonia

Materials and Methods

Experimental design and seed material

A field experiment was conducted during 2019-2020 in a research farm in Saveh, Iran, to examine growth, yield, and physiological responses sugar beet cultivars to priming treatments and salinity stress. The experiment followed a factorial split-plot design based on a randomized complete block design (RCBD), with three replications. The main plots were arranged for irrigation with salty water to induce salinity stress at three levels: less than 2 dS/m (as a control), 8 dS/m, and 16 dS/m sourced from a natural salinity source. Sugar beet cultivars including ‘Shokofa’, ‘Sina’, ‘Paya’, ‘Torbata’, and ‘Aria’, along with three levels of seed priming including control (no priming), hydro-priming, and osmo-priming (zinc sulfate) were examined in sub-plots.

In the autumn, the farm preparation, including deep fall plowing to a depth of 30 to 35 cm and preliminary leveling operations, were carried out, and the determination of the soil texture and the amount of nutrients present in it was carried out, the results of which are presented in Table 1. All these cultivars were obtained from the Sugar Beet Seed Institute (Table 2).

Before applying priming treatments, all seeds were disinfected with 10% sodium hypochlorite for 3 min and then washed with distilled water. Previous studies showed that the best priming time for sugar beet seeds is 12 h, and these data were used in the experiment (Shokohian and Omidi 2021). After disinfection, seeds were treated with distilled water (as hydro-priming) and zinc sulfate (0.5%) for 12 h at a temperature of 15 ℃. Based on the results of Shokohian and Omidi (2021), the zinc concentration and the priming time were selected. Then the treated seeds were dried at room temperature for 12 h.

The experimental plots were set up with 4 rows each, and each row was 6 m long with a 60 cm gap between them. The space between plants within each row was kept at 17 cm, and the seed planting depth was between 3 to 4 cm. All plots were planted at the same time on March 30, 2019, and they were irrigated immediately after planting. After the seedlings grew to the 4-6 leaf stage, thinning was carried out by leaving a distance of 17 cm between plants. Drip irrigation method was to the plots. The second thinning was conducted one week after the first one, which adjusted the distance between plants on the cultivation lines to 15 and 20 cm. As a result, the field density was approximately 100,000 plants per hectare.

After the final thinning, salinity treatments were applied by irrigating with natural saline water having electrical conductivity levels of 8 ± 1.5 and 16 ± 1.5 dS/m. Irrigation was performed based on the crop's root system at a depth of 0.5 meters, in accordance with the crops' field capacity (FC). The amount of water used was irrigated daily to refill the soil profile of the field up to a depth of 0.5 m within the limits of FC. The soil moisture content was monitored at regular intervals during the experiment to control the amount of water in the soil profile. In order o prevent the accumulation of salt in the field soil after a week of irrigation with saline water, a heavy irrigation stage was performed with ordinary water. In this case, the electrical conductivity is almost within the range of the determined treatments (Shahverdi et al., 2018).

To manage the *Chaetocnema tibialis* pest during the 6-8 leaf stage of sugar beet cultivation, the field was treated with fenvalerate insecticide at a concentration of 20% and a rate of 0.75 liters per ha. Following the thinning process, as well as cultivation and weed control measures, 125 kg of nitrogen were applied.

The physiological traits were measured by taking samples from the youngest leaves before cutting them. This involved measuring the photosynthetic pigments, as well as the malondialdehyde content (MDA) and proline levels.

Measurement of photosynthetic pigments

To measure the photosynthetic pigments, Lichtenthaler and Buschmann (2001) method was used. This method 0.25 g of fresh leaf is extracted using 5 mL of 80% acetone. The extract was centrifuged at 11000 rpm for 10 min. The extract's optical density (O.D.) was measured at 646.8 and 663.2 nm wavelengths. The amount of pigments was calculated according to the following equations (Shahverdi et al., 2019):

$$\text{Chl a } (\mu\text{g/g FW}) = 12.7\ (\text{O. D of } 663) - 2.69\ (\text{O. D of } 645) \times \frac{v}{w \times 1000}$$

$$\text{Chl b } (\mu\text{g/g FW}) = 22.9\ (\text{O. D of } 645) - 4.68\ (\text{O. D of } 663) \times \frac{v}{w \times 1000}$$

$$\text{Total Chl } (\mu\text{g/gr FW}) = 20.2\ (\text{O. D of } 645) + 8.02\ (\text{O. D of } 663) \times \frac{v}{w \times 1000}$$

where w: the fresh weight by grams for extracted tissue; v: the final size of the extract in 80% acetone; O.D: optical density at a specific wavelength.

Proline assay

The proline content was determined using the method described by Bates et al. (1973). First, approximately 0.5 g of fresh leaves were homogenized with 10 mL of 3% aqueous sulfosalicylic acid. The resulting solution was filtered through Whatman paper No. 2. Next, 2 mL of the filtrate was mixed with 2 mL each of acid-ninhydrin and glacial acetic acid in a test tube. The mixture was then incubated in a water bath at 100 ℃ for 1 h. After incubation, the reaction mixture was extracted with 4 mL of toluene, cooled to room temperature, and the absorbance was measured at 520 nm using a spectrophotometer.

Malondialdehyde assay

Malondialdehyde (MDA) contents were measured by the method of Heath and Packer (1968). According to this method, 0.25 g of fresh leaves were ground in a mortar containing 5 mL of 0.1% trichloroacetic acid (TCA). The resulting extract was centrifuged at 10,000 g for 5 min. In the next step, 250 µL of the supernatant solution obtained from the centrifuge was mixed with 1 mL of MDA solution containing 20% trichloroacetic acid and 0.5% thiobarbituric acid (TBA). The resulting mixture was heated for 30 min at a temperature of 95 ℃ in a bath. Then it was immediately cooled in ice, and the mixture was centrifuged for 10 min at 10000 rpm. The absorption intensity of this solution was read using a spectrophotometer at a wavelength of 532 nm. The absorbance of other non-specific pigments was determined at 600 nm and subtracted from the resulting value.

Growth and yield attributes

The field's irrigation was discontinued three weeks before the harvest, and the sugar beet crop was subsequently harvested during the initial weeks of November. During the harvesting process, the roots were collected by removing the margins. To determine the dry weight of both the roots and aerial parts (all the leaves were removed before

Table 3
ANOVA of the effect of salinity stress and seed priming on the morpho-physiological and yield characteristics of sugar beet cultivars

SOV	df	Mean Square (MS)								
		Chlorophyll a	chlorophyll b	chlorophyll total	Proline	MDA	LAI	Root yield	Aerial yield	Biological yield
Block (B)	2	2.33ns	0.0052ns	1.87ns	0.046ns	0.022ns	0.061ns	1.27ns	0.0038ns	273.08ns
Salinity (S)	2	397.5**	9.78**	1391.0**	0.79**	5.65**	7.77**	31.42**	2.96**	7033.5**
B (S)	4	3.98	0.11	5.03	0.059	0.107	0.13	0.730	0.22	155.3
Priming (P)	2	23.56*	0.50ns	59.0*	0.101*	2.76**	2.24**	13.25**	0.346ns	2655.9**
Genotype(G)	4	6.17ns	0.10ns	3.75ns	0.009ns	0.14ns	0.038ns	2.57**	0.772**	637.9**
S×P	4	29.07**	0.42ns	58.35**	0.136**	0.47*	0.939**	2.08*	5.08**	214.61ns
S×G	8	8.60ns	0.73**	41.41**	0.033ns	0.14ns	0.714**	0.272ns	0.332ns	59.45ns
P×G	8	7.99ns	0.39ns	34.72*	0.008ns	0.096ns	0.414**	0.246ns	0.608**	100.69ns
S×P×G	16	18.19**	0.57**	65.48**	0.032ns	0.137ns	0.728**	1.09*	1.08**	344.7**
Error	84	6.32	0.20	14.52	0.021	0.148	0.143	0.665	0.196	138.6
CV (%)	-	18.34	16.42	17.53	15.76	22.54	19.18	12.68	14.26	22.46

ns, * and **: non-significant and significant at the 5% and 1% probability levels, respectively.

harvest), fresh samples of 5 plants from each treatment were taken and then placed in an oven at 70 ℃ for 48 h. Afterward, the dried samples were weighed.

To measure the leaf surface, the leaves of 5 plant samples were separated from each treatment and determined with a leaf surface measuring device; then, the average leaf surface of a single plant was calculated.

The biological yield of a plant can be measured by considering its aerial and root organ yields. Specifically, the aerial yield can be calculated by determining the total dry weight of the aerial organs, including the leaves per unit area. Additionally, the biological yield can also consider the total root weight per unit area, along with the aerial yield (Kandil et al., 2020).

Statistical Analysis

The Statistical Analysis System (SAS) software, version 9.2, was used to perform an Analysis of Variance (ANOVA), and Duncan's multiple range test was used to determine significant differences among treatment means at a significance level of $p<0.05$. Additionally, the Pearson correlation coefficient was calculated using SAS software to evaluate the relationships between growth, yield, and physiological characteristics.

Results

Chlorophyll content

The study found that salinity stress and the interaction of salinity, priming, and genotype significantly affected chlorophyll a, b, and total. In addition, the effects of priming and salinity × priming were significant on chlorophyll a and total, as shown in Table 3. The highest chlorophyll-a content (19.21 µg/g FW) was observed in the seeds of the 'Aria' cultivar that were hydro-primed under non-stress conditions. Similarly, hydro-priming of 'Sina' cultivar seeds under 8 dS/m salinity produced the highest chlorophyll-a content. The 'Turbata' genotype showed the highest increase in chlorophyll content compared to the non-prime treatment under high salinity levels (16 dS/m) when subjected to osmo-priming. On the other hand, non-priming and hydro-priming of 'Aria' genotype seeds under high salinity levels had the lowest chlorophyll-a content (10.55 and 8.19 µg/g FW, respectively) while osmo-priming of this genotype under 16 dS/m salinity increased chlorophyll content, as shown in Fig. (I).

As shown in Fig. (II), the highest chlorophyll-b content was achieved through the non-priming of ‘Shokofa’ genotype, hydro-priming of ‘Aria’ genotype, and osmo-priming of ‘Paya’ genotype

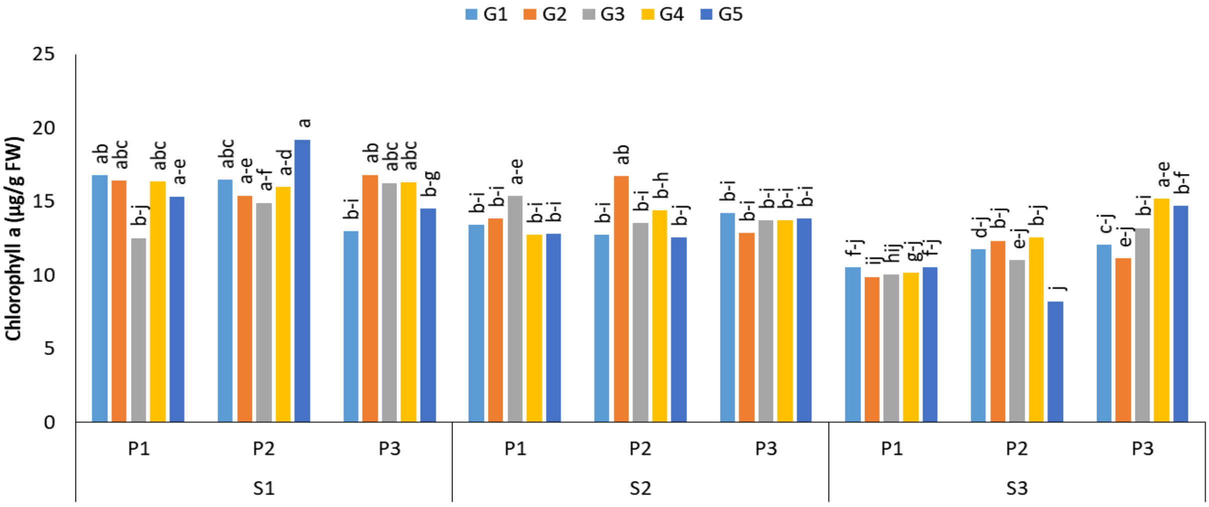

Fig. I. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on chlorophyll-a content of sugar beet

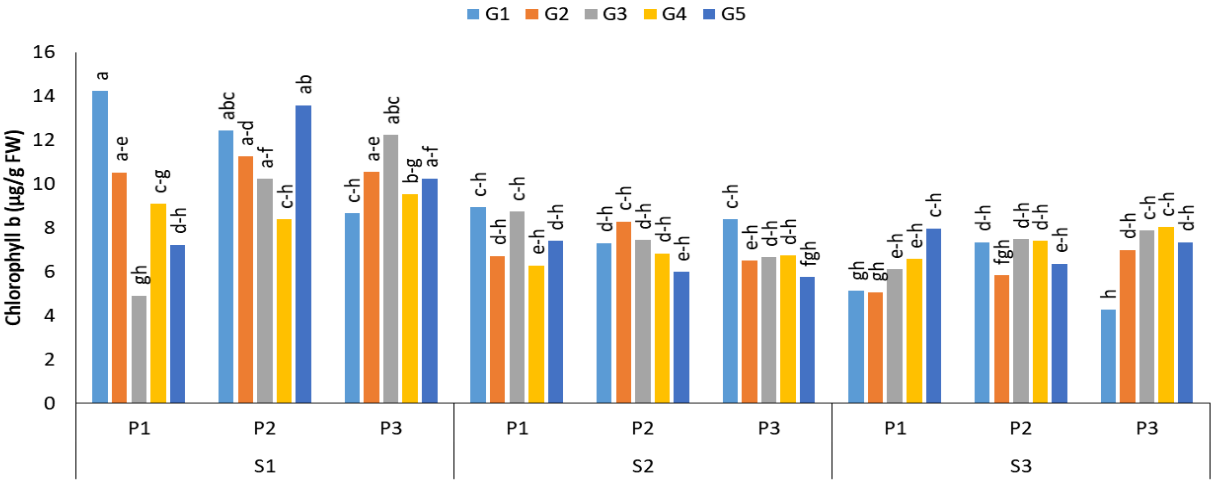

Fig. II. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on chlorophyll-b content of sugar beet

under the non-salinity stress (14.24, 13.59, and 12.24 µg/g FW, respectively). On the other hand, non-priming and osmo-priming of 'Shokofa' genotype under 16 dS/m salinity showed the lowest chlorophyll-b content (5.13 and 4.27 µg/g FW, respectively).

Results showed that non-priming of 'Shokofa' genotype, hydro-priming of 'Aria' genotype, and osmo-priming of 'Paya' genotype under non-salinity conditions had the highest total chlorophyll content (31.04, 32.80, and 28.50 µg/g FW, respectively). Osmo-priming of 'Paya', 'Turbata', and 'Aria' cultivars under severe salt stress conditions (16 dS/m) led to an increase in total chlorophyll content compared to the non-primed seeds. Hydro-priming of 'Aria' genotype under 16 dS/m salinity showed the lowest total chlorophyll (14.57 µg/g FW) (Fig. III).

Proline content

According to the ANOVA, proline content was affected by salinity stress, seed priming, and interaction of salinity ×priming (Table 3). As shown in Fig. (IV), the highest proline content was related to the hydro-and osmo-priming of seeds under high salinity stress (1.91 and 1.23 µmol/g FW,

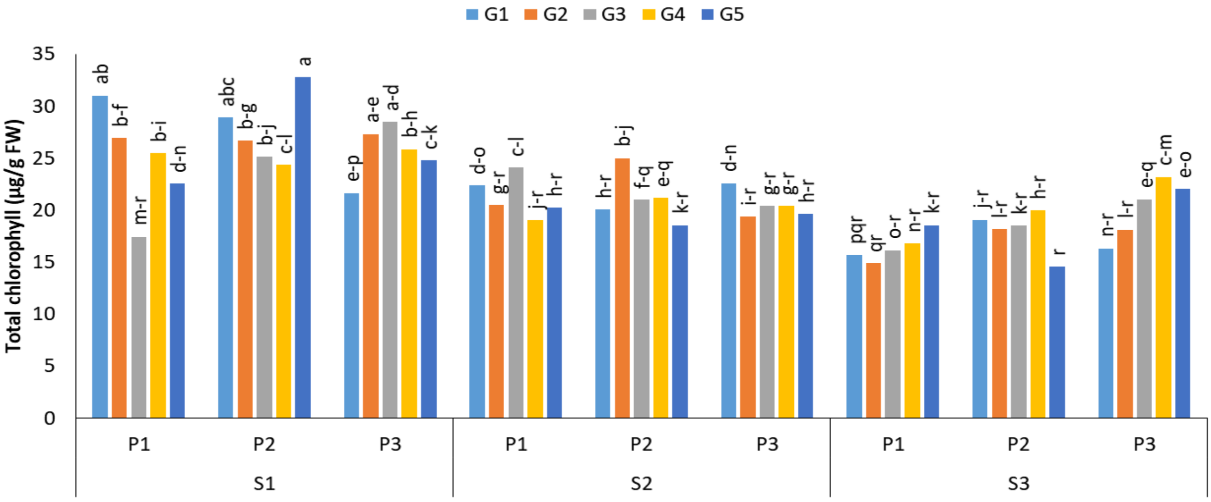

Fig. III. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on total chlorophyll content of sugar beet

respectively). On the other hand, the lowest proline content was obtained in the seed hydro-priming under non-stress conditions (0.65 µM/g FW).

MDA content

The data analysis revealed that salinity stress, priming, and the interaction of salinity and priming had a significant effect on the MDA content, as shown in Table 3. The mean comparison results indicated that the highest MDA content (5.46 nM/ml) was observed in the seeds that were subjected to osmo-priming under high salt stress conditions. Fig. (V) shows that the non-priming and hydro-priming treatments under non-saline conditions had the lowest MDA content (2.05 and 2.02 nM/ml, respectively).

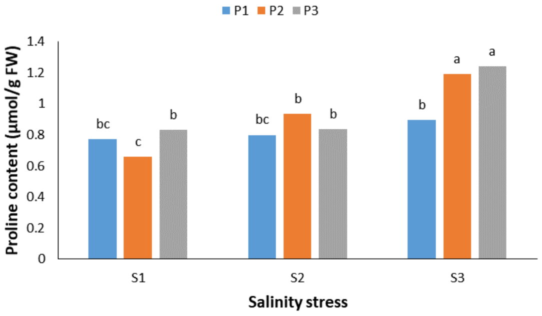

Fig. IV. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m) and priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), on proline content of sugar beet

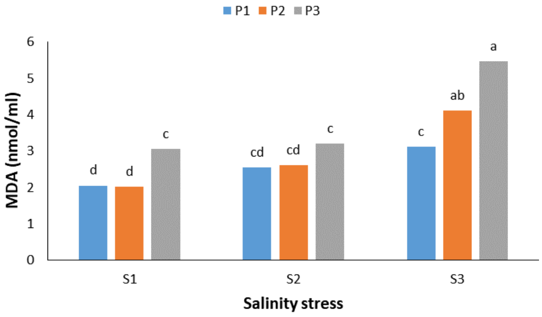

Fig. V. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m) and priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), on MDA content of sugar beet

Leaf area index (LAI)

Results indicated that salinity stress, seed priming, as well as the interaction effects of these two factors, and the triple interaction effect of salinity stress × seed priming × genotype had a significant impact on LAI (p<0.01), as shown in Table 3. In mean comparison, the highest LAI was related to ‘Shokofa’ seed hydro-priming under a high salt stress level (7.63). It is worth noting that many of the priming treatments and cultivars exhibited the highest LAI at the control salinity level and were grouped statistically alongside the best treatment. The non-priming of ‘Paya’ genotype under severe salt stress conditions (16 dS/m) led to the lowest LAI with an average of 0.92 (Fig. VI).

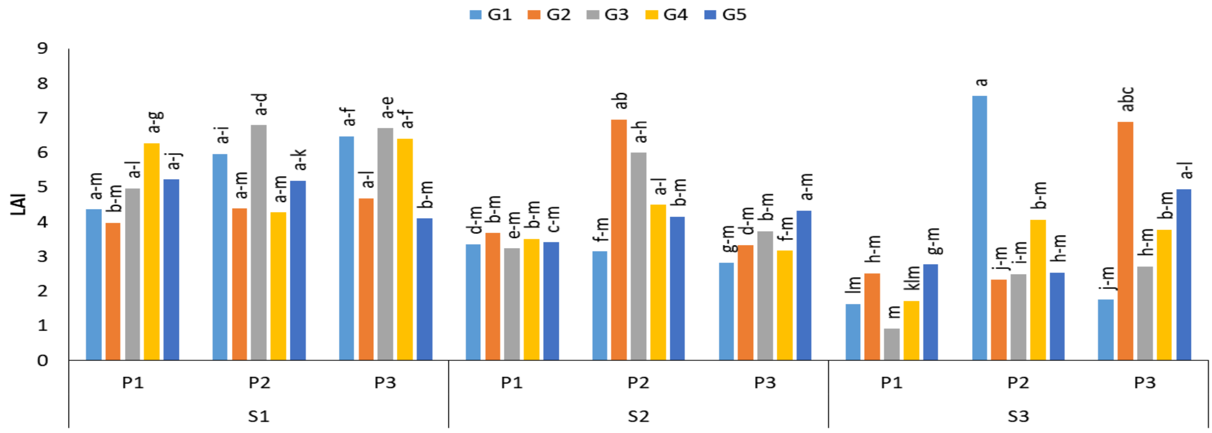

Fig. VI. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on LAI of sugar beet

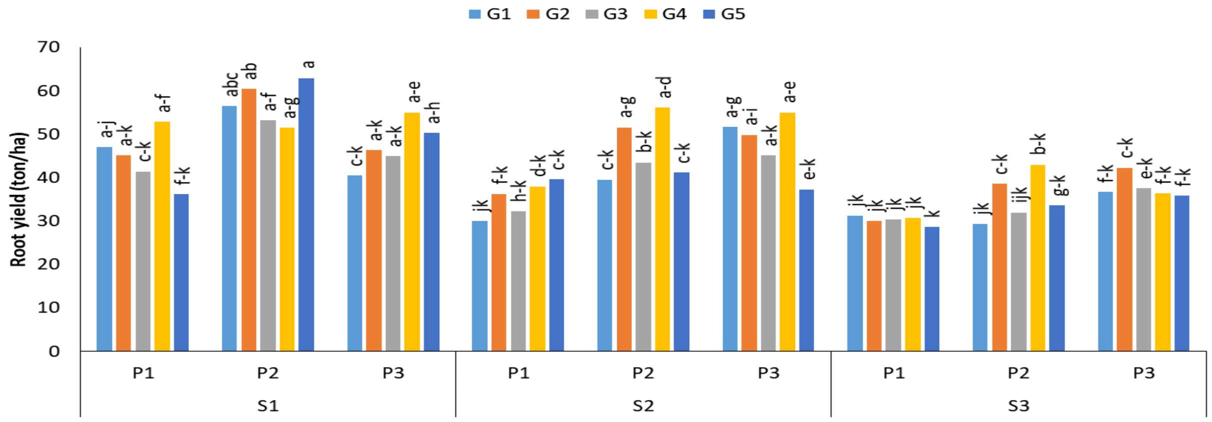

Fig. VII. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on sugar beet root yield

Root yield

The effects of salinity stress, seed priming, and genotype were significant at 1% probability level. The interaction effect of salinity, priming, and genotype was significant at 5% probability level on root yield (Table 3). As shown in Fig. (VII), the highest root yield (62.9 tons/ha) was observed in the hydro-priming of ‘Aria’ genotype seeds under non-salinity stress conditions. In addition, all sugar beet cultivars under non-stress and hydro-priming conditions had the highest root yield. The results also showed that severe salinity stress led to a decrease in the average root yield in all studied cultivars, so that the lowest root yield (28.5 tons/ha) was observed in non-priming of ‘Aria’ genotype under severe salinity stress conditions (16 dS/m).

Aerial yield

The research findings indicated that salinity stress, genotype, salinity × priming, priming × genotype, and salinity × priming × genotype had significant effects on aerial yield ($p<0.01$), as shown in Table 3. According to Fig. (VIII), the highest aerial yield (17.13 tons/ha) was obtained from the osmo-priming treatment of 'Turbata' genotype seeds with zinc sulfate under non-saline conditions. In addition, the osmo-priming of ‘Paya’ and ‘Aria’ cultivars under stress-free conditions, as well as hydro-priming seeds of ‘Shokofa’, ‘Sina’, and ‘Aria’ cultivars under non- salinity stress conditions had the highest aerial yield. Under severe salt stress conditions, the lowest aerial yield was observed in the osmo-priming treatment of 'Aria' genotype seeds, which yielded 5.16 tons/ha.

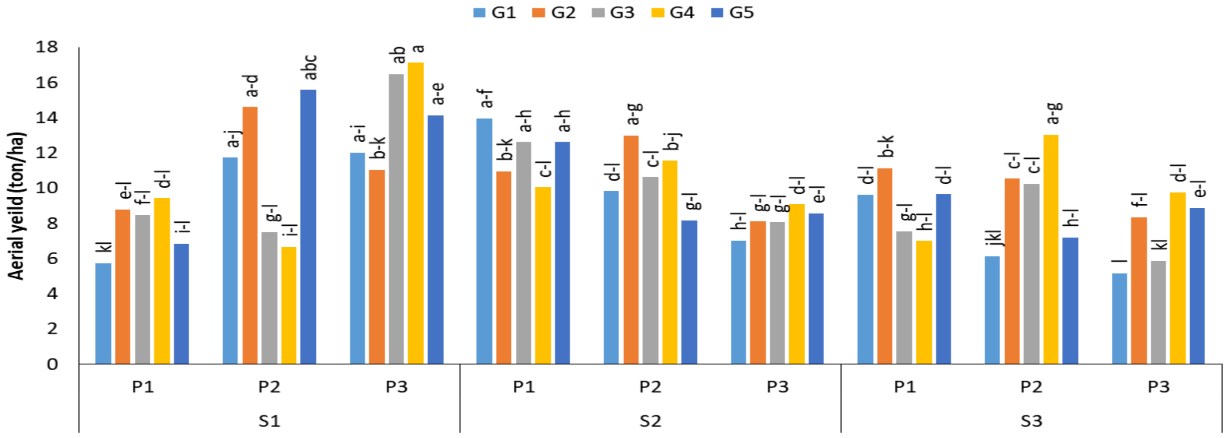

Fig. VIII. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on sugar beet aerial yield

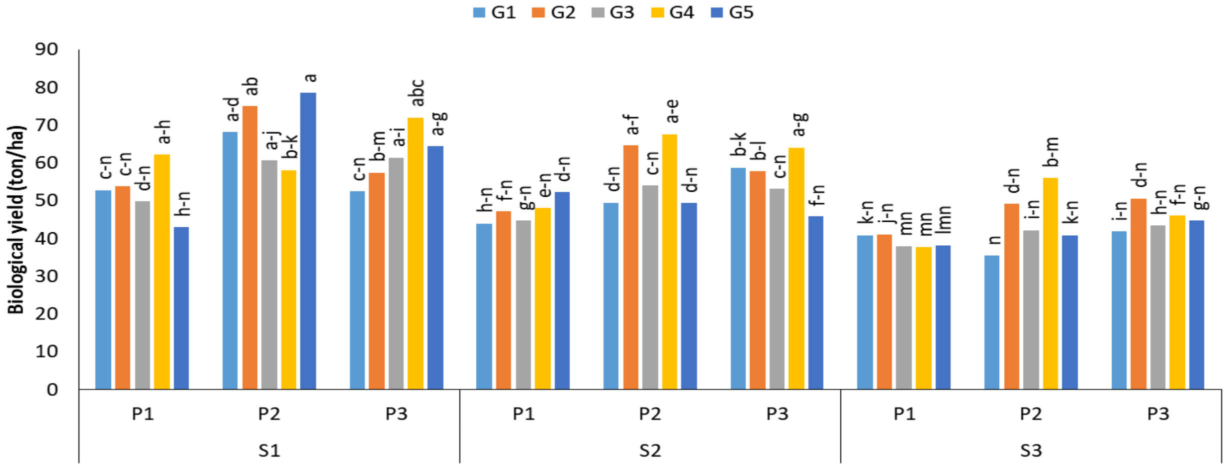

Fig. IX. Interaction effects of salinity stress (S1: control <2 dS/m, S2: 8 dS/m, and S3: 16 dS/m), priming (P1: non-priming as control, P2: hydro-priming, and P3: osmo-priming), and cultivars (G1: Shokofa, G2: Sina, G3: Paya, G4: Turbata, and G5: Aria) on sugar beet biological yield

Biological yield

The effect of salinity stress, priming, genotype, and the interaction of these three factors significantly impacted the biological yield (sum of root and aerial yield) at 1% probability level, as shown in Table 3. The highest biological yield (78.5 tons/ha) was observed in the hydro-priming of ‘Aria’ genotype seeds under non-salinity stress (Fig. IX). Also, the hydro-priming of ‘Shokofa’, ‘Sina’, and ‘Paya’ cultivars had the highest mean of this trait under non-stress conditions. Moreover, under moderate salinity stress (8 dS/m), hydro-priming and osmo-priming of ‘Turbata’ genotype seeds had the highest biological yield. The results showed that severe salinity stress (16 dS/m), especially without seed priming, led to a decrease in biological yield, so that the lowest biological yield was related to hydro-priming of ‘Shokofa’ seeds under severe salinity stress (35.5 tons/ha).

Discussion

The current study aimed to assess the morpho-physiological and yield-related responses of various sugar beet cultivars to seed priming treatments under salt stress conditions in field settings. Severe salinity (16 dS/m) significantly changed physiological traits, including photosynthetic pigments, proline, and MDA. Through this, root yield, LAI, and aerial yield decreased. Salinity problems for higher plants are due to a large amount of sodium salts, especially sodium chloride or sodium sulfate; these salinity sources are spread in the soils of dry and coastal areas and surface and underground water sources (Shahid et al., 2020).

Previous studies have suggested that salt-resistant cultivars can accumulate mineral ions, particularly potassium, under salt stress. This ability helps reduce the osmotic potential of the cells, allowing for increased water absorption compared to root cells. Consequently, these plants can better maintain water levels in their tissues when under stress (Shahverdi et al., 2020). Several experiments have demonstrated that salinity stress can inhibit protein synthesis, reduce the relative water content of leaves, and impair root function in sugar beet plants. These effects ultimately lead to a decrease in yield and reduced tolerance to salinity (Shokohian and Omidi, 2021). Most plants can prevent the reduction of turgor pressure by osmotic adjustment when they are exposed to salinity. Following the occurrence of momentary water shortages and to create a balance between the amount of water evaporated from the leaf surface (transpiration) and the amount of water through the roots, plants close the stomata by increasing the production of abscisic acid and limit CO_2 (Shahid et al., 2020; Farzami Sepehr et al., 2022; Gholamzadeh Alam et al., 2022).

Proline significantly increased with increasing salinity level compared to the control. In addition to eliminating free radicals during salt stress, proline stabilizes phospholipid membranes (Gohari et al., 2021). It seems that in the tolerant cultivars, the level of toxic ions in the cytosol does not reach a level that causes a significant increase in the amount of proline. In contrast, the sensitive cultivar increases the amount of ions in the cytosol due to its inability to manage toxic ions (distribution in the vacuole), and cells are forced to increase proline to protect themselves and fight against toxic ions and membrane phospholipids oxidation and cellular damage (Aghighi Shahverdi et al., 2017). In stress conditions, proline, dry matter, leaf sodium, and root length traits increased significantly compared to non-stress conditions. Still, potassium, leaf area, relative water content, leaf water loss, and root dry matter decrease (Khorshid et al., 2020). It seems that among osmolytes, proline reacts to stress faster than other substances (Khorshid et al., 2018). Wu et al. (2013) observed that in the tolerant variety of sugar beet, accumulation of proline and soluble sugars in roots and shoots is higher than in the other two varieties. Proline has been found to play a vital role in osmotic regulation, acting as a regulator between the cytoplasm and vacuole. By regulating osmosis, proline can stabilize protective and antioxidant enzymes, thereby helping to reduce membrane vulnerability to reactive oxygen species. Furthermore, proline helps to protect the cellular structure under stress conditions (Yousif et al., 2010; Huang et al., 2009). As a result, proline is considered an adaptive substance that can increase plant adaptation to stress conditions (Anoshee and Farzami Sepehr, 2016; Khorshid et al., 2020).

Salinity stress increased MDA production in sugar beet leaves, probably due to lipid peroxidation that occurs under such conditions. As a result, the MDA content increases in response to salinity stress (Peykani and Farzami Sepehr, 2018). Superoxide radicals produced by dehydration cause lipid peroxidation. The result of peroxidation of membrane lipids will be compounds such as MDA, propanol, hexane, etc. These substances are used as an indicator to measure the peroxidation of membrane lipids. In this research, salt stress led to an increase in the amount of MDA (Yu, 2019). An increase in the concentration of MDA under the influence of salinity stress has been reported in different plants (Mohamed et al., 2021). Salinity stress can cause the destruction of cell membranes and lead to the production of MDA. This occurs due to the breakdown of cell membrane fats and can serve as a useful criterion for evaluating the response of different cultivars to salinity stress (Peykani and Farzami Sepehr, 2018; Mohamed et al., 2021).

Findings revealed that sodium chloride had a negative impact on leaf development, and the leaf area index (LAI) was more significantly affected by salt stress than the number of leaves. This reduction in LAI was mainly due to the lack of leaf development and the cessation of their growth (Efisue and Dike, 2020). The effect of salinity on the LAI was more remarkable than its effect on the dry matter because salt accumulation in the aerial organs occurs through transpiration, mostly in old leaves. Such a situation may be caused by the reduction in the growth and development of leafless cells due to the accumulation of salt in the leaf, the reduction of useful photosynthetic energy in the growth of organs (increased respiration), or the weak transfer of nutrients effective in the growth of leaves through the root. Not finding the number of leaves is the distribution of the absorbed harmful salts between the leaf organs and reducing the effect of these salts (Asadi et al., 2013; Shahid et al., 2020). The results of the study of other researchers also confirm such changes and mechanisms (Shahverdi et al., 2020). Salinity stress significantly reduced the dry weight of roots and shoots. In the past, the cause of reduced root yield in saline conditions was unknown. After extensive research, it was found that the decrease in plant growth and yield was attributed to either the inhibition of photosynthesis or a lack of nutrition caused by the toxicity of certain elements. Plant responses to salinity and water deficit are similar in many situations. Salinity reduces the ability of plants to absorb water and quickly causes a decrease in plant growth, and symptoms similar to water stress appear in the plant (Khorshid et al., 2020).

The reduction in root dry weight observed under salinity stress conditions may be attributed to a disturbance in the absorption of essential nutrients required for growth, resulting from a reduction in the development of the root system. One of the negative impacts of salinity on plant growth is the disruption of photosynthetic assimilate availability (Shahverdi et al., 2020). In saline conditions, the sugar beet plant spends a certain proportion of its energy on tissue maintenance, and the rest is spent on vegetative stages such as shoot formation; overall, less energy is allocated for root growth (Abbasi et al., 2019). Such findings were also observed in the results of other studies (Asadi et al., 2013). Researchers studied sugar beet's morphological and physiological reactions at different salt levels. They concluded that with the increase in salinity, the dry weight of the whole shoot, the dry root weight, and the LAI decreased drastically. The yield components and vegetative traits also showed different reactions to salinity (Khorshid et al., 2020). A study investigated the effect of three salinity levels on 28 sugar beet cultivars. With increasing salinity, the amount of sodium and the ratio of sodium to potassium increased, but the amount of potassium decreased. In the tolerant cultivars, sodium and the sodium to potassium ratio in sugar beet leaves were higher than in the sensitive cultivars (Pakniyat and Armion, 2007).

Seed priming has been demonstrated to be an effective method for imparting stress tolerance to plants, involving the treatment of seeds with natural and/or synthetic compounds before germination (Moreno et al., 2018). Various priming techniques have been employed to improve salinity tolerance in numerous plant species, with several priming agents proving to be effective. Therefore, no standard methods to treat seeds or target osmotic or ionic effects of salt stress are clearly defined. In the present study, hydro-priming and osmo-priming had the highest physiological and yield attributes. The superiority of hydro-priming as a treatment in increasing plant growth parameters compared to other treatments may be attributed to the absorption of more water by seeds compared to other treatments; in fact, they reached the highest percentage of germination, as reported by (Shokohian and Omidi, 2021). Seed priming has been suggested as one of the most useful physiological approaches to adapting glycophyte species to saline conditions. Pavia et al. (2019) found that zinc treatments reduced non-regulated energy dissipation caused by environmental stress, and protected plants against permanent damage to the photosynthetic apparatus. They improved the recovery of wheat plants after stress relief. Meanwhile, the positive effects of seed priming, such as faster, higher, and more uniform germination, were observed under both optimal and suboptimal salinity conditions, resulting in overall improved plant growth (Moreno et al., 2018; Shokohian and Omidi, 2021).

Significant differences were found among sugar beet cultivars with respect to physiological and yield traits under different salinity levels. Specifically, 'Aria' genotype had a high mean total chlorophyll content under stress and non-stress conditions. Additionally, this genotype exhibited superior root yield under non-stress conditions. In contrast, 'Sina' and 'Torbata' had a higher mean root yield under salt stress conditions. The results showed that severe salinity (16 dS/m) decreased yield traits, including root, shoot, and biological yield.

On the other hand, seed priming (hydro- and osmo-priming) increased the mean values of these traits. Under salinity stress conditions, seed priming (hydro- or osmo-priming) led to the change of physiological characteristics (chlorophyll content, proline, MDA) and, in this way, moderated the adverse effects of salinity stress on yield traits. Hydro-priming is a recommended method for seed treatment due to its numerous advantages over osmo-priming. These benefits include more significant positive effects on seed germination and growth, ease of implementation, and availability of the required materials. Additionally, hydro-priming is more cost-effective than osmo-priming, making it a more practical choice for farmers and seed producers. Based on the findings, the cultivars 'Sina', 'Torbata', and 'Aria', as well as hydro-priming of seeds are recommended for cultivation in areas with saline soils.

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