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Research Article

Sugar beet (*Beta vulgaris* L.) germination indices and physiological properties affected by priming and genotype under salinity stress

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Abstract

Seed priming has proved to be an effective method in imparting stress tolerance to plants using natural and/or synthetic compounds to treat the seeds before germination. The present study was designed to investigate the physiological mechanism of seed priming with $ZnSO_4$ (osmopriming) and distilled water (hydropriming) on sugar beet genotypes ('Shokofa', 'Sina', 'Paya', 'Turbata', and 'Aria') germination indices, seedling growth parameters, and biochemical properties under salinity stress (0, 2, 5, and 12 dS/m NaCl). A significant reduction in germination percentage (33.23%), germination rate (77.2%), chlorophyll a, b, and total contents (43.9, 31.9, and 39.9%, respectively) while, a significant increase in radical, plumule, and seedling length (57.1, 44.4, and 51.2%, respectively), seedling vigour index (48.9%), superoxide dismutase activity (61.3%), proline (54.0%) and sugar (56.3%) contents were achieved at 12 dS/m NaCl in compared to the control treatment. Seed hydropriming and osmopriming caused significant improvements in photosynthetic pigments, antioxidant enzyme activity, and proline content reflected in high germination percentage and rate as well as seedling vigour index and reduced mean germination time under salinity. 'Paya' and 'Aria' genotypes had a superiority according to the germination percentage and seedling vigour index, respectively. The hydropriming of 'Paya' genotype resulted in the highest germination percentage (95%) under high level of salinity (12 dS/m) which 11.84% increase compared to the control treatment. Hydropriming of 'Sina' seeds showed the highest chlorophyll a and total, and carotenoids under non-stress conditions (22.89, 31.65, and 2116.6 µg/g FW). Also, hydropriming by increases chlorophyll b content led to the modulation of the negative effects of high salinity stress (12 dS/m). In conclusion, different seed priming treatments in sugar beet seeds improved the salinity tolerance by physiological characteristics nonetheless hydropriming was the most effective treatment to get higher germination indices in 'Paya' and 'Aria' genotypes.

Keywords: hyrdopriming; osmopriming; photosynthetic pigments; proline content; salinity tolerance

Abbreviations: GP, germination percentage; GR, germination rate; RL, radical length; PL, plumule length; SL, seedling length; SVI, seedling vigor index; Chl, chlorophyll; ROS, reactive oxygen species; SOD, superoxide dismutase

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Introduction

Sugar beet (*Beta vulgaris* L.) is one of the most recently domesticated crops, belongs to the order Caryophyllales (Skorupa *et al*., 2019). It is an important root crop in the world for sugar production, where its taproots are used. Sugar beet is the second most important source of sugar worldwide, after sugar cane, providing annually million tonnes of sugar for consumption and beet pulp for animal feed. The world production of sugar from sugar beet in 2018 was approximately 42 million metric tons, accounting for nearly 30% of the world's sugar supply (Lv *et al*., 2019). Sugar beet pulp is especially rich in polysaccharides, such as cellulose (20-24%), hemicellulose (25-36%), and pectic substances (15-25%).

Among abiotic stresses, high concentrations of salt in the soil can result in severe detrimental factors, such as poor germination, seedling establishment, and crop yield (Carvalho *et al*., 2011). Salinity is an increasing problem affecting crop productivity in many irrigated arid and semi-arid areas of the world. Plant growth is affected, and detrimental effects include (i) reduced water availability, due to an osmotic effect from high concentrations of soluble salts in the root medium, (ii) ion toxicity, as a result of the accumulation of Na^+ and Cl, (iii) oxidative stress, resulting from an overproduction of reactive oxygen species (ROS) and (iv) acute K^+ deficiency as a result of massive K^+ leak from depolarized cells (Moreno *et al*., 2018). In general, the adaptive responses of plants to salt stress can be grouped into three categories: osmotic stress tolerance, ion exclusion, and tissue tolerance to salinity (Yang and Guo, 2018). Feghhenabi *et al*. (2020) reported that the response to salinity stress at each stage of growth varies not only among plant species but also among genotypes or cultivars. Most crop species are sensitive to salinity stress at early growth stages, including the germination and seedling establishment stages. During germination and emergence, plant survival is likely the most vital indicator of salinity tolerance, whereas afterward yield and growth reduction may be considered as the final tolerance criterion (Saadat and Homaee, 2015).

Nowadays, seed priming has become common practice to increase the rate and uniformity of seed germination and emergence, and many patented seed priming treatments targeting horticultural species (tomato, pepper, onion, sugar beet, lettuce, and *Brassica*) are commercially available (Paparella *et al*., 2015). Seed priming is an efficient method for increasing seedling vigour index (SVI) and synchronization of germination, as well as the growth of seedlings of many crops under stressful conditions (Aghighi Shahverdi *et al*., 2017). In this process, one interesting approach to the question of the ameliorative effects of priming solutions on stress tolerance is that seed priming with nutrients or hormones can also result in alleviated oxidative stress (Subramanyam *et al*., 2019). Seed priming as a pre-sowing technique can improve radicle emergence, germination percentage (GP), germination rate (GR), SVI, seedling establishment, and yield by making changes in metabolic activities in the seeds of many crops (Mosavikia *et al*., 2020). The effectiveness of seed priming depends on the plant species, the type and concentration of priming solution, the priming duration, and temperature and storage conditions (Aghighi Shahverdi *et al*., 2017; Khaing *et al*., 2020). During pre-germination metabolic activities, structural (membrane protection during imbibition) and genetic repair, RNA and protein synthesis and antioxidant mechanism take place in primed seed, which ensures its proper germination and seedling development (Saddiq *et al*., 2019). Hydropriming is the simplest approach to hydrate seeds and minimize the use of chemicals. It reported that hydropriming treatments increased the GP, GR, radical length (RL), as well as plumule, radical, and seedling dry weight (Toklu, 2015). The results of Anwar *et al*. (2020) suggested that seed priming with GA_3 and KNO_3 synergistically promoted the photosynthetic pigments and nutrients uptake in cucumber seedlings, thus leading to improve plant growth.

Zinc is an important microelement essential for plants and humans. It is the cofactor of many enzymes like DNA, RNA polymerases, and zinc finger proteins (Rehman *et al*., 2018). Its deficiency in agricultural soils is common all over the developed and developing countries especially in drought and salt areas (Mahmood *et al*., 2019). Besides, priming seeds in zinc-containing solutions were shown to increase zinc content in the primed seeds and/or to contribute to a better seedling growth and yield. Pavia *et al*. (2019) reported that the priming improves germination and seed reserve utilization, growth, antioxidant responses, and membrane

stability at early seedling stage of Saudi sorghum varieties under drought stress. Many studies have developed salt-tolerant lines or cultivars using conventional plant breeding, but seed priming is a simple, promising, and non-GMO technique to pre-condition stress tolerance in plants before germination. The objectives of this study were to (i) characterize the germination and physiological response to different salinity stress, hydropriming and osmopriming and (ii) investigate whether hydropriming and osmopriming induce tolerance to salinity in seeds of sugar beet genotypes.

Materials and Methods

Plant material and treatments

To determine the effect of different primes on germination indices and physiological traits of sugar beet genotypes, an experiment was conducted at Shahed University, Tehran, Iran, in 2019. Three factorial experiments were carried out to assess the effects of priming (unprimed as control, hydropriming, and osmopriming with zinc sulfate ($ZnSO_4$)) on germination indices and physiological characteristics of five genotypes of sugar beet ('Shokofa', 'Sina', 'Paya', 'Turbata', and 'Aria') under salt stress (0, 2, 5, and 12 dS/m NaCl) in a completely randomized design with three replications. Seeds of sugar beet genotypes were obtained from Sugar Beet Seed Institute, Karaj, Iran. Before experimenting, to determine of best priming duration was a pilot experiment, where five priming durations (0, 8, 16, 24, and 32 hours) for each treatment (hydropriming and osmopriming) separately, were tested. The results showed that the best duration was 12 hours, that was used this data in the experiment. ISTA recommends germinating sugar beet seeds on between paper or top of the paper at constant or alternating temperatures, 25 or 20/30 °C, respectively. In the experiment, the seed of sugar beet was immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility. Then they were soaked in distilled water (as hydropriming) and $ZnSO_4$ (0.5%) suspension for about 12 hours at 15 °C. Treated seeds were shade-dried for 12 hours. Zinc sulfate wad was purchased from Sigma-Aldrich, USA. Then the 50 seeds were placed in a Petri dish (24 × 1.5 cm) with one piece of sterilized filter paper (Whatman No.1) and 12 ml of distilled water (for control) or salinity solution based on various treatments was added to each Petri dish. Van't Hoff formula (1) was used prepared for salinity solution (Aghighi Shahverdi *et al.,* 2017).

Formula 1) $\Psi = -MIRT$

Whereas Ψ: osmotic potential according to Bar; M: molarity; I: Van't Hoff factor = 2 for NaCl; R: constant number = 0.08206 bar; T: temperature according to Kelvin

Germinated seeds were counted on the second day was done daily and finally at the end of the testing period (14 days) was calculated GP, mean germination time (MGT), GR, germination uniformity (GU), and SVI according to the following formulas (Parmoon et al 2015; Aghighi Shahverdi et al 2017).

Germination percentage	$GP = (N \times 100) / M$
Mean time of germination	$MTG = \sum)Ni) / \sum N$
Germination rate	$GR = \sum Ni / Ti$
Germination uniformity	$GU = 1/N^*$
Seed vigor index	SVI= GP× Mean (SL)

Whereas N = sum of germinated seeds at the end of the experiment, M = total planted seeds, Ti = number of days after germination, N* = the number of days that germination reached 10 to 90%, SL= Seedling length.

Relative water content

The relative water content of the leaves was determined using the methods of Shaw *et al.* (2002). Each disc (diameter 10 mm) was placed into a separate glass-stoppered tube. Having ensured that there was no excess water on the discs, the fresh weight of each was recorded. The discs were then floated on 2 ml of distilled water for 24 h in natural daylight. At the end of this period, the fully turgid samples were rapidly surface dried with

filter paper and re-weighed. These discs were then dried at 60 °C for 24 h and the dry weights established. The relative turgidity was calculated.

Photosynthetic pigments

For measuring the content of photosynthetic pigments was used Lichtenthaler and Buschmann (2001) method. According to this method, 0.25 g of fresh tissue was extracted using 5 ml 80% acetone. The extract was centrifuged at 11000 rpm for 10 min. The optical density (O.D.) of the extract was measured at wavelengths 646.8, 663.2, and 470 nm. The amount of pigment present in each sample was calculated according to the following equations (Shahverdi *et al.*, 2019):

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

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

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

$$\text{Carotenoids (µg/gr FW)} = (1000 \times A470 - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b})/198$$

Whereas 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.

Anthocyanin content

After being thoroughly extracted in 3 ml methanol-HCl (1% HCl, v/v), the samples were left at 4 °C in the refrigerator for 2 days. Later on, he extracts were filtered and the total anthocyanin content was measured by a UV-visible spectrophotometer as the difference between the absorbance at 530 and 657 nm wavelength and placed in the A530-A657 formula to eliminate the chlorophyll content in the extract, defined quantitatively as OD 530 g^{-1} fresh weight (Mancinelli, 1990).

Protein and superoxide dismutase assay (SOD)

Samples were frozen in liquid nitrogen and stored at -30 °C. One g of frozen sample was homogenized in a mortar with 5 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol and 2% polyvinyl pyrrolidone. The homogenate was centrifuged at 15,000 g for 25 min and the supernatant was used for protein and SOD assay. Protein percentage measurement according to the method of Bradford (1976). For SOD activity measurement, the method of Beauchamp and Fridovich (1971) was used which is briefly described here. About 3 ml of the reaction mixture, containing 0.1 ml of 200 mM methionine, 0.01 ml of 2.25 mM nitro blue tetrazolium, 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml distilled water and 0.05 ml of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm.

Proline assay

Proline was determined according to the method described by Bates *et al.* (1973). Approximately 0.5 g of fresh seedling was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Then, this aqueous solution was filtered through Whatman's paper No. 2 and finally, 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer.

Sugar content

Samples of fresh seedlings were weighed (0.2 g) and homogenized using 70% ethanol. Then they were filtered and pigments were removed by the use of benzene. An aliquot of 0.2 ml of seedling extract was added to 1.0 ml of 0.2% anthrone to react in a water bath for 10 min at 100 °C. Soon after, the test tube 3 was cooled in an ice bath and then the absorbance was reared at 620 nm, according to Yemm and Folkes (1953).

Statistical analysis

Analysis of variance (ANOVA) was carried out using Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2) and Duncan was used to measuring significant differences among treatment means at $p < 0.05$. The Pearson correlation coefficient was used to measure relationships between germination and physiological traits by using SAS software *vr.*9.2.

Results

Germination parameters

The effects of genotypes, salinity, and priming as well as interaction genotype × salinity × priming was significant on GP, MGT, GR, GU, RL, PL, SL, and SVI. Salinity, in 12 dS/m level, reduced GP, GR, RL, PL, SL, and SVI in different sugar beet genotypes but increased MGT and GU (Table 1). Results indicated that a moderate level of salinity (5 dS/m) had the highest seedling growth parameters such as RL, PL, SL, and SVI compared to the non-stress conditions. In other words, NaCl concentration up to 5 dS/m was caused by increases seedling growth parameters (Table 1). In general, the results showed that seed priming increased the mean of germination parameters compared to unprimed seeds (Table 1). For example, the hydro-priming of 'Paya' genotype seeds resulted in the highest GP (95%) under high level of salinity (12 dS/m) which 11.84% increase compared to the control treatment (Table 2).

As shown in Table 2, in the interaction effects of genotype, salinity, and priming, the highest MGT was related to seed priming with $ZnSO_4$ in 'Sina' and 'Aria' genotypes under non-stress condition (3.86 and 3.9 days, respectively). On the other hand, the lowest MGT was found in the hydro- and osmopriming of 'Paya' genotype under high level of salinity (0.155 and 0.09 days).

Results indicated that the hydropriming of 'Paya' sugar beet seeds increase GR (84.9%) under high level of salinity compared to the control treatment. Osmopriming by $ZnSO_4$ resulted in the lowest GR (0.26 seed per day) in 'Sina' genotype under non-stress conditions (Table 2).

Results indicated that the salinity decreases GU, but priming treatment increases the mean of these traits (Table 1). According to the results of interaction effects (Table 2), GU ranged from 0.14 to 0.715. The highest GU was related to primed G2 ('Sina' genotype) seed by $ZnSO_4$ under non-stress conditions, and the lowest of these traits was in G3 ('Paya' genotype) under high level of salinity and all priming treatments.

The results of ANOVA showed that the genotype, salinity, and priming significantly affected seedling growth parameters such as RL, PL, SL, and SVI. In terms of seedling growth parameters, 'Aria' genotype had a significant advantage over other genotypes (Table 1). In interaction effects, the highest RL (88.75 mm) and SL (144.0 mm) were related to osmopriming of 'Paya' genotype seeds under 5 dS/m salinity, while the highest PL (67.0 mm) and SVI (20.91) was found in hydropriming of G5 genotype ('Aria') seeds under 5 dS/m. The results indicated that the lowest means of these traits was achieved in unprimed 'Paya' genotype seed under high level of salinity (Table 2).

Table 1. Effect of different levels of salinity stress (0, 2, 5, and 12 dS/m) and priming treatments on germination indices of sugar beet (*Beta vulgaris* L.) genotypes

Treatments	GP	MGT	GR	GU	RL	PL	SL	SVI
	(%)	(day)	(seed/day)		(mm)			
Genotypes (G)								
G1	71.14 b	1.26 d	1.38 b	0.19 d	37.56 b	38.11 a	75.67 b	10.14 b
G2	55.62 e	2.10 b	0.67 c	0.30 a	42.22 a	36.75 a	78.97 ab	9.87 bc
G3	86.66 a	0.63 e	2.44 a	0.15 e	37.31 b	31.40 b	68.71 c	9.31 c
G4	64.89 c	1.94 c	0.63 c	0.23 c	18.29 c	28.08 c	46.37 d	6.82 d
G5	59.27 d	2.56 a	0.44 d	0.28 b	41.87 a	38.08 a	79.95 a	12.05 a
Salinity levels (S) (dS/m)								
Control	81.50 a	0.93 d	2.37 a	0.17 d	18.26 d	20.46 c	38.73 d	5.38 c
2	72.41 b	1.57 c	0.82 b	0.20 c	36.23 c	37.57 b	73.80 c	10.59 b
5	61.75 c	1.96 b	0.72 b	0.25 b	44.73 a	43.07 a	87.80 a	12.05 a
12	54.41 d	2.33 a	0.54 c	0.30 a	42.58 b	36.83 b	79.41 b	10.53 b
Priming (P)								
Unprimed seed (control)	64.06 b	1.23 c	0.96 b	0.18 c	10.27 c	19.17 c	29.45 c	4.27 c
Hydro-priming	80.37 a	1.75 b	1.37 a	0.24 b	51.42 a	44.06 a	95.48 a	13.16 a
$ZnSO_4$	58.12 c	2.12 a	1.01 b	0.28 a	44.66 b	40.22 b	84.88 b	11.48 a
Statistics								
G × S	**	**	**	**	**	**	**	**
G × P	**	**	**	**	**	**	**	**
S × P	**	**	**	**	**	**	**	**
G × S× P	**	**	**	**	**	**	**	**

Means followed by the same letter in each column are not significantly different according to Duncan test at 5 % level
**: significant at α= α=0.01.
G1: Shokofa; G2: Sina; G3: Paya; G4: Turbata; G5: Aria
GP: germination percentage; MGT: Mean time germination; GR: germination rate; GU: germination uniformity; RL: radical length; PL: plumule length; SL: seedling length; SVI: seedling vigor index

Table 2. Interaction effects of salinity and priming on germination indices of sugar beet (*Beta vulgaris* L.) genotypes

Genotypes	Salinity	Priming	GP (%)	MGT (day)	GR (seed/day)	GU	RL (mm)	PL (mm)	SL (mm)	SVI
G1	S1	C	75 i.l	1.44 n.r	0.7 i.m	0.19 l.r	13.5 o.t	30 m.t	43.5 q.u	6.21 p.s
		H	60 opq	1.42 n.r	0.7 i.m	0.215 j.p	62.5 de	46.25 g.k	108.75 d.h	13.85 f.k
		S	43.75 uv	2.79 efg	0.36 lm	0.315 e.h	52.5 fgh	36.25 k.q	88.75 j.m	12.13 j.m
	S2	C	71.25 klm	1.22 q.v	0.82 g.m	0.19 l.r	6 tuv	29 n.t	35 s.x	4.72 r.u
		H	55 qrs	1.84 k.n	0.54 lm	0.235 jkl	65 de	58.25 a.e	123.25 bcd	15.97 c.f
		S	60 opq	2.02 j.m	0.5 lm	0.23 j.m	62.5 de	62.75 abc	125.25 bc	17.57 bcd
	S5	C	83.75 d.g	1.01 r.y	1.05 f.m	0.17 n.r	10.5 q.v	33.5 m.r	44 q.t	6.25 p.s
		H	72.5 j.m	1.24 q.u	0.82 g.m	0.185 l.r	62.5 de	47.6 e.k	110.1 c.g	14.83 e.i
		S	75 i.l	1.05 r.y	0.95 f.m	0.18 l.r	57 efg	57.25 a.g	114.25 c.f	15.33 d.g
	S12	C	85 c.f	0.75 v.y	1.43 e.i	0.16 pqr	4.5 tuv	6.5 yz	11 z	1.51 wx
		H	83.75 d.g	0.215 z	5.66 c	0.15 r	35 j	34 m.r	69 no	8.74 nop
		S	88.75 a.e	0.165 z	3.08 d	0.145 r	19.25 m.q	16 u.y	35.25 s.x	4.63 r.u
G2	S1	C	58.75 pqr	1.79 l.o	0.56 klm	0.225 j.n	8.5 r.v	30.5 m.t	39 q.v	5.17 q.t
		H	22.5 x	3.28 bcd	0.3 lm	0.475 c	65.75 de	37 k.o	102.75 f.j	10.83 lmn
		S	11.25 y	3.86 a	0.26 m	0.715 a	63.5 de	32.5 m.s	96 g.k	7.62 opq
	S2	C	77.5 g.k	1.16 q.v	0.86 g.m	0.18 l.r	8.5 r.v	23.5 r.w	32 s.y	4.48 s.v
		H	43.75 uv	2.69 fgh	0.375 lm	0.315 e.h	81.25 ab	51.25 d.j	132.5 ab	18.01 bc
		S	20 x	3.55 ab	0.28 lm	0.535 b	60 def	41.25 j.m	101.25 f.j	10.86 lmn
	S5	C	88.75 a.e	0.79 u.y	1.51 e.h	0.16 pqr	9.5 r.v	26 p.v	35.5 s.x	5 r.u
		H	52.5 rst	2.25 i.k	0.44 lm	0.26 ijk	62 de	59.25 a.d	121.25 b.e	16.63 cde
		S	50 stu	2.26 h.k	0.44 lm	0.265 h.k	61.25 de	45.25 h.k	106.5 e.i	14.33 e.j
	S12	C	91.25 abc	0.925 t.y	1.08 f.l	0.16 pqr	6.5 tuv	13.5 w.z	20 w.z	2.93 t.x
		H	83.75 d.g	0.675 wxy	150 e.h	0.16 pqr	50 ghi	43 i.l	93 h.l	12.48 i.m
		S	67.5 mn	2 j.m	0.5 lm	0.215 j.p	30 jkl	38 k.n	68 nop	10.07 mno
G3	S1	C	83.75 d.g	1.01 r.y	1 f.m	0.165 o.r	12 p.v	19.5 t.x	31.5 s.y	4.41 s.v
		H	77.5 g.k	1.31 p.t	0.87 g.m	0.18 l.r	82.5 ab	54 c.i	136.5 ab	19.31 ab
		S	75 i.l	1.12 r.w	1.04 f.m	0.185 l.r	50 ghi	45.5 h.k	95.5 g.k	12.83 g.l
	S2	C	87.5 b.e	0.605 y	1.69 ef	0.155 qr	11 q.v	21.5 s.x	32.5 s.y	4.44 s.v

		H	92.5 ab	0.19 z	2.07 e	0.145 r	80 b	53.6 c.i	133.6 ab	17.49 bcd
		S	80 f.i	1 r.y	1.05 f.m	0.17 n.r	63.75 de	57.5 a.f	121.25 b.e	16.55 cde
	S5	C	90 a.d	0.755 v.y	1.34 e.k	0.155 qr	10.5 q.v	17.5 u.y	28 t.z	3.96 s.w
		H	92.5 ab	0.09 z	1.48 e.i	0.14 r	5 tuv	16.5 u.y	21.5 w.z	2.75 t.x
		S	83.75 d.g	0.755 v.y	1.37 e.j	0.165 o.r	88.75 a	55.25 b.h	144 a	19.5 ab
	S12	C	91.25 abc	0.64 xy	1.57 efg	0.15 r	3 v	4 z	7 z	0.98 x
		H	95 a	0.155 z	7.3 a	0.14 r	35 j	19.5 t.x	54.5 opq	7.15 pqr
		S	91.25 abc	0.09 z	6.85 b	0.145 r	6.25 tuv	12.5 w.z	18.75 xyz	2.38 u.x
G4	S1	C	72.5 j.m	1.46 n.r	0.68 i.m	0.195 l.r	12 p.v	15 w.z	27 u.z	3.78 s.w
		H	45 uv	3.36 bc	0.29 lm	0.355 de	24 lmn	30.5 m.t	54.5 opq	8.58 nop
		S	46.25 tuv	3.42 bc	0.29 lm	0.355 de	26 klm	47 f.k	73 mn	11.86 j.m
	S2	C	76.25 h.l	1.41 n.r	0.72 h.m	0.185 l.r	7.5 s.v	13 w.z	20.5 w.z	2.92 t.x
		H	58.75 pqr	2.07 i.l	0.48 lm	0.235 jkl	44 i	37.5 k.o	81.5 k.n	11.34 klm
		S	45 uv	2.64 fgh	0.39 lm	0.3 f.i	33.5 jk	58.5 a.e	92 i.l	12.55 h.m
	S5	C	82.5 e.h	1.35 o.t	0.74 h.m	0.18 l.r	7.5 s.v	10.5 yxz	18 yz	2.67 t.x
		H	60 opq	1.63 l.q	0.61 j.m	0.22 j.o	21.5 l.o	26.5 o.u	48 qrs	6.32 p.s
		S	63.75 nop	2.87 d.g	0.35 lm	0.27 hij	20.5 m.p	52.5 c.i	73 mn	12.52 h.m
	S12	C	78.75 f.j	1.03 r.y	1.03 f.m	0.18 l.r	3.5 uv	4 z	7.5 z	1.04 x
		H	75 i.l	1.09 r.x	0.92 f.m	0.18 l.r	12.5 p.t	34 m.r	46.5 qrs	6.28 p.s
		S	75 i.l	0.955 s.y	1.07 f.m	0.175 m.r	7 s.v	8 yz	15 yz	1.97 vwx
G5	S1	C	71.25 klm	1.93 j.m	0.52 lm	0.21 k.q	22.5 lmn	30.5 m.t	53 pqr	7.93 op
		H	42.5 v	2.85 d.g	0.335 lm	0.325 efg	75.5 bc	59 a.d	134.5 ab	18.35 bc
		S	31.25 w	3.9 a	0.265 lm	0.515 bc	68 cd	39 k.n	107 d.i	15.1 d.h
	S2	C	78.75 f.j	2.47 ghi	0.46 lm	0.23 j.m	21.5 l.o	25 q.v	46.5 qrs	8.52 nop
		H	46.25 tuv	3.42 bc	0.29 lm	0.35 def	67 d	66 ab	133 ab	21.5 a
		S	33.75 w	3.21 b.e	0.31 lm	0.39 d	59.5 def	47.5 e.k	107 d.i	13.92 f.k
	S5	C	83.75 d.g	1.6 m.q	0.62 j.m	0.185 l.r	16 o.s	17 u.y	33 s.y	5.13 q.t
		H	52.5 rst	3.06 c.f	0.315 lm	0.305 e.i	64 de	67 a	131 ab	20.91 a
		S	55 qrs	3 c.f	0.325 lm	0.295 ghi	47 hi	32 l.s	79 lmn	12.72 h.l
	S12	C	80 f.i	1.21 q.v	0.83 g.m	0.18 l.r	11 q.v	13.5 w.z	24.5 v.z	3.49 t.x
		H	70 lm	2.33 hij	0.44 lm	0.23 j.m	33.5 jk	40.5 j.n	74 mn	11.93 j.m
		S	66.25 mno	1.75 l.p	0.57 j.m	0.21 k.q	17 o.r	20 t.x	37 r.w	5.17 q.t

Means followed by the same letter in each column are not significantly different according to Duncan test at 5 % level

G1: Shokofa; G2: Sina; G3: Paya; G4: Turbata; G5: Aria

C: control (non-priming); H: Hyrdopriming; S: $ZnSO_4$

GP: germination percentage; MGT: Mean time germination; GR: germination rate; GU: germination uniformity; RL: radical length; PL: plumule length; SL: seedling length; SVI: seedling vigor index

Physiological and biochemical characteristics

Genotypes, salinity, and priming as well as interaction of them significantly affected RWC, chlorophyll a, b, and total, carotenoids, anthocyanin, SOD, protein, proline, and sugar contents (Table 3). Salinity stress decreased RWC, while priming increased it. The highest RWC was related to 'Turbata' seeds primed by $ZnSO_4$ under 2 and 5 dS/m salinity levels (90.45 and 85.7%) which increased by 36.5% and 33.0%, respectively, compared to the control treatment. Also, in 'Aria' genotype, all priming treatment showed the highest RWC under non-stress conditions (Table 4).

High level of salinity stress was caused by decreasing photosynthesis pigments such as chlorophyll-a, b, and total as well as carotenoid contents by 43.9, 31.9, 39.9, and 20.4% compared to the control treatment (0 dS/m). Hydropriming of 'Sina' seeds showed the highest chlorophyll-a, total, and carotenoids under non-stress conditions (22.89, 31.65, and 2116.6 µg/g FW). Also, hydropriming by increases chlorophyll b content led to the modulation of the negative effects of high salinity stress (12 dS/m) (Table 4).

As shown in Table 4, $ZnSO_4$ priming of 'Shokofa' seeds significantly increased (79.3%) the anthocyanin content under salinity stressed plant compared to the non-stress conditions. The lowest mean of anthocyanin content was achieved in the hydropriming of 'Shokofa' (1.84 µg/g FW) and unprimed of 'Sina' (1.91 µg/g FW) seeds under non-stress conditions.

Results indicated that salinity stress significantly increases SOD activity (Table 3). Based on these findings, osmopriming of 'Shokofa' genotype had the highest SOD activity (90.3 U/mg protein) under high

level of salinity (12 dS/m) and the lowest mean (9.9 U/mg protein) was related to 'Turbata' genotype under unprimed and non-stress conditions (Table 4).

Genotype, salinity, and priming treatments significantly affected seedling protein content. Salinity decreased protein content while seed priming increased compared to the control treatment (Table 3). The highest protein content (12.35%) was related to 'Aria' genotype under unprimed and non-stress conditions, whereas the lowest this trait (2.1%) was in unprimed seeds of 'Sina' genotype under high level of salinity (12 dS/m) (Table 4).

Results showed that the main and interaction effects of genotype, salinity, and priming were significant on proline content (Table 3). Results illustrated that the salinity and priming treatments significantly increases seedling proline content, so that the highest proline content (2.15 µmol/g FW) was achieved in the osmopriming by $ZnSO_4$ of 'Shokofa' genotype under severe salinity treatment, and the lowest mean was related to the unprimed of 'Sina' seeds under 2 dS/m salinity (0.337 µmol/g FW) (Table 4).

Significant sugar accumulations were observed in sugar beet seedlings at all salinity concentrations and priming treatments (Table 3). In the interaction effects, the highest sugar content was found 'Shokofa' seed primed by $ZnSO_4$ under high level of salinity (33.2 mg/g FW). The sugar accumulation at 12 dS/m in 'Shokofa' rose to about 6-fold that of the control treatment (Table 4). On the other hand, the lowest mean of this trait was observed in the unprimed of 'Turbata' genotype under non-stress conditions (4.54 mg/g FW) (Table 4).

Table 3. Effect of different levels of salinity stress (0, 2, 5, and 12 dS/m) and priming treatments on physiological characteristics of sugar beet (*Beta vulgaris* L.) genotypes

Treatments	RWC	Chl a	Chl b	Total Chl	Carotenoids	Anthocyanin	SOD activity	Protein content	Proline content	Sugar content
	(%)	(µg/g FW)					(U/mg protein)	(%)	(µmol/g FW)	(mg/g FW)
Genotypes (G)										
G1	52.15 b	6.42 d	2.59 d	9.02 d	5.65 d	5.16 a	25.35 a	6.80 d	0.68 a	10.13 a
G2	46.23 b	9.09 c	3.65 c	12.75 c	7.82 c	2.63 e	19.76 c	7.24 c	0.55 c	8.15 c
G3	42.43 b	5.01 e	1.95 e	6.96 e	5.07 e	3.02 d	21.35 b	6.79 d	0.59 b	8.72 b
G4	66.73 a	9.53 a	4.49 a	14.02 a	9.36 b	4.83 b	17.96 d	7.99 b	0.51 d	7.28 e
G5	69.65 a	9.26 b	4.02 b	13.28 b	9.51 a	4.63 c	17.84 d	8.56 a	0.50 d	7.52 d
Salinity levels (S) (dS/m)										
Control	86.33 a	9.92 a	4.20 a	14.12 a	8.32 a	3.39 d	13.04 d	8.19 a	0.40 d	5.68 d
2	62.77 b	8.67 b	3.38 b	12.05 b	7.81 b	4.84 a	15.39 c	8.00 b	0.46 c	6.63 c
5	55.19 b	7.31 c	2.91 c	10.17 c	7.19 c	3.81 c	19.68 b	7.02 c	0.55 b	8.13 b
12	43.06 b	5.56 d	2.86 c	8.48 d	6.62 d	4.18 b	33.70 a	5.97 d	0.87 a	13.00 a
Priming (P)										
Control	57.81 b	5.68 c	2.17 c	7.85 c	5.39 c	3.57 c	16.28 c	6.76 b	0.46 c	6.76 c
Hydro-priming	66.51 a	8.45 b	4.30 a	12.75 b	8.68 a	3.66 b	19.81 b	7.82 a	0.55 b	8.19 b
$ZnSO_4$	61.18 a	9.46 a	3.55 b	13.01 a	8.38 b	4.94 a	25.26 a	7.86 a	0.68 a	10.13 a
Statistics										
G × S	**	**	**	**	**	**	**	**	**	**
G × P	**	**	**	**	**	**	**	**	**	**
S × P	NS	**	**	**	**	**	**	**	**	**
G × S× P	**	**	**	**	**	**	**	**	**	**

Means followed by the same letter in each column are not significantly different according to Duncan test at 5 % level

NS: non-significant;**: significant at α= α=0.01.

G1: Shokofa; G2: Sina; G3: Paya; G4: Turbata; G5: Aria

Chl a: chlorophyll a; Chl b: chlorophyll b; Total Chl: total chlorophyll; SOD: superoxide dismutase.

Table 4. Interaction effects of salinity and priming on physiological characteristics of sugar beet (*Beta vulgaris* L.) genotypes

Genotypes	Salinity	Priming	RWC (%)	Chl a (µg/g FW)	Chl b (µg/g FW)	Total Chl (µg/g FW)	Carotenoid (µg/g FW)	Anthocyanin (µg/g FW)	SOD (U/mg protein)	Protein (%)	Proline (µmol/g FW)	Sugar (mg/g FW)
G1	S1	C	48.5 h.n	7.18 y	4.37 hi	11.54 op	673.5 a*	2.64 w.z	13.1 stu	8.04 n.q	0.399 yza*	5.62 yza*
		H	50.65 g.l	8.24 r	2.71 p.t	10.95 qr	778.3 u	1.84 z	11.6 u.x	8.61 hij	0.379 z.c*	5.31 z.c*
		S	73.6 bcd	7.76 w	3.58 j.m	11.33 pq	730.4 x	2.34 xyz	16.4 nop	8.8 gh	0.487 qrs	7.12 st
	S2	C	55 f.j	6.94 z	2.84 n.s	9.78 tuv	583.4 hi*	3.03 r.w	15.2 o.r	6.5 uvw	0.448 uvw	6.4 u.x
		H	61.65 d.g	7.21 y	3.09 m.q	10.3 s	711.4 y	2.28 yz	13.6 rst	8.27 k.o	0.425 v.y	6.04 v.y
		S	60.85 d.g	7.2 y	2.71 p.t	9.91 stu	220.2 u*	12.8 a	25 g	7.46 r	0.68 g	10.16 g
	S5	C	66.7 cde	6.15 de*	2.11 t.w	8.26 y	487.9 mn*	5.46 h	20.6 jk	5.14 zab*	0.569 lmn	8.31 j.o
		H	67 cde	6.07 e*	2.12 t.w	8.19 y	589.6 g*	3.31 p.s	19.5 kl	6.69 tu	0.559 mno	8.1 l.p
		S	29.45 opq	6.76 a*	2.54 q.v	9.3 vw	667.2 b*	3.6 nop	28.9 e	7.05 s	0.77 e	11.5 e
	S12	C	42 i.p	3.5 m*	1.02 yz	4.52 c*	388 s*	5.95 fg	21.7 ij	4.8 cde*	0.592 klm	8.67 jkl
		H	37.15 k.q	4.41 k*	2.27 r.w	6.69 a*	513 j*	4.54 jk	27.8 ef	4.91 bcd*	0.74 ef	11.04 ef
		S	33.3 m.q	5.69 g*	1.75 wx	7.43 z	442.1 p*	4.97 i	90.3 a	5.34 yz	2.15 a	33.2 a
G2	S1	C	26.7 pq	8.67 n	0.93 yz	9.6 uv	589.7 g*	1.91 z	15.4 opq	7.89 pq	0.451 t.w	6.45 uvw
		H	55.35 f.i	22.89 a	8.76 b	31.65 a	2116.6 a	2.19 yz	15.2 o.r	10 c	0.461 s.v	6.6 tuv
		S	59.15 e.g	10.46 h	2.8 o.s	13.27 jk	812.9 s	2.27 yz	16.8 no	9.28 e	0.497 pqr	7.28 rs
	S2	C	65.8 c.f	7.91 v	0.25 a*	7.49 z	483.8 no*	2.39 xyz	21.2 ij	5.06 ab*	0.582 lm	8.51 j.m
		H	53.3 f.k	9.13 l	3.41 j.n	12.54 lm	924 n	2.38 xyz	15.4 opq	7.98 opq	0.464 stu	6.66 tu
		S	59.45 e.g	8.93 m	3.06 m.q	11.99 no	810.9 s	2.45 xyz	17.4 mn	8.64 hi	0.514 pq	7.48 qrs
	S5	C	37.8 j.q	6.17 d*	0.25 a*	5.88 b*	391.4 s*	2.64 w.z	23.4 gh	5.05 abc*	0.63 hij	9.29 hi
		H	49.65 g.m	8.84 m	3.43 j.n	12.27 mn	860 q	2.8 u.z	17.6 mn	6.87 st	0.524 opq	7.43 qrs
		S	49.15 g.m	8.44 p	3.15 k.p	11.6 op	791.4 t	2.61 w.z	22.5 hi	8.23 k.o	0.62 ijk	9.29 hi
	S12	C	36 l.q	3.77 l*	0.27 a*	4.05 d*	210 v*	2.96 r.y	24.6 g	4.56 e*	0.65 ghi	9.7 gh
		H	37.05 k.q	5.6 h*	17.44 a	23.04 d	658.1 c*	3.22 p.t	20.5 jk	6.67 tu	0.57 ml	8.46 j.n
		S	25.35 pq	8.34 q	1.31 xy	9.64 uv	736.6 w	3.74 no	26.5 f	6.59 uv	0.71 f	10.68 f
G3	S1	C	28 opq	4.45 k*	2.25 s.w	6.7 a*	489.3 lm*	2.64 w.z	10.3 xy	7.86 q	0.337 d*	4.64 de*
		H	50.45 g.l	6.81 a*	2.25 s.w	9.06 wx	585.6 gh*	2.56 xyz	11.2 v.y	8.6 hij	0.369 a.d*	5.16 a.d*
		S	83.75 ab	9.64 k	3.31 j.o	12.95 kl	757.9 v	2.78 u.z	14.5 qrs	9.06 ef	0.445 uvw	6.45 uvw
	S2	C	51.5 g.l	2.89 n*	1.06 yz	3.94 d*	405 r*	2.72 v.z	11.7 u.x	6.4 uvw	0.369 a.d*	5.16 a.d*
		H	36.2 l.q	5.91 f*	2.27 r.w	8.19 y	580.1 i*	2.86 t.y	11.6 u.x	8.27 k.o	0.379 z.c*	5.31 z.c*
		S	54.5 f.k	7.34 x	2.64 p.u	9.99 stu	681.7 z	3.06 r.v	17.4 mn	8.64 hi	0.51 pqr	7.48 qrs
	S5	C	18.5 r	2.2 o*	0.94 yz	3.14 e*	301.8 t*	2.96 r.y	11.7 u.x	4.76 cd*	0.369 a.d*	5.16 a.d*
		H	38.6 j.q	4.78 j*	2.62 p.v	7.41 z	493.1 kl*	2.92 s.y	14.5 qrs	6.55 uvw	0.445 uvw	6.35 u.x
		S	55 f.j	5.89 f*	2.64 p.u	8.54 y	596 f*	3.25 p.t	21.4 ij	8.09 m.q	0.599 jkl	8.88 ij
	S12	C	38.5 j.q	1.03 p*	0.71 yz	1.75 f*	205.2 w*	3.13 r.v	19.3 kl	2.1 f*	0.556 mno	7.84 o.r
		H	32.85 n.q	3.74 l*	0.65 z	4.38 cd*	479.4 o*	3.58 n.q	77.7 b	5.76 x	1.86 b	28.7 b
		S	21.3 q	5.51 i*	2.03 vw	7.54 z	516 j*	3.8 mn	34.2 d	5.44 y	0.88 d	13.4 cd
G4	S1	C	57.4 e.g	6.38 bc*	4.79 gh	11.17 pqr	610.9 d*	3.24 p.t	9.9 y	7.41 r	0.333 d*	4.54 c*
		H	61.7 d.g	8.97 m	8.1 c	17.07 g	1347.9 d	9.2 b	11.9 u.x	8.92 fg	0.349 cd*	5.31 z.c*
		S	83.15 ab	9.67 k	4.3 hi	13.97 i	1072.4 g	8.23 c	12.8 utv	9.57 d	0.395 yza*	5.62 yza*
	S2	C	50.55 g.l	7.32 x	2.86 n.r	10.18 st	683.7 z	4.41 kl	11.6 u.x	6.89 st	0.366 a.d*	5.16 a.d*

		H	80.05 abc	13.14 d	5.94 f	19.09 e	1215.7 e	6.38 e	13.6 rst	8.49 ijk	0.405 x.a*	5.83 xyz
		S	90.45 a	18.23 c	7.54 d	25.77 c	1683.9 c	6.82 d	14.7 qrs	10.1 c	0.474 r.u	6.35 u.x
	S5	C	45.45 h.o	5.65 gh*	2.89 n.q	8.53 y	417.2 q*	3.03 r.w	15.2 o.r	6.29 w	0.445 uvw	6.45 uvw
		H	66.45 c.f	12.15 e	5.13 g	17.28 g	1191.4 f	4.76 ijk	20.1 jkl	8.4 i.l	0.579 lm	8.26 k.o
		S	85.7 a	11.51 g	3.39 j.o	14.9 h	1045.7 i	3.36 o.r	23.8 gh	8.17 l.p	0.66 gh	9.24 hi
	S12	C	38.55 j.q	3.52 m*	3.12 l.q	6.64 a*	298 t*	2.31 xyz	20.3 jk	5.18 yza*	0.569 lmn	8 m.q
		H	65.2 c.f	8.06 tu	2.04 uvw	10.1 st	779.7 u	3.22 p.t	24.3 g	8.08 m.q	0.668 g	8.82 ijk
		S	76.1 bcd	9.77 j	3.84 ij	13.6 ij	894.3 p	3.02 r.x	36.8 c	8.36 j.m	0.94 c	13.7 c
G5	S1	C	91.12 a	8.49 o	3.53 j.m	12.02 no	604.1 e*	3 r.x	12.2 t.w	12.35 a	0.356 bcd*	4.9 b.e*
		H	84.3 a	9.18 l	3.67 jk	12.85 kl	814.2 s	3.03 r.w	11 wxy	9.09 ef	0.353 cd*	4.75 cde*
		S	87.05 a	20.05 b	7.7 cd	27.75 b	495.8 k*	3.02 r.x	12.6 u.w	8.14 l.p	0.392 y.b*	5.47 y.b*
	S2	C	60.05 d.g	8.13 st	3.42 j.n	11.55 op	976.8 l	5.07 i	13.6 rst	11.7 b	0.415 w.z	5.99 wxy
		H	87.5 a	8.15 s	3.74 jk	11.89 no	830.6 r	3.19 q.u	12.5 t.w	8.32 k.n	0.405 x.a*	6.35 u.x
		S	74.65 bcd	11.63 f	6.54 e	18.16 f	932.9 m	3.53 n.q	15.4 opq	7.33 r	0.468 stu	6.5 uvw
	S5	C	61.6 d.g	6.95 z	5.07 g	12.02 no	987.4 k	5.71 gh	15 pqr	9.28 e	0.441 u.x	6.5 uvw
		H	80.9 abc	7.99 u	3.34 j.o	11.33 pq	899.3 o	4.8 ij	16.8 no	8.19 l.o	0.494 qrs	7.64 p.s
		S	75.95 bcd	10.08 i	3.91 ij	13.99 i	1066.8 h	5.98 fg	23.4 gh	6.52 uvw	0.628 j	9.49 h
	S12	C	56.5 fgh	6.33 c*	2.05 uvw	8.38 y	997.3 j	6.23 ef	18.6 lm	7.91 pq	0.54 npo	7.89 n.q
		H	60.35 d.g	7.78 w	3.02 m.q	10.8 r	996.1 j	4.1 lm	28.9 e	7.57 r	0.75 e	11.5 e
		S	45.72 h.o	6.46 b*	2.24 s.w	8.69 xy	1818.8 b	7.92 c	33.5 d	6.32 vw	0.86 d	13.1 d

Means followed by the same letter in each column are not significantly different according to Duncan test at 5 % level
Genotypes (G1: Shokofa; G2: Sina; G3: Paya; G4: Turbata; G5: Aria)
C: control (non-priming); H: Hyrdopriming; S: zinc-sulphate

Correlation coefficients

As shown in Table 5, GP was significantly and positively correlated with RG, seedling growth parameters, photosynthetic pigment contents, and protein content while negatively correlated with MGT, UG, SOD activity, proline, and sugar contents. The correlation results illustrated that the SOD activity and proline and sugar contents were negatively and significantly correlated with the pigment contents.

Table 5. Correlation coefficients among germination indices and physiological characteristics of sugar beet genotypes under salinity stress and priming treatments

	GP	MGT	GR	UG	RL	PL	SL	SVI	RWC	Chl a	Chl b	Total chl	Caro	Antho	SOD	Protein	Proline
MGT	-0.90**																
GR	0.47**	-0.59**															
UG	-0.91**	0.86**	-0.36**														
RL	0.50**	-0.40**	0.16*	0.42**													
PL	0.43**	-0.41**	0.37**	0.44*	0.78**												
SL	0.50**	-0.43**	0.36**	0.47**	0.96**	0.92**											
SVI	0.43**	0.44**	0.33**	0.30**	0.91**	0.93**	0.97**										
RWC	0.11ns	-0.16ns	0.14ns	0.13ns	0.04ns	0.09ns	0.06ns	0.08ns									
Chl a	0.71**	-0.65**	0.35**	0.69**	0.35**	0.35**	0.37**	0.34**	0.19ns								
Chl b	0.35**	-0.34**	0.21*	0.30*	0.25*	0.22*	0.25*	0.24*	0.08	0.49**							
Total chl	0.64**	-0.60**	0.34**	0.56**	0.36**	0.35**	0.37**	0.34**	0.21*	0.91**	0.81**						
Caro	0.53**	-0.52**	0.30**	0.40**	0.20ns	0.23*	0.22*	0.21*	0.07	0.69**	0.45**	0.68**					
Antho	0.04ns	0.12ns	0.05ns	0.01ns	-0.01ns	0.11ns	0.03ns	0.08ns	0.01ns	0.03ns	0.07ns	0.06ns	0.1ns				
SOD	-0.28*	0.34**	-0.60**	0.20ns	-0.14ns	-0.21*	-0.18ns	-0.18ns	-0.12ns	-0.20ns	-0.19ns	-0.21*	-0.20ns	0.10ns			
Protein	0.50**	-0.54**	0.38**	0.12ns	0.40**	0.41**	0.43**	0.42**	0.37**	0.58**	0.37**	0.56**	0.54**	0.03ns	-0.40**		
Proline	-0.28*	0.34**	-0.60**	0.22*	-0.12ns	-0.19ns	-0.16ns	-0.16ns	-0.13ns	-0.2ns	-0.2ns	-0.22*	-0.1ns	0.09ns	0.99*	-0.34*	
Sugar	-0.27*	0.33**	-0.60**	0.22*	-0.12ns	-0.19ns	-0.15ns	-0.16ns	-0.13	-0.2ns	-0.2ns	-0.22*	-0.1ns	0.1ns	0.99**	-0.34*	0.99**

ns: non-significant; * and **: significant at 5 and 1% probability levels

Discussion

The aims of this study were to (i) characterize the germination and physiological response to different salinity stress, hydropriming and osmopriming ($ZnSO_4$) and (ii) investigate whether hydropriming and osmopriming induce tolerance to salinity in seeds of sugar beet genotypes. Results indicated that the salinity stress and priming significantly affected germination indices (GP, MGT, GR, SL, PL, RL, and SVI) and physiological attributes (RWC, photosynthetic pigments, anthocyanin, SOD activity, protein, and proline contents. As shown in our results, germination indices including GP, GR, RL, PL, SL, and SVI decreased and increased in sugar beet genotypes submitted to salinity and priming treatment, respectively, compared to the control. Gheidary *et al.* (2017) reported that the salinity can also affect different stages of plant life such as germination, length of root, and stem because as salinity increases water absorption by the seed is decreased which shows the inhibitory effect of salinity on seed germination. More other researchers also reported the deleterious effects of salinity (NaCl) on seed germinability and seedling growth of many crops (Aghighi Shahverdi *et al.*, 2017). The reasons for reduced germination percentage can be attributed to primary water uptake reduction and the adverse effect of osmotic potential and ion toxicity on physiological processes of seed germination (Javadi *et al.*, 2016; Aghighi Shahverdi *et al.*, 2017). Notable point a small increase in NaCl concentration in comparison to the control (non-stress), caused increase seed germination percentage. Aghighi Shahverdi *et al.* (2017) founded the reason for this may be due to the stimulatory effect of sodium chloride, which has the effect of stimulating out of root in seed and increases the germination percentage at low concentration of salt. In this regard, reported that seed priming more efficient to improve the performance of cucumber seedling in bio-saline water (Matias *et al.*, 2015).

Seed priming has proved to be an effective method in imparting stress tolerance to plants using natural and/or synthetic compounds to treat the seeds before germination (Moreno *et al.*, 2018). Different priming techniques have been used to enhance salinity tolerance in several species and several priming agents have proved to be effective. Therefore, no standard methods to treat seeds or target osmotic or ionic effects of salt stress are clearly defined. For example, improved germination parameters in salinity stress resulted from osmopriming seeds with solutions of low water potential for Amaranthus, while for quinoa, this effect was achieved from hydropriming and osmopriming seeds with solutions of high-water potential (Moreno *et al.*, 2018). In the present study, hydropriming had the highest germination indices among different seed priming treatments. Seed hydropriming and osmopriming caused significant improvements in GU, reflected in high GP, high GR and reduced MGT under salinity. The superiority of hydropriming as a treatment in increasing plant germination indices compared to other treatments may be due to the effect of the absorption of more water by seeds compared to other treatments, they reached the highest percentage of germination as was reported by Gheidary *et al.* (2017). Similar results were reported by Kaur *et al.* (2002) who found applying hydropriming on seeds increases RL and stem the growth of pea plant. It was reported that $ZnSO_4$ seed priming treatments may be useful tools due to their positive effects on GR and GP of lentil. Moreno *et al.* (2018) reported that the primed tolerance to moderate salinity was achieved for *Chenopodium quinoa* and *Amaranthus caudatus* species, the salinity threshold for germination to occur was slightly broadened. Finally, seed priming has two beneficial effects, firstly caused will increase germination indices such as GP, GR, seedling growth parameters, and SVI and secondly, moderate the adverse effects of salinity stress due to NaCl, that between priming treatments, hydropriming had more positive effects on germination indices.

Seed priming has been suggested as one of the most useful physiological approaches to adapt glycophyte species to saline conditions. Pavia *et al.* (2019) concluded that the Zn treatments lessened the non-regulated energy dissipation caused by environmental stress, protected the plants against irreversible damages to the photosynthetic apparatus and enabled a better recovery of wheat plants after stress relief. The well-known effect from priming regarding faster, higher, and uniform germination was observed under optimal and suboptimal salinity conditions, which represent an overall improvement in seed vigor (Moreno *et al.*, 2018). Aghighi Shahverdi *et al.* (2017) reported that the priming enhanced seed performances are related to the repair and the

build-up of nucleic acid, enhanced synthesis of protein, repair of membranes and improves the antioxidant system. Mahmood *et al.* (2019) illustrated that increased levels of stress caused a decrease in GP, GR, and SVI of all chickpea cultivars while $ZnSO_4$ seed priming treatment enhances the values of all these attributes under all drought levels. Increasing SVI as a result of priming with $ZnSO_4$ or distilled water can result in a significant increase in SL and GP (the two parameters involved in the calculation of SVI), that maybe are because of the role of these treatments in cell division and cell elongation or cell division and growth meristematic (Noor-Un-Nisa Memon *et al.*, 2013; Aghighi Shahverdi *et al.*, 2017).

This study revealed that seedling growth from $ZnSO_4$ primed seeds showed an increase in values of all germination attributes. According to previous research, it has been reported that in wheat plants Zn plays an effective role in the production of increased dry weight (Mahmood *et al.*, 2019). While having comparison with results provided on seed priming effect on growth parameters of maize, it has been revealed that $ZnSO_4$ seed priming treatment influenced positively shoot and root length of maize and pulses (Ambika and Balakrishnan, 2015). An increased root length of plants grown from seeds primed with $ZnSO_4$ as compared to unprimed seeds could be the result of extensibility in the cell wall of the embryo (Mahmood *et al.*, 2019). Also, the same researcher reported that seed priming with $ZnSO_4$ solution decreased the resistance mechanism of endosperm envelope against growth permitting turgor threshold for germination as compared to non-primed seeds resulting increased shoot and root length (Mahmood *et al.*, 2019).

In the present study, salinity caused reduces of chlorophyll contents (Table 3). While seed priming led to an increase in the mean of photosynthetic pigments under control and salinity stress conditions (Table 4). It seems that one of the reasons for the good tolerance of sugar beet, in the germination stage, to salinity stress is to moderate the negative effects of stress using priming treatments. Increasing the amount of photosynthetic pigments thus seed priming is stated in different reports (Noor-Un-Nisa Memon *et al.*, 2013; Aghighi Shahverdi and Omidi 2015; Aghighi Shahverdi *et al.*, 2017). The reduction of photosynthesis pigment is one of the major consequences of salinity or drought stresses in plants. This decrease may be due to stomatal or non-stomata limitations (Pavia *et al.*, 2019). Stomatal closure is usually considered the determining factor in the reduction of CO2 assimilation and photosynthesis reduction during environmental stress such as salinity or drought (Abid *et al.*, 2016). Nonetheless, ¸ non-stomatal limitations may also occur at moderate and severe drought events (Brito *et al.*, 2018). In our study, reduction of photosynthesis pigment contents such as chlorophyll a, b, total as well as carotenoids were observed by increasing NaCl concentration (Table 3). Generally, Mosavikia *et al.* (2020) concluded that limited stomatal conductance, diminished activities of carbon fixation enzymes, reduced quantities of photosynthetic pigments, and destruction of photosynthetic apparatus are among the key factors limiting the process of photosynthesis under salinity stress conditions.

On the other hand, the results of the current experiment showed that all sugar beet genotypes primed with $ZnSO_4$ gave higher values of all chlorophyll pigment (chlorophyll a and total chlorophyll) as compared to unprimed seeds. This increase may be due to Zn which functions as a structural, functional, and catalytic component of enzymes, proteins and also a co-factor for normal growth and development of biosynthetic pigments (Samreen *et al.*, 2017). The results presented are the agreement with the findings of Mahmood *et al.* (2019). The absorption of excess energy in the photosynthetic apparatus may be the main cause of ROS in large concentrations it could be escaped by degrading the pigments responsible for their absorption (Mahmood *et al.*, 2019).

In the current study, the higher accumulation of carotenoid in 'Sina' sugar beet genotype cultivars under non-stress conditions could result in a positive effect on germination and seedling growth by affecting RWC and chlorophyll content as reported by Talebi *et al.* (2013). Carotenoids have a critical role as photoprotective compounds by quenching triplet and singlet oxygen derived from excess light energy, thus limiting membrane damage (Mahmood *et al.*, 2019). However, the more enhanced increase in chlorophyll and carotenoid content in 'Sina' sugar beet genotype raised from seeds primed with distilled water as compared to unprimed seeds under control conditions.

Enzymatic and non-enzymatic antioxidants response are defensive mechanisms to overcome oxidative stress (Demidchik, 2015). Proline and sugar, non-enzymatic antioxidants response, are known to act as an osmolyte/osmoprotectant agent under drought or salinity stress (Mosavikia *et al*., 2020). The osmolyte has an important role in scavenging free radicals, osmotic pressure adjustment, stabilizing sub-cellular structures, and storing carbon and nitrogen (Gorzi *et al*., 2017). The results of the current study concluded that a significant increase in the free proline and sugar contents as well as the activity of SOD enzyme under salt stress especially in 12 dS/m NaCl level. Salinity stress in this experiment caused reduces of photosynthetic pigment contents and protein content while increased of SOD activity, proline and sugar contents. However priming treatment, by increases means of these traits causing moderated the adverse effect of salinity stress on biochemical characteristics of the sugar beet seedling, so that reduction of the amount of chlorophyll was minor in terms of priming treatments in high salinity levels (Table 3). Proline acts as a signaling/regulatory molecule and in the case of salt stress will be able to enhance the resistance of the plant to salinity (Javadi *et al*., 2016). A positive relationship between proline accumulation and the antioxidant level in the plant that the ROS caused by stress increases and leads to the accumulation of free proline in the plant reported by Zhang *et al*. (2006) which is in line with the findings of the correlation between traits in the present study. Aghighi Shahverdi *et al*. (2017) reported that the seed priming by increasing the activity of the enzyme proline 5-carboxylase synthase, as a key enzyme in the synthesis of proline increased synthesis of these secondary metabolites in the high salinity stress conditions.

Wang *et al*. (2013) reported that the antioxidant activity of SOD increased under salinity stress that the answer is crop tolerance to salinity conditions. Similar results were obtained in the present study. Salinity increases the activity of antioxidant enzymes were all priming treatments. Ambika and Balakrishnan (2015) reported that seed priming treatment caused an increase in the activity of ROS scavenging enzymes to enhance plant strength and viability. Metabolic activities are expected to increase remarkably in seeds following their priming which may lead to the higher activity of ROS as secondary products of mitochondrial respiration. There is strong evidence that antioxidant enzymes and free radicals are abundantly produced within seeds during germination, and are cooperatively tackled by enzymatic reactions (Noor-Un-Nisa Memon *et al*., 2013; Aghighi Shahverdi *et al*., 2017). The enhanced expression and activity of antioxidant enzymes as recorded in our studies have been proposed as part of seed strategy to cope with ROS produced during seed priming (Chiu *et al*., 2005). Hydropriming is the simplest approach to hydrate seeds and minimize the use of chemicals. However, if the seeds are not accurately hydrated, the hydration rate cannot be exactly controlled. It was observed that hydropriming practically ensured rapid and uniform germination accompanied by low abnormal seedling percentage.

Conclusions

Salinity (NaCl) stress harmed seed germination and seedling growth of sugar beet genotypes. While seed priming (especially hydropriming) under high salinity level was caused by highly improved germination parameters and reduced germination time to promote early seed germination and harmonized growth. Also, seed priming with $ZnSO_4$ and distilled water has improved germination indices and seedling physiological parameters such as photosynthetic pigments, proline, sugar, anthocyanin, and the activity of antioxidant enzyme. Generally, the most pronounced effect of seed priming was related to hydropriming and 'Aria' sugar beet genotype. The finding of this study leads to the conclusion that seed priming with $ZnSO_4$ and distilled water by improving physiological mechanisms such as synthesis of photosynthetic pigments, antioxidant enzyme activity, proline amino acid, soluble sugar, carotenoids and anthocyanin moderated the negative effects of high salinity stress and increased GP, GR, and seedling growth parameters such as RL, PL, SL, and SVI. Also, genotypes 'Paya' and 'Aria' showed high tolerance and genotype 'Shokofa' showed low tolerance to salinity stress at the germination stage.

Authors' Contributions

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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