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Antioxidant and Biochemical Alterations in Sea Beet (*Beta vulgaris* subsp. *maritime* (L.) Arcang.) and Sugar Beet (*Beta vulgaris* L.) Exposed to Salt Stress

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ABSTRACT

A pot experiment was conducted using a randomized complete block design with a factorial arrangement and 3 replications. The treatments consisted of genotype (15 sea beet genotypes and two cultivated beets of one susceptible and one tolerant to stress), and salinity (four NaCl concentrations including 0, 50, 100, 200 and 400 mM) on the 35-days-old beet seedlings for 55 days. The following parameters and traits were recorded: activities of superoxide dismutase, catalase and glutathione peroxidase, malone dialdehyde, di-tyrosine, di-hydroxy guanosine, proline, and total soluble sugars. The results showed a highly significant effect of salinity treatments on the traits studied. Moreover, with increasing stress intensity, the effects of salinity on these traits increased. At least five genotypes of sea beet were clearly superior than the cultivated beet for producing a lower constitutive level of MDA, DT and 8-OH-dG destruction biomarkers, but higher activities of SOD, CAT and GPX enzymes, and proline, total soluble sugars, and glycine betaine contents were recorded under salt stress conditions. These results strongly suggest that the wild salt-tolerant sea beet possess distinct advantages over the sugar beet counterparts for protection mechanism against oxidative damage by maintaining a higher inherited and induced activity of enzymatic/ non-enzymatic antioxidant activities. Therefore, it can be concluded that under salt stress, sea beet has a significant potential for the physiological/biochemical variation in salinity tolerance, which can be exploited for improving salinity tolerance in sugar beet cultivars.

Keywords: Antioxidants, Biomarkers, Oxidative stress, Salinity tolerance.

INTRODUCTION

Cultivated beet (*Beta vulgaris* ssp. *vulgaris*), that is cultivated nowadays in many parts of the world, originated and is still affected by continuous introgression from its wild ancestor sea beet (*Beta vulgaris* ssp. *maritima*) (Biancardi *et al*., 2005; Francis, 2007). Different parts of Iran, especially western parts of the country, are among the natural habitats of this plant (Biancardi *et al*., 2012). The knowledge of the genetic diversity and relationships within and among crop species and their wild relatives is also essential for the efficient use of plant genetic resource, in order to introgress desirable traits into cultivated species and improve crop quantity and quality (Arzani and Ashraf, 2016).

Salinity is one of the most important abiotic stresses that annually causes huge loss and damage to crop plants worldwide (Arzani and Ashraf, 2016; Akrami *et al*., 2018). Excess Na^+ and Cl^- ions in saline soil usually retard crop growth and development through osmotic stress, ionic toxicity, and

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oxidative damage (Munns and Tester, 2008; Arzani and Ashraf, 2016). Plants vary in their ability to cope with salinity, being capable of growth in a wide diversity of habitats ranging from non-saline environments to salt marshes. Differences in salt tolerance exist not only between species but also amongst genotypes of certain species (Dadkhah and Grrifits, 2006).

Some plants stimulate oxidative stress due to the destruction caused by salinity and drought, resulting in the production and accumulation of various toxic oxygen such as superoxide ($O_2^{\bullet-}$), Hydroxyl radicals (•OH), singlet Oxygen ($^1O_2$), and Hydrogen peroxide (H_2O_2). The ROS are produced during normal aerobic metabolism by the interaction between O_2 and electrons leaking from electron transport chains in the chloroplasts and mitochondria (Halliwell and Gutteridge, 1999). ROSs are highly harmful to organisms at high concentrations. ROSs at low/moderate concentration act as a messenger to signal intracellular messages they transmit many responses in plant cells. When ROSs content is more than the defense mechanisms, a cell is normally said to be in a certain state, which is called oxidative stress (Mozaffari and Fathollahy, 2020). The enhanced production of ROS during environmental stresses threatens the cells because it causes oxidation of proteins, peroxidation of lipids, nucleic acids destruction, enzyme inactivation, as well as the activation of PCD, that is, programmed cell death. They ultimately lead to the death of the cells (Srivastava and Dubey, 2011; Yao *et al*., 2012; Akrami and Arzani, 2019; Mozaffari and Fathollahy, 2020).

One of the products of oxidative degradation of proteins is Di-Tyrosine (DT), which is used as a biomarker for identifying plant species susceptible or resistant to biotic and abiotic stresses. Dityrosine is enhanced directly with increased oxidative stress.

When oxidative stress occurs, peroxidation of unsaturated fatty acids and lipids increases the free radical attack of lipids, resulting in Malondialdehyde (MDA) production (Hossain *et al*., 2017; Mozaffari and Fathollahy, 2020). MDA is one of the final products of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Mozaffari and Fathollahy, 2020). Two common sites of ROS attack on the phospholipid molecules are the unsaturated (double) bond between two carbon atoms and the ester linkage between glycerol and the fatty acid. ROS attack the polyunsaturated fatty acids, which are normally present in membrane phospholipids. It has been demonstrated that salt treatment increases lipid peroxidation or induces oxidative stress in plant tissues (Hernandez *et al*., 1994). Lipid peroxidation requires active O_2 uptake and involves the production of superoxide radical ($O_2^{\bullet-}$) (Fridovic, 1986). The other highly reactive chemical species include singlet Oxygen ($^1O_2$), Hydroxyl free radical (•OH) and H_2O_2 all of which initiate lipid peroxidation (Fridovic, 1986; Hossain *et al*., 2017).

The ROSs and their produce agents cause many damages, including degradation, deformation, oxidation of deoxyribose, DNA fractures, mutations, and other lethal genetic effects in the DNA molecule, and ROS are a major source of DNA damage (Imlay and Linn, 1988; Mozaffari and Fathollahy, 2020). The cell organelles such as nuclear, mitochondrial, and chloroplastic DNA are especially prone to oxidative damages caused by ROSs. Exposure to environmental stresses such as salinity leads to enhanced DNA degradation in plants (Liu *et al*., 2000). •OH addition to double bonds occurs when DNA bases are attacked, while hydrogen abstraction from deoxyribose causes sugar damage (Dizdaroglu, 1993). The •OH is known to react with all purine and pyrimidine bases and the deoxyribose backbone (Halliwell and Gutteridge, 1999). Among the various products from the DNA bases which are generated by Hydroxyl radical (•OH), one can name saturated products, urea, C-8 hydroxylation of guanine to form 8-oxo-7,8 dehydro-2′-deoxyguanosine, not to mention

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hydroxymethyl urea and adenine ring-opened (Tsuboi *et al*., 1998). 8-OH-DeHydroxyguanine (8-OH-dG) is the most observed product.

Plants possess complex anti-oxidative defense system comprising of non-enzymatic and enzymatic components to scavenge ROS. Both of these defense systems are vital for the survival and activity of aerobic organisms. Antioxidant enzymes are one of the important defense systems of organisms in coping with oxidative stress. Plants scavenge ROSs by inducing activity of various Antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPX). If there is an efficient anti-oxidative system, which consists of the enzymatic antioxidants and non-enzymatic detoxification or removal of excess ROS can be achieved (Noctor and Foyer, 1998). Generally, there is a direct correlation between increasing tolerance of plants to various environmental stresses and maintaining a high antioxidant capacity to scavenge the toxic Oxygen Species (ROSs) (Chen *et al*., 2010; Mozaffari and Fathollahy, 2020).

The non-enzymatic antioxidant systems such as solvent in water (ascorbic acid and glutathione) and solvent in fat (α-tocopherol, β-carotene, Phenolics, Flavonoids), proline and glycine betaine within cell can play an important role in reducing the Active Oxygen Species (AOS) caused by oxidative stress in the plant and modifying the destructive effects of salt stress (Agarwal and Pandey, 2004).

To date, several studies have been done to evaluate the physiological responses of sugar beet cultivars under conditions of salinity (Abbasi *et al*. 2015; Hossain *et al*., 2017; Wu *et al*. 2016; Wang *et al*., 2017; Wu *et al*. 2019). Recently, protection mechanisms of sugar beet and other crop plants against salt stress have been elucidated by the molecular and genetic investigations (Abbasi *et al*. 2015, Akrami and Arzani, 2019; Sahashi *et al*., 2019) However, there is limited information on the physiological behaviors of sea beet against salinity (Biancardi *et al*., 2012).

Therefore, the main purpose of our research was to investigate enzymatic/ non-enzymatic antioxidant defense systems and biochemical destruction biomarkers in Iranian native sea beet and sugar beet under salt stress.

MATERIALS AND METHODS

To evaluate antioxidant and biochemical alterations in sea beet (*Beta maritima*) and sugar beet (*Beta vulgaris*) exposed to salt stress, an experiment was conducted in a Completely Randomized Design (CRD) with the factorial arrangement and 3 replications. The treatments consisted of a genotype factor including 15 sea beet samples from Khuzestan, Ilam, Kermanshah, Kurdistan, and Azerbaijan, and two agronomic cultivars including susceptible (22393-196 cultivar) and resistant (7233-P.29×MSc2 cultivar) to salt stress (Characteristics and Geographical origin of wild and cultivated beets studied in the experiment are shown in Table 1). Also, treatments included salt concentration factor consisting of four levels: 0, 50, 100, 200, and 400 mM NaCl. Experimental traits included levels of activity of Superoxide Dismutase enzymes (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX), Malone Di-Aldehyde (MDA), Di-Tyrosine (DT), di-Hydroxy Guanosine (8-OH-dG) biomarkers content, proline, total soluble sugars, and glycine betaine content. Sowing of wild beets genotypes and sugar beet cultivars was carried out in vented plastic pots (in order to drain the pots) with 28 ×21.5×30 cm size. After soil preparation, soil testing and fertilization (e.g. urea, ammonium phosphate, and potassium sulfate), 10 seeds were planted in each pot. At the fourth leaf stage, the thinning was carried out and the number of seedlings decreased to five plants per pot. Salinity treatment was applied 35 days after sowing in combination with

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Table 1. Characteristics and geographical origin of wild and cultivated beets studied in the experiment.

Genotype or Variety	Type	Ploidy and Germ	Stress Level	Geographical coordinate (E: East and N: North)
7233-P.29×MSc2	Cultivar	Triploid, Monogerm	Salt Tolerance	Iranian Sugar Beet Seed Institute (SBSI)
22393-196	Cultivar	Diploid, Monogerm	Salt Sensitive	Iranian Sugar Beet Seed Institute (SBSI)
Khuzestan 1	Wild	Multigerm	Under testing	E“07’24°48 N“57’22°32
Khuzestan 2	Wild	Multigerm	Under testing	E“09’40°48 N“13’19°31
Khuzestan 3	Wild	Multigerm	Under testing	E“15’18°48 N“21’20°30
Ilam 1	Wild	Multigerm	Under testing	E“22’25°46 N“15’38°33
Ilam 2	Wild	Multigerm	Under testing	E“04’16°47 N“39’41°32
Ilam 3	Wild	Multigerm	Under testing	E“11’25°47 N“33’59°32
Kermanshah 1	Wild	Multigerm	Under testing	E‘4°47 N‘18°34
Kermanshah 2	Wild	Multigerm	Under testing	E“45’34°45 N“56’30°34
Kermanshah 3	Wild	Multigerm	Under testing	E“55’57°47 N“15’30°34
Kurdistan 1	Wild	Multigerm	Under testing	E‘50°46 N‘20°35
Kurdistan 2	Wild	Multigerm	Under testing	N“22’52°35 E“10’36°47
Kurdistan 3	Wild	Multigerm	Under testing	N“37’31°35 E“35’10°46
Azerbaijan 1	Wild	Multigerm	Under testing	N“47’45°36 E“20’43°45
Azerbaijan 2	Wild	Multigerm	Under testing	N“19’33°37 E“21’04°45
Azerbaijan 3	Wild	Multigerm	Under testing	N“01’33°38 E“08’57°44

irrigation water and continued until 90 days after planting, at which time different traits were evaluated. To measure the antioxidant enzymes and biomarkers, the beet leaves were first sampled. Samples were stored at -80°C in a freezer immediately after harvesting. The samples were then frozen in nitrogen (liquid) and powdered in Chinese molds. A 0.1 g powder sample was homogenized with 1 mL of 100 mM potassium phosphate buffer with pH 8.0 containing 0.1 mg EDTA and polyvinylpyrrolidone on ice.

The extract from each sample was centrifuged at 1,400 rpm at a temperature of 4°C for 30 minutes using a Hettich centrifuge machine. The upper unpopulated solution (Supernatant) was harvested in sterile vials and used as enzymatic extracts to measure the activity of leaf antioxidant enzymes. All extraction steps were performed to measure the amount of enzyme activity on ice.

Enzyme Assay

Sairam *et al*. (2002) method was used to determine the activity of Superoxide Dismutase (SOD) and Catalase (CAT) enzymes. Also, Paglia and Valentine (1997) method was used to measure the Glutathione Peroxidase (GPX) enzyme activity.

Biomarker Assay

The amount of MDA, D-T, and 8-OH-dG biomarkers were measured by Madhava and Sresty (2000), Orhanl *et al*. (2004), and Bogdanov *et al*. (1999), respectively. The total soluble sugars, proline, and glycine betaine contents were determined according to Bates *et al*. (1973), Shlegil (1986), and Grattan and Grieve (1992), respectively.

Statistical Analysis

Statistical analyses were undertaken using the SPSS Statistics v. 25.0 (IBM Corp., USA), while the graphics were prepared using the package Excel v. 2016. The treatment mean values were compared by Duncan’s multiple range test at 0.05 level of probability.

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RESULTS

The results showed that the effects of salinity on SOD, CAT and GPX activities, MDA, DT, 8-OH-dG biomarkers, proline, glycine betaine, and total soluble sugars contents were highly significant ($P< 0.01$) (Table 2). These traits enhanced with increasing salt concentration. The activity of SOD, CAT, GPX enzymes, MDA, DT, 8-OH-dG biomarkers, proline and total soluble sugars contents in 400 mM NaCl treatment were 3.6, 12.6, 2.8, 1.68, 1.77, 2.24, 3.4 and 2.32 times higher than non-stress treatment, respectively (Table 3).

SOD, CAT, and GPX

There was a highly significant difference between the genotypes for all the traits ($P< 0.01$) (Table 2). Sea beet genotypes including Khouzestan-2 and 3, Kermanshah-2 with 133.93, 133.2 and 132.73 nmol H_2O_2 protein^{-1} min^{-1}, respectively, had the highest SOD activity (Table 4). Khouzestan-1, Ilam-1 and Azerbaijan-3 with the mean of 122.93, 120.93 and 119.8 nmol H_2O_2 protein^{-1} min^{-1}, respectively, were in the next rank (Table 4). In addition, SOD content in the salt-resistant and sensitive sugar beet cultivars were 110.33 and 53.67 nmol H_2O_2 protein^{-1} min^{-1}, respectively (Table 4).

The SOD activity in Kermanshah-2 and Khouzestan-1, 2 and 3, Ilam-1, Azerbaijan-3 genotypes in 100 and 400 mM NaCl were 2.58 and 5.43 times higher than the non-stress, respectively (Figure 1).

The highest CAT activity belonged to Khuzestan-2 and Kermanshah-2 with 341.5 and 331.7 nmol H_2O_2 protein^{-1} min^{-1}, respectively, and ranked the first. Also, Khuzestan-3, Ilam-1, and Khuzestan-1 with 329.8, 315.7 and 312.1 NanoMol H_2O_2 protein^{-1} min^{-1} were ranked next in a common statistical group (c) (Table 4). The CAT activity in salt tolerant and sensitive cultivar were 299.4 and 261.73 nmol H_2O_2 protein^{-1} min^{-1}, respectively (Table 4). The CAT activity in Khuzestan-1, 2, 3 and Ilam-1 in 400 mM NaCl treatment was increased 13.9, 13.3, 13.6 and 11.6 times compared with the non-stress treatment, respectively (Figure 1).

Khuzestan-3 and Kermanshah-2 sea beet genotypes had the highest activity of GPX with 28.58, 27.45 and 26.83 nmol H_2O_2 protein^{-1} min^{-1}, respectively (Table 4). In this regard, Kermanshah-2, Khuzestan-1, and Ilam-1 sea beet genotypes were assigned the following ranks with 26.83, 26.59 and 26.42 NanoMol H_2O_2 protein^{-1} min^{-1}, respectively (Table 3). Also, data analysis showed that in these genotypes, GPX enzyme activities in the 50, 200 and 400 mM treatments were approximately 2, 3 and 4 times higher than non-stress, respectively (Figure 1).

MDA, Di-Tyrosine (DT), and 8-OH-dG

Results showed that Ilam-1 and Khuzestan-2 have the lowest MDA biomarker with 34.77 and 33.37 μMol g^{-1} protein, respectively (Table 4). MDA content in Khuzestan-1 and 3 and Kermanshah-2 was 36.47, 36.71 and 36.75, and μMol g^{-1} protein, respectively (Table 4). MDA of wild beet genotypes was lower than the sensitive and resistant sugar beet cultivars (Table 4).

In general, MDA increased in all genotypes by increasing salt concentration (Figure 2). The highest content of biomarkers belonged to sensitive sugar beet cultivar, Kurdistan-3 and Kermanshah-3 with 71.60, 69.60, and 68.53 μMol g^{-1} protein, respectively (Figure 2).

Genotypes Ilam-1 and Kermanshah-2 had the lowest di-tyrosine with 11.75 and 11.64 μMol g^{-1} protein, respectively (Table 4). Khouzestan-3, 1 and 2 had the lowest di-tyrosine with 12.36, 12.44 and 12.42 μMol g^{-1} protein, respectively (Table 4). The other genotypes (except Kermanshah-3, Kordestan-3, and sensitive sugar beet cultivar), were like salt tolerant cultivar (Table 4). Also, di-tyrosine content of these genotypes under 100 and 400 mM NaCl

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Table 2. Mean of squares effect of Genotype and Salt stress on experimental traits.

Source Of Variance (SOV)	df	Anti-oxidative enzymes (Nano Mol H_2O_2 protein^{-1} min^{-1})			Biochemical destroyed biomarkers (µMol g^{-1} protein)			Osmo-regulators (mg g^{-1} FW)		
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione Peroxidase (GPX)	Malon Dialdehyde (MDA)	Di-Tyrosine (DT)	Dehydroxi Guanosine (8-OH-dG)	Proline	Total sugar soluble	Glycine betaine
Genotype (G)	16	7335.1**	7182.6**	292.7**	444.8**	50.67**	21.18**	59.03**	552.83**	29.22**
Salt stress (S)	4	156957.4**	2583922.3**	3709.6**	3868.7**	517.7**	333.7**	1335.2**	15851.7**	1166.7**
G×S	64	1394.9**	906.9**	33.7**	47.2**	5.54**	3.19**	8.78**	86.46**	3.82**
Exp Error	170	30.6	207.8	3.2	4.06	0.66	0.16	0.68	15.04	0.16
CV (%)	-	5.28	4.83	7.84	4.79	5.67	4.85	7.35	7.00	12.56

* and **: Significant at the 5 and 1% levels of probability, respectively.

Table 3. Comparison of different levels of salinity for biochemical traits of the examined beets.[a]

Salt Concentration (mM NaCl)	Anti-oxidative enzymes (Nano Mol H_2O_2 protein^{-1} min^{-1})			Biochemical destroyed biomarkers (µMol g^{-1} protein)			Osmo-Regulators (mg g^{-1} FW)		
	Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione Peroxidase (GPX)	Malondialdehyde (MDA)	Di-Tyrosine (DT)	Dehydroxi Guanosine (8-OH-dG)	Proline	Total Sugar soluble	Glycine betaine
0	51.7^{e}	50.0^{e}	12.07^{e}	31.49^{e}	10.41^{e}	5.31^{e}	4.93^{e}	33.6^{e}	1.72^{e}
50	67.3^{d}	155.6^{d}	17.24^{d}	36.2^{d}	12.35^{d}	6.86^{d}	6.98^{d}	43.75^{d}	4.42^{d}
100	98.2^{c}	262.2^{c}	23.21^{c}	41.23^{c}	13.49^{c}	7.87^{c}	12.49^{c}	55.06^{c}	7.98^{c}
200	140.6^{b}	394.2^{b}	27.69^{b}	48.0^{b}	16.27^{b}	9.74^{b}	15.04^{b}	66.61^{b}	10.86^{b}
400	187.6^{a}	630.2^{a}	33.79^{a}	53.09^{a}	18.49^{a}	11.89^{a}	16.78^{a}	77.91^{a}	13.61^{a}

[a] Means with same letters showed no significant difference (P<= 0.05).

Table 4. Mean comparison of beet genotypes on antioxidant enzymes and biomarkers activity, proline, soluble sugars and glycine betaine contents.[a]

Beet genotypes	SOD (NanoMol H_2O_2 protein^{-1} min^{-1})	CAT (NanoMol H_2O_2 protein^{-1} min^{-1})	GPX (NanoMol H_2O_2 protein^{-1} min^{-1})	MDA (µMol g^{-1} protein)	DT (µMol g^{-1} protein)	8-OH-dG (µMol g^{-1} protein)	Proline (mg g^{-1} FW)	Total soluble sugars (mg g^{-1} FW)	Glycine betaine (mg g^{-1}Dw)
Azerbayjan 1	111.07 cd	293.3 d	23.73 cd	42.05 de	14.15 d	8.49 ef	11.43 ef	51.06 ef	7.18 f
Azerbayjan 2	110.93 cd	296.6 d	22.71 def	41.81 e	14.67 cd	8.45 ef	11.47 e	55.42 d	7.65 e
Azerbayjan 3	119.80 b	291.5 d	22.05 ef	41.68 e	14.26 d	8.89 d	10.67 h	50.59 fg	7.85 de
Sensitive Sugar beet	53.67 g	261.7 f	15.25 g	52.56 a	17.45 ab	10.19 b	7.67 i	44.23 h	4.38 h
Ilam 1	120.93 b	315.7 c	26.42 b	34.77 g	11.75 f	7.00 h	13.64 ab	65.52 a	9.27 a
Ilam 2	108.27 d	276.1 e	21.39 f	44.11 c	14.27 d	8.52 ef	11.05 e-h	55.94 d	8.11 d
Ilam 3	111.20 cd	291.7 d	24.47 c	42.05 de	14.96 c	8.28 f	10.97 fgh	53.67 de	7.99 d
Kermanshah 1	109.73 cd	292.2 d	22.79 de	41.87 e	14.20 d	8.63 e	10.77 gh	55.05 d	8.48 c
Kermanshah 2	132.73 a	331.7 ab	27.45 ab	36.75 f	11.64 f	7.37 g	13.73 ab	62.48 bc	9.22 a
Kermanshah 3	61.67 f	272.3 e	13.79 h	50.80 b	17.71 a	9.81 c	7.81 i	48.00 g	5.52 g
Khuzestan 1	122.93 b	312.1 c	26.59 b	36.47 f	12.44 e	6.65 i	13.28 bc	64.51 ab	8.92 b
Khuzestan 2	133.93 a	341.5 a	26.83 b	33.37 g	12.42 e	6.74 i	13.77 a	62.13 bc	9.01 ab
Khuzestan 3	133.20 a	329.8 b	28.58 a	36.71 f	12.36 e	6.75 i	13.03 c	60.25 c	8.77 b
Kordestan 1	113.13 c	289.7 d	23.85 cd	43.27 cde	14.18 d	8.27 f	11.22 efg	54.46 d	7.33 f
Kordestan 2	108.07 d	301.1 d	24.08 cd	43.55 cd	14.79 cd	8.53 ef	10.69 h	54.10 d	8.01 d
Kordestan 3	92.73 e	277.1 e	14.66 gh	50.00 b	16.97 b	10.76 a	7.93 i	47.85 g	5.51 g
Resistance sugar beet	110.33 cd	299.4 d	22.99 de	42.32 de	14.72 cd	8.40 ef	12.07 d	56.32 d	7.99 d

[a] Means with the same letters showed no significant difference (P<= 0.05).

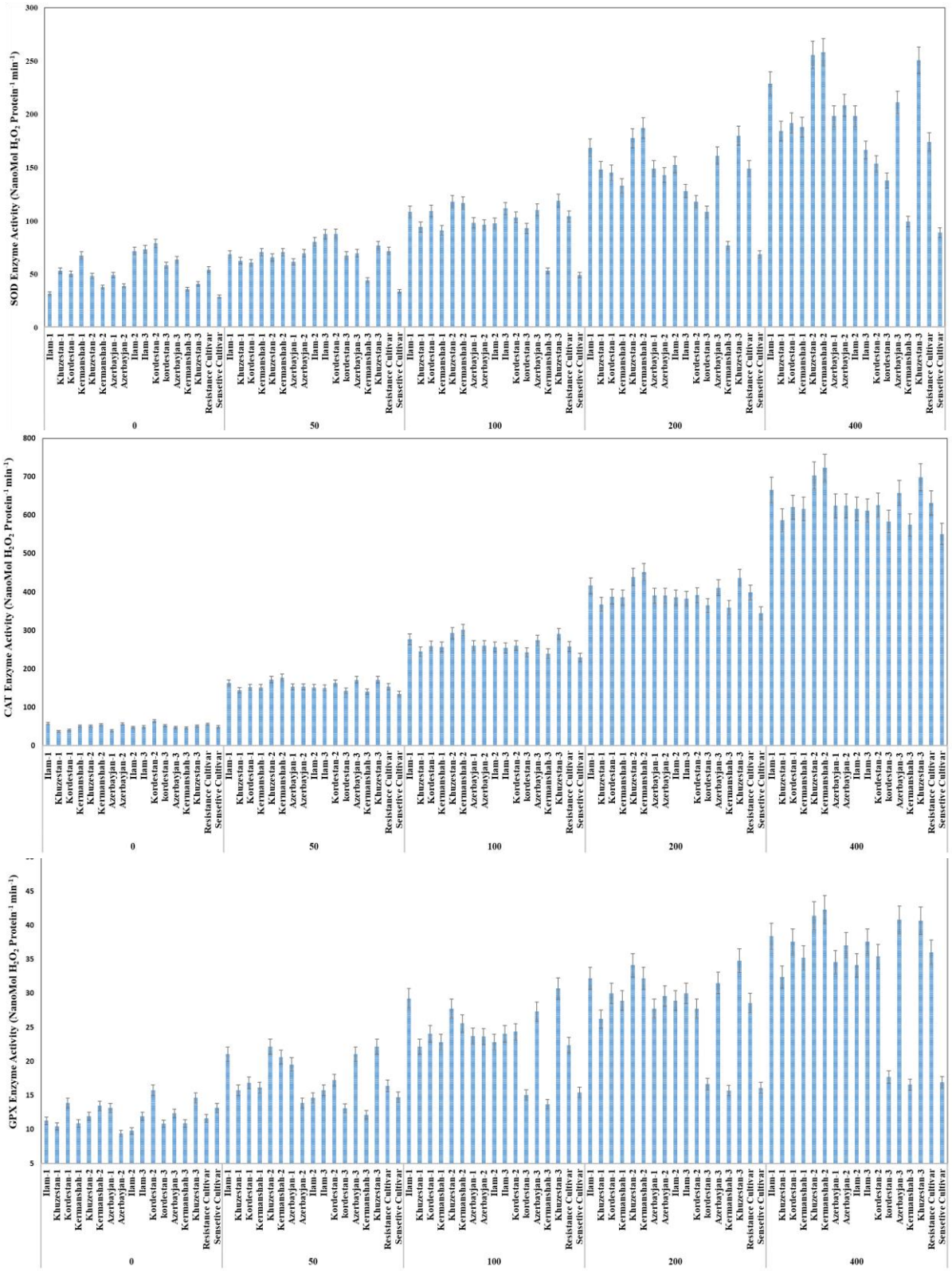

Figure 1. Interaction effect of beet genotypes and salt concentration (0, 50, 100, 200 and 400 mMol NaCl) o SOD, CAT and GPX enzyme activity.

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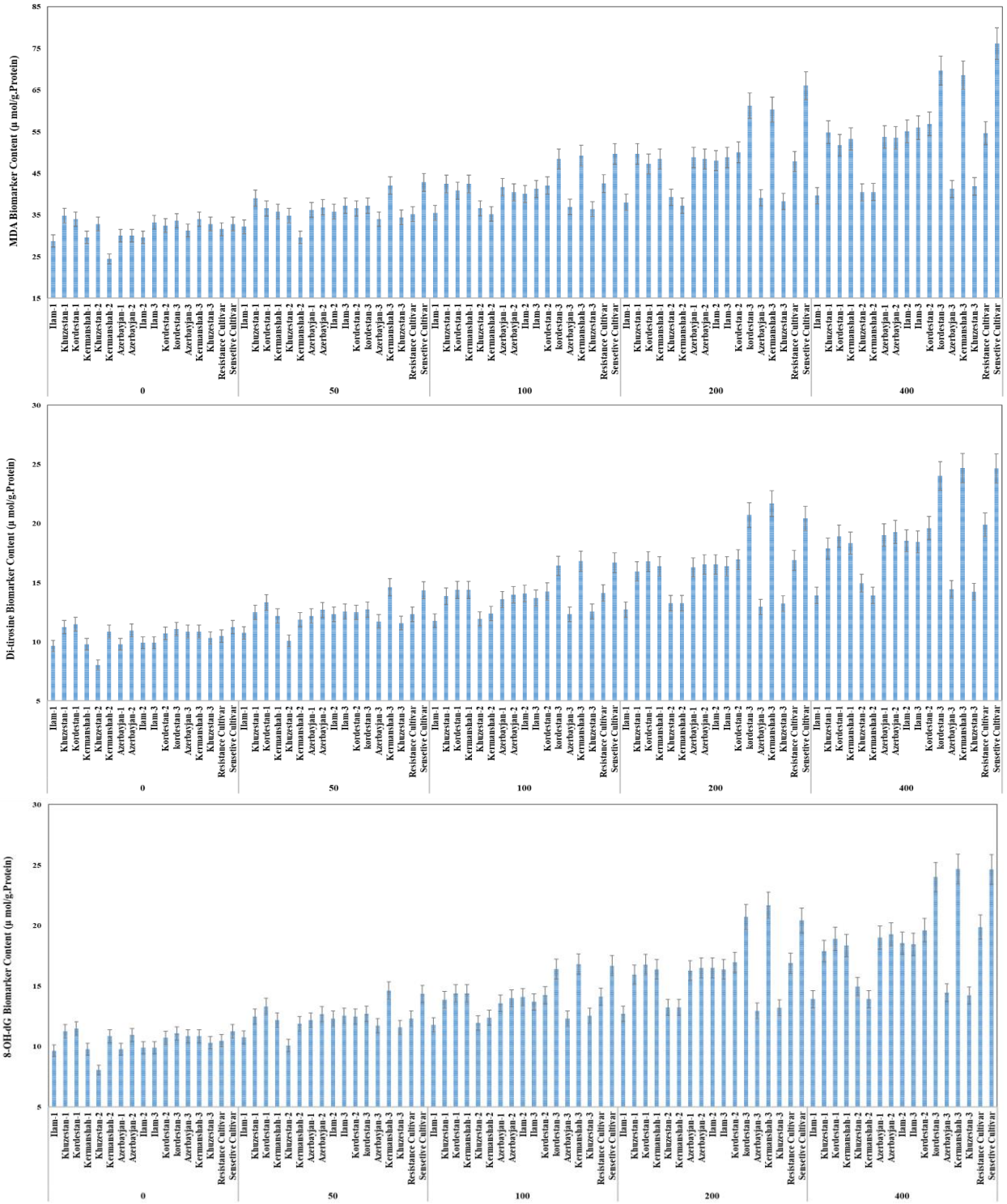

Figure 2. Interaction effect of beet genotypes and salt concentration (0, 50, 100, 200 and 400 mMol NaCl) on MDA, di-tyrosine, and 8-OH-dG biomarker content.

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treatments was 1.23 and 1.44 times less than salt sensitive cultivar, respectively (Figure 2).

Khuzestan-1, 2 and 3 had lowest of 8-OH-dG with 6.75, 6.65, and 6.74 µMol g^{-1} protein, respectively (Table 4). The 8-OH-dG in these genotypes increased by 1.39 and 1.77 in treatments of 100 and 400 mM compared to non-stress conditions, respectively (Figure 2). Also, 8-OH-dG content most sea beet except Kurdistan-3 with 10.76 µMol/g protein, Kermanshah-3 with 9.81 µMol/g protein and Azerbaijan-3 with 8.89 µMol/g protein was like resistant sugar beet cultivar (Table 4).

Proline, Total Soluble Sugars, and Glycine Betaine

Results showed that Khuzestan-2, Kermanshah-2, and Ilam-1 had the highest proline contents with 13.77, 13.73 and 13.64 mg g^{-1} FW, respectively (Table 4). In these genotypes, the proline content in 50, 100, 200 and 400 mM NaCl treatment was 1.37, 2.55, 3.25 and 3.69 times more than non-stress treatment, respectively (Figure 3). Khuzestan-1 and 3 with 13.28 and 13.03 mg g^{-1} FW were in the next rank (Table 4). Proline content in salt-resistant sugar beet was 12.07 mg g^{-1} FW (Table 4). The proline content in resistant cultivar under 100 and 400 mM NaCl treatments was, respectively, 2.53 and 3.4 times higher than the sensitive cultivar (Figure 3).

Genotypes Ilam-1 and Khuzestan-1 had the highest Total Soluble Sugars (TSS) with 65.52 and 64.51 mg g^{-1} FW, respectively (Table 4). Kermanshah-2 and Khuzestan-2 with 62.48 and 62.13 mg g^{-1} FW had a significant TSS (Table 4). Meanwhile, TSS content in salt-resistant sugar beet was 56.32 mg g^{-1} FW, and this difference was statistically different from the mean of the mentioned genotypes (Table 4). The TSS content Ilam1, Khuzestan-2 Azerbaijan-3, and Kermanshah-2 in 400 mM treatment were 2.61, 2.89, 2.8 and 2.49 times more than sensitive cultivar, respectively (Figure 3).

Genotypes Ilam-1, Kermanshah-2, and Khuzestan-2 had the highest glycine betaine content with 9.28 and 9.21, and 9.02 mg g^{-1} FW, respectively (Table 4). Khuzestan-1, 3 with 8.92 and 8.77 mg g^{-1} FW had a significant glycine betaine content (Table 4). Meanwhile, total soluble sugars content in salt resistance cultivar was 8.00 mg g^{-1} FW, and this difference was statistically different from the mean of the mentioned genotypes (Table 4). The glycine betaine content of Ilam-1, Khuzestan-2, Azerbaijan-3, and Kermanshah-2 in 400 mM treatment were 2.61, 2.89, 2.8 and 2.49 times more than non-stress condition, respectively (Figure 3).

DISCUSSION

Oure results showed that salinity treatments were effective on the activity of antioxidant enzymes in wild genotypes and sugar beet cultivars. The activity of antioxidant enzymes was increased with increasing stress intensity. Pour-Aboughadareh *et al.* (2020) observed that water deficit stress caused a significant decrease in the shoot biomasses but resulted in an increase in the activity of all antioxidant enzymes and relative expression of antioxidant enzyme-encoding genes. Oxidative stress is a complex set of mechanisms that cause major damage to plants due to environmental stresses (Shabala, 2012). Increased activity of antioxidant enzymes due to salinity stress in sugar beet (Wang *et al*., 2017), spinach (Muchate *et al*., 2019) safflower (Ghasemi *et al*., 2020; Shaki *et al*., 2020) and sunflower (Bakhoum *et al*., 2020; Lalarukh and Shahbaz, 2020). The results showed that five genotypes of sea beets (Ilam-1, Kermanshah-2, Khuzestan-1, 2 and 3) had higher activity of antioxidant enzymes and fewer biomarkers than salt tolerant sugar beet cultivar. This shows that under salt stress conditions, the least oxidative damage

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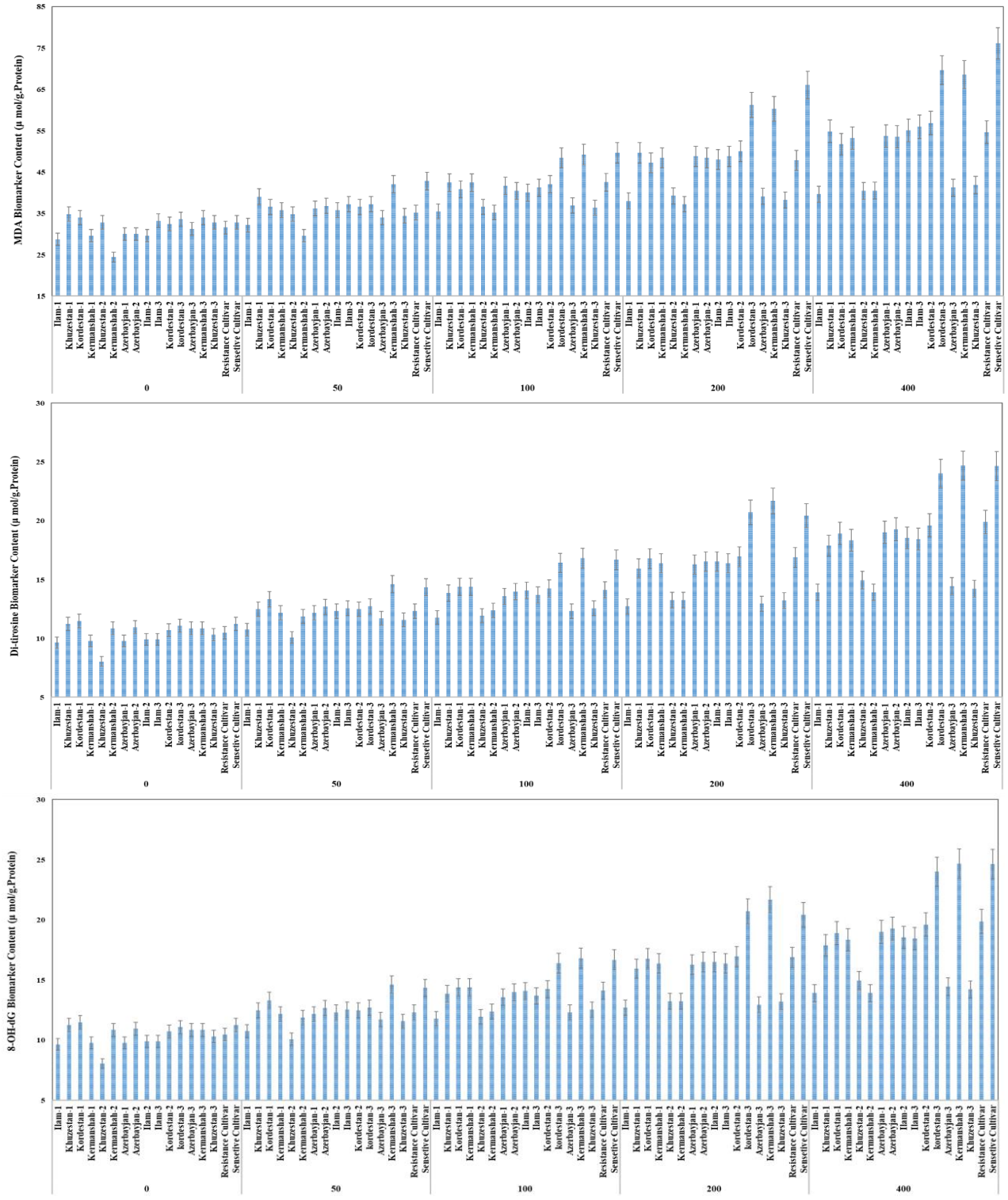

Figure 3. Interaction effect of genotype and salt concentration (0, 50, 100, 200 and 400 mMolar NaCl) on proline, total sugar soluble, and glycine betaine content.

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occurs in these samples. However, the activity of antioxidant enzymes and biomarkers of other sea beets, except for Kermanshah-3 and Kurdistan-3, in most cases was close to salt-resistant cultivar. This indicates that sea beet has a high potential for antioxidant activity, and the ability of these plants to control oxidative stress is one of the most important mechanisms against salinity. So far, little research has been done on the capabilities of sea beets against oxidative stress. However, Bor *et al*. (2003) by studying the changes of lipid peroxidation and antioxidant activity in salinity stress conditions in B. *vulgaris*, Ansa cultivar and B. *maritima* genotypes, and genotype TR51196 indicated that among all treatments, genotype B. *maritima*, had less lipid peroxidation and increased activity of SOD, CAT, and GR enzymes than sugar beet cultivar.

Our results showed that CAT activity was significantly higher than other enzymes under salt stress. CAT breaks down two molecules hydrogen peroxide (as a substrate) into one molecule of oxygen (Von Ossowski *et al*., 1993) and two molecules of water substance.

The results showed that salinity caused a significant increase in proline content. Proline content in salinity stress was 3.4-fold. Armion (2001) investigated the effects of salinity stresses on B. *maritima* and B. *vulgaris* genotypes. Increasing salinity levels increased the amount of proline in both wild beet genotypes and sugar beet cultivars, but this increase was higher in sea beets than in sugar beet. They concluded that B. *maritima* uses proline accumulation as a powerful tool for adjusting osmotic potential under saline conditions.

Wang *et al*. (2017) indicated that the activity of antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Peroxidase (GPX), and Malondialdehyde (MDA) biomarker content in sugar beet genotypes showed increasing patterns with increase in salt concentrations. Moreover, osmoregulators such as free amino acids, proline, and betaine increased in concentration as the external salinity increased.

Increase in the proline content under stress conditions has also been reported in sugar beet (Wu *et al*., 2016; Wu *et al*. 2019), spinach (Muchate *et al*., 2019), melon (Akrami *et al*., 2018), sunflower (Mushke *et al*., 2019), and safflower (Shaki *et al*., 2018). In fact, increasing proline under salt stress is considered as an evolutionary property in plants, as it is an effective combination of regulating and adjusting osmotic pressure and, from another point of view, a non-enzymatic antioxidant (Taiz and Zeiger, 2018). Proline also acts as a protective molecule, including the protection of enzymes (especially antioxidant enzymes) against dehydration, and plays an important role in plant cells in compatibilizing and protective responses to stress (Tuteja and Singh, 2012; Jenks and Hasegawa, 2014). Water and salt stresses provoke the accumulation of many amino acids in sugar beet, but the increase in proline was greater than the others. However, it was noticed that sugar beet was tolerant to low levels of salt (about 150 mM NaCl) and more salt could increase proline accumulation (Gzik, 1996). Asadi Nasab *et al.* (2013) demonstrated that higher concentrations of sodium chloride decreased osmotic potential. An increase of proline neither affected electrolyte leakage nor plant water status. Therefore, in sugar beet, the antioxidant role of proline could not provide plasma membranes protection against damage caused by salt stress.

Salinity caused a significant increase in glycine betaine in beet. Glycine betaine in salinity was 7.9 fold more than normal condition. Glycine betaine is a "compatible solutes" and is one of the most important plant remedies for osmotic regulation, and one of the most toxic, non-toxic asbestos-free and osmotic modifiers in many plant species such as spinach, beet, and in particular, halophytes in response to increase in different types of environmental stresses

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(Abbas *et al*., 2012; Shabala, 2012; Wang *et al*., 2017). Glycine betaine also plays a crucial role in cell wall stability, maintaining membrane fluidity, neutralizing the toxicity of the active oxygen species, and stabilizing proteins and enzymes (Gorham, 1995; Shabala, 2012). Studies in various plant species that grow under salt stress have shown that the use of external proline and glycine betaine provides osmotic protection and facilitates growth (Jenks and Hasegawa, 2014).

Total soluble sugars increased with increasing salinity stress and increased 2.3 times in severe stress (400 mM NaCl) compared to non-stress treatment. Soluble sugars include alcoholic sugars (e.g. Glycerol, Inositol, and Pinitol), simple sugars (e.g. Glucose and Fructose) and compound sugars (e.g. Trehalose, Raffinose, and Fructus) are also compatible organic matters. The accumulation of soluble carbohydrates in response to salt stress is mainly due to osmotic regulation and cell membrane protection (Hopkins and Huner, 2009; Tuteja and Singh, 2012; Akrami *et al*., 2018).

An increased total soluble sugars under stress conditions reported in sugar beet (Wu *et al*., 2016) and melon (Akrami and Arzani, 2018). Increased soluble sugars concentration because of salinity can be attributed to increasing enzyme activity, especially amylase, under stress conditions. But the more likely reason is that cells, by consuming energy, try to withstand against ion imbalance under stress conditions (Jenks and Hasegawa, 2014), and this is an evolutionary feature that has been created over many tens of years in sea beets that have been continuously exposed to these tensions (Biancardi *et al*., 2012). Between the studied genotypes, Ilam-1, Kermanshah-2, and Khuzestan-1, 2 and 3 had the highest proline, glycine betaine, and total soluble sugars contents under salt stress conditions. The proline, glycine betaine, and total soluble sugars contents of these sea beets were higher than salt tolerant sugar beet cultivars, especially in severe salt stress treatments.

CONCLUSIONS

Based on the experimental results, at least 5 sea beet genotypes (Ilam-1, Kermanshah-2, Khuzestan-1, 2 and 3) were identified that had higher antioxidant enzymes, proline, glycine-biotin, soluble sugars, and lower biomarker activity than sugar beet salt tolerant cultivar, under salt stress conditions. The results also showed that the increase in antioxidant enzymes by preventing an increase in oxidative stress is one of the mechanisms used by sea beet under salinity stresses. The findings also showed that accumulation of proline, glycine, and soluble sugars are important strategies for regulating osmotic pressure in the sea beet investigated. Based on the findings, it can be concluded that sea beet (*Beta maritima*) have high physiological diversity and potential to combat with salt stress and can be exploited to develop sugar beet (*Beta vulgaris*) cultivars with greater tolerance to salt-stress.

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تغییرات آنتی اکسیدانی و بیوشیمیایی در چغندر دریایی (*Beta maritima*) و چغندر قند (*Beta vulgaris*) در معرض تنش شوری

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چکیده

یک آزمایش گلدانی با استفاده از آزمایش فاکتوریل در قالب طرح بلوک کامل تصادفی با ۳ تکرار انجام شد. تیمارها شامل ژنوتیپ (۱۵ ژنوتیپ چغندر دریایی و دو چغندر زراعی حساس و متحمل به تنش) و شوری در چهار غلظت: ۵۰ ، ۱۰۰ ، ۲۰۰ و ۴۰۰ میلی مولار NaCl بر روی نشاءهای چغندر ۳۵ ساله به مدت ۵۵ روز بود. صفات اندازه‌گیری شده شامل: فعالیت آنزیم های سوپراکسید دیسموتاز (SOD)، کاتالاز (CAT) و گلوتاتیون پراکسیداز (GPX)، بیومارکرهای مالون دیالدئید (MDA)، دی تیروزین (DT)، دی هیدروکسی گوانوزین (8-OH-dG)، پرولین و قندهای محلول کل بود. نتایج نشان داد که تیمارهای شوری تاثیر قابل توجهی بر صفات مورد بررسی داشت. علاوه بر این، با افزایش شدت تنش، تاثیر شوری بر این صفات افزایش یافت. در شرایط تنش شوری، حداقل پنج ژنوتیپ چغندر دریایی به‌طور مشخصی با تولید بیومارکرهای تخریب کننده MDA، DT و8-OH-dG کمتر، اما فعالیت بالای آنزیم‌های SOD، CAT و GPX و پرولین، قندهای محلول کل و گلایسین بتائین نسبت به سایر ژنوتیپ‌های دیگر برتری داشتند. این نتایج قویا نشان دادند که چغندر وحشی متحمل به تنش شوری دارای مزایای مشخصی نسبت به همتایان چغندرقند برای مکانیسم محافظت در برابر آسیب ناشی از تنش اکسیداتیو با حفظ قابلیت توارث و فعالیت آنتی اکسیدانی از نوع آنزیمی و غیر آنزیمی بالا، است. بنابراین، می‌توان نتیجه گرفت که چغندر دریایی در شرایط تنش شوری دارای پتانسیل قابل توجهی برای تغییرات فیزیولوژیکی و بیوشیمیایی در تحمل به شوری است که می‌توان برای بهبود تحمل به تنش شوری در ارقام چغندرقند از آن استفاده کرد.

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