Pak. J. Bot., 54(6): 2001-2011, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-6(32)

DIFFERENTIAL ADAPTATION OF ROOTS AND SHOOTS TO SALT STRESS CORRELATES WITH THE ANTIOXIDANT CAPACITY IN MUSTARD (*BRASSICA JUNCEA* L.)

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Abstract

Salt adaptive mechanisms of the shoots and roots in mustard *(Brassica juncea* L.) were studied by examination of their growth parameters, biomass, photosynthesis, malondialdehyde (MDA) content and some key antioxidants. Mustard seedlings were treated at four levels of salt (0, 50, 100 and 200 mM NaCl) at various times of exposure. Severe salt stress significantly inhibited the growth of shoots by causing a reduction in the leaf area and dry and fresh weights. The inhibitory effect of salt on the shoots positively correlated with the decrease in chlorophyll content and performance index and negatively correlated with the content of MDA in leaves. Higher salinity for the roots under stress proved to positively affect growth. The root-shoot ratio, number of first-order lateral roots and the lateral root density were higher than those of the control group by 26.1%, 28.7% and 58.5%, respectively. The levels of MDA remained the same. Coordination of the antioxidant enzymes ensures the plants are highly effective at scavenging reactive oxygen species (ROS). These results strongly suggest that the antioxidant system is involved in the adaptive regulation of root growth to avoid the harmful effects of high soil salinity.

Key words: Salt stress, *Brassica juncea* L., Morphology, Chlorophyll fluorescence, Antioxidant enzyme activity.

Abbreviations: APX: ascorbate peroxidase; CAT: catalase; DAT: days after treatment; MDA: malondialdehyde; PI_{ABS}: performance index; PVPP: polyvinylpyrrolidone; ROS: reactive oxygen species; RSA: root system architecture; SOD: superoxide dismutase.

Introduction

Salinity is an increasingly serious issue for global agriculture, which inhibits the growth of plants and reduces the productivity of crops. Twenty percent of the 230 million hectares of irrigated croplands are affected by salts, and this proportion increases dramatically each year owing to unsuitable irrigation practices (Deinlein *et al*., 2014). It is estimated that 50% of the world's arable land will be salinized by 2050 (Jamil *et al*., 2011). Therefore, it is urgent to improve the tolerance of crops to salt. One way to help to ensure higher agricultural production is to explore novel salt-tolerant germplasms.

Salt stress increases the concentration of sodium and chloride ions, thus, leading to nutritional imbalance and even plant death (Zahedi *et al*., 2012). Salt stress reduces the plant height, leaf area and relative water content and affects the thickness of the whole leaf and biomass (Uddin *et al*., 2005, Purty *et al*., 2008). Salinity accelerates the degradation of chloroplasts and then inhibits the synthesis of chlorophyll (Ma *et al*., 2012). Leaf chlorophyll is involved in the capture, absorption and transfer of light energy in photosynthesis, and the decrease in the content of chlorophyll negatively correlated with plant yield (Feng *et al*., 2014).

Plant roots are closely associated with nutrients and water uptake and are the first contact tissue that responds to stress signals. Multiple figures determine the root system architecture (RSA), particularly salinity (Osmont *et al*., 2007, Galvan-Ampudia & Testerink, 2011). Plants have established a sophisticated mechanism to adapt to salt stress conditions, such as regulating the plant RSA (Galvan-Ampudia & Testerink, 2011). A study in *Arabidopsis thaliana* reported that salt stress markedly promotes the elongation of lateral roots (Wang *et al*., 2009). In *Brassica napus*, stress stimulates changes in root morphology, including the growth and development of root hairs on lateral roots, which leads to an additional increase in the root surface area compared with plants that are not stressed. To some extent, the increase of root surface area indicates that plants can absorb more water and nutrients from the surrounding rhizosphere, and this change induced by stress in root morphology serves as an adaptation strategy (Arif *et al*., 2019). The natural variation of RSA enables its use as a modern breeding strategy to improve the efficiency of uptake of water and nutrients, and further increase crop yields (White *et al*., 2013, He *et al*., 2019).

ROS obviously accumulates under stress conditions. To keep the ROS in balance and not harm the plant, the plant activates its antioxidant system to eliminate the deleterious ROS. It has been documented that the antioxidant enzyme activity was positively related to salt resistance in rice (*Oryzae sativa*) (Khan *et al*., 2002), chickpea (*Cicer arietinum*) (Rasool *et al*., 2013) and maize (*Zea may*) (Neto *et al*., 2006). ROS are necessary for cellular proliferation and differentiation, even though excessive amounts of ROS inhibit the synthesis of proteins and chlorophyll, resulting in wilting or death under severe stress (Mittler, 2017). A recent study in *Brassica napus* revealed that in addition to hormones, ROS can also regulate the growth and development of roots (Feigl *et al*., 2019).

Mustard has outstanding economic value and is commonly used as an oil crop, source of leafy greens, spice, fodder and green manure (Hooks *et al*., 2019). In recent years, abiotic stresses (limited moisture supply, high transpiration and continuous high temperature) have intensified the salinization of soil and further inhibited the growth of mustard in the Ukraine. Most previous studies on *Brassica* have focused on assessing the differences in morphology, physiology and gene expression between different cultivars in response to salt stress (Uddin *et al*., 2005, Hooks *et al*., 2019, Singh *et al*., 2019), while few studies have been conducted on the morphological and physiological mechanisms of the adaptation of different tissues of mustard when subjected to salt stress. Therefore, our goal was to investigate the effects of antioxidant enzymes and mechanisms of morphological adaptation in the roots and shoots of mustard seedlings subjected to salinity. Different adaptations of tissues contribute to an understanding of the mechanism of tolerance to salinity and will provide a better understanding for future breeding programs to better enable plants to respond to stress.

Material and Methods

Experimental materials and culture conditions: The mustard variety FELICIA was provided by Sumy National Agricultural University, Sumy, Ukraine. Mustard seeds were surface sterilized and germinated for five days. Eight seedlings were transplanted into each plastic pot that was filled with 5 L Hoagland's solution. These seedlings were cultured in an artificial climate chamber at 28 ± 2°C, 14-h light/ 10-h night photoperiod and 45% relative humidity. The Hoagland's solutions that contained up to 50, 100 and 200 mM NaCl were regarded as subjecting the plants to low, moderate and severe salt stress, respectively. All the nutrient solutions were changed twice weekly to prevent fungal contamination. Morphological and physiological indices were measured on days 3, 7 and 10 after treatment (DAT).

Morphology and biomass of the seedlings: The leaves and roots of five plants from each treatment were separated after 3, 7 and 10 DAT. An Epson Perfection V800 Photo scanner (Epson America, lnc., Long Beach, CA, USA) was used to scan the roots and shoots of seedlings, and WinRHIZO 2007 (Regent Instruments. Inc., Quebec, Canada) was used to analyze the scanning results, including the total root length, total surface area, and the projected area of leaves among others. The number of first-order lateral roots was counted manually. The fresh weights were directly determined, and the plants were dried at 80 °C for 48 h to determine their dry weight.

$$\text{The first-order lateral root density } (cm^{-1}) = \frac{\text{Number of first} - \text{Order lateral roots}}{\text{Lateral root zone}}$$

$$\text{Root: shoot ratio (dry weight) } (\%) = \frac{\text{Root dry weight}}{\text{Shoot dry weight}} \times 100$$

$$\text{Dry weight/Fresh weight ratio of shoot (root) } (\%) = \frac{\text{Shoot (root) dry weight}}{\text{Shoot (root) fresh weight}} \times 100$$

Chlorophyll concentration: The relative chlorophyll content of five expanded leaves from each treatment was measured using a Dualex Scientific (Force-A, Orsay, France).

Chlorophyll fluorescence: A portable fluorometer (PEA, Hansatech Instruments Ltd, King's Lynn, UK) was used to determine the maximal photochemical efficiency (F_v/F_m) and performance index (PI_{ABS}). Five leaves were selected from each treatment as replicates, and all the treated leaves were placed in the dark for half an hour before measurement.

Enzyme assays and protein determination: To avoid potential differences of the content of antioxidant enzymes in different plant positions, all the leaves were excised from the third or fourth fully expanded leaves at the bottom of the plant, and the roots were collected from the taproot tips. One-half gram each of lyophilized leaves and roots were homogenized with 5 mL of 100 mM potassium phosphate buffer (pH 7.5) that contained 1 mM EDTA and 1% polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 12,000 g for 20 min at 4°C, and the crude extract was collected to assay the protein, enzyme activities and lipid peroxidation.

The content of soluble protein was measured using Coomassie brilliant blue G250 staining (Bradford, 1976). A total of 30 µl supernatant and 170 µl of Coomassie brilliant blue G250 were mixed, and the absorbance was read at 595 nm using bovine serum albumin as a standard. The activity of superoxide dismutase (SOD) was assayed as described by Beauchamp (1971) at 560 nm. The activity of peroxidase (POD) was determined using guaiacol as the substrate (Kochba *et al*., 1977). The absorbance of the mixture was determined at 470 nm within 3 min. The activity of catalase (CAT) was determined as described by Neto (2006) with modifications. The activity of CAT was calculated based on the rate of disappearance of H_2O_2 in 240 nm of ascorbate. The activity of ascorbate peroxidase (APX) was determined as described by Nakano & Asada (1981), and the absorbance of the mixture was measured at 290 nm.

Lipid peroxidation (MDA): The content of MDA was determined using TBA (Rao & Sresty, 2000). The assay mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10000 g for 20 min, the absorbance of the supernatant was measured at 450 nm, 532 nm and 600 nm.

Statistical analysis

A statistical analysis was conducted with SPSS 22 (IBM, Armonk, NY, USA). Different lowercase letters differ significantly based on a Duncan's multiple range test, and $p<0.05$ was used as the significance level. Pearson's correlation coefficient (r) was used to test the significant correlation between physiological characteristics.

Results

Phenotype of mustard: NaCl induced a prominent reduction in the traits of the shoots of mustard as shown in (Table 1). The reduction in leaf area was greater when subjected to severe salt stress and reached 33.2%, 71.1% and 92.8% on 3, 7 and 10 DAT, respectively. A low concentration of salt slightly increased the leaf area compared with the control by 7.2% only on 3 DAT. Salt stress reduced the stem length compared with plants that were not subjected to salt stress, and the stem length was significantly reduced by 22.4% and 50.4% with moderate and severe salt stress on 10 DAT, respectively.

Salt stress also affected the RSA of seedlings (Table 2, Fig. 1). The plants were stressed for 3 days, and severe salt stress reduced the root growth and development. However, the low concentration of salt increased the growth of mustard. Compared with plants that were not subjected to salt stress, the total root length, number and density of the first-order lateral roots that were treated with 50 mM NaCl markedly increased by 21.2%, 36.3% and 23.7% on 3 DAT, respectively. Other traits of RSA also increased, but they did not differ significantly. Despite the dramatic inhibition of the growth of seedling roots after 10 days of salt exposure, the number and density of first-order lateral roots following treatment with 200 mM NaCl were higher than those under normal conditions by 28.7% and 58.5%, respectively. These results clearly showed that salt stress modulates RSA in mustard.

Fresh and dry weights of mustard seedlings: The fresh and dry weights of plants gradually decreased for both shoots and roots as the treatment and level of stress were prolonged (Table 1). These data showed that the dry weights of roots decreased by 24.3%, 43.5% and 80.3%, and the dry weights of shoots decreased by 12.1%, 38.7% and 84.1% when the plants were exposed to three levels of salt for 10 days. We observed the same results on the fresh weight of the roots and shoots, which indicated that the biomass gradually decreased for both shoots and roots when treated with the three salt concentrations. However, during the early stages of salt stress, low salt stress promoted the growth of seedlings, and the fresh and dry weights of the shoots increased by 10.1% and 8.7%, and those of the roots by 33.3% and 23.1%, respectively. Therefore, the response of plants to salt stress depends on concentration and time. The dry-fresh ratio of shoots subjected to severe salt stress was higher than those subjected to low and moderate stress. Moreover, the root-shoot ratio of severe salt stress significantly increased by 26.1% compared with the control during the later stages of salt treatment. In addition, the root-shoot ratio did not change when subjected to low and moderate levels of stress.

Chlorophyll content: All the salt treatments resulted in a decrease in the content of chlorophyll, which positively correlated with the concentration of salt. In addition, the chlorophyll content of moderate and severe salt stress decreased with the extension of the time of stress, from 10.8% and 12.3% on 3 DAT to 15.6% and 29.8% on 10 DAT, respectively. Low salt stress did not significantly affect the content of chlorophyll (Fig. 2).

Chlorophyll fluorescence: The maximal photochemistry of PSII (F_v/F_m) and performance index (PI_{ABS}) serve as important parameters of chlorophyll fluorescence. Mustard leaves grown with and without stress exhibited an insignificant change in the F_v/F_m, and the value was distributed at approximately 0.8 (Fig. 3A). However, the PI_{ABS} decreased significantly as the concentration of NaCl increased compared with that of the control plants (Fig. 3B). In addition, PI_{ABS} reached its minimum under severe stress.

MDA content: The content of MDA in the leaves and roots indicated the degree of peroxidation of plants (Fig. 4). The concentration of MDA in the roots increased with the duration of low and moderate stress compared with the control plant, and the accumulation of MDA reached its highest levels during the later stage of stress. Notably, the content of MDA decreased when the plants were subjected to severe salt stress, and the lowest value appeared on day 10 of this stress. The content of MDA in salt-stressed leaves increased on 3 DAT, but the difference was not significant. The content of MDA decreased or was not affected at low and moderate salt stress on 7 and 10 DAT, while the content of MDA was higher than that of the control when the plants were subjected to severe salt stress and reached its maximum value of 199.5% on 10 DAT.

Enzyme activity: The change in the activities of antioxidant enzymes (SOD, POX, APX and CAT) are shown in (Fig. 5). The activity of SOD induced by salt stress differed significantly in the roots and leaves of mustard seedlings. The activity of SOD in all of the treatments in roots was higher than that of the plants that were not subjected to salt stress. The specific activity of SOD dramatically increased with the levels of salt by 61.4%, 61.4% and 114.3%, and reached its maximum value on 3 DAT. With the extension of time of stress, the activities of SOD in the roots subjected to low and severe salt stress were 33.0% and 34.4% greater on DAT 10, respectively. Among the groups of leaves treated with NaCl, the activity of SOD activity was 23.9%, 23.1% and 58.1% on 7 DAT than in the controls, while it remained almost unchanged on both 3 and 10 DAT. The other treatments decreased by 18.4% with low salt stress on 3 DAT and by 40.0% at severe salt stress on 10 DAT, respectively.

Table 1. Effects of NaCl treatment on the biomass and growth of mustard seedlings.

DAT (d)	NaCl	Shoot					Root			Root: Shoot ratio (DW)(%)
		Leaf area (cm^2)	Stem length (cm)	Fresh weight (mg)	Dry weight (mg)	DW/FW ratio (%)	Fresh weight (mg)	Dry weight (mg)	DW/FW ratio (%)	
3	Control	24.68±7.56a	8.97±1.18a	907.80±275.79ab	68.67±6.43a	8.33±0.02a	303.40±89.54a	17.33±1.53b	6.8±0.04a	25.47±0.04ab
	Low salt stress	26.46±5.21a	7.16±1.02b	999.80±176.04a	74.67±6.51a	7.7±0.02a	404.40±106.06a	21.33±2.08a	5.38±0.02a	28.77±0.04a
	Moderate salt stress	16.88±2.71b	6.88±0.64b	692.80±144.37b	45.40±2.62b	6.96±0.01a	297.60±86.14a	13.63±1.82c	5.01±0.01a	29.94±0.02a
	Severe salt stress	8.97±1.26c	7.12±1.67b	404.80±67.32c	37.13±2.42b	10.11±0.03a	107.20±32.43b	7.20±0.60d	7.03±0.03a	19.43±0.02b
7	Control	64.54±14.73a	13.10±1.54a	2633.67±761.02a	287.67±19.86a	11.63±0.04a	815.67±187.01a	58.17±2.47a	7.38±0.02ab	20.24±0.01c
	Low salt stress	54.91±7.88a	8.06±1.25b	2571±310.60a	250.33±12.50b	10.1±0.01a	761.50±137.34a	48.33±2.52b	6.73±0.01b	19.31±0c
	Moderate salt stress	23.80±1.8b	8.02±1.29b	1405.50±182.32b	137.83±19.36c	10.17±0.02a	463±57.01b	42.33±3.06c	10.32±0.02a	33.91±0.01a
	Severe salt stress	10.43±1.86c	7.81±1.05b	534.80±53.77c	55.07±4.50d	10.24±0a	194±35.93c	13.83±0.65d	7.08±0.01ab	25.21±0.02b
10	Control	105.16±37.54a	14.20±1.88a	3977±1620.73a	367.67±15.95a	11.75±0.02a	1524.75±490.47a	85±10.54a	6.6±0a	23.08±0.02b
	Low salt stress	70.29±22.92b	14.89±2.75a	4069±1845.56a	323±23.64b	7.09±0.03b	1061.80±271.65b	64.33±6.11b	5.83±0.01a	20.02±0.03b
	Moderate salt stress	30.36±5.9c	11.02±1.51b	2422.80±397.70a	225.70±7.88c	10.06±0.01ab	834.20±197.39b	48.07±1.66c	5.72±0.01a	21.3±0b
	Severe salt stress	7.57±2.57c	7.04±1.49c	574.20±141.92b	58.33±4.73d	12.35±0.02a	283.60±30.55c	16.67±0.78d	5.85±0a	28.71±0.03a

Note: Means ± SD, n = 5. Values in a column followed by different lowercase letters are significantly different at $p<0.05$ according to Duncan's multiple range test.
CK: Control; DW: Dry weight; FW: Fresh weight. DAT: Days after treatment

Table 2. Effects of NaCl treatment on the root system architecture of mustard seedlings.

DAT (d)	NaCl	Total root length (cm)	Total root surface area (cm^2)	Total root diameter (mm)	Total root volume (cm^3)	Number of first-order lateral roots	Length of primary root (cm)	First-order lateral root district (cm)	First-order lateral root density (cm^{-1})	Total of lateral root length (cm)
3	Control	577.41±101.01b	40.78±11.94a	0.22±0.01a	0.23±0.08a	63.4±6.77b	9.96±1.84a	7.82±1.66a	8.34±1.65b	567.45±148.19a
	Low salt stress	699.69±87.43a	48.17±7.09a	0.22±0.01a	0.26±0.06a	86.4±11.72a	10.54±2.37a	8.54±1.62a	10.32±1.78a	689.15±186.5a
	Moderate salt stress	529.44±95.07b	37.17±10.28a	0.22±0.01a	0.21±0.06a	68.8±5.72b	9.62±0.91a	7.11±0.59a	9.71±0.86ab	519.86±153.14a
	Severe salt stress	249.69±71.6c	18.02±5.53b	0.23±0.02a	0.1±0.04b	39.2±6.8c	10.19±0.71a	7.98±0.93a	5.1±0.51c	240.96±74.1b
7	Control	1267.04±167.82a	101.34±18.44a	0.25±0.01a	0.64±0.15a	82.25±3.2b	15.54±1.64a	12.65±2.19a	6.53±0.94b	1269.06±226.89a
	Low salt stress	1161.26±203.6a	93.53±21.5a	0.26±0.02a	0.6±0.11a	94.5±14.53ab	10.15±1.72b	8.39±1.78b	11.47±2.02a	1179.81±377.33a
	Moderate salt stress	933.61±102.8b	64.95±10.93b	0.22±0.01b	0.36±0.08b	101.5±7.59a	10.62±2.16b	8.24±2.17b	12.76±2.4a	934.27±115.9a
	Severe salt stress	563.48±67.6c	37.44±5.02c	0.21±0.01b	0.2±0.03c	92.25±5.19ab	9.37±0.89b	8.49±0.81b	11.1±0.62a	554.11±66.92b
10	Control	1826.31±194.1a	172.35±39.53a	0.3±0.03a	1.31±0.43a	79.25±5.74c	11.9±3.81a	9.8±1.45a	7.93±0.76b	1826.11±373.31a
	Low salt stress	1601.87±291.18ab	117.2±45.26b	0.26±0.03b	0.74±0.22b	84.25±9.43bc	8.89±1.21b	7.35±1.18b	11.53±2.77ab	1472.98±716.97ab
	Moderate salt stress	1485.51±135.7b	106.3±22.09b	0.23±0.01c	0.61±0.13bc	97.25±7.18ab	8.87±1.14b	7.84±1.15b	12.52±1.4a	1451.47±342.57ab
	Severe salt stress	808.99±105.8c	53.09±7.21c	0.21±0.01c	0.28±0.05c	102±15.98a	9.26±0.93ab	8.48±1.18ab	12.57±3.79a	799.73±105.22b

Note: Means ± SD, n = 5. Values in a column followed by different lowercase letters are significantly different at p<0.05 according to Duncan's multiple range test. DAT: Days after treatment

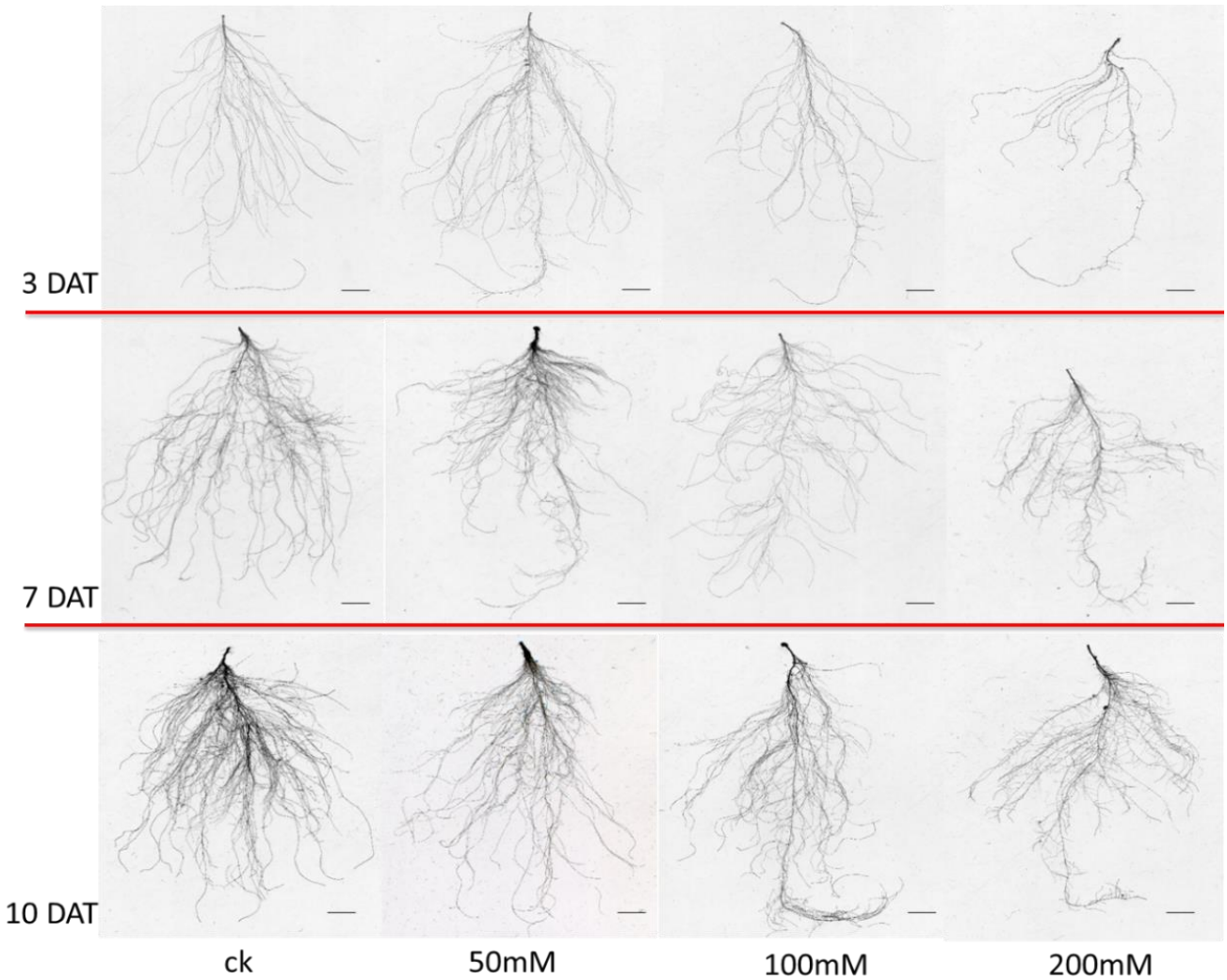

Fig. 1. Effects of salt stress on the RSA of mustard seedlings. DAT: days after treatment. RSA: root system architecture.

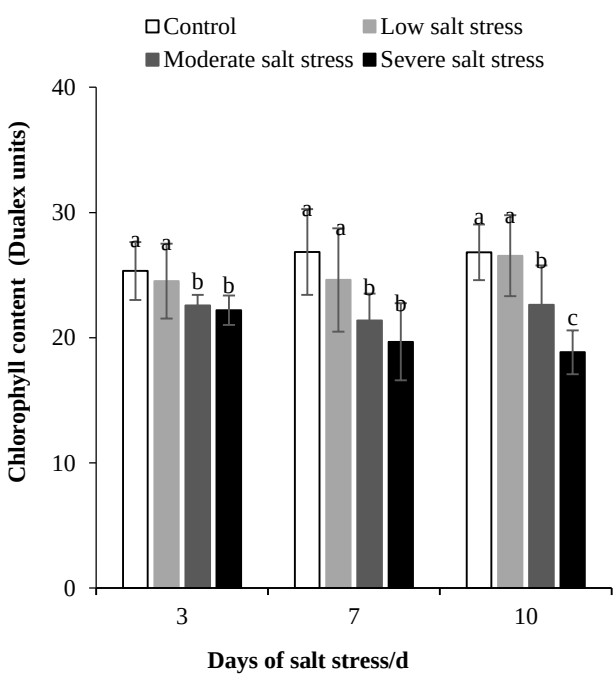

Fig. 2. Changes in chlorophyll content under salt stress (0, 50, 100, and 200 mM NaCl for 3,7and 10 d). Means followed by different lowercase letters differ significantly according to Duncan's multiple range test, $p<0.05$, n = 5.

The activity of POD in stressed leaves and roots differed significantly during the experimental period. Salt induced a rapid increase in the activity of POD in the roots and maintained a high level throughout the treatment period. The activity of POD of the root treatment group increased by 122.5%, 286.1% and 267.7% at 10 DAT compared with the control treatment group, respectively. The activity of POD in leaves increased by 36.9%, 97.0% and 169.5% with the NaCl treatments after 10 days, respectively, and there was no significant difference compared with the control at both 3 and 7 DAT with the exception of the group treated with low salt stress on 3 DAT. In addition, the activity of POD in roots increased markedly compared with that in the leaves.

The levels of root APX activity increased with the increments of NaCl on 3 DAT by 19.4%, 31.8% and 50.2%, respectively, and the maximum activity increased by 54.7% with severe salt stress on 7 DAT. The APX activity in the roots changed slightly on 10 DAT but did not differ significantly compared with the control plants. A similar result was observed for the activity of APX in leaves. The concentrations of salt (100 and 200 mM NaCl) rapidly induced the activity of APX on 3 DAT by 67.1% and 71.7%, respectively. The activity of APX did not differ significantly under all the treatments on both 7 and 10 DAT, with the exception of a rapid increase in the treatment of a low concentration on 7 DAT.

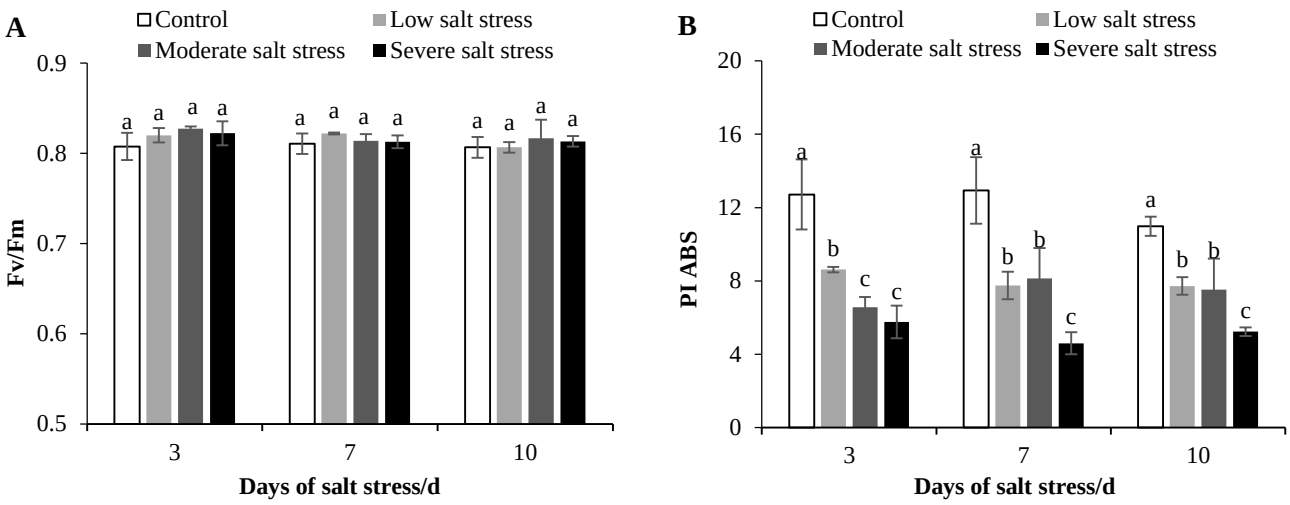

Fig. 3. Changes in the parameters of chlorophyll fluorescence of mustard seedling under salt stress (0, 50, 100, and 200 mM NaCl for 3, 7 and 10 d), A: Fv/Fm; B: PI_{ABS}. Means followed by different lowercase letters differ significantly according to Duncan's multiple range test, $p<0.05$, n = 5.

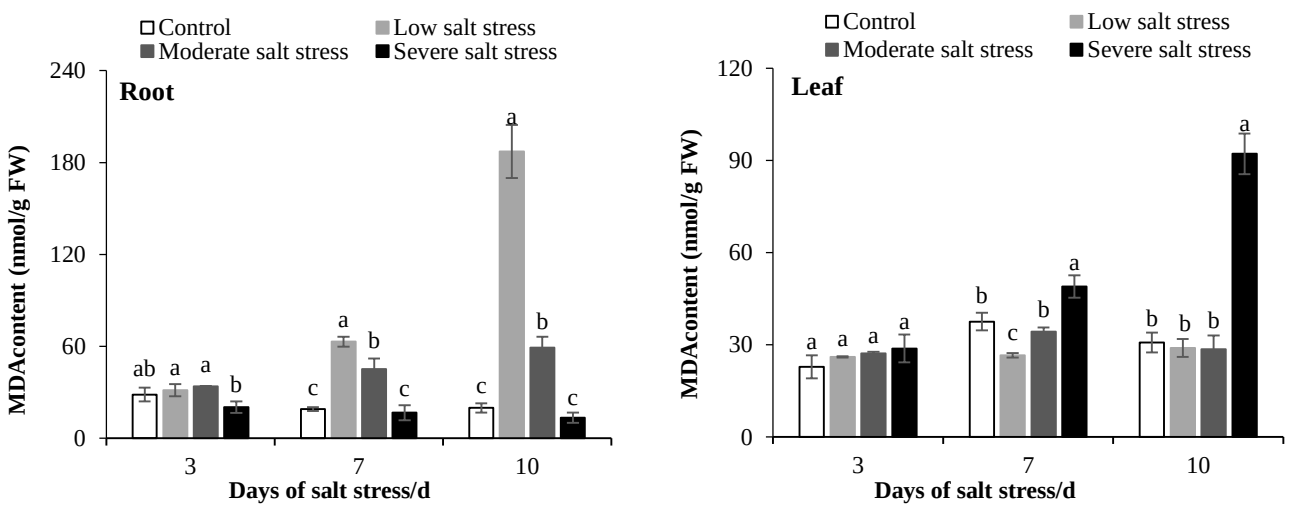

Fig. 4. Changes in the content of MDA of mustard seedlings under salt stress (0, 50, 100, and 200 mM NaCl for 3, 7 and 10 d). Means followed by different lowercase letters differ significantly according to Duncan's multiple range test, $p<0.05$, n = 3.

Moderate and severe salt stress rapidly increased the activity of CAT in the roots during all the treatment days and peaked by 713.2% and 293.1% on 10 DAT, respectively. However, the activity of CAT in the roots of low salt treatment did not increase significantly until 10 DAT. NaCl induced a surge of increase in the activity of CAT in leaves compared with the treatment without salt stress during the experimental period. The activity of CAT of the leaves was the highest by 212.4% and 255.2% on 3 DAT following treatment with low and moderate salt, respectively. Salt-induced CAT maintained a high level in both the roots and leaves throughout the stress period.

Soluble protein: The content of protein in all the salt treatments differed significantly (Fig. 6). With the exception of low salt stress, in which the content of protein decreased or did not change significantly on 3 and 7 DAT, treatment with moderate and severe salt stress caused an increase in the concentration of protein in the roots. In addition, the content of protein increased with the stress time, which was 32.5%, 64.2% and 49.1% compared with the treatment on 10 DAT that lacked salt, respectively. In contrast, the highest content of protein in the leaves was noted under salt-treated conditions on 3 DAT, which were 103.9%, 76.9% and 70.1% over the control, respectively. The change in content of protein in the leaves decreased during the experiment.

Correlation Analysis: A correlation analysis of the shoot physiological characteristics under stress indicated that the dry and fresh weight of shoots as determined by the leaf area and stem length, and the content of chlorophyll positively correlated with the leaf area and protein. The activity of SOD was positively regulated by the content of chlorophyll and the dry and fresh weights of the shoot. However, the activity of POD negatively correlated with the leaf area and shoot biomass (Table 3).

The increase in total lateral length of roots resulted in an increase in the total root length. SOD and the root biomass were positively correlated. MDA negatively correlated with the density and number of first-order lateral roots. The protein positively correlated with CAT and MDA (Table 4).

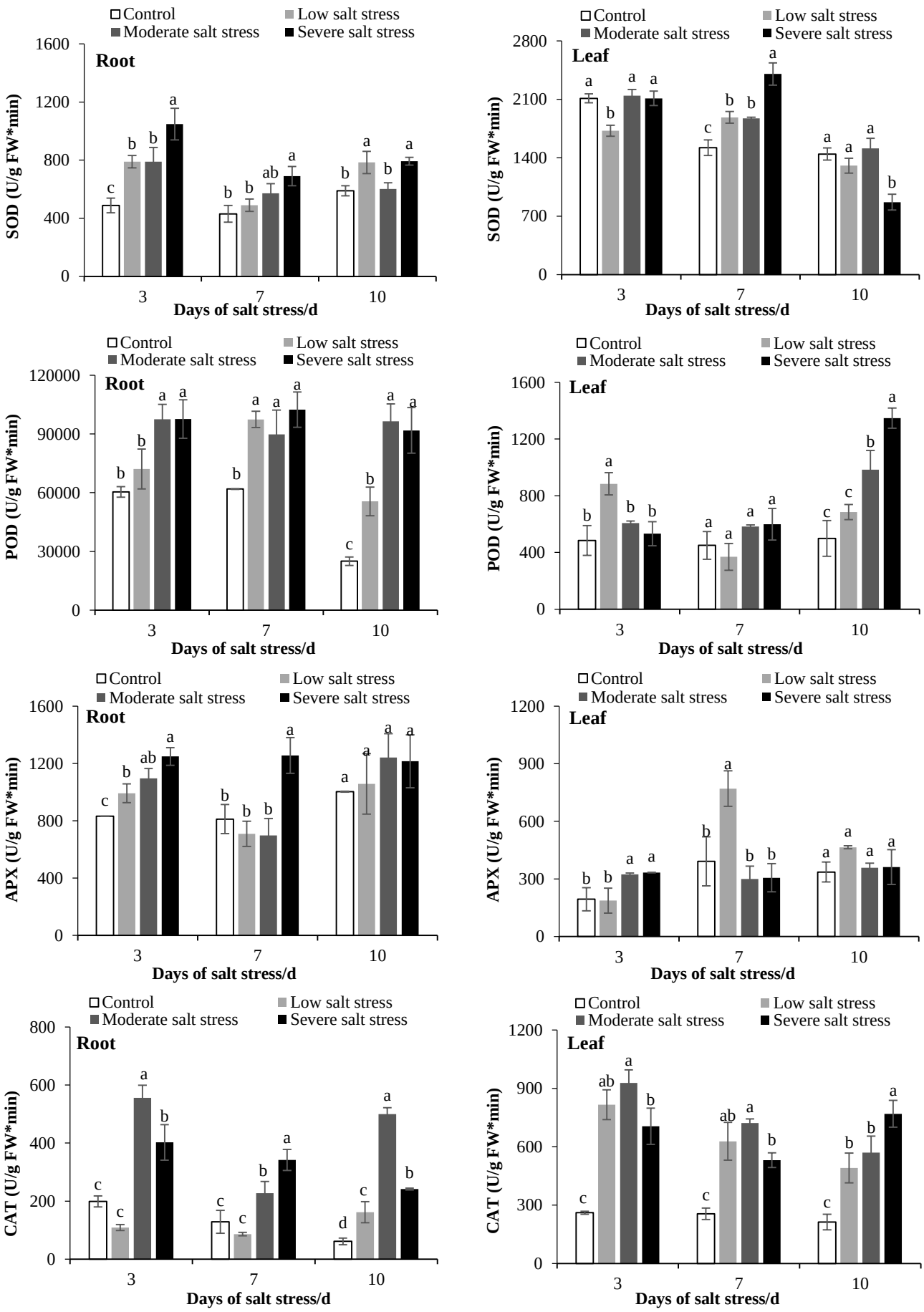

Fig. 5. Changes in the activities of SOD, POD, APX and CAT in the leaves and roots of seedlings (0, 50, 100, and 200 mM NaCl for 3, 7 and 10 d). Means followed by different lowercase letters differ significantly according to Duncan's multiple range test, $p<0.05$, n = 3.

Table 3. Pearson's correlation coefficient (r) for the relationships among the physiological characteristics of shoot under NaCl treatments in mustard.

Item	SFW	LA	SDW	SL	Chl	PI	APX	CAT	SOD	POD	MDA
LA	0.85**										
SDW	0.43	0.27									
SL	0.75**	0.59**	0.48*								
Chl	0.37	0.48*	-0.04	0.19							
PI	-0.31	-0.55	-0.27	-0.20	-0.35						
APX	0.42	0.32	0.56	0.28	-0.54	-0.14					
CAT	-0.71	-0.22	-0.48	-0.67	0.46	-0.47	-0.27				
SOD	0.81*	0.56	0.86**	0.62	-0.24	0.00	0.60	-0.76*			
POD	-0.82*	-0.77*	-0.93**	-0.66	0.21	0.05	-0.75*	0.54	-0.85**		
MDA	-0.70	-0.51	-0.87**	-0.48	0.04	-0.24	-0.46	0.75*	-0.92**	0.81*	
Protein	-0.14	0.29	0.31	-0.32	0.91**	-0.07	-0.28	0.36	-0.10	-0.02	-0.15

Note: LA, Leaf area; SDW, Shoot dry weight; SFW: Shoot fresh weight; SL: Stem length; Chl: Chlorophyll; PI_{ABS}: Performance index; APX: Ascorbate peroxidase; POD, Peroxidase; SOD, superoxide dismutase; CAT, Catalase; MDA, Malondialdehyde. *p<0.05. **p<0.01

Table 4. Pearson's correlation coefficient (r) for the relationships among the physiological characteristics of root under NaCl treatments in mustard.

Item	RFW	TRL	DW	TLRL	PRL	NLR	DLR	APX	POD	SOD	CAT
TRL	0.23										
RDW	0.34	-0.03									
TLRL	0.41	0.60**	0.13								
PRL	-0.20	-0.39	0.14	-0.25							
NLR	-0.06	0.01	-0.28	-0.04	0.29						
DLR	-0.19	0.31	-0.52*	0.28	0.07	0.73**					
APX	0.19	0.36	0.39	-0.15	-0.19	0.12	-0.01				
POD	-0.65	0.17	-0.69	-0.07	-0.29	0.14	0.17	-0.20			
SOD	0.72*	0.08	0.88**	0.09	0.11	-0.51	-0.55	0.16	-0.84**		
CAT	0.15	-0.36	0.15	-0.28	-0.41	-0.80*	-0.71*	-0.05	0.02	0.29	
MDA	0.01	-0.27	0.07	-0.39	-0.48	-0.91**	-0.85**	-0.10	0.06	0.24	0.91**

Note: RDW, Root dry weight; RFW: Root fresh weight; TRL, Total root length; TLRL: Total lateral root length; PRL, Primary root length; NLR: Number of first-order lateral root; DLR: Density of first-order lateral root; APX: Ascorbate peroxidase; POD, Peroxidase; SOD, Superoxide dismutase; CAT, Catalase; MDA, Malondialdehyde. *p<0.05. **p<0.01

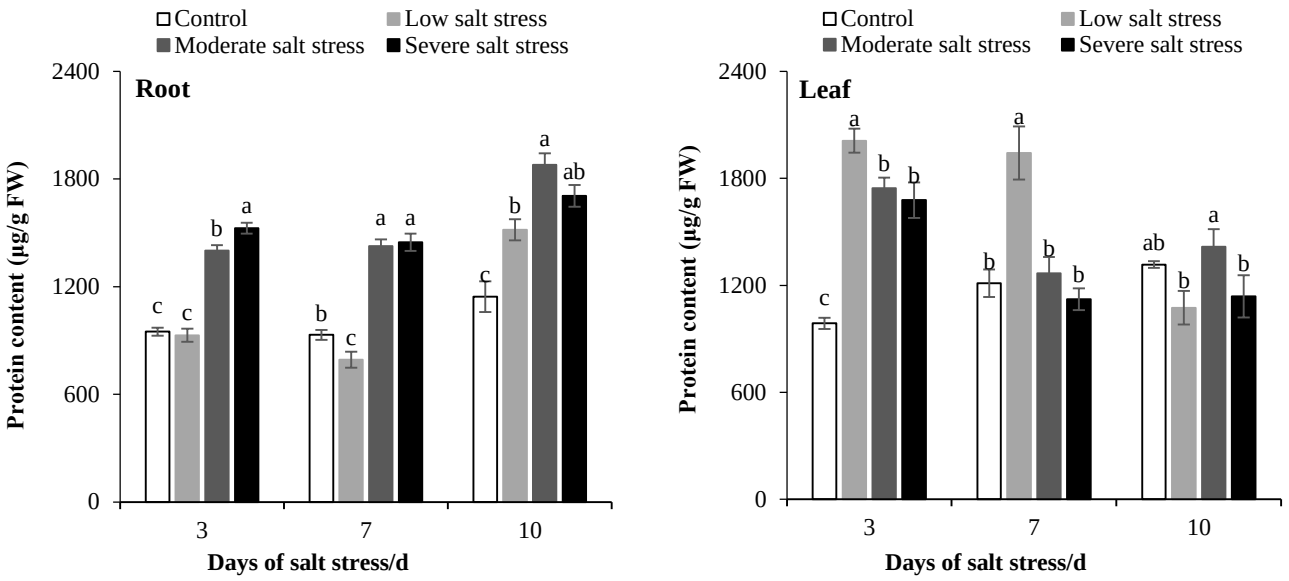

Fig. 6. Changes in the content mustard seedling protein subjected to salt stress (0, 50, 100, and 200 mM NaCl for 3, 7 and 10 d). Means followed by different lowercase letters differ significantly according to Duncan's multiple range test, $p<0.05$, n = 3.

Discussion

Salinity is the major factor for adversity factors, and negatively impacts the global environment and economy (Munns, 2005). The adaptability of mustard to salt stress is a comprehensive reflection of many factors. Plant morphology, leaf characteristics, photosynthesis, RSA, antioxidant enzyme activity and biomass allocation are important indicators that reveal differences in the tolerance of plants to salt and are also crucial indicators that reflect the tolerance of plants to salt.

Changes in biomass are a comprehensive reflection of the response of a plant to salt stress and a direct indicator of the plants to salt tolerance (Levitt, 1980). Previous studies suggested that a 50% decrease in biomass was a critical survival threshold (Alshammary *et al*., 2004). Our results indicated that the reduction in seedling dry weight was 14.3%, 40.7% and 83.6% under 50, 100 and 200 mM NaCl, respectively. Thus, 100 mM NaCl was a survival threshold for mustard seedlings. The distribution of biomass in different tissues and organs reflects the response of plants to stress. In this study, the plant biomass was inhibited by salt stress on 10 DAT, while the root-shoot ratio increased significantly by 26.1% following treatment with severe stress, indicating that more dry matter accumulates in the roots under severe stress (Table 1). Increasing the root-shoot ratio is a strategy by which plants respond to salt stress. Previous studies on elevated root-shoot ratios under stress have been reported in maize (*Zea mays*) (Ren *et al*., 2020) and pepper (*Capsicum annuum*) (Tang *et al*., 2020), suggesting that plants preferentially transport photosynthetic products to roots under severe stress, which helps to maintain root growth and increase the total surface area of root absorption.

Photosynthesis is undoubtedly the most important physiological process that affects plant growth and biomass. Chloroplasts are one of the sites in which ROS are primarily formed. The reasons for the decrease in photosynthesis by the accumulation of ROS include the destruction of chlorophyll structure, a decrease in the content of chlorophyll and the inhibition of PSII. Our results indicated that NaCl stress negatively affected the content of chlorophyll and PI_{ABS}. In addition, the reduction of leaf area caused by salt stress positively correlated with the content of chlorophyll (Table 3). Therefore, we hypothesized that salt stress inhibited photosynthesis and then reduced the shoot growth and biomass. PI_{ABS} and F_v/F_m can reflect the reaction center activity of PS Ⅱ, and the change in their values can reflect the inhibition of active centers by stress (Strasser *et al*., 2000). However, our results showed that F_v/F_m did not change under salt stress. These results were consistent with previous research in rapeseed (*Brassica napus*) (Hooks *et al*., 2019) and wheat (*Triticum sp.*) (Mehta *et al*., 2010). As previously reported, PI_{ABS} was suggested to be a more effective photosynthetic parameter than F_v/F_m under conditions of stress (Appenroth *et al*., 2001, Van Heerden *et al*., 2003). Thus, PI_{ABS} can be useful markers to screen mustard genotypes and identify salt-tolerant genotypes. The decrease of leaf area under salt stress is closely related to the chlorophyll content.

Plant roots are the primary part of the response to the stress, and the modification of RSA has been identified as an adaptive mechanism (Dorairaj *et al*., 2020). *Brassica* is composed of a main root (support and fixed) and lateral roots (absorption moisture and nutrients) (Arif *et al*., 2019). Stress conditions can have both negative and positive effects on the development of lateral roots (Sun *et al*., 2017). In this study, salinity reduced the growth and development of mustard seedling roots, particularly at severe salt stress but increased the number and density of first-order lateral roots by 28.7% and 58.5% on 10 DAT, respectively (Table 2). These results are consistent with those of quinoa (*Chenopodium quinoa*) (Panuccio *et al*., 2014), which suggested that the expansions of plant cells and lateral buds occurred because osmotic stress inhibited the uptake of water by the plant roots. The number and

density of the first-order lateral roots increased the root surface area to some extent. Considering the function of lateral roots, the increase in root surface area further improved the ability of plants to absorb water and nutrients, which, in turn, can be considered a strategy for plants to adapt to stress (Arif *et al*., 2019). This result was also demonstrated by a significant increase in the root-shoot ratio when the plants were subjected to severe salt stress, which indicated that the increase in the number and density of first-order lateral roots positively affected the accumulation of dry matter by the root.

As a product of membrane lipid peroxidation, the content of MDA positively correlated with membrane lipid damage (Chen *et al*., 2011). In our experiment, the content of MDA in the roots did not change and increased in leaves with severe salt stress compared with those that were not subjected to treatment with salt (Fig. 4). The specific changes in the content of MDA demonstrated that the leaves and roots had different mechanisms of adaptation to salt stress. There are two possible explanations for the result that the levels of MDA did not change when the plants were under severe salt stress. Wang *et al*., (2014) and Pan *et al*., (2006) suggested that the content of MDA only increased during the early hours of a high-concentration treatment and then dropped to a level close to that of the plants that were not subjected to stress. Another reason was that the highly effective antioxidant enzymes removed the toxicity of ROS and reduced the damage to membrane lipids. Combined with the fact that the root-shoot ratio significantly increased under severe salt stress, this suggested that effective activities were owing to the latter hypothesis.

Salt tolerance is related to the efficient anti-oxidative system that includes antioxidant compounds and several antioxidative enzymes (Neto *et al*., 2006). SOD is considered to be a key ROS scavenger owing its conversion of superoxide anion ($O_2^{\cdot-}$) to H_2O_2 and acts as the first line of defense against ROS. In contrast, other enzymes, such as POD, APX, and CAT, have main functions to detoxify H_2O_2 and can be induced by H_2O_2 to increase their activity (Mittova *et al*., 2004). The activity of SOD of roots maintained a higher level than the control and reached its peak on day 3 under saline conditions. The activities of CAT, APX, and POD also increased rapidly. In contrast, different trends of variation were observed in the leaves. The activity of SOD in leaves only significantly increased on 7 DAT, while the activity of POD increased on 10 DAT (Fig. 5). The synergistic effect of antioxidant enzymes in roots slowed down the production of ROS and improved the adaptability of roots to salt. Similar results were observed in rice (Nounjan *et al*., 2012) and sesame *(Sesamum indicum)* (Koca *et al*., 2007).

In addition, the activity of CAT tended to increase in both the roots and leaves treated with salt, and the activity of POD maintained a relatively high level in the roots throughout the experiment. It could be assumed that CAT and POD play an important role in scavenging ROS. Similar results were showed that two cultivars of sesame that are strongly tolerant to stress have higher activities of POD and CAT (Koca *et al*., 2007). Alternatively, efficient ROS detoxification in plants may suggest that maintaining a certain level of ROS may be necessary for cell proliferation and differentiation (Mittler, 2017). A hydroponics study proved that zinc stress stimulated an increase in the lateral roots in *B. juncea* and *B. napus* (Feigl *et al*., 2016). Altogether, this research suggested that the antioxidant system increased the number and density of lateral roots, which in turn enhanced the tolerance of roots to higher levels of salt.

Conclusions

This study suggested that roots have a more effective mechanism of adaptation than shoots that were subjected to high salinity. Its mechanisms of adaptation included those of root morphology and the activation of an efficient antioxidative system. In addition, our results indicated that 100mM NaCl was a survival threshold for mustard seedlings, and PI_{ABS} can be considered a good indicator for screening mustard genotypes. Understanding the mechanisms of the adaptation of mustard roots and shoots to salt could be of great importance. It may provide a theoretical basis for further analysis on genotypes of mustard that are tolerant to salt.

Acknowledgements

This work was supported by Program for Innovative Research Team (in Science and Technology) in University of Henan Province (21IRTSTHN023), China.

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(Received for publication 11 February 2021)