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Comparative effects of salt and alkali stress on photosynthesis and root physiology of oat at anthesis

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Abstract: The effects of different concentrations of salt and alkali on root physiology and photosynthesis in oat (*Avena sativa* L.) at anthesis were compared. Treatment with salt and particularly with alkali, decreased yield and grain weight. Both alkali and salt treatments decreased the photosynthetic rate (Pn), stomatal conductance and chlorophyll content, with the effect on Pn and the chlorophyll content more marked after treatment with alkali. The decrease in Pn induced by salt and mild alkali (50 mmol L^{-1}) contributed to a decrease in stoma opening. Under moderate and severe concentrations of alkali (100 and 150 mmol L^{-1} respectively), other factors rather than stoma closure were responsible for limiting Pn. The transpiration rates (Tr) in alkali-treated plants were higher than in salt-treated plants. We also observed decreases in both root volume and root dry weight, and increases in root activity in alkali- and salt-treated plants. Severe alkali and salt concentrations (150 mmol L^{-1}) decreased the root-shoot ratio. A positive correlation between yield and root dry weight and a negative correlation between root activity and Pn were observed. Alkali and salt treatments increased superoxide dismutase (SOD) and peroxidase (POD) activities and the malondialdehyde (MDA) content, but decreased catalase (CAT) activity. Yield was negatively correlated with MDA, and the MDA content was greater under alkali treatments than after salt treatment. We conclude that alkali treatments had more severe effects on oat plants at anthesis than salt treatments.

Key words: oat; salt stress; alkali stress; photosynthesis; root physiology; anthesis

INTRODUCTION

Soil salinization and alkalization have very adverse effects on agricultural production [1]. About 20% of the world's cultivated land is threatened by salinity, with this figure rising to 50% for irrigated land [2]. In China, especially in Inner Mongolia, salinity and alkalinity threaten more than 50% of the available arable land. Many studies have investigated the physiological mechanisms of plants in response to salinity and found that salinity reduces the osmotic potential of the soil solution, causing water stress, and that excess Na^+ disrupts the ion balance and causes ion stress, leading to oxidative stress, which inhibits respiration and photosynthesis, causing nutrition deficiency [2]. Recent studies have demonstrated that salt stress can affect physiological processes, including osmotic adjusting materials (soluble sugars, proline, polyamines), antioxidant defense mechanisms, ion compartmentation and hormonal regulation [3].

In the past few decades, studies have focused on salt and/or mixed salt-alkali stress on plants, with little attention paid to the effects of alkalinity alone, despite the more severe effects of alkalinity as compared to salinity [4]. Understanding of the physiological responses of crop plants to alkalinity is of paramount importance to the selection of genotypes with improved tolerance to alkali stress.

The polyamines putrescine (Put), spermidine (Spd) and spermine (Spm), which play essential roles

in osmotic adjustment, have also been found to play a role in the antioxidant system by eliminating reactive oxygen species (ROS) when plants are experiencing salt and high temperature stresses [5,6]. We hypothesized that polyamines affect the activity of antioxidant enzymes during alkali stress.

Studies have shown that the effects of salinity on crops are different during various growth stages [5,7]. Therefore, it is essential to differentiate between the physiological mechanisms that respond to stress at each growth stage. However, most studies have concentrated on the seedling growth stage and not on the reproductive growth stage, with the physiological changes induced by salinity or alkalinity at the seedling stage described for wheat, rice, barley, oat, tomato and *Suaeda glauca* [8-12]. The typical reproductive stage, anthesis, is the key growth period that influences crop yield and it warrants further study.

Oat (*Avena sativa* L.) is a moderately salt-tolerant crop that has the potential to improve saline and alkaline soils as a biological agent. In Baicheng City (China), field tests showed that oat plants could produce seeds even under severe salt treatment (200 mmol L^{-1} NaCl:Na_2SO_4=1:1) and could tolerate soil pH values up to 9.0. Studies on the response mechanisms of oat plants to alkalinity and salinity are limited, as reduced oat production has restricted research efforts for more than 50 years [13] and the physiology of oat plants at anthesis during salt and alkali stress is unreported.

Roots absorb nutrients and water and play a role in the synthesis of amino acids, organic acids and organophosphorus compounds [14,15]. In this study, we investigated the comparative effects of salt and alkali concentrations on oat root physiology at anthesis and determined whether this plays an important role in both yield and salt and alkali tolerance of oat plants.

MATERIALS AND METHODS

Experimental design

This study was conducted under greenhouse conditions at the Inner Mongolia Agricultural Science Center from May 2011 to August 2012. The Canadian Eastern Cereal and Oilseed Research Center provided seeds of the salt- and alkali-tolerant oat genotype Vao-9 for this study. Twenty Vao-9 seeds were sown in sand-filled (6 kg), 20-cm diameter pots and subsequently thinned to 15 plants per pot. The oat plants were watered with Hoagland nutrient solution every three days [16]. The average temperature was 16-28°C during the day and 10-15°C during the night, and the relative humidity was 50-60%. There were three replicates. Two experimental treatments were applied: salinity (NaCl and Na_2SO_4 at a 2:1 molar ratio) and alkalinity (Na_2CO_3 and $NaHCO_3$ at a 1:2 molar ratio). When oats were at the two-leaf stage, plants were treated with 200 mL (per plot) of increasing salt (0, 50, 100 and 150 mmol L^{-1}) and alkali (0, 50, 100 and 150 mmol L^{-1}) concentrations, or with water (control plants). To prevent salt shock, the salt and alkali treatments were initiated with low concentrations. Treatments were applied every two days, and there were four stress treatments in total (Table 1). Root samples were collected and stored in liquid nitrogen at anthesis for further analysis.

Table1. The frequency and concentration of salt and alkali at the two-leaf stage. All four treatments were applied at the two-leaf stage.

Treatment times	Salt (mmol L^{-1})				Alkali (mmol L^{-1})			
First time	0	12.5	25	37.5	0	12.5	25	37.5
Second time	0	25	50	75	0	25	50	75
Third time	0	37.5	75	112.5	0	37.5	75	112.5
Fourth time	0	50	100	150	0	50	100	150

Photosynthesis

The net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) of oat plants were measured using an Li-6400 portable photosynthesis apparatus (LICOR, USA) at anthesis. Photosynthetic characteristics were measured using the second functional leaves. A stoma limitation value (Ls) was calculated using the formula: Ls=1-Ci/Ca, where Ca is the CO_2 concentration (378-389 mmol mol^{-1}). The chlorophyll content was measured using a SPAD meter (502Plus) [17].

Root activity, root volume, root dry matter and the root-shoot ratio

Root activity was measured in accordance with the triphenyl tetrazolium chloride (TTC) method [18].

TTC is reduced by dehydrogenases that are considered an indicator of root activity. Root volume was determined using the immersion method [19]. For dry weight determination, roots and aerial parts of six oat plants were separated using scissors and placed into a drying oven at 105°C for 30 min, followed by 80°C for 24 h. The ratio of root dry weight to shoot dry weight was calculated to determine the root-shoot ratio.

Antioxidant enzyme activities and MDA content

SOD, POD and CAT activities and the MDA content were determined according to the methods of Bai et al. [20]. Root samples (1 g) were suspended in 10 mL of precooled (4°C) extraction buffer (0.05 mol L^{-1}, pH 7.8, phosphate buffer) and then ground to a fine powder using a ceramic mortar and pestle. The homogenate was centrifuged at 3000 *x* g at 4°C for 1 min. The crude extract was then centrifuged at 13000 *x* g at 4°C for 20 min.

The content of MDA, which is an end-product of lipid peroxidation, was determined using the thiobarbituric acid (TBA) reaction. Two mL of supernatant and 2 mL of 0.5% (w/v) TBA in 10% (w/v) trichloroacetic acid (TCA) were mixed. The mixture was heated at 100°C for 30 min, after which it was cooled in an ice bath. Absorbances of the supernatants were recorded at 532 nm and 600 nm.

SOD activity was assayed by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm. The reaction mixture contained 2 mL of 15 mmol L^{-1} L-methionine, 0.1 mL of 0.1 mmol L^{-1} NBT, 0.1 mL of 0.1 mmol L^{-1} riboflavin and 2 mL of 0.003 mmol L^{-1} EDTA. The reaction was started by adding 0.1 mL of the supernatant. A complete reaction mixture without enzyme addition, which produced maximal color, served as the control. Distilled water served as a blank. Absorbance was recorded at 560 nm.

POD activity was determined using a reaction mixture of 50 mL of phosphate buffer (0.05 mol L^{-1}, pH 6), 28 µL of guaiacol and 19 µL of H_2O_2. Three mL of the mixture and 1 mL of the supernatant were mixed. Absorbance was recorded at 470 nm.

CAT activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm. The reaction mixture contained 1.7 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.8), 1.7 mL of 0.1 mmol L^{-1} EDTA-Na_2 and 0.2 mL of 0.1 mol L^{-1} H_2O_2. The reaction was started by adding 0.1 mL of the supernatant and the decrease in absorbance at 240 nm for 5 min was recorded.

Polyamine contents

Put, Spd, and Spm were measured as described [21]. Samples (2 g) were suspended in 4 mL of precooled (4°C) extraction buffer (5% (v/v) aqueous perchloric acid) and then ground to a fine powder using a ceramic mortar and pestle. The homogenate was centrifuged at 15000 *x* g at 4°C for 30 min. Then, 500 µL of the supernatant, 7 µL of benzoyl chloride and 1 mL of NaOH (2 mol L^{-1}) were mixed. The mixture was vortexed vigorously and then incubated for 20 min at 37°C. Two mL of saturated NaCl solution were added to stop the reaction, and 2 mL of ether was used for the benzoyl-polyamine extractions. Finally, 1 mL of the ether phase was vacuum-dried and dissolved in 100 mL of methanol.

The polyamine content was analyzed using an high-performance liquid chromatography (HPLC) system (Waters 1500) supplemented with a 2487-nm dual wavelength UV-Vis detector with a Spursil C18 column (Waters, 150×mm, 4 µm). The mobile phase consisted of a methanol:water ratio of 60:40 (V/V), a flow rate of 0.7 mL min^{-1} and a detection wavelength of 230 nm. The column temperature was maintained at 30°C.

Yield

Oat plants in the control, alkali- and salt-treated groups were harvested at maturity and dried at 50°C for 60 h, after which the yield and components were measured. Panicles were hand-threshed. The empty grains were separated from filled grains by immersion in water. The numbers of spikelets and grains per spike of 15 oat plants were averaged. The 100-grain yield and yield (20 oats) were weighed with an electronic balance.

Statistical analysis

The data were expressed as the means±SE (n=3). Correlation and one-way analysis of variance (ANOVA)

Table 2. Effects of alkali and salt on oat yield and components in pot experiment.

Treatment	Grain weight (g)	Grain number per spike	Effective panicles	Yield (g)
Control	2.01±0.13a	6.01±0.26b	5.76±0.03a	11.91±0.48a
50 mmol L^{-1} salt	1.93±0.062a	9.10±1.00a	5.80±0.22a	9.68±0.28b
100 mmol L^{-1} salt	1.88±0.12ab	8.28±0.98a	6.10±0.13a	7.90±0.26b
150 mmol L^{-1} salt	1.59±0.06b	4.26±0.01bc	6.27±0.05a	4.90±0.76cd
50 mmol L^{-1} alkali	1.81±0.06a	6.92±0.31a	6.84±0.65a	8.43±0.31b
100 mmol L^{-1} alkali	1.60±0.06b	8.92±0.48a	7.28±1.00a	6.30±0.21c
150 mmol L^{-1} alkali	1.47±0.05b	3.42±0.33c	5.98±0.03a	4.28±0.31d

Values are means±SE (n=3). Different letters in a column denote significantly different average values at the 0.05 level of probability.

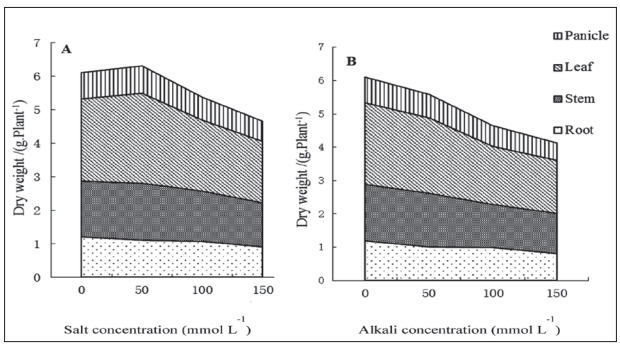

Fig.1 Effects of salt stress (**A**) and alkali stress (**B**) on dry weights of panicle, leaf, stem and root.

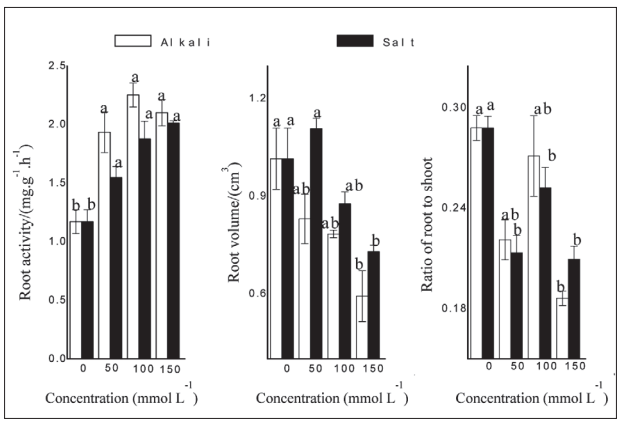

Fig. 2 Effects of salt and alkali treatments on root activity, volume and on the root/shoot ratio at anthesis. Each value is the mean of three replicates; vertical bars: ±standard error. Different letters denote significant differences at the 0.05 level of probability.

were calculated using SAS 9.0. P<0.05 was regarded as a significant difference, P<0.01 was regarded as a highly significant difference.

RESULTS

Yield and its components

Salt and alkali treatments significantly decreased oat yield. Adverse effects of alkalinity on yield were more severe than those of salinity. The yield was reduced by 29.26%, 47.13% and 64.11% by 50, 100, and 150 mmol L^{-1} alkali treatments, respectively. A decrease in yields of 18.75%, 33.66% and 58.83% occurred under the 50, 100, and 150 mmol L^{-1} salt treatments, respectively. There was no difference in spike number per panicle among the four levels of stress treatment. Grain number per spike of salt-treated plants increased by 51.07% and 37.46% as compared to 14.87%, 48.05% for the alkali-treated plants (Table 2).

Root activity, root volume, root dry matter and the root-shoot ratio

Salt and alkali treatments caused a decrease in root dry matter and root volume (Figs. 1 and 2). Root dry matter was positively correlated with the yield (Table 6). The lowest root dry matter was observed at the 150-mmol L^{-1} concentration. The root-shoot ratio was decreased by 50, 100 and 150 mmol L^{-1} of salt and alkali treatments. The root activity increase ranged from 48.1%-87.5% under 50-, 100- and 150-mmol L^{-1}alkali treatments, and from 33.3%-66.7% under 50-, 100- and 150-mmol L^{-1} salt treatments when compared to the control. (Figs. 1 and 2). The root volume was lower after alkali treatments than after salt treatments, and root activity was higher after alkali treatments than after salt treatments (Fig. 1).

Table 3. The effect of salt and alkali treatments on photosynthesis of oat leaves at anthesis.

Treatment	SPAD	Pn (μmol $m^{-2}·s^{-1}$)	Gs (mmol mol^{-1})	Tr (mmol $m^{-2}·s^{-1}$)	Ci (μmol mol^{-1})	Ls	Pn/Ci
Control	42.23±0.63a	8.19±0.02a	0.14±0.002a	4.46±0.18b	257.88±13.49a	0.32±0.01d	0.032±0.001b
50 mmol L^{-1} salt	40.08±0.66a	6.13±0.05b	0.06±0.005b	1.87±0.02e	174.08±12.62c	0.53±0.01b	0.035±0.001a
100 mmol L^{-1} salt	39.98±1.20ab	6.02±0.07b	0.06±0.006b	1.86±0.01e	156.35±12.76c	0.58±0.01ab	0.038±0.001a
150 mmol L^{-1} salt	37.91±1.40b	4.35±0.06d	0.06±0.005b	1.59±0.01e	157.95±16.18c	0.58±0.02a	0.028±0.001c
50 mmol L^{-1}alkali	36.59±0.47b	5.83±0.08c	0.06±0.005b	5.73±0.03a	222.20±12.08b	0.42±0.01c	0.026±0.001c
100 mmol L^{-1}alkali	35.50±0.95b	4.34±0.07d	0.05±0.004bc	3.29±0.13c	261.70±16.78a	0.32±0.02d	0.017±0.001d
150 mmol L^{-1}alkali	33.67±0.53b	3.40±0.14e	0.03±0.001d	2.56±0.05d	281.11±12.21a	0.27±0.03d	0.012±0.001e

SPAD – chlorophyll contents. Values are means±SE (n=3). Different letters in a column denote average values different at the 0.05 level of probability.

Table 4. The effects of salt and alkali treatments on the antioxidant enzymes in root at anthesis.

Treatment	SOD ($Ug^{-1}min^{-1}$)	POD ($Ug^{-1}min^{-1}$)	CAT ($Ug^{-1}min^{-1}$)	MDA ($nmolg^{-1}$)
Control	2015.±104c	3.24±0.03b	10.04±1.31a	4.12±0.14c
50 mmol L^{-1} salt	3632±106b	5.99±0.55a	9.18±0.49ab	6.06±0.31bc
100 mmol L^{-1} salt	3999±72b	5.99±0.36a	8.75±0.11ab	7.33±1.44b
150 mmol L^{-1} salt	5527±70a	4.96±0.48ab	7.55±0.38b	11.19±0.63a
50 mmol L^{-1} alkali	3469±395b	4.35±0.16a	6.39±0.34b	8.56±0.44b
100 mmol L^{-1} alkali	3442±177b	3.05±0.16b	8.72±1.27ab	8.99±1.90ab
150 mmol L^{-1} alkali	5378±37a	2.63±0.05b	3.99±0.02c	11.19±0.79a

Values are means±SE (n=3). The different letters within a column denote significantly different average values at the 0.05 level of probability.

Photosynthesis

As salt and alkali concentrations increased, Pn and gs decreased. The extent of the decrease after alkali treatments was greater than after the salt treatments ($p<0.05$). Pn was positively correlated with the yield and root dry matter ($P<0.05$) (Table 6). Mild alkali treatment (50 mmol L^{-1}) caused an increase in the Tr of plants, while the 100-and 150-mmol L^{-1} alkali treatments and the 50-, 100- and 150-mmol L^{-1} salt treatments decreased the Tr. The Tr was higher in plants under alkali stress than under salt stress. When compared to the control, the 100- and 150-mmol L^{-1} alkali concentrations significantly increased Ci, while the 100- and 150-mmol L^{-1} salt treatments led to a decrease in Ci (Table 3).

Compared to the control, all salt treatments and the mild alkali treatment (50 mmol L^{-1}) increased the Ls, while the severe alkali treatment (150 mmol L^{-1}) caused a decrease. Compared to the control, the 50- and 100-mmol L^{-1} salt concentrations caused increases in Pn/Ci, while the 150-mmol L^{-1} salt concentration and the 50-, 100- and 150-mmol L^{-1} alkali concentrations caused a decrease. The chlorophyll contents (SPAD value) decreased under both stresses (Table 3). There was a significant positive correlation between the yield and chlorophyll contents, and a negative correlation between the chlorophyll and MDA contents ($P<0.05$) (Table 6). Correlation analysis also showed that Pn was positively correlated with chlorophyll content (Table 7).

Antioxidant enzymes

Salt and alkali treatments stimulated an increase in SOD and POD activities in roots. The exception was a decrease in POD activity in the 150-mmol L^{-1} alkali-treated plants. CAT activity under both stresses decreased ($P<0.05$). SOD activity was negatively correlated with yield, and CAT activity was positively correlated with yield under salt treatments (Table 6). Of the four salt treatments, the highest SOD activity occurred at 150 mmol L^{-1}, and the highest POD activity occurred at 100 mmol L^{-1}, with increases of 174.26% and 85.26%, respectively, as compared to the control. Of the four alkali treatments, the highest SOD activity was observed at 150 mmol L^{-1}, and the highest POD activity at 50 mmol L^{-1}, with increases of 166.83% and 34.37%, respectively, as compared to the control. SOD, POD and CAT activities in salt-treated roots were higher than in alkali-treated roots. Salt and alkali treatments led to an accumulation of MDA, although it was lower in salt-treated roots than

Table 5. The effects of salt and alkali treatments on polyamines in roots at anthesis.

Treatment	Put (nmolg^{-1})	Spd (nmol g^{-1})	Spm (nmolg^{-1})	(Spd+Spm) Put
Control	266.20±8.43a	41.42±1.77d	8.47±0.83d	0.19±0.02e
50 mmol L^{-1}Salt	220.79±6.73b	92.59±5.94b	14.30±1.49c	0.48±0.04c
100 mmol L^{-1}Salt	171.75±2.50c	103.39±2.61b	21.40±0.50bc	0.73±0.01b
150 mmol L^{-1}Salt	159.69±0.54c	82.40±10.27b	23.83±1.04b	0.67±0.07b
50 mmol L^{-1}Alkali	206.30±8.49b	53.98±3.32d	16.30±1.90c	0.34±0.01d
100 mmol L^{-1}Alkali	153.30±3.02c	69.30±7.05c	23.19±0.70b	0.60±0.06bc
150 mmol L^{-1}Alkali	146.98±6.31c	120.29±7.78a	28.40±1.27a	1.01±0.02a

Values are means±SE (n=3). Different letters in a column denote significantly different average values at the 0.05 level of probability.

Table 6. Correlation analysis of yield and physiological indexes for oat leaves and roots at anthesis.

Parameter	Salt	Alkali
SPAD	0.95*	0.96*
Pn	0.97*	0.97*
SOD	-0.99*	-0.94
POD	-0.46	0.44
CAT	0.99*	0.78
MDA	-0.988*	-0.978*
RA	-0.86*	-0.76
RW	0.98*	0.95*
RV	0.78	0.98*
Put	0.95	0.98*
Spd	-0.58	-0.90
Spm	-0.96*	-0.99*

Asterisks (*) indicate significant correlation, *(P<0.05). RA – Root activity; RW – root dry weight; RV – root volume; Pn – net photosynthetic rate; SOD – superoxide dismutase; POD – peroxidase; CAT – catalase; Put – putrescine; MDA – malondialdehyde; Spd – spermidine; Spm – spermine.

Table 7. Correlation analyses of physiological indices of oat leaves and roots at anthesis

Item	SPAD	Pn	SOD	POD	CAT	MDA	RA
SPAD	1						
Pn	0.98*	1					
SOD	-0.96*	-0.99*	1				
POD	-0.76	-0.61	0.55	1			
CAT	0.92	0.98*	-0.99*	-0.45	1		
MDA	-0.96*	-0.97*	0.98*	0.60	-0.97*	1	
RA	-0.97*	-0.96*	0.94	0.65	-0.94	0.99*	1
RW	0.97*	0.95*	-0.97*	-0.35	0.99*	-0.96*	-0.88
RV	0.96*	0.97*	-0.96*	-0.06	0.74	-0.98*	-0.75
Put	0.95*	0.93*	-0.96*	-0.65	0.95*	-0.99*	-0.37
Spd	-0.83	-0.69	0.95*	0.98*	-0.96*	0.71	0.77
Spm	-0.95*	-0.93*	0.95*	0.61	-0.96*	0.99*	0.99*

Asterisks (*) indicates significant correlation (P<0.05). RA – root activity; RW – root dry weight; RV – root Volume; Pn – net photosynthetic rate; SOD – superoxide dismutase; POD – peroxidase; CAT – catalase; MDA – malondialdehyde; Put – putrescine; Spd – spermidine; Spm – spermine.

in alkali-treated roots (Table 4). There was a significant negative correlation between the MDA content and yield (Table 6). Correlation analysis also showed that MDA contents correlated negatively with both Pn and chlorophyll contents (Table 7).

Polyamines

Salt and alkali treatments decreased the Put content but increased Spd and Spm contents (P<0.05). There was a positive correlation between Put and yield (Table 6). Spm correlated negatively with MDA, yield and chlorophyll contents (P<0.05) (Table 7). As alkalinity and salinity increased, an improvement of the (Spd+Spm)/Put ratio was observed (Table 5). Of the salt treatments, the 100- and 150-mmol L^{-1} concentrations caused the largest increases in Spd and Spm contents, by 149.59% and 181.21%, respectively, as compared to the control. Of the alkali treatments, the 150-mmol L^{-1} concentration caused the largest increase in Spd and Spm contents, by 190.40% and 235.16%, respectively, as compared to the control. The extent of the increase under alkali treatment was larger than that under the salt treatment.

DISCUSSION

Effects of salt and alkali treatments on yield

Both salt and alkali treatments lowered oat yield and grain weight. The yield, grain number per spike and grain weight of alkali-treated plants were all lower than in salt-treated plants. This result is consistent with Yang et al. [22], and the higher pH levels, which may cause lower yields, in alkali-treated plants previously reported [23].

Effects of salt and alkali treatments on photosynthesis

There was a significant positive correlation between yield and Pn, measured in the second functional leaves, indicating that the decrease in yield under salt and alkali treatments is related to photosynthesis at the flowering stage.

In recent years, studies have mostly concentrated on the effects of salt and mixed salt-alkali stresses on photosynthesis. The decrease in Pn induced by exposure to a mixed salt-alkali stress in barley and oat was most likely the result of stomatal closure [20]. Studies related to alkali treatment are scarce [24,25].

Some studies have reported that the relative magnitude of stoma closure and non-stoma factors in limiting Pn might depend on stress severity. Non-stoma factors lead to a reduction in chloroplast activity and changes in some biochemical processes [26]. Consistent with this result, it was found that Pn, Ci, and gs were decreased and Ls increased in response to mild alkalinity (50 mmol L^{-1}). However, at moderate (100 mmol L^{-1}) and severe (150 mmol L^{-1}) alkali concentrations, Ci increased, but Ls did not change significantly when compared to the control. This suggests that stoma closure limited the photosynthetic rate under mild alkali treatment (50 mmol L^{-1}), and that other factors, rather than stoma closure, limited Pn under moderate (100 mmol L^{-1}) and severe (150 mmol L^{-1}) alkali concentrations at the flowering stage. The non-stomatal factors include the cumulative effects of water potential, osmotic potential and different biochemical composition. These results are not consistent with those of Yang [27], who reported that barley Ci decreased under severe alkali concentrations. This inconsistency may be due to differences in species.

Some studies have reported that stoma closure is the main driver of Pn reduction caused by salinity [28]. However, other studies have reported that salinity has little effect on Ci, and that non-stomatal factors mainly limit the photosynthetic rate [27]. In this study, salinity decreased Pn, Ci and gs, but stoma closure mainly limited the Pn of oat plants at the flowering stage.

Salinity caused higher Pn/Ci levels than alkalinity, suggesting that the effect of alkali treatment on the activities of photosynthesis-carbon assimilation enzymes was greater than that of salt treatment. Both salt and alkali treatments caused a significant decrease in the chlorophyll content, and the extent of the reduction in alkali-treated oats was larger than that in salt-treated oats. This result is similar to that of Yang [29], who reported that salt and alkali treatments decreased the chlorophyll content of wheat, perhaps because of an improvement in chlorophyll-degradation enzyme activity or deficiency in metal ions. A positive correlation between the chlorophyll contents and Pn was observed, indicating that the reduction in chlorophyll content mainly limits the photosynthetic rate. At moderate (100 mmol L^{-1}) and severe (150 mmol L^{-1}) salt and alkali concentrations, Tr decreased. However, Tr levels of alkali-treated plants were higher than in salt-treated plants, suggesting that alkalinity can facilitate the increase in transpiration, which could reduce leaf temperature.

Effects of salt and alkali treatments on root activity, root volume, root dry matter and the root-shoot ratio

In recent years, the relationship between plant aerial parts and roots has received considerable attention, and it was concluded that root growth can affect shoot growth and yield production [30]. To further elucidate the correlation between roots and photosynthesis and the effects of salinity and alkalinity on their relationship, the root dry matter, root volume, root activity, antioxidant enzyme activities and polyamine contents of roots were measured at four salt and alkali concentrations at the flowering stage.

There was a positive correlation between root dry matter and yield. Similar results have also been reported in rice [31]. In this study, root activity correlated negatively with yield in salt-treated plants, and root volume correlated positively with yield in alkali-treated plants, suggesting that at the flowering stage, root physiology influenced the yield. The changes in root physiology caused by salinity and alkalinity stresses might affect the absorption of water and nutrients, which would subsequently influence yield.

Further, salinity and alkalinity decreased root dry matter and root volume. This result is in agreement

with Burcu et al. [32], who reported that salinity decreased root dry matter in wheat. The extent of the decrease observed in alkali-treated plants was greater than in salt-treated plants, indicating that the adverse effects of alkalinity on roots were more severe than those of salinity. Under both stresses, root dry matter negatively correlated with SOD activity and with the MDA content, indicating that ROS is one of the main factors responsible for root dry matter reduction under these stresses.

Under severe salt and alkali concentrations (150 mmol L^{-1}), the root-shoot ratio decreased, suggesting that the inhibitory effect of severe stress on the roots was much greater than on aerial parts. This may be due to the energy consumption of growing roots, which is twice that of the aerial parts. Unnecessary energy consumption caused by root redundancy could limit the yield [33]. Thus, a decrease in the root-shoot ratio increases salinity and alkalinity tolerance. In conclusion, tolerance to both stresses depends on the coordination between roots and the growth of aerial plant parts.

Effects of salt and alkali treatments on antioxidant enzymes

There was a significant negative correlation between the MDA contents and yield. This suggests that lipid peroxidation caused by ROS limited the yield under saline and alkaline conditions. Thus, the increase in SOD and POD activities could contribute to the improvement of oat yield.

Many studies have investigated the effects of salt on the antioxidant system. Some researchers have claimed that salinity causes ROS accumulation, which leads to damage caused by lipid peroxidation. Many higher plants resist ROS by increasing antioxidant enzyme activities, thereby improving salt tolerance. In this study, salt and alkali treatments increased SOD and POD activities but decreased CAT activity. This result is not consistent with that of Burcu et al. [32], who reported that salinity increases POD activity but decreases SOD and CAT activities in wheat roots.

SOD is the first line of defense against ROS. SOD catalyzes the reaction of $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$, after which H_2O_2 is broken down by POD and CAT. In this study, the increase in MDA content after salt and alkali treatments points to ROS accumulation. Compared to the salt treatments, the higher MDA contents after alkali treatments suggested that alkali caused more severe lipid peroxidation. The increase in SOD and POD activities could reduce lipid peroxidation and improve the resistance to salt and alkali. The function of POD is similar to that of CAT, and the improvement in POD activity could make up for the limitation of CAT activity caused by stress [34].

Effects of salt and alkali treatments on polyamines

Polyamines affect plant growth and play important roles in plant resistance to drought and salt treatments [35]. However, only a few studies have examined the mechanism of polyamine response to alkali treatment. Some reports have shown that salinity causes the accumulation of Put in nonphotosynthetic organs [15], whereas some researchers have reported that salinity decreases the Put content and increases the Spm content [21]. In this study, the alkali treatment decreased the Put content and increased the Spd and Spm contents in roots. The improvement of the (Spd+Spm)/Put ratio was observed under alkali treatment. Studies have proved that the increase in polyamine contents and (Spd+Spm)/Put could improve salt resistance [36]. Thus, the improvement of Spd, Spm and (Spd+Spm)/Put may contribute to increased alkali tolerance in oats.

Goyal [5] and Zhang [21] have reported that polyamines also participate in the elimination of ROS and are positively correlated with the activity of antioxidant enzymes. In this study, there was a significant positive correlation between Spm content and SOD activity, and a positive correlation between MDA content and Spm for both salt and alkali treatments. This phenomenon suggests that Spm enhances the membrane stability of oats exposed to salt and alkali treatments.

CONCLUSION

Alkali and salt had different effects on oat physiology at anthesis. Stoma closure limited Pn after salt treatments, while other factors, rather than stoma closure, limited Pn under moderate (100 mmol L^{-1}) and severe (150 mmol L^{-1}) alkali treatments. The extent of

the decrease in yield and root dry matter was greater after alkali treatments than after salt treatments. In both salt- and alkali-treated plants, the root-shoot ratio decreased, possibly in an attempt to save energy for increased stress tolerance. In plant roots, the increase in SOD activity contributed to catalyzing superoxide radicals to hydrogen peroxide, and the improvement of POD activity helped eliminate the peroxide. Spm contents, which positively correlated with SOD activity, contributed to the elimination of ROS. Root dry matter and root volume correlated positively with yield, and root factors contributed to yield production. Our data suggest that photosynthesis and root physiology at anthesis were closely related to yield production under increased salt and alkali conditions. The adverse effects on oat plants of alkali were greater than those of salt. Therefore, future research should focus on the effects of alkaline conditions on oat production.

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