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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES TO NACl SALINITY STRESS IN THREE *ROEGNERIA* (POACEAE) SPECIES

JIHONG XIE[1,2#], YATING DAI[1,2#], HUAIBIN MU[1,2], YING DE[1,2], HAO CHEN[3], ZINIAN WU[1,2], LINQING YU[1,2] AND WEIBO REN[1,2*]

[1]*Grassland Research Institute, the Chinese Academy of Agricultural Sciences, Hohhot 010010, China*
[2]*Key Laboratory of Grassland Resources and Utilization, Ministry of Agriculture, the Chinese Academy of Agricultural Sciences, Hohhot 010010, China*
[3]*College of Science, Inner Mongolia University of Technology, Hohhot 010051, China*
[#]*Jihong-Xie and Yating-Dai contributed equally to this work*
**Corresponding author's e-mail: rppcaucau@163.com; Tel: +86-0471-4926906; Fax: +86-0471-4926906*

Abstract

This study aimed to identify the most tolerant species under salinity stress, amongst *Roegneria turczaninovii* (Drob.) Nevski var. *macrathera* Ohwi (*R. turczaninovii*), *Roegneria stricta* Keng (*R. stricta*) and *Roegneria komarovii* (Nevski) Nevski (*R. komarovii*). The seeds of three species were exposed to different NaCl concentrations (0, 75, 100, 125, 150, 175, 200, 225, 250 or 275 mmol/L) and the germination percentage (GP) was calculated after 7 days. Meanwhile, seedlings grown under normal condition at the two-leaf stage were subjected to 500 mL of NaCl solution (0, 30, 60, 90, 120, 150 or 180 mmol/L) for 7 days. Then the physical indicators such as plant height, root length, contents of chlorophyll (Chl), malondialdehyde (MDA), proline, soluble sugars, relative water content (RWC); and the biochemical changes including activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in three species under different concentrations of NaCl were determined. As a result, GP, plant height, root length, contents of Chl and RWC were reduced with the increase of concentration of NaCl, while MDA, proline, soluble sugars, SOD, POD and CAT were increased. *R. komarovii* achieved a higher GP; contents of Chl, RWC, proline, soluble sugars; SOD, POD and CAT activities; but a lower MDA content, compared to the other two species. Significant differences between any two species were detected ($P < 0.05$). *R. komarovii* is more resistant and tolerant in response to salinity stress than *R. turczaninovii* and *R. stricta*.

Key words: *Roegneria kamoji*; NaCl; Salinity stress; Tolerance; Chlorophyll; MDA; Antioxidant enzymes.

Introduction

Sodium chloride (NaCl) salinity is one of the most common abiotic stresses that inhibit plant growth and reduce the productivity (Jouyban, 2012; Ahmad *et al*., 2014). Reportedly, the loss of more than 60% of the potential yield of crops is caused by salinity stress in the environment (Munns, 2002; Tuteja *et al.*, 2013). Besides, salinity stress is the causative factor for osmotic stress and nutritional imbalance in plants (Hu *et al.*, 2012). Currently, the different species, genotypes and ecotypes of crops are very important sources for the identification of the tolerant and resistant variety to abiotic stresses such as salinity stress (Babakhani *et al.*, 2013). For instance, Sadeghi (2012) find that Afzal cultivar of *Hordeum vulgare* L. is more suitable to grow in the saline soils among 2 barley cultivars by determining several physiological characteristics under different salinity concentrations. In addition, Rao *et al*., (2013) determine the tolerance in 10 varieties of *Triticum aestivum* L. by measuring several physiological indexes and the antioxidative enzymes activities under salinity stress. Besides, 10 commercial tomato cultivars are exposed to the salinity stress to identify the nutritional and biochemical indicators for the most resistant variation, via examination of the contents of biochemical elements (Juan *et al.*, 2005).

Roegneria C. Koch a large genus, belongs to the tribe Triticeae of Poaceae, which is widely spread in Eurasia and the Americas (Li *et al.*, 2006). The genus was firstly researched as a model plant that named as *R.caucasica* C. Koch in 1848 (Baum *et al.*, 1991). There are 130 species in this genus and almost 79 of them are distributed in China, mainly in the northwest, southwest and north China. Having the advantages of good palatability, high protein content and high digestion rate, *Roegneria* C. Koch is beneficial to the economic and ecological environment as a kind of forage grasses (Yan *et al.*, 2009; Chang *et al.*, 2011). Several researches have investigated the morphology, cytology and molecular marker technologies, such as random amplification polymorphism DNA, amplified fragment length polymorphism and simple sequence repeat in *Roegneria* C. Koch (Cai, 2000; Peng *et al.*, 2012). Moreover, a recent study obtains a DREB-like gene *RcDREB1* in *Roegneria ciliar* and further explores its functions (Zhao *et al.*, 2013). However, the tolerance and resistance to salinity stress of plants in this genus are rarely involved. Therefore, we detected the physiological and biochemical changes in three *Roegneria* C. Koch species under different NaCl concentrations, including *Roegneria turczaninovii* (Drob.) Nevski var. macrathera Ohwi (*R. turczaninovii*), *Roegneria stricta* Keng (*R. stricta*) and *Roegneria komarovii* (Nevski) Nevski (*R. komarovii*), which are the common species in northwest China, to assess their resistance to salinity stress, and thus to identify high quality species for the development of agriculture and animal husbandry.

Materials and Methods

Plant material: The three species of *R. turczaninovii*, *R. stricta* and *R. komarovii* were cultivated respectively in Taibus Banner, Xilin Gol League, inner Mongolia in 2005 and harvested in 2006.

Seed germination: The seeds were disinfected with 0.1% $HgCl_2$ for 5 min and then rinsed with distilled water for 3-5 times. Subsequently, the seeds were cultivated on the MS medium that were mixed with different concentrations of NaCl (75, 100, 125, 150, 175, 200, 225, 250 and 275 mmol/L). The control group (CK) was cultivated on the MS medium without NaCl. All the seeds were cultivated in vitro under the condition of constant 25°C and 12 h of daylight for 7 days. Germination was determined in every 2 days during the whole germination period, based on the following formula:

$$\text{Germination percentage (GP)} = G_n/T_n \times 100\%$$

where G_n indicates the number of germinated seeds (in 6 days in this experiment) and T_n indicates the number of the totally tested seeds.

Seedling growth and the determination of root length and plant height: Seedlings were cultured in the plastic pots (20 cm diameter × 15 cm height), which contained the compounds of field soil, sandy soil, turf soil and vermiculite (1:1:1:1). Each pot was filled with 1.5 kg compounds and was drenched with running water. Then 30 seeds were placed into each pot by hill-drop drilling in a controlled environment chamber, with annual microclimate parameters of 4-30°C, 15-60% relative humidity and under the cycles of 16 h of light (110-120 umol m^{-2} s^{-1}) and 8 h of dark. Fifteen robust seedlings were chosen for final singling of seedlings at the two-leaf stage. There were 3 pots under each NaCl treatment (30, 60, 90, 120, 150 and 180 mmol/L), and the seedlings in each pot were watered with 500 mL NaCl solution at three-leaf stage for 7 days. The 3 pots in CK group were watered with distilled water for 7 days. The shoot height and root length were measured according to the method of Cha-um et al (Cha-um *et al*., 2006). Ten seedlings were chosen randomly from each pot in each treatment, and the roots were rinsed with distilled water. The root length and plant height were measured by a steel tape.

Determination of physical and biochemical indicators: Leaves were cut into small pieces and placed in liquid nitrogen and then stored at -80°C for further experiments. Each sample pool was made up of 10 individual seedlings. The chlorophyll (Chl) contents, chl-a and chl-b were determined according to the methods of Shabala *et al* (Shabala *et al*., 1998). Herein, a little modification was made that we increased the sample weight into 0.2 g (instead of 100 mg) to facilitate to Chl extraction and the content calculation. The 0.2g fresh leaf collected from the second and third nodes of the shoot tip were put into a 25 mL glass vessel. Then 10 mL 95.5% acetone were added and were blended using a homogenizer. To prevent evaporation, the vessel was well sealed with parafilm and then stored at 4°C for 48 h. The contents of Chl *a* and Chl *b* were determined at 663 nm and 645 nm wavelengths using a UV-2450 spectrophotometer (Shimadzu, Japan).

The concentration of malondialdehyde (MDA) was calculated using the thiobarbituric acid method with minor adjustments (Kramer *et al*., 1991; Chen *et al*., 2012).

For the proline measurement, the method of Bates *et al* (Bates *et al*., 1973) was utilized with a minor modification. In brief, the 0.2 g fresh leaf samples were ground in a mortar with liquid nitrogen. Then the homogenized powder was mixed with 5 mL sulpho-salicylic acid (3% w/v) and incubated in boiling water bath for 10 min, and then centrifuged at 15,000 g for 10 min till the mixture cooled to room temperature. Afterwards, 2 mL extracted supernatant was reacted in 3 mL acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6 M orthophosphoric acid) and 2 mL glacial acetic acid, and then incubated in 100°C for 40 min. The termination was conducted in an ice bath. After adding 4 mL toluene and warming at 25°C, the chromophore was measured at 520 nm in a UV-2450 spectrophotometer (Shimadzu, Japan). L-proline was used as a standard.

Soluble sugars were calculated using the anthrone colorimetric method (Zhang *et al*., 2006). The fresh leaf samples (0.1 g) were added to a test tube with 10 mL distilled water and then was incubated in boiling water bath for 30 min. Then 1 mL supernatant was added to a 20 mL graduated test tube with 1.5 mL distilled water and 5 mL anthranone solution (1 g anthranone dissolved in 1 L of 80% sulphuric acid soluble). Then the whole mixture was whirled and the absorbance was detected at 630 nm wavelength using the UV-2450 spectrophotometer (Shimadzu, Japan).

The relative water content (RWC) was also measured. About 10 of the functional leaves from different individual seedlings were accurately weighed as W1 using an electronic analytical balance (TP-114, Denver Instrument, Denver, CO, USA). Subsequently, after soaking in distilled water for 6-8 h and then (the surface water) drying by the filter paper, the leaves were weighed as W2. Finally, leaves were disposed at 105°C for 15 min for fixation and then dried in an oven at 80°C until a constant dry weight was gained (W3). The RWC was calculated with the following equation: RWC = (W1-W3)/(W2-W3).

The antioxidant enzyme activities, including guaiacol peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) in the leaf tissue were determined. POD activity was determined using the guaiacol method (Zhang & Qu, 2003), CAT activity was determined by the H_2O_2 reduction-UVabsorption method and SOD activity was determined with the tetrazolium (NBT) method (Ning *et al*., 2009).

Statistical analysis: All data were shown as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed Duncan's multiple-range test (Duncan, 1957) was applied to compare the differences under different NaCl concentration treatments, respectively in *R. turczaninovii*, *R. stricta* and *R. komarovii*, by using the software of SAS SPSS 19.0 (SPSS Inc, Chicago, USA). The *P* value of 0.05 was set as the cut-off criterion. Additionally, the two-way ANOVA using SPSS was performed to evaluate whether NaCl treatment and species have a significant effect on the response and whether there are significant interactions between both factors. The separate comparisons between two species were also carried out by least-significant-difference (LSD) test when the F test showed a pronounced significance ($p<0.05$) (Jones *et al*., 2013). All the above treatments were biologically repeated 3 times.

Results

Seed germination of three species under different NaCl salinity stress: In the CK groups, GPs in *R. turczaninovii*, *R. stricta* and *R. komarovii* were 94.67 ± 2.31%, 98.67 ± 2.31% and 100%, respectively. The GPs showed a decreasing trend with the increase of NaCl concentrations in all the three species. Moreover, the GP reached a lower level under the higher NaCl concentration (> 220 mmol/L), indicating that the higher NaCl concentration would inhibit the seed germination. As shown in Fig. 1, there were no significant differences between NaCl-treated groups and CK groups on the GPs in each species with a lower NaCl concentration (less than 100 mmol/L) (p>0.05). However, when the NaCl concentration elevated to 125 mmol/L, GP of *R. turczaninovii* (81.33 ± 2.31%) was significantly reduced compared to CK (p<0.01), while that of *R. stricta* (94.67 ± 2.31%) and *R. komarovii* (94.67 ± 2.31%) were not pronouncedly affected (p>0.05). When the concentration was increased as high as 175 mmol/L, the GPs of three species were all distinctly reduced, compared to the CK (p<0.01). We also found that the GP of *R. komarovii* was 8.67 ± 7.02% with 275 mmol/L NaCl concentration, while that of *R. turczaninovii* or *R. stricta* was near zero. Overall, the GP trend under salinity stress in three species was: *R. komarovii* > *R. stricta* > *R. turczaninovii*. The two-way ANOVA indicated that the NaCl treatment (df = 9, F = 251.986, p<0.001), species (df = 2, F = 76.713, p<0.001) and the interactions of the two factors (df = 18, F = 7.308, p<0.001) all had a pronounced difference to the salt response. Notably, LSD test showed a remarkable significance between any two species (p<0.05, Table 1).

Plant height and root length of three species under different NaCl salinity stress: There were no pronounced differences between NaCl-treated groups and CK groups on plant height (Fig. 2A) and root length (Fig. 2B) in three species under a lower NaCl concentration (less than 60 mmol/L) (p>0.05). Meanwhile, the plant height and root length of *R. turczaninovii* and *R. stricta* were significantly lower than the CK groups (p<0.01), under the NaCl concentration of more than 60 mmol/L. The same results were appeared in *R. komarovii*, with the NaCl concentrations more than 120 mmol/L. The change trends of the two indicators in three species were: *R. komarovii* > *R. stricta* > *R. turczaninovii*. Two-way ANOVA revealed that NaCl treatment (Plant height: df = 6, F = 122.193, p<0.001; Root length: df = 6, F = 45.479, p<0.001) and species (Plant height: df = 2, F = 56.412, p<0.001; Root length: df = 2, F = 49.511, p<0.001), but not the interaction of them (Plant height: df = 12, F = 1.517, p = 0.134; Root length: df = 12, F = 0.565, p = 0.864), had remarkable effect on these two indicators under salt response. For the factor of species, the LSD test suggested a statistical significance (p<0.05, Table 1).

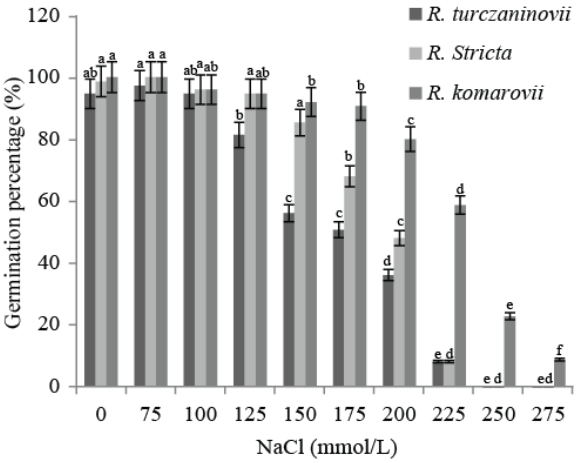

Fig. 1. Germination percentage of *R. turczaninovii*, *R. stricta* and *R. komarovii* species under 0, 75, 100, 125, 150, 175, 200, 225, 250 and 275 mmol/L NaCl.
Values are means (±SD) of three biological replicates. Different letters represent statistically significant differences (p<0.01).

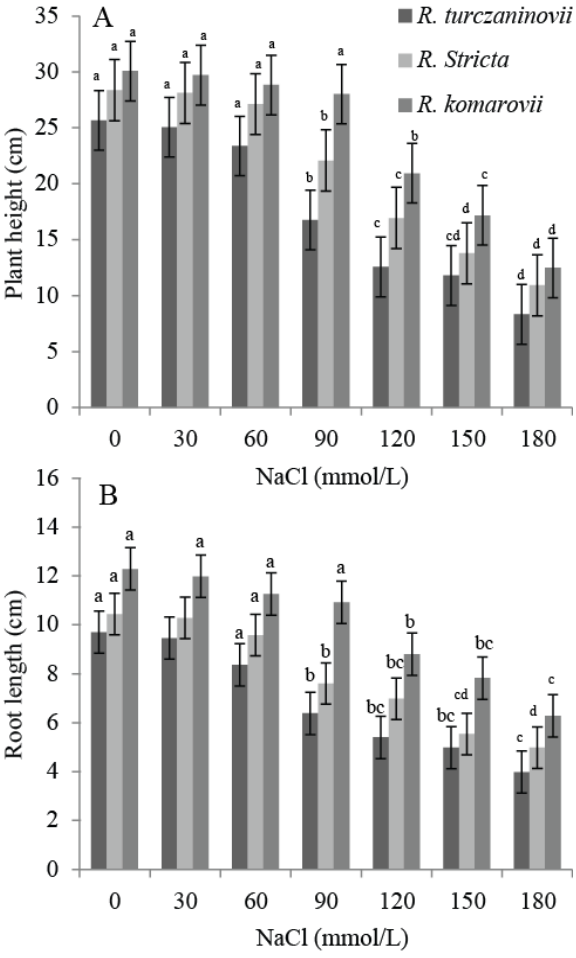

Fig. 2. Plant height and root length of *R. turczaninovii*, *R. stricta* and *R. komarovii* species under 0, 30, 60, 90, 120, 150 and 180 mmol/L NaCl.
Values are means (±SD) of three biological replicates. Different letters represent statistically significant differences (p<0.01).

Table 1. Effects of NaCl treatments and species on the response in different indicators by two-way analysis of variance.

Indicators	*R. turczaninovii*	*R. Stricta*	*R. komarovii*	*P**
GP	59.867(57.266, 62.467)b	51.867(49.266, 54.467)c	74.333(71.733, 76.934)a	< 0.001
Plant height	17.649(16.821, 18.476)c	21.049(20.221, 21.876)b	23.894(23.066, 24.722)a	< 0.001
Root length	6.894(6.461, 7.327)c	7.914(7.481, 8.347)b	9.906(9.473, 10.339)a	< 0.001
MDA	0.118(0.117, 0.119)a	0.111(0.110, 0.112)b	0.099(0.099, 0.100)c	< 0.001
Proline	0.927(0.884, 0.970)c	1.150(1.107, 1.193)b	1.340(1.297, 1.383)a	< 0.001
RWC	0.602(0.569, 0.636)c	0.659(0.625, 0.692)b	0.751(0.717, 0.784)a	< 0.001
Soluble sugar	23.083(22.463, 23.704)a	20.028(19.408, 20.648)b	14.768(14.148, 15.388)c	< 0.001
SOD	109.330(106.565, 112.095)c	126.869(124.104, 129.634)b	156.174(153.409, 158.939)a	< 0.001
POD	2793.81(2702.12, 2885.50)c	3326.57(3234.88, 3418.26)b	3796.48(3704.78, 3888.17)a	< 0.001
CAT	25.398(24.689, 26.106)c	27.909(27.200, 28.617)b	29.931(29.222, 30.639)a	< 0.001
Ca	1.584(1.575, 1.592)c	1.817(1.808, 1.825)a	1.761(1.753, 1.770)b	< 0.001
Cb	0.770(0.745, 0.796)c	1.066(1.040, 1.091)b	1.344(1.319, 1.370)a	< 0.001
Cab	2.351(2.325, 2.383)c	2.882(2.853, 2.912)b	3.106(3.076, 3.135)a	< 0.001

GP: Germination Percentage; MDA: Malondialdehyde; RWC: Relative Water Content; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; Ca: chlorophyll-a; Cb: Chlorophyll-b; Cab: Chlorophyll-a and Chlorophyll-b; *, Overall differences among three species; a, b, c: Comparison of the differences between two species, different letters represent the significant differences (p<0.05)

Table 2. Chlorophyll contents of *R. turczaninovii*, *R. stricta* and *R. komarovii* under different concentration of NaCl.

NaCl (mmol/L)	*R. turczaninovii*			*R. stricta*			*R. komarovii*		
	Chla	Chlb	Chl	Chla	Chlb	Chl	Chla	Chlb	Chl
0	2.03 ± 0.001a	1.12 ± 0.007a	3.14 ± 0.008a	2.06 ± 0.025a	1.54 ± 0.014a	3.59 ± 0.032a	2.04 ± 0.0248a	1.83 ± 0.014a	3.88 ± 0.010a
30	2.00 ± 0.007b	1.02 ± 0.008b	3.02 ± 0.010b	2.06 ± 0.019a	1.46 ± 0.022a	3.52 ± 0.008a	2.03 ± 0.005a	1.82 ± 0.012a	3.85 ± 0.014ab
60	1.91 ± 0.001c	0.87 ± 0.008c	2.79 ± 0.007c	2.04 ± 0.022a	1.25 ± 0.248b	3.29 ± 0.261b	1.96 ± 0.026b	1.81 ± 0.023a	3.77 ± 0.005b
90	1.64 ± 0.008d	0.79 ± 0.001d	2.43 ±0.007d	1.92 ± 0.019b	1.05 ± 0.016c	2.97 ± 0.016c	1.89 ± 0.002c	1.18 ± 0.011b	3.07 ± 0.010c
120	1.60 ± 0.013e	0.63 ± 0.003e	2.23 ± 0.014e	1.84 ± 0.011c	0.95 ± 0.003c	2.78 ± 0.013d	1.71 ± 0.029d	1.10 ± 0.023c	2.81 ± 0.051d
150	1.00 ± 0.009f	0.50 ± 0.013f	1.51 ± 0.022f	1.66 ± 0.027d	0.70 ± 0.023d	2.36 ± 0.050e	1.53 ± 0.021e	1.02 ± 0.011d	2.55 ± 0.011e
180	0.91 ± 0.017g	0.45 ± 0.012g	1.36 ± 0.029g	1.15 ± 0.0005e	0.52 ± 0.027e	1.67 ± 0.027f	1.16 ± 0.043f	0.66 ± 0.077e	1.82 ± 0.120f

R. turczaninovii: *Roegneria turczaninovii* (Drob.) Nevski var. macrathera Ohwi; *R. stricta*: *Roegneria stricta* Keng; *R. komarovii*: *Roegneria komarovii* (Nevski) Nevski; Chla: chlorophyll a; Chlb: chlorophyll b; Chl: chlorophyll. Different letters indicate statistically significant differences (p<0.01)

Physiological responses of three species under NaCl salinity stress: Several physiological indicators including Chl, MDA, proline, RWC and soluble sugars were measured to assess the influence of NaCl on the three species. As shown in Table 2, the Chl content was decreased with the increased NaCl concentration (p<0.01). The change trend of Chl content in three species under different NaCl concentrations was: *R. komarovii* > *R. stricta* > *R. turczaninovii*. The contents of MDA and soluble sugars in leaves showed significant differences between the NaCl-treated group (under any concentration) and the CK group in three species (p<0.01) (Fig. 3A and 3D). The contents of soluble sugars in *R. turczaninovii*, *R. stricta* and *R. komarovii* were increased to 24.17 (mg/g) ± 1.82, 33.40 (mg/g) ± 1.09 and 37.74 (mg/g) ± 3.35, respectively, at 180 mmol/L NaCl concentration, in comparison with their respective CK groups (10.70 (mg/g) ± 0.51, 12.62 (mg/g) ± 0.97 and 13.64 (mg/g) ± 0.62). The overall trends of MDA content in three species under NaCl concentrations were: *R. komarovii* < *R. stricta* < *R. turczaninovii*. Under the NaCl concentration of 60 mmol/L, the contents of proline in *R. komarovii* (0.74 ± 0.08 (mg/g)) and *R. stricta* (0.62 ± 0.44 (mg/g)) were significantly increased comparing with their CKs (0.051 (mg/g) ± 0.02 and 0.047 (mg/g) ± 0.04, respectively) (p<0.01), while that in *R. turczaninovii* did not show any remarkable difference between this concentration and the CK group. Under 120 mmol/L NaCl concentration, the content of proline revealed a significant higher level than CK (p<0.01) in *R. komarovii* (Fig. 3B). The RWC in three species were reduced with the increased NaCl concentration (Fig. 3C). Notably, the RWC under the NaCl concentration of 60 mmol/L were significantly lower in *R. turczaninovii* (66.3% ± 0.12) and *R. stricta* (77.53% ± 0.02), than their CK groups (89.4% ± 0.01 and 9.033% ± 0.03, respectively) (p<0.01), whereas in *R. komarovii*, the pronounced differences between NaCl treatment group and CK was detected under higher NaCl concentrations with more than 120 mmol/L. Two-way ANOVA of all the above indicators indicated that NaCl treatment (Chl: df = 6, F = 1054.477, p<0.001; MDA: df = 6, F = 994.950, p<0.001; proline: df = 6, F = 557.460, p<0.001; soluble sugars: df = 6, F = 227.477, p<0.001; RWC: df = 6, F = 67.317, p<0.001), species (Chl: df = 2, F = 711.823, p<0.001; MDA: df = 2, F = 564.323, p<0.001; proline: df = 2, F = 93.994, p<0.001; soluble sugars: df = 2, F = 187.311, p<0.001; RWC: df = 2, F = 20.371, p<0.001) and the interaction of them (except for the indicator of RWC) (Chl: df = 12, F = 10.955, p<0.001; MDA: df = 12, F = 49.555, p<0.001; proline: df = 12, F = 5.354, p<0.001; soluble sugars: df = 12, F = 7.742, p<0.001; RWC: df = 12, F = 0.624, P = 0.809) achieved a significant difference to the salt response. LSD test showed a pronounced significance between any two species in the above indicators (p<0.05, Table 1).

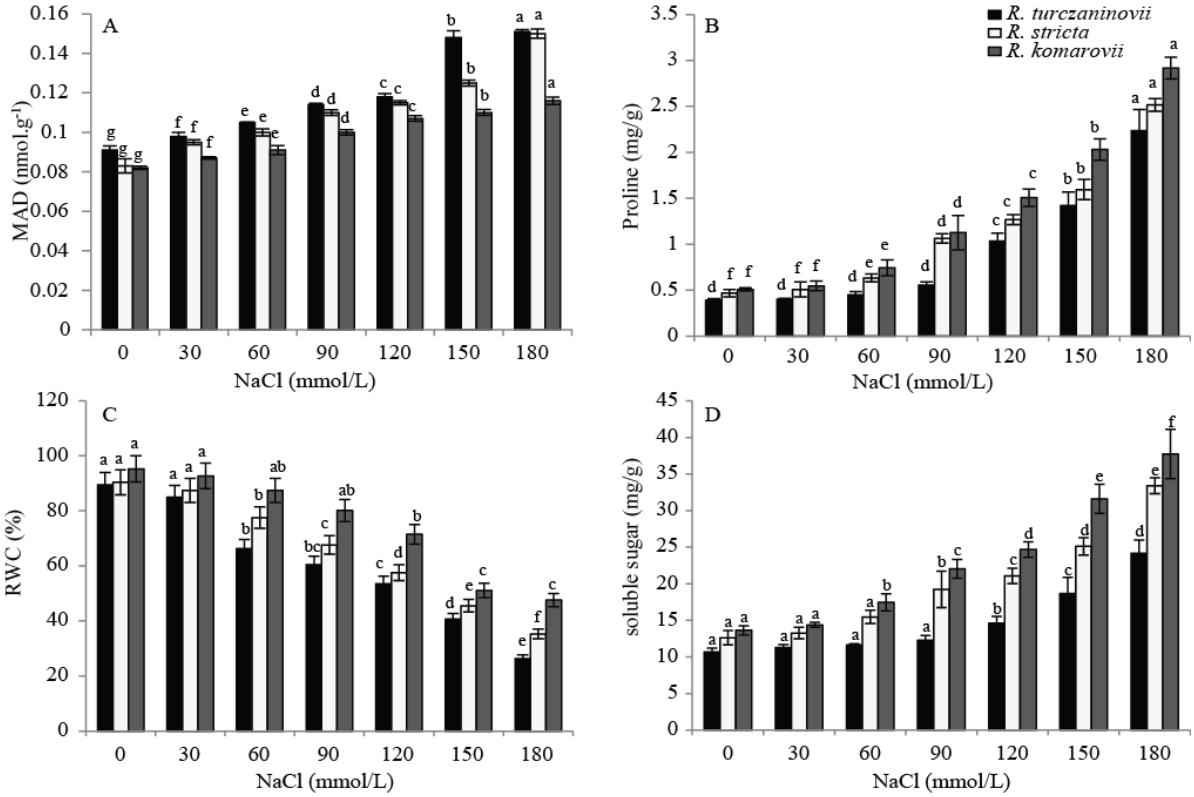

Fig. 3. Contents of malondialdehyde (MDA), proline, relative water content (RWC) and soluble sugars of *R. turczaninovii*, *R. stricta* and *R. komarovii* species under 0, 30, 60, 90, 120, 150 and 180 mmol/L NaCl.
Values are means (±SD) of three biological replicates. Different letters represent statistically significant differences (p<0.01).

Biochemical responses of three species under NaCl salinity stress: The alterations of antioxidant enzymes activity under various NaCl concentrations in the three species are shown in Fig. 4. As a result, with the increase of NaCl concentration, the activities of SOD and POD in all species showed a first increasing and then decreasing trend, while the activity of CAT revealed a constant increasing trend. Under the 60 mmol/L NaCl, the SOD and POD activities in *R. turczaninovii* and *R. stricta* were significantly higher than the CKs (p<0.01), while the significance was detected under 90 mmol/L NaCl in *R. komarovii* (p<0.01). With regard to the CAT activity, *R. komarovii* showed a significant higher level than CK with the 90 mmol/L NaCl concentration, while the significant differences were exhibited in other two species under 150 mmol/L NaCl, comparing to their CKs (p<0.01). In total, the trends of the activities of SOD, POD and CAT in three species under different salinity stresses were: *R. komarovii* > *R. stricta* > *R. turczaninovii*. Meanwhile, the peak level of SOD in *R. turczaninovii*, *R. stricta* and *R. komarovii* were 136.92 ± 0.52, 153.18 ± 0.52 and 156.16 ± 1.11 ($U.g^{-1}.min^{-1}FW$), respectively under 60, 90 and 180 mmol/L NaCl concentrations (Fig. 4A). The peak levels of POD in *R. turczaninovii*, *R. stricta* and *R. komarovii* were 3498.67 ± 30.29, 4331.33 ± 178.35 and 4902 ± 362.15 ($U.g^{-1}.min^{-1}FW$), respectively under 60, 90 and 120 mmol/L NaCl concentrations (Fig. 4B). The peak levels of CAT under 180 mmol/L NaCl concentration in *R. turczaninovii*, *R. stricta* and *R. komarovii* were 32.5 ± 2.21, 36.32 ± 2.84 and 40.89 ± 3.22 ($U.g^{-1}.min^{-1}FW$), respectively (Fig. 4C). The two-way ANOVA of the three indicators showed that NaCl treatment (SOD: df = 6, F = 48.567, p<0.001; POD: df = 6, F = 41.040, p<0.001; CAT: df = 6, F = 86.751, p<0.001), species (SOD: df = 2, F = 298.381, p<0.001; POD: df = 2, F = 121.910, p<0.001; CAT: df = 2, F = 41.869, p<0.001) and the interaction of them (except for the indicator of CAT) (SOD: df = 12, F = 6.305, p<0.001; POD: df = 12, F = 10.158, p<0.001; CAT: df = 12, F = 1.759, p = 0.088) had a remarkable significance to the salt response. LSD test showed a pronounced significance between any two species in the above indicators (p<0.05, Table 1).

Discussion

Salinity has great adverse effects on the plant growth (Kanokwan *et al*., 2015). In the present study, we determined a series of physiological and biochemical indicators under salinity stress with diverse NaCl concentrations in three *Roegneria* species. As a result, *R. komarovii* showed a higher level on GP, Chl, proline, RWC and antioxidant enzymes activity, but a lower MDA content than the other two species, under high concentrations of NaCl.

A spectrum of defense responses are involved in the salinity tolerant plant species. Germination is a crucial period sensitive to high salinity. High germination capacity is the primary step to resist salinity stress (Deng *et al*., 2014; Yang *et al*., 2014). Reportedly, Abpakhsh is the most tolerant to water salinity, which had the highest GP among

ten sesame cultivars (*Sesamum indicum* L.) (Bahrami & Razmjoo, 2012). The cultivar sharkord is proposed as the most tolerant and sensitive to salinity stress among four grasspea varieties, due to it has the most GP at a high NaCl concentration (215 mmol/L) (Mahdavi & Sanavy, 2007). Consistent with these previous studies, our findings indicated that *R. komarovii* achieved a higher GP than *R. turczaninovii* and *R. stricta* under the same NaCl concentration. Notably, at a high NaCl concentration (275 mmol/L), the GPs of *R. turczaninovii* and *R. stricta* were nearly reduced to zero, while that of *R. komarovii* was 8.67 ± 7.02%, indicating that *R. komarovii* had a greater resistance to salinity than the other two species.

MDA, proline and soluble sugars are the indexes of lipid peroxidation and osmotic adjustment in biological materials (Ghoulam *et al.*, 2002; Zhang *et al.*, 2012). Salinity stress could result in the oxidative damages in plant tissues, which could increase cell membrane permeability and thus lead to tremendous loss of inorganic nutrients and disorders of cellular metabolism (Zhang *et al.*, 2012). Elevated MDA level is detected in various salt sensitive plant species such as perennial ryegrass (Hu *et al.*, 2012). On the other hand, it has been convinced that the stress tolerance in *ALDHs* transgenic arobidopsis is associated with the reduction of MDA (Zhang *et al.*, 2007). As MDA is much lower in cv. *Cumhuriyet* than in cv. *Orhangazi* under salinity stress, the cv. *Cumhuriyet* is considered as the more tolerant species (Koca *et al.*, 2007). Rapid accumulation of free proline is a positive response to abiotic stresses in various plant species (Feng *et al.*, 2002; Ashraf & Foolad, 2007; Cha-Um & Kirdmanee, 2009; Zhang *et al.*, 2012). In the *Pisum sativum* L., the salt tolerant EC 33866 had a more content of proline than the susceptible variety Puget (Ahmad *et al.*, 2008). Accumulation of soluble sugars is also a positive response to salt stress because it can reduce cell osmotic potential, maintain cell turgor pressure and prevent cell dehydration (Yakushiji *et al.*, 1996; Mohammadkhani & Heidari, 2008). In our study, in comparison with *R. stricta* and *R. turczaninovii* species, *R. komarovii* had a lower MDA content, but a higher amount of proline and soluble sugars under the same NaCl concentration, indicating that *R. komarovii* was the most resistant to salinity stress among the three species.

Chl biosynthesis is the crucial factor to achieve oxygenic photosynthesis (Tsukatani & Masuda, 2015). The reduced Chl content is found in *Cucumis sativus* with NaCl treatment. It is speculated that the reduction under salinity stress might be due to the increased activity of chlorophyll-degrading enzyme chlorophyllase, the decreased uptake of Mg element, or the stomatal closure (Fariduddin *et al.*, 2013). Furthermore, through the Chl fluorescence measurements, Moradi & Ismail (2007) discovered a decreased electron transport rate in rice under salt stress. The higher Chl content is associated with the higher tolerance of salt stress. Three wheat genotypes, viz. Lu-26s, Sarsabz and KTDH, with higher amounts of Chl are considered as tolerant varieties under saline conditions (Shirazi *et al.*, 2009). Consistent with this result, our findings revealed that *R. komarovii* had the highest Chl content amongst the three species under higher NaCl concentration, demonstrating that *R. komarovii* was more tolerant to salinity stress than the other two species.

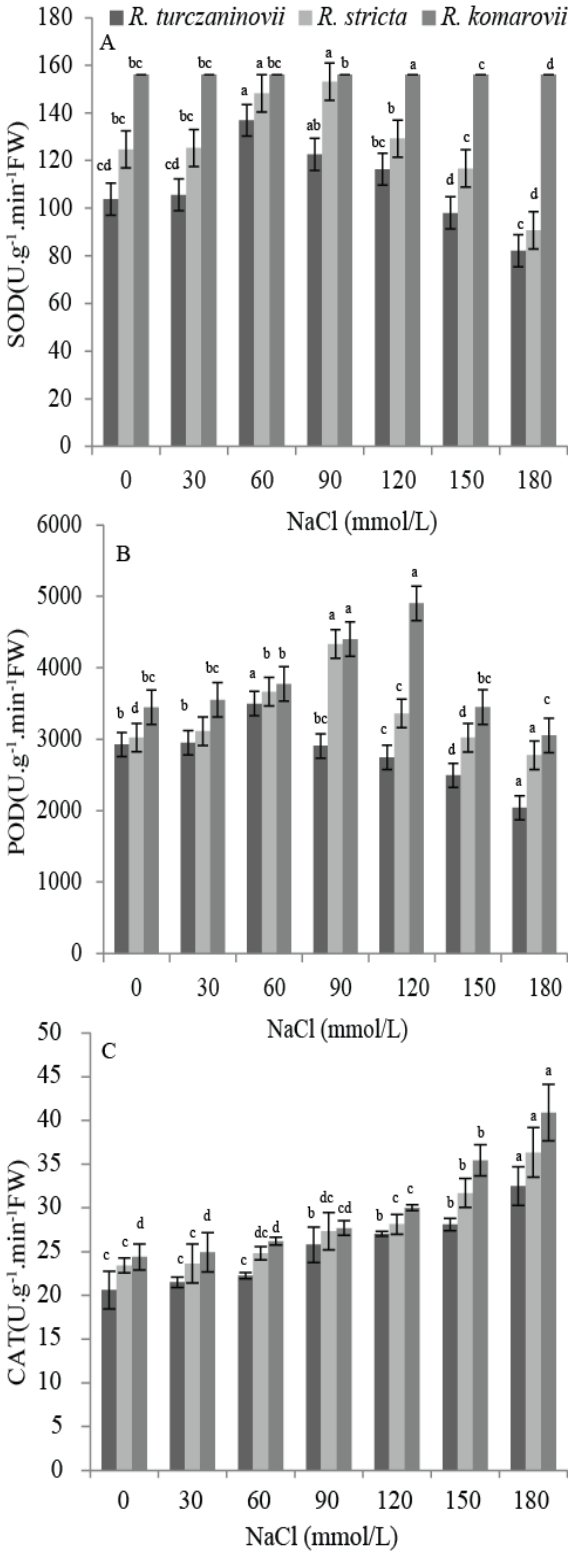

Fig. 4. Activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of *R. turczaninovii*, *R. stricta* and *R. komarovii* species under 0, 30, 60, 90, 120, 150 and 180 mmol/L NaCl.
Values are means (±SD) of three biological replicates. Different letters represent statistically significant differences (p<0.01).

The environmental stresses such as salinity stress can cause the oxidative damage via inducing the generation of active oxygen species. In response to the adverse stresses, plants are evolved to develop a physiological defense system together with the increase of antioxidant enzymes such as SOD, POD, CAT and glutathione reductase (GR) (Joseph & Jini, 2011; Sharma *et al*., 2012). Several studies have reported the increased activities of SOD, POD and CAT under drought, salinity and high temperature stresses in tolerant wheat genotypes (Ruzhen, 1997; Sairam *et al*., 1997; Sairam *et al*., 2001). Moreover, a study finds that the increased capacity of antioxidative system (e.g. increased SOD, CAT and GR activity) in rice root might contribute to the improved tolerance to salt stress (Khan & Panda, 2008). In this study, activities of SOD, POD and CAT in *R. komarovii* species were all significantly higher than those in *R. turczaninovii* and *R. stricta* under salinity stress. Besides, the fluctuations of antioxidant enzymes in *R. komarovii* were lower than those in *R. turczaninovii* and *R. stricta*, when compared with their respective CK groups, which indicated that *R. komarovii* was more resistant to salinity stress than *R. turczaninovii* and *R. stricta*.

Conclusion

In conclusion, NaCl has adverse effects on growth of three *Roegneria* C. Koch species in a concentration-dependent manner. *R. turczaninovii* is more tolerant than *R. stricta* and *R. komarovii* in responses to salinity stress.

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