

Effects of exogenous application of salicylic acid on drought performance of medicinal plant, *Fritillaria przewalskii* Maxim

Effets de l'application exogène d'acide salicylique sur la résistance à la sécheresse d'une plante médicinale, *Fritillaria przewalskii* Maxim

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Article abstract

This study investigated the effects of salicylic acid (SA) foliar application on *Fritillaria przewalskii* under drought stress condition. Plants were subjected to three irrigation regimes, 75–80% control (CK), 60–65% medium stress (M) and 40–45% severe stress (S) of the field capacity and three levels of SA, 0.0, 0.5 and 1.0 mM. Relative water content (RWC), proline content, total soluble carbohydrates, chlorophyll “a” (Chl a), chlorophyll “b” (Chl b), chlorophyll “a + b” (Chl a + b), carotenoids contents, malondialdehyde (MDA) contents and activities of several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) activity were measured. RWC, soluble carbohydrates, Chl b, Chl a + b, MDA content and activities of antioxidant enzymes (SOD, POD, CAT, GR and APX) were significantly affected by water deprivation without SA. Exogenous SA significantly increased the content of RWC, total leaf soluble carbohydrates, leaf proline and Chl b at moderate water deficit and severe water deficit. MDA content was decreased significantly by exogenous SA. The activities of antioxidant enzymes (SOD, POD, CAT, GR and APX) were also significantly affected by exogenous SA. However, the content of Chl a, Chl a + b, and carotenoids were not significantly affected by exogenous SA.

Effects of exogenous application of salicylic acid on drought performance of medicinal plant, *Fritillaria przewalskii* Maxim

Ruili Ma^{1,2,3}, Shengrong Xu^{1,2,3}, Yuan Chen^{2✉}, Fengxia Guo², Rui Wu², Samuel Anim Okyere⁴, Fusheng Wang³, Yanming Jing³ and Xingzheng Wang³

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This study investigated the effects of salicylic acid (SA) foliar application on *Fritillaria przewalskii* under drought stress condition. Plants were subjected to three irrigation regimes, 75-80% control (CK), 60-65% medium stress (M) and 40-45% severe stress (S) of the field capacity and three levels of SA, 0.0, 0.5 and 1.0 mM. Relative water content (RWC), proline content, total soluble carbohydrates, chlorophyll "a" (Chl a), chlorophyll "b" (Chl b), chlorophyll "a + b" (Chl a + b), carotenoids contents, malondialdehyde (MDA) contents and activities of several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) activity were measured. RWC, soluble carbohydrates, Chl b, Chl a + b, MDA content and activities of antioxidant enzymes (SOD, POD, CAT, GR and APX) were significantly affected by water deprivation without SA. Exogenous SA significantly increased the content of RWC, total leaf soluble carbohydrates, leaf proline and Chl b at moderate water deficit and severe water deficit. MDA content was decreased significantly by exogenous SA. The activities of antioxidant enzymes (SOD, POD, CAT, GR and APX) were also significantly affected by exogenous SA. However, the content of Chl a, Chl a + b, and carotenoids were not significantly affected by exogenous SA.

Keywords: salicylic acid, drought stress, antioxidative enzyme, osmolytes, chlorophyll.

[Effets de l'application exogène d'acide salicylique sur la résistance à la sécheresse d'une plante médicinale, *Fritillaria przewalskii* Maxim]

Cette étude a examiné les effets de l'application foliaire d'acide salicylique (AS) sur *Fritillaria przewalskii* en condition de sécheresse. Les plantes ont été soumises à trois régimes d'irrigation : 75-80 % comme contrôle (CK), 60-65 % de stress moyen (M) et 40-45 % de stress sévère (S) de la capacité au champ et trois niveaux d'AS : 0,0, 0,5 et 1,0 mM. Les teneurs en eau relative (RWC), en proline, glucides solubles totaux, chlorophylle « a » (Chl a), chlorophylle « b » (Chl b), chlorophylle « a + b » (Chl a + b), teneurs en caroténoïdes et malondialdéhyde (MDA) ont été mesurées, ainsi que les activités de plusieurs enzymes antioxydantes telles que la superoxyde dismutase (SOD), la peroxydase (POD), la catalase (CAT), la glutathion réductase (GR) et l'ascorbate peroxydase (APX). La RWC, les glucides solubles, Chl b, Chl a + b, la teneur en MDA et les activités des enzymes antioxydantes (SOD, POD, CAT, GR et APX) étaient significativement affectées par la privation en eau sans AS. L'AS exogène a considérablement augmenté la teneur en RWC, en glucides solubles dans les feuilles, en proline et en Chl b en déficit hydrique modéré et sévère. Le contenu en MDA a été réduit de manière significative par l'AS exogène. Les activités des enzymes antioxydantes (SOD, POD, CAT, GR et APX) étaient également significativement affectées par les AS exogènes. Cependant, les teneurs en Chl a, Chl a + b et les caroténoïdes n'étaient pas significativement affectées par les AS exogènes.

Mots clés : acide salicylique, sécheresse, enzyme antioxydante, osmolytes, chlorophylle.

1. Qinghai Provincial Key Laboratory of Qinghai-Tibet Plateau Biological Resources, China.
2. College of Agronomy, College of Life Science and Technology, Gansu Agricultural University, Lanzhou 730070, China; corresponding author e-mail: China.cygcx@163.com.
3. Dingxi Academy of Agricultural Sciences, Dingxi 743000, China.
4. CSIR-Oil Palm Research Institute, Box 74, Kade Ghana.

INTRODUCTION

Fritillaria przewalskii Maxim. (Liliaceae) is a perennial medicinal plant found in the alpine meadow and grasslands in the west of the Tibetan plateau (3000 m to 5000 m above sea level). Its growth environs in the high mountains make it highly susceptible to drought stress as it requires adequate supply of water for growth and development (Chang *et al.* 2010). The changing climate and decreasing rainfall in recent years threaten the growth and development of this medicinal plant. Therefore, the search for drought tolerance strategies is requested to determine if this plant can adapt to a limited water supply environment.

Studies have shown that the plant adopts various mechanisms to survive in water stress environments. Osmolytes such as proline and soluble carbohydrates accumulate in the plant cells to renovate cell turgor during drought stress. Proline keeps the cells safe by scavenging for reactive oxygen species (ROS), while the plant adjusts using carbohydrates to maintain its metabolism and saving energy under drought (Khalid *et al.* 2010).

In one study, Kadkhodaie *et al.* (2014) observed that during oxidative stress triggered by drought, plant cells adapt by increasing ROS. Plants use specific mechanisms to scavenge ROS, including activation of antioxidant enzymes, non-enzymatic antioxidants, such as carotenoids, glutathione, ascorbic acid, and proline. ROS causes membrane lipid peroxidation and chlorophyll degradation. The capability of a plant to synthesize more chlorophyll under water shortage may improve drought tolerance (Pinhero *et al.* 2000). However, during the progression of drought stress and ROS accumulation, it results in protein oxidation, membrane lipid peroxidation and even can cause the death of plants (Liu *et al.* 2015).

Salicylic acid (SA) has been identified to play a key role in plant growth, development and defense responses through the provision of protection under biotic and abiotic stress conditions. Its regulation of chlorophyll, protein synthesis, transpiration, photosynthesis, protective enzymes such as superoxide dismutase (SOD) and peroxidase (POD), which together increase plant tolerance to environmental stresses has been identified (Mutlu *et al.* 2009). Janda *et al.* (2014) have also noted the role of SA in antioxidant enzymes of plant leaves under water stress and degraded ROS.

The effects of drought stress on plants could be alleviated by exogenous application of SA (Askari and Ehsanzadeh 2015). However, the exact mechanisms of SA to mitigate drought stress in medicinal plants which are found in varying environmental conditions are poorly understood. This work seeks to evaluate drought-induced responses and SA application responses of *F. przewalskii*. Data obtained will be useful in unveiling basic mechanisms involved in drought tolerance in medicinal plants. Therefore, this study was conducted to research drought stress on physiological and antioxidant responses of *F. przewalskii*. to determine whether foliar application/spray with SA could mitigate the adverse effect of drought stress.

MATERIALS AND METHODS

Plant culture and growth conditions

A pot experiment was conducted in a rain shelter under natural light (outdoor) at Honglou Experimental Station of Gansu Agricultural University, China (36°09'N, 103°69'E), during

the 2016 growing season. *F. przewalskii* Maxim. cultivar which represents local populations in zhangxian in Gansu province of China (4000 m above sea level) was used in this study. Three-year-old seed bulbs were collected on 10 March 2016. Each pot was planted with thirty bulbs. Thirty uniform bulbs were surface sterilized and sown 3 cm deep in free-draining plastic pots, 60 cm height x 25 cm diameter. The initial moisture at field capacity was calculated weighing the pots at field capacity, SW1, then drying soil at 105°C, and weighed again to obtain the dried soil weight, SW2. The soil moisture at field capacity was obtained from the Eq. (1).

$$\text{Soil Moisture at Field Capacity (\%)} = \left(\frac{\text{SW1} - \text{SW2}}{\text{SW1}} \right) \times 100 \quad (1)$$

The experiment was designed with three replications (six pots per replication). Each pot was filled with 25 kg of top soil (20% sand, 30% silt, 50% clay). The soil had organic matter content of 31.80 g kg⁻¹, total nitrogen (N) contents of 2.44 g kg⁻¹, available phosphate (P₂O₅) level of 18.00 mg kg⁻¹ and available potassium (K₂O) level of 124.24 mg kg⁻¹. Thinning was carried out 10 days after germination at 25 seedlings per pot for the studies. Each pot was irrigated to 75-80% of the field capacity (FC) until the start of the treatments.

Irrigation regimes and SA treatments

The drought stress was imposed 20 days after germination, three regimes of drought stress were used and no interactive treatment was applied. Control (75-80% of FC, CK), moderate (60-65% of FC, M) and severe (40-45% of FC, S) (Askari and Ehsanzadeh 2015). Drought stress was applied by withholding watering for 10 days (Han *et al.* 2014). All pots were weighed and irrigated daily up to the water level of each treatment.

SA (2-hydroxybenzoic acid, molecular weight 138.1, Sigma-Aldrich) was dissolved in distilled water at the 0.5 mM and 1.0 mM concentration and sprayed on plants twice (Askari and Ehsanzadeh 2015), 30th and 31st days after germination. The SA solution was sprayed on each plant with a manual sprayer to run-off to ensure that the same quantity was applied to each plant. CK plants were sprayed with distilled water. Thirty-two days after germination, the following measurements were carried out on plants in all treatments. The pots with different treatments were rotated on every other day to ensure that all the pots received equal radiation and other environmental exposures till the end of the experiment.

Leaf relative water content and osmolytes

Relative water content

Leaf relative water content (RWC) was determined as described by Smart and Bingham (1974) method. Three-year-old seed bulbs have only one leaf in that period and RWC (%) was determined with this leaf. Leaf was harvested from six plants with each replication (one plant per pot), fresh weights (FW) were estimated immediately after excision. Dry weights (DW) were estimated after drying the leaf samples in an oven for 72 h at 70°C until it achieved constant weight. Finally, RWC was calculated by the Eq. (2).

$$\text{RWC (\%)} = \left(\frac{\text{FW} - \text{DW}}{\text{FW}} \right) \times 100 \quad (2)$$

Total leaf soluble carbohydrates content

Total leaf soluble carbohydrates were measured using the method of Irigoyen *et al.* (1992). A 500 mg subsample (a plant has only one leaf, leaf was harvested from thirty plants with

each treatment [one or two leaves randomly harvested per pot] and mixed them, dried after, the dried samples were precisely weighed) of dried leaves with each treatment was homogenized with 5 ml of 95% ethanol. Then, 0.1 ml of alcoholic extract was mixed with 3 ml of anthrone (150 mg anthrone, 100 ml of 72% sulfuric acid). The samples were placed in a boiling water bath for 10 min. The light absorption of the samples was determined spectrophotometrically at 625 nm. Total leaf soluble carbohydrates were estimated using glucose standard curve (Irigoyen *et al.* 1992).

Leaf proline content

Free proline content in the leaves was measured using the method of Delauney and Verma (1993). A 500 mg fresh leaf subsample (sampling methods as before, the fresh samples were precisely weighed), then were grinded in 10 ml of 3% aqueous sulfosalicylic acid and the extract was filtered. Two millilitres of the extract was added in the test tube with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid. The resultant mixture was boiled in a water bath at 100°C for one hour. After cooling the mixture on ice, 4 ml of toluene was added and mixed thoroughly, the toluene phase was separated and its absorbance measured at 520 nm using a HITACHI U1800 spectrophotometer against toluene blank.

Leaf chlorophyll and carotenoids contents

The contents of chlorophyll and carotenoids in fresh leaves were measured spectrophotometrically using the method of Sheng *et al.* (2015). One gram of fresh leaf tissue in each

treatment was ground using mortar and pestle containing 10 ml of acetone (80%). The absorption of the leaf extract solution was recorded at 662 nm and 645 nm (for Chl a and Chl b respectively) and at 470 nm (for carotenoids). Finally, the results were expressed as milligrams of pigment per gram of leaf fresh weight.

Measurement of antioxidant enzymes activities

Enzyme extraction

To measure antioxidant enzymes activities, the samples were prepared as described by Sheng *et al.* (2015). A 500 mg fresh leaf subsample (sampling methods as before) was frozen in liquid nitrogen and finely ground with pestle in a chilled motor, the frozen powder was added to 10 ml of 100 mM phosphate buffer (KH₂PO₄/K₂HPO₄) pH 7.0, containing 0.1 mM Na₂EDTA and 0.1 g of polyvinylpyrrolidone. The homogenate was filtered through cheese cloth then centrifuged at 15,000 g for 10 min at 4°C. The supernatant was re-centrifuged at 18,000 g for 10 min and then the resulted supernatant was stored at 4°C and the assay used to determine the following antioxidant enzymes.

The activity of superoxide dismutase (SOD)

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photo reduction of nitro blue tetrazolium (NBT) using the method of Sheng *et al.* (2015). The amount of enzyme that inhibited 50% of NBT photo reduction at 560 nm was expressed as one unit of SOD activity.

Table 1. Results of two factor analysis of variance (two-way ANOVA) with three replications for physiological indicators of *F. przewalskii* Maxim

Experimental indicators	Variance sources of two-way ANOVA					
	Factor A: FC (3 levels)		Factor B: SA (3 levels)		Interaction of FC×SA	
	<i>F</i>	<i>P</i> -Value	<i>F</i>	<i>P</i> -Value	<i>F</i>	<i>P</i> -Value
Relative water	487.63**	0.000	83.60**	0.000	45.15**	0.000
Carbohydrates	44.58**	0.000	17.04**	0.000	4.06*	0.016
Proline	1740.30**	0.000	198.20**	0.000	2.04	0.131
Chl a	0.16	0.852	0.89	0.428	0.20	0.935
Chl b	6.96**	0.006	4.30*	0.030	0.75	0.573
Chl a + b	5.06*	0.018	5.74*	0.012	1.04	0.413
Carotenoids	0.54	0.593	0.65	0.533	0.35	0.838
SOD	630.31**	0.000	90.22**	0.000	62.22**	0.000
POD	11.32**	0.001	14.42**	0.000	10.99**	0.000
CAT	27.52**	0.000	12.08**	0.000	12.11**	0.000
GR	19.95**	0.000	10.96**	0.001	14.07**	0.000
APX	35.43**	0.000	6.39**	0.008	5.17**	0.006
MDA	66.62**	0.000	10.38**	0.001	2.21	0.109

Note: * following the *F* value indicate significant difference at $p < 0.05$ level, ** following the *F* value indicate greatly significant difference at $p < 0.01$ level.

FC (field capacity) 3 levels: CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficits, 40-45% FC.

SA (salicylic acid) 3 levels: 0.0, 0.5, and 1.0 mM concentration.

The activity of peroxidase (POD)

Peroxidase activity was determined according to the method of Sheng *et al.* (2015) by the oxidation of guaiacol in the presence of H₂O₂. The increase in absorbance due to the formation of tetraguaiacol was recorded at 470 nm. The enzyme activities were calculated and expressed as unit min⁻¹ g⁻¹ fresh weight.

The activity of catalase (CAT)

Catalase activity was measured according to the method of Sheng *et al.* (2015). The activity of catalase was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H₂O₂ consumption. The enzyme activities were calculated and expressed as unit min⁻¹ g⁻¹ fresh weight.

The activity of glutathione reductase (GR)

Glutathione reductase activity was measured according to the method of Sheng *et al.* (2015), which depends on the rate of decrease in the absorbance of NADPH at 340 nm. The enzyme activities were calculated and expressed as unit min⁻¹ g⁻¹ fresh weight.

The activity of ascorbate peroxidase (APX)

Ascorbate peroxidase activity was assayed according to the method of Sheng *et al.* (2015) by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized. The enzyme activities were calculated and expressed as unit min⁻¹ g⁻¹ fresh weight.

Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content using the thiobarbituric method of Sheng *et al.* (2015). It was expressed as nmol of MDA formed using extinction coefficient of 155 mM⁻¹ cm⁻¹ and the results expressed as μmol (MDA) g⁻¹ fresh weight.

Statistical analyses

Each replicate consisted of six pots, each pot containing 25 seedlings and all the treatments were replicated three times. The data was analyzed statistically by two-way ANOVA using the Statistical Package for Social Science (SPSS 19.0) program. The LSD was used to test the difference between treatments.

RESULTS**Effect of salicylic acid on leaf relative water content and osmolytes**

The content of RWC (relative water content), total leaf soluble carbohydrates and leaf proline were significantly affected by field capacity (FC) at three levels ($p < 0.01$), and RWC, total leaf soluble carbohydrates and leaf proline were also significantly affected by spraying salicylic acid (SA) at three levels ($p < 0.01$) (Tables 1 and 2). RWC ($p < 0.01$) and total leaf soluble carbohydrates ($p < 0.05$) were significantly affected by the interaction of FC and SA, while the leaf proline ($p > 0.05$) was not signi-

Table 2. Difference significance of main effect based on LSD multiple comparison results of two factor analysis of variance (two-way ANOVA) with three replications for physiological indicators of *F. przewalskii* Maxim

Experimental indicators	Factor A: FC (3 levels)			Factor B: SA (3 levels)		
	CK	M	S	0	0.5	1.0
Relative water	84.97 ± 1.56 ^{6aA}	78.29 ± 3.21 ^{bB}	60.14 ± 11.26 ^{cC}	68.97 ± 17.47 ^{aA}	74.82 ± 10.34 ^{bB}	79.60 ± 5.92 ^{cC}
Carbohydrates	230.56 ± 24.82 ^{aA}	321.33 ± 23.94 ^{bB}	385.67 ± 21.67 ^{cC}	288.22 ± 67.44 ^{aA}	308.89 ± 70.64 ^{bB}	340.44 ± 65.02 ^{cC}
Proline	54.89 ± 2.76 ^{aA}	63.11 ± 5.84 ^{bB}	69.22 ± 6.89 ^{cC}	57.89 ± 4.34 ^{aA}	62.56 ± 7.40 ^{bB}	66.78 ± 9.35 ^{bB}
Chl a	0.92 ± 0.04	0.93 ± 0.04	0.91 ± 0.07	0.90 ± 0.03	0.92 ± 0.05	0.94 ± 0.06
Chl b	0.33 ± 0.04 ^{aA}	0.27 ± 0.05 ^{abAB}	0.25 ± 0.06 ^{bB}	0.25 ± 0.07 ^{aA}	0.28 ± 0.05 ^{abAB}	0.32 ± 0.04 ^{bB}
Chl a + b	1.25 ± 0.06 ^{aA}	1.20 ± 0.07 ^{abAB}	1.16 ± 0.09 ^{bB}	1.16 ± 0.09 ^{aA}	1.20 ± 0.06 ^{abAB}	1.25 ± 0.06 ^{bB}
Carotenoids	0.22 ± 0.03	0.24 ± 0.04	0.22 ± 0.04	0.21 ± 0.04	0.23 ± 0.03	0.24 ± 0.04
SOD	288.22 ± 22.17 ^{aA}	340.00 ± 12.74 ^{bB}	339.00 ± 6.80 ^{bB}	331.78 ± 15.80 ^{aA}	310.00 ± 37.70 ^{bB}	325.44 ± 26.82 ^{cC}
POD	53.81 ± 4.84 ^{aA}	59.18 ± 6.50 ^{bB}	54.41 ± 3.55 ^{aA}	56.88 ± 7.56 ^{aA}	52.07 ± 3.03 ^{bB}	58.45 ± 2.56 ^{aA}
CAT	32.50 ± 5.74 ^{aA}	41.84 ± 5.42 ^{bB}	40.25 ± 5.50 ^{bB}	41.53 ± 5.81 ^{aA}	34.91 ± 5.22 ^{bB}	38.15 ± 7.95 ^{cAB}
GR	3.27 ± 0.75 ^{aA}	4.18 ± 0.56 ^{bB}	4.02 ± 0.62 ^{bB}	4.16 ± 0.59 ^{aA}	3.45 ± 0.59 ^{bB}	3.86 ± 0.89 ^{aAB}
APX	1.86 ± 0.63 ^{aA}	3.20 ± 0.40 ^{bB}	2.47 ± 0.41 ^{bB}	2.76 ± 0.50 ^{aA}	2.56 ± 0.97 ^{aAB}	2.20 ± 0.60 ^{bB}
MDA	28.73 ± 2.21 ^{aA}	36.87 ± 3.21 ^{bB}	42.24 ± 4.69 ^{cC}	38.49 ± 7.77 ^{aA}	33.14 ± 4.61 ^{bB}	36.20 ± 6.59 ^{aAB}

Each experiment data in this table indicators mean ± SE. Different small and capital letters in the mean physiological indicators in the same row within each factor indicate significant and great significant significance at $p < 0.05$ and $p < 0.01$, respectively, based on ANOVA-LSD multiple comparison results (LSD_{0.05} = 1.729, LSD_{0.01} = 2.369).

FC (field capacity): CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficits, 40-45% FC. SA (salicylic acid): 0.0, 0.5, and 1.0 mM concentration.

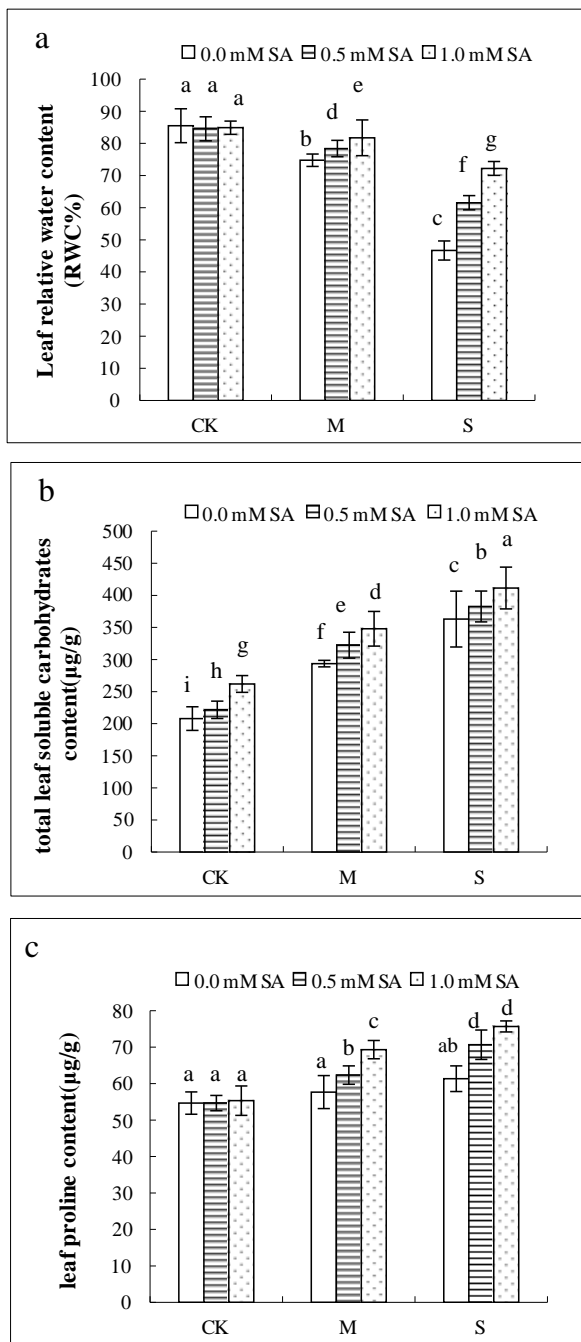


Figure 1. Changes in leaf relative water content (a), total leaf soluble carbohydrates (b), and leaf proline (c) under different levels of salicylic acid (SA: 0.0, 0.5, and 1.0 mM) and field capacity (FC: CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficit, 40-45% FC). Vertical bars represent the standard error of the average of three replications and different lowercase letters represent the significant differences at the level of 5% based on ANOVA-LSD multiple comparison results.

ificantly affected (Table 1). Without foliar application of SA, water deficit significantly decreased leaf relative water content (RWC) compared to control condition (CK), and increased the content of soluble carbohydrates (Fig. 1). It was observed that at moderate and severe stresses, 0.5 mM and 1.0 mM levels of SA significantly increased RWC, leaf proline, and total soluble carbohydrates compared to 0.0 mM SA, while 1.0 mM SA had the highest values of RWC (81.72%) at moderate (M), 1.0 mM SA had the highest values of leaf proline ($75.63 \mu\text{g g}^{-1}$) and leaf soluble carbohydrates ($411.33 \mu\text{g g}^{-1}$) at severe (S) (Fig. 1).

Effect of salicylic acid on chlorophyll and carotenoids contents

The content of Chl b and Chl a + b were significantly affected by field capacity (FC) at three levels ($p < 0.01$), Chl b and Chl a + b were also significantly affected by spraying salicylic acid (SA) at three levels ($p < 0.01$), the content of Chl a and carotenoids was not affected by FC and SA ($p > 0.05$) (Tables 1 and 2). The interaction of FC and SA have no significant difference for Chl a, Chl b, Chl a + b and carotenoids ($p > 0.05$) (Table 1). With no foliar application of SA, Chl b and Chl a + b contents were significantly reduced as compared to the CK in response to water deficit at S (severe), whereas Chl a and carotenoids contents remained unchanged when grown under drought at M and S compared to CK condition (Fig. 2, Table 2). At M and S, 0.5 mM and 1.0 mM levels of SA did not significantly affect the content of Chl a, Chl a + b and carotenoids compared to 0.0 mM SA, while at M and S, the content of Chl b was significantly increased by 0.5 mM and 1.0 mM levels of SA compared to 0.0 mM SA (Fig. 2).

Effect of salicylic acid on antioxidant enzymes activity

The antioxidant enzymes activity of SOD, POD, CAT, GR, APX and the content of MDA were significantly affected by field capacity (FC) at three levels ($p < 0.01$), and were also significantly affected by spraying salicylic acid (SA) at three levels ($p < 0.01$) (Tables 1 and 2). All antioxidant enzymes activity ($p < 0.01$) were significantly affected by the interaction of FC and SA, while the content of MDA was not significantly affected (Table 1).

Without foliar application of SA, at M and S, MDA content was significantly higher compared to CK with decreased soil moisture (Fig. 3). However, exogenous application of SA at 0.5 mM level significantly decreased at S, lower than 1.0 mM SA.

Water deficit under M significantly increased SOD (8.80%), POD (20.99%), CAT (18.39%), and APX (21.32%) activity compared to CK at 0.0 mM levels of SA (Fig. 4). However, at S, with the exception of SOD activity which increased 9.14% relative to CK at 0.0 mM levels of SA, POD (1.66%), CAT (7.34%), GR (16.55%) and APX (10.69%) activity decreased. At M, 0.5 mM SA also led to a significant decrease in SOD, CAT, GR and POD activity as compared with 0.0 mM levels of SA, with the exception of APX activity which remained unchanged. However, all traits of antioxidant enzymes increased in M and S at 0.5 mM and 1.0 mM levels of SA compared to CK. The extent of the increase was greater at plants applied with 1.0 mM in comparison with 0.5 mM of SA, with the exception of APX activity, where 0.5 mM of SA increased higher than 1.0 mM SA (Fig. 4).

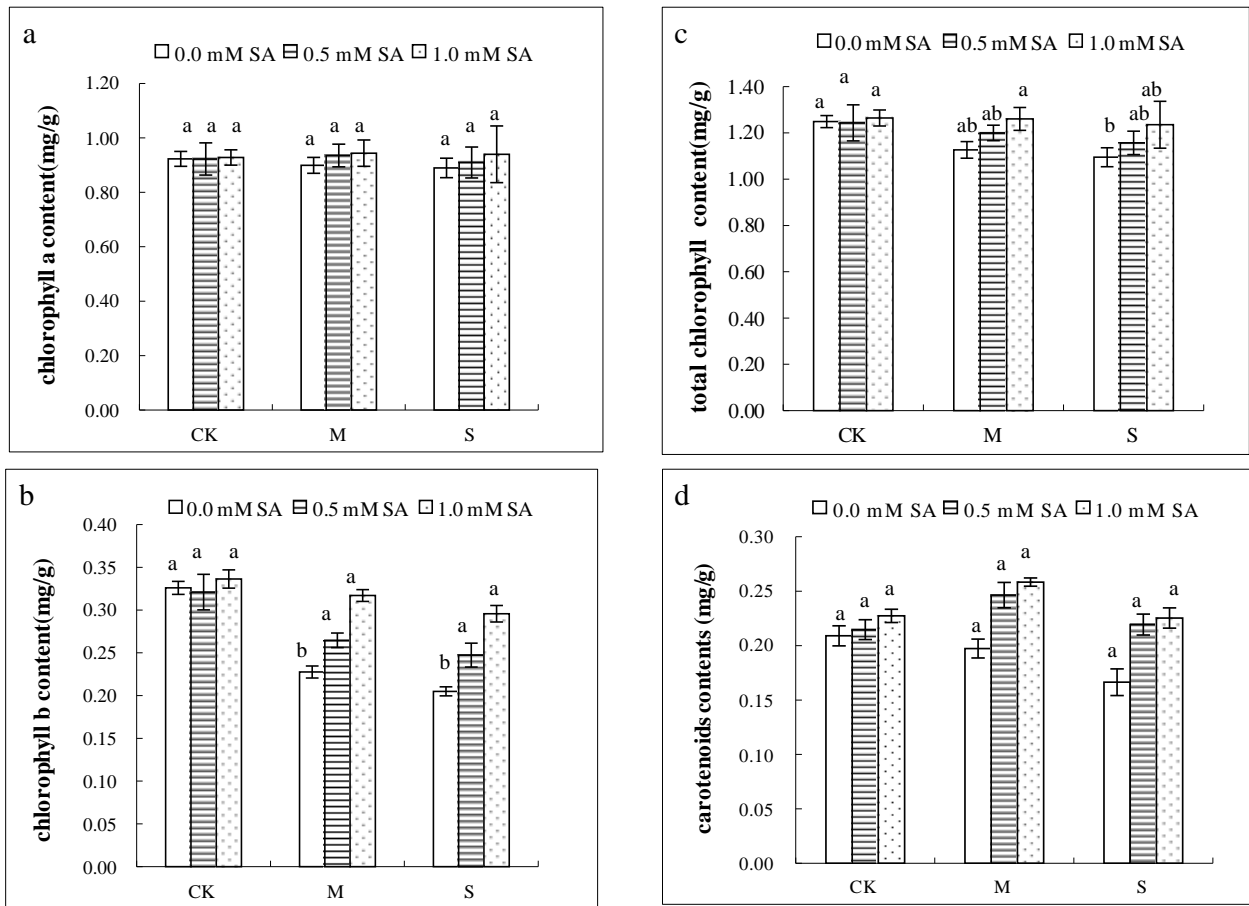


Figure 2. Changes in chlorophyll a (a), chlorophyll b (b), chlorophyll a+b (c), and carotenoids content (d) under different levels of salicylic acid (SA: 0.0, 0.5, and 1.0 mM) and field capacity (FC: CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficit, 40-45% FC). Vertical bars represent the standard error of the average of three replications and different lowercase letters represent the significant differences at the level of 5% based on ANOVA-LSD multiple comparison results.

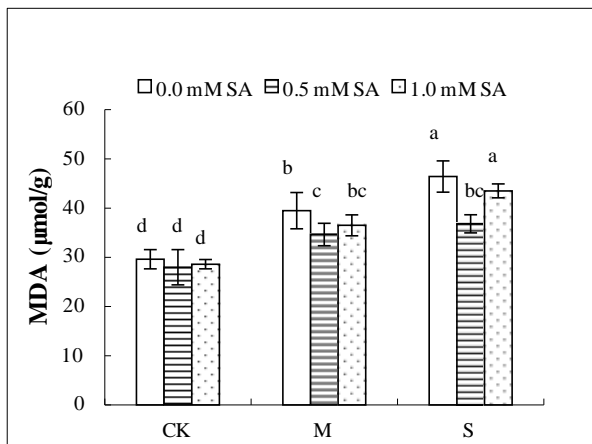


Figure 3. Changes in MDA levels under different levels of salicylic acid (SA: 0.0, 0.5, and 1.0 mM) and field capacity (FC: CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficit, 40-45% FC). Vertical bars represent the standard error of the average of three replications and different lowercase letters represent the significant differences at the level of 5% based on ANOVA-LSD multiple comparison results.

DISCUSSION

Water deficit can seriously affect plant growth and in order to maintain normal growth, plant relies on keeping a high water content in their protoplasts, achieved by osmotic adjustment. Plants under drought stress have been found to accumulate osmolytes such as carbohydrates and proline to increase tolerance to water deficit (Porcel and Ruiz-Lozano 2004). Delauney and Verma (1993) suggested that accumulation of proline in plant may be part of a general adaptation response to stresses and often may provide an adaptive advantage under water deficit conditions. In our experiment, it was observed that RWC significantly decreased with reduced soil moisture at 0.0 mM levels of SA compared to the CK plant, while accumulation of proline and soluble carbohydrates increased considerably as a consequence of drought stress. It could be inferred that *F. przewalskii* in order to prevent injury against drought stress when grown under water deprivation produced more carbohydrates and proline to increase tolerance. It is generally accepted that RWC is positively correlated with plant tolerance to stresses such as drought. In the present study, exogenous SA application significantly increased RWC under drought stress. Decreasing protein synthesis or increasing proteolysis led to accumulation of proline in plant cells. The accumulation of proline at drought stress conditions not only

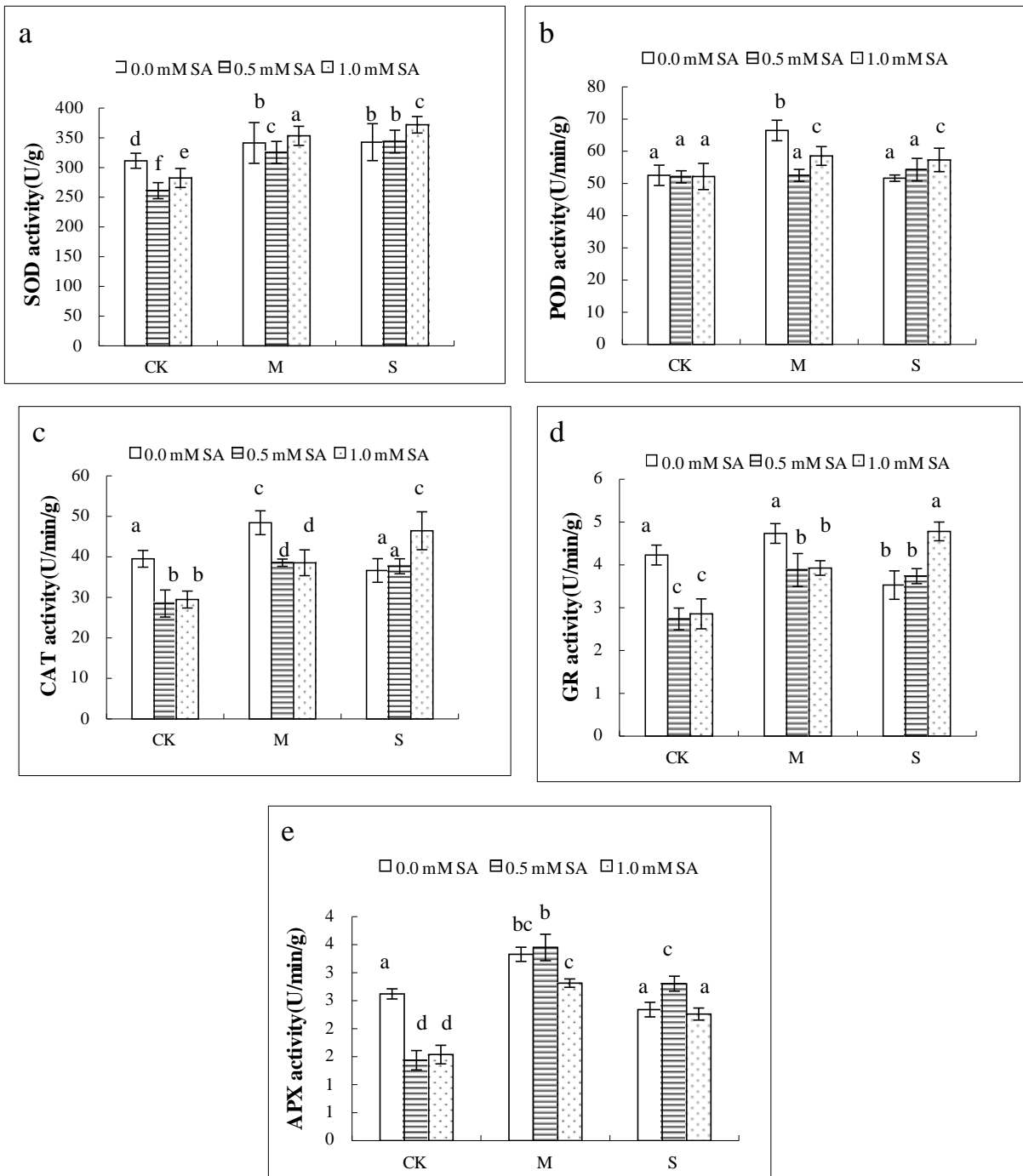


Figure 4. Changes in SOD (a), POD (b), CAT (c), GR (d), and APX (e) under different levels of salicylic acid (SA: 0.0, 0.5, and 1.0 mM) and field capacity (FC: CK, control, 75-80% FC; M, moderate water deficit, 60-65% FC; S, severe water deficit, 40-45% FC). Vertical bars represent the standard error of the average of three replications and different lowercase letters represent the significant differences at the level of 5% based on ANOVA-LSD multiple comparison results.

can protect the cell by buffering the cellular redox potential and stabilizing subcellular structures, but also can confer enzyme protection and increase membrane stability (Delauney and Verma 1993). Exogenous SA response to a variety of biotic and abiotic stresses leads to improve the plants resistance to stresses (Janda *et al.* 2014). This study also showed that exogenous SA increased RWC, leaf proline and total

soluble carbohydrates content at 0.5 mM and 1.0 mM levels compared to 0.0 mM SA in M and S. It indicated that SA regulated cellular processes by increasing osmolytes such as proline and soluble carbohydrates. These therefore led to increase RWC under water deprivation, so exogenous SA contributes to plants drought tolerance when plants are subjected to water deficit conditions. Porcel and Ruiz-Lozano

(2004) found that proline and total soluble sugars in leaves of soybean increased under drought stress were associated with a decrease in RWC. In *Aeluropus lagopoides*, RWC decreased by more than 20% when grown under drought condition, while soluble sugars and proline content increased (Mohsenzadeh *et al.* 2006). These findings on different plant species are in agreement with the findings on *F. przewalskii*.

The leaf chlorophyll content is an important physiological index related to the photosynthetic performance of plants. According to Singh and Dubey (1995), reduction in leaf chlorophyll content could be due to the lipid protein ratio to pigment-protein complexes changed, which increases the activity of chlorophyllase which leads to chlorophyll degradation and inhibits synthesis of photosynthetic pigments. Carotenoids also play a critical role in the plant antioxidant defense, but they are very susceptible to oxidative destruction and in severe stress, long-term chlorosis may appear (Singh and Dubey 1995). This study found out that Chl a, Chl a + b and carotenoids contents were not significantly affected in response to water deficit as compared to the CK, 0.0 mM SA. This suggests that photosynthetic capacity was not reduced under water deficit. The chlorophyll and carotenoids contents decreased under drought have been reported in some species by Munne-Bosch and Alegre (2000). The extent to which the chlorophyll and carotenoids contents decrease is dependent on the duration and severity of the drought stress. SA has been reported to enhance the activity of photosynthetic enzyme (Khodary 2004). Consequently, the efficiency of the photosynthetic apparatus was not increased due to SA treatments of the plants under drought stress. This may indicate that SA might not alleviate the adverse effects of drought stress via augmenting photosynthesis in *F. Przewalskii*.

Environmental constraints such as drought stress injure plants directly, and indirectly increase reactive oxygen species generation in the cells (Mutlu *et al.* 2009). So, at least in part, plant drought tolerance may depend on the enhancement of the antioxidative defense system. When plants are under environmental stress conditions, the concentrations of antioxidant enzymes become higher (Janda *et al.* 2014). Therefore, measuring the enzymes of the antioxidant defense systems can be an indirect method of evaluating plant oxidative stress. SOD is one of the most important antioxidant enzymes and plays an important role in the defense mechanism against ROS toxicity (Mutlu *et al.* 2009). When the cellular antioxidant defense is deficient or ROS is generated in excess, free radical chain reactions can occur and cause membrane lipid peroxidation which leads to MDA production. So, measuring MDA is a common method of determining lipid oxidation (Kadkhodaie *et al.* 2014.) In the present study, the MDA content increased with decreased soil moisture at 0.0 mM level of SA. Exogenous application of SA led to significantly decreased in MDA, SA increased ability of cellular defense antioxidant stress. This is in agreement with those who reported that drought stress increases the ROS in the leaves of plants, which can be indicated by the concentration of MDA (Mutlu *et al.* 2009). In plant cells, different antioxidative enzymes such as POD, CAT, GR and APX may take part in regulating the intracellular level of ROS toxicity. Janda *et al.* (2014) reported that applied exogenous SA at suitable concentrations can enhance the efficiency of antioxidant systems in plants. Drought induced harmful effects could be alleviated on fennel and the seed yield increased by foliar application of SA at high levels (Askari and Ehsanzadeh 2015). War *et al.* (2011) proposed that SA application increases activity of antioxidative enzymes and certain protein-based defensive compounds in plant. This study revealed that drought induces an oxidative stress, the activity of antioxidant system under moderate drought

stress induces SOD, CAT, CAT, GR and APX activities at 0.0 mM SA, whereas their activities were significantly decreased in severe drought stress. These antioxidant enzymes seemed to be crucial in lowering the extent of damage that might have been initiated by ROS. The results also showed that under certain water deficit conditions, the plant could resist oxidative stress and protect the plant from drought stress, but when the plant suffered from severe water deficit, the increase of ROS resulted from the increase of lipid peroxidation. Plants are damaged by drought stress. Under drought stress, exogenous SA at 0.5 mM and 1.0 mM levels could increase the antioxidant enzyme activity of the plants compared with the control plants. Exogenous SA significantly increased activity of antioxidant enzymes under drought stress at 0.5 mM and 1.0 mM levels of SA compared to CK plants. The modification of the antioxidant status of plant cells by SA application was previously investigated with pathogens (Mutlu *et al.* 2009). Therefore, application SA might alleviate the adverse effects of drought stress via increasing activity of antioxidative enzymes. Thus, it could be concluded that there was a strong correlation between drought tolerance and antioxidant system activity. These results give a considerable reason to believe that drought tolerance of *F. przewalskii* seems to be linked to an increase in the activity of antioxidant enzymes. This was in agreement with other reported by investigators Janda *et al.* (2014). Therefore, SA plays an important role in adaptation to certain levels of drought stresses by foliar spray. Senaratna *et al.* (2000) had emphasized the significance of exogenous application SA in agricultural crops, and they indicated that seeds of bean and tomato applied SA led to expression higher tolerance to heat, chilling, and drought stresses.

CONCLUSIONS

In conclusion, SA plays an important role in plant defense system, harmful effects of water stress on *F. przewalskii* could be alleviated by foliar spray with SA. Hence, SA application could contribute to plant drought tolerance under water deficit conditions. This effect not only might be due to the observable increase in RWC, leaf proline, and soluble carbohydrates contents, but also significantly increased the activities of antioxidant enzymes that all these traits enabled *F. przewalskii* against drought stress.

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COMPLIANCE WITH ETHICAL STANDARDS

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