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Adaptive responses of *Populus przewalskii* to drought stress and SNP application

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Abstract In this study we used the cuttings of *Populus przewalskii* Maximowicz as experimental material and sodium nitroprusside (SNP) as nitric oxide (NO) donor to determine the physiological and biochemical responses to drought stress and the effect of NO on drought tolerance in woody plants. The results indicated that drought stress not only significantly decreased biomass production, but also significantly increased hydrogen peroxide content and caused oxidative stress to lipids and proteins assessed by the increase in malondialdehyde and total carbonyl contents, respectively. The cuttings of *P. przewalskii* accumulated many amino acids for osmotic adjustment to lower water potential, and activated the antioxidant enzymes such as superoxide dismutase, guaiacol peroxidase and ascorbate peroxidase to maintain the balance of generation and quenching of reactive oxygen species. Moreover, exogenous SNP application significantly heightened the growth performance of *P. przewalskii* cuttings under drought treatment by promotion of proline accumulation and activation of antioxidant enzyme activities, while under well-watered treatment the effect of SNP application was very little.

Keywords Antioxidant enzymes · Drought stress · Osmotic adjustment · Proline accumulation

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Abbreviations

APX	Ascorbate peroxidase
C=O	Carbonyl content
GPX	Guaiacol peroxidase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
NO	Nitric oxide
ROS	Reactive oxygen species
SNP	Sodium nitroprusside
SOD	Superoxide dismutase

Introduction

Drought stress is a major limitation for plant survival and growth in the arid and semi-arid regions (Kramer and Boyer 1995). Several physiological and biochemical mechanisms are involved in the adaptation to drought by plants (Centritto 2005). One way many plants cope with osmotic stress is to synthesize and accumulate compounds termed osmoprotectants, including certain polyols, sugars, amino acids, betaines and related compounds (Bohnert and Jensen 1996). Free proline, one of the most important osmolytes, has been observed in response to a wide range of abiotic and biotic stresses in plants. The mechanism of proline action has not been fully understood yet, but it is suggested that the increased accumulation permits osmotic adjustment, as well as provides protection for enzymes and biological membranes (Sharma et al. 1998; Basak et al. 2001). Therefore, it is considered to be one of the first metabolic responses to stress, and also function as a second messenger (Hare and Cress 1997).

Another common effect of drought stress is the disturbance between the generation and quenching of reactive

oxygen species (ROS) (Smirnoff 1993). ROS, such as superoxide radicals ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH), are highly reactive and in the absence of effective protective mechanism, can seriously damage plants by lipid peroxidation, protein degradation, breakage of DNA and cell death (Beligni and Lamattina 1999). To keep the levels of active oxygen species under control, plants possess antioxidative systems which are composed of metabolites such as ascorbate, glutathione, tocopherol, etc., and enzymatic scavengers such as superoxide dimutases, peroxidases and catalases (Asada 1999). There are many cases that plants growing in hostile environments exhibit increased oxy-stress enzyme activities to combat the deleterious effect of ROS (Jebara et al. 2005).

Nitric oxide (NO) is a water and lipid soluble small gas molecule and in recent years it has emerged as a major signaling molecule of ubiquitous importance (Durner et al. 1999). Many previous studies have reported its presence in the plant kingdom and its involvement in growth, development (Beligni and Lamattina 1999) and defense responses to drought stress (Leshem 1996; Haramaty and Leshem 1997), osmotic stress (Xing et al. 2004), heat stress (Leshem et al. 1998), heavy metal toxicity (Hsu and Kao 2004), disease resistance (Delledonne et al. 1998), and apoptosis (Pedroso et al. 2000). As a free-radical molecule, NO is either protective or toxic depending on its concentration and different tissues it acts (Beligni and Lamattina 1999). The application of exogenous NO to whole plants or cell cultures has allowed obtaining valuable information on how this molecule affects some physiological and biochemical processes.

Altogether, we hypothesized that there could be a large set of parallel changes in the physiological and biochemical responses when plants were exposed to drought stress, exogenous NO application in a certain concentration could enhance the drought tolerance in plants. In this study we used the cuttings of *Populus przewalskii* Maximowicz as experimental material and sodium nitroprusside (SNP) as NO releaser to determine the physiological and biochemical responses to drought stress and the effect of NO on drought tolerance in trees.

Materials and methods

Plant material and experiment design

The cuttings of *P. przewalskii* Maximowicz were collected from Aba region (32°57′N, 101°56′E, 3,290 m altitude), Sichuan Province, Southwest China. The mean annual rainfall and mean annual temperature in this region are 712 mm and 3.3°C, respectively. After sprouting and growing for about 1 month, the healthy cuttings of approximately equal height were selected and replanted into 5-l plastic pots filled with homogenized soil. And then they were grown in a naturally lit greenhouse under the semi-controlled environment with a temperature range of 18.0–32.0°C and relative humidity range of 50–80% during 1 May to 1 August 2005.

The experimental designs were completed as follows: 60 pots under well-watered treatment were watered to 100% of field capacity by supplying an amount of water equal to transpiration losses every day, another 60 pots under drought treatment were maintained at 25% of field capacity by watering every day. In each watering treatment, half of the cuttings were sprayed with 10 ml exogenous 0.2 mM SNP (Sigma, St Louis, MO, USA) per day, another half of the cuttings were sprayed with 10 ml water per day as control. Each treatment included five replications and six cuttings per replication were used. Following periods of rapid growth, an empirical relationship between plant fresh weight (Y, g) and plant height (X, cm): $Y = 0.975 + 0.112X$ ($R^2 = 0.968$, $P < 0.001$) (Li et al. 2004), was used to correct pot water for changes in plant biomass.

Growth measurements

All cuttings were harvested at the end of the experiment and the total leaf area was determined by a Portable Laser Area Meter (CI-203, CID Inc., Camas, WA). Biomass samples were dried (80°C, 48 h) to constant weight and weighed.

Detection of indices of stress damage

The relative water content (RWC) of leaves was calculated according to the following formula: 100 × [(fresh weight – dry weight)/(saturated weight – dry weight)]. Saturated weight was determined after incubation of the leaf in water for 24 h at room temperature. Dry weight was measured following oven drying at 80°C until constant weight. H_2O_2 content was measured colorimetrically as described by Jana and Choudhuri (1982). H_2O_2 was extracted by homogenizing 0.5 g leaf samples with phosphate buffer (50 mM, pH 6.5) containing 1 mM hydroxylamine. The homogenate was centrifuged at 6,000×*g* for 20 min. To determine H_2O_2 content, the extracted solution was mixed with 0.1% titanium chloride in 20% (v/v) H_2SO_4. The mixture was then centrifuged at 10,000×*g* for 25 min. The absorbance was measured at 415 nm. Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2. Leaf oxidative damage to lipids was expressed as equivalents of MDA contents. About 0.5 g leaf segments were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12,000×*g* for 10 min. After that, 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA was

added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10,000×*g* for 10 min, the absorbance of the supernatant at 450, 532 and 600 nm was determined. The MDA content was calculated according to Hodges et al. (1999). Oxidative damage to protein was quantified as total protein carbonyl content as described by Levine et al. (1994). One gram leaves were homogenized in 100 mM sodium phosphate buffer (pH 7.4) containing 1 mM EDTA, 2 mM dithiothreitol, 0.2% (v/v) Triton X-100 and 1 mM PMSF. Homogenates were filtered through two layers of Miracloth and centrifuged 10,000×*g* for 20 min and the supernatants were incubated with 0.03% (v/v) Triton X-100 and 1% (w/v) streptomycin sulphate for 20 min to remove the nucleic acids. After then centrifuging at 2,000×*g*, supernatants (200 µl) were mixed with 300 µl of 10 mM DNPH (2,4-dinitrophenylhydrazine) in 2 M HCl. After 1 h incubation at room temperature, proteins were precipitated with 10% (w/v) trichloroacetic acid (TCA) and the pellets were washed three times with 500 µl of ethanol/ethylacetate (1:1). The pellets were finally dissolved in 6 M guanidine hydrochloride in 20 mM potassium phosphate buffer and the absorption at 370 nm was measured.

Contents of free proline and total amino acids determination

Free proline was extracted and determined as described by Bates et al. (1973); 0.5 g leaves were homogenized in a mortar after the addition of a small amount of quartz sand and 10 ml of a 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was centrifuged at 3,000×*g* for 20 min. The supernatant was treated with acid ninhydrin (2.5 g ninhydrin/100 ml of a solution containing glacial acetic acid, distilled water and *ortho*-phosphoric acid 85% at a ratio of 6:3:1) boiled for 1 h, and the reaction was terminated in a water bath of room temperature (25°C) for 10 min. Then absorbance at 520 nm was determined using L-proline as standard. Contents of proline were expressed as µg g^{-1} dry weight (DW).

The quantitative measurement of total amino acids of the supernatant was done using the ninhydrin reaction (Correia et al. 2005). Two milliliters of buffered ninhydrin solution (0.8 g of ninhydrin and 0.12 g of hydrindantin dissolved in 30 ml of 2-methoxyethanol plus 10 ml of acetate buffer 4 M, pH 5.5) were added to 1 ml of supernatant and heated in a boiling water bath for 15 min. The mixture was cooled to room temperature. Three milliliters of 50% ethanol were added and the absorbance was read at 570 nm after 10 min. The amount of amino acids was determined by reference to a standard curve previously prepared with arginine.

Antioxidant enzyme activities assay

Frozen leaf segments about 0.5 g were crushed into a fine power under liquid nitrogen. Soluble proteins were extracted by homogenizing the powder in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone, with the addition of 1 mM ASA in the case of APX assay. The homogenate was centrifuged at 12,000×*g* for 20 min at 4°C and then the supernatant was used for the following enzyme assays. (1) SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) as described by Giannopolitis and Ries (1977). The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0.1 ml enzyme extract. The reaction mixtures were illuminated for 15 min at light intensity of 75 µmol m^{-2} s^{-1}. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. (2) GPX (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation (extinction coefficient 26.6 mM^{-1} cm^{-1}) at 470 nm by H_2O_2. The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), 16 mM guaiacol and 0.1 ml of 10% H_2O_2 in a 3 ml volume. The reaction was initiated by adding 50 µl enzyme extract and was followed for 3 min (Lin and Wang 2002). (3) APX (EC 1.11.1.11) activity was analyzed by following the decrease in A290 (extinction coefficient 2.8 $mM^{-1}cm^{-1}$) for 1 min in 3 ml of a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM ASA (sodium ascorbate), 2.5 mM H_2O_2 and 200 µl enzyme extract (Nakano and Asada 1981). In addition, total soluble protein contents were determined as described by Bradford (1976), using bovine serum albumin as a calibration standard.

Statistical analysis

Analyses of variance (ANOVA) for all variables from measurements were used for testing the possible differences between the treatments. Pearson's correlation coefficients were calculated to determine the relationships between variables using individual data. Statistical analysis was done with SPSS 11.0 for windows statistical software package.

Results

Morphological properties

Drought significantly decreased these morphological properties including shoot height (Sh), basal diameter (Bd),

Table 1 Shoot height (Sh), basal diameter (Bd), total biomass (Tb), leaf number (Ln), total leaf area (Tla) and average leaf area (Ala) in *P. przewalskii* cuttings as affected by drought stress and exogenous SNP application

	Shoot height (cm)	Basal diameter (mm)	Total biomass (g)	Leaf number	Total leaf area (dm^2)	Average leaf area (cm^2)
Water	104.67 ± 3.44 b	8.65 ± 0.59 a	27.29 ± 2.82 b	32.30 ± 4.72 a	2138.3 ± 176.4 a	66.72 ± 4.23 a
Water + SNP	123.07 ± 3.08 a	8.94 ± 0.67 a	33.75 ± 2.13 a	33.33 ± 3.58 a	2384.3 ± 185.5 a	72.96 ± 11.26 a
Drought	36.00 ± 9.51 d	3.17 ± 0.29 c	4.98 ± 0.34 d	15.20 ± 4.15 b	498.9 ± 34.3 c	32.32 ± 3.36 c
Drought + SNP	69.20 ± 11.12 c	6.16 ± 0.35 b	11.24 ± 0.28 c	22.40 ± 1.52 b	941.1 ± 90.9 b	42.02 ± 5.89 b
Fw	0.000	0.000	0.000	0.000	0.000	0.000
Fs	0.000	0.000	0.004	0.356	0.028	0.016
Fw × s	0.037	0.000	0.960	0.210	0.502	0.529

Values (means of five replicates ± SE) followed by different letters are significantly different from each other at the $P < 0.05$ level

Fw watering effect; *Fs* SNP effect; *Fw* × *s* watering × SNP interaction effect

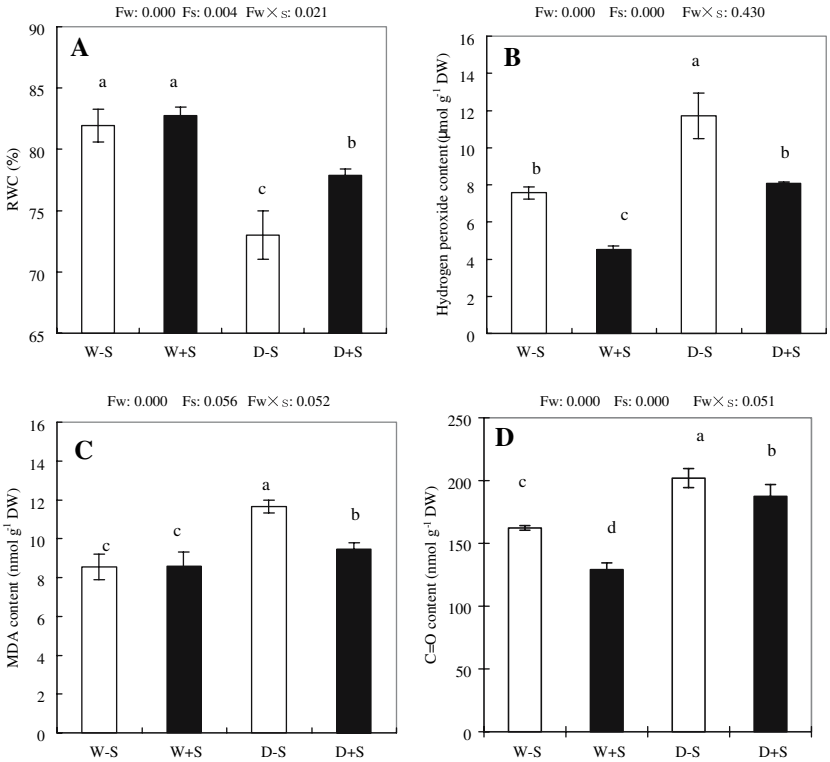

Fig. 1 RWC (**a**), H_2O_2 (**b**), MDA (**c**) and C=O (**d**) contents of *P. przewalskii* cuttings as affected by drought stress and exogenous SNP application. Values (five mean ± SE) followed by different letters are significantly different from each other at the $P < 0.05$ level. *Fw*, watering effect; *Fs*, SNP effect; *Fw* × *s*, watering × SNP interaction effect. *W*, well-watered treatment; *D*, drought treatment; *open square*, no SNP application; *filled square*, 0.2 mM SNP application

total biomass (Tb) and leaf number (Ln), total leaf area (Tla) and average leaf area (Ala) ($P < 0.001$). Under well watered treatment SNP application showed no obvious effect, while it significantly increased these parameters under drought treatment ($P < 0.001$) (Table 1).

Stress indices

Drought also significantly decreased RWC and increased the H_2O_2, MDA and C=O contents ($P < 0.001$). Under-well watered treatment SNP application had no obvious effect on RWC, and MDA, while it significantly increased RWC and decreased MDA content under drought treatment. SNP application significantly decreased the C=O and H_2O_2 contents under both well watered and drought treatments ($P < 0.001$) (Fig. 1).

Proline and total amino acids contents

Drought significantly increased the contents of free proline and total amino acids ($P < 0.001$). SNP application significantly increased total amino acids under both well watered and drought treatments. SNP application also significantly increased proline content under drought treatment, while little effect on proline content under well-watered treatment (Fig. 2).

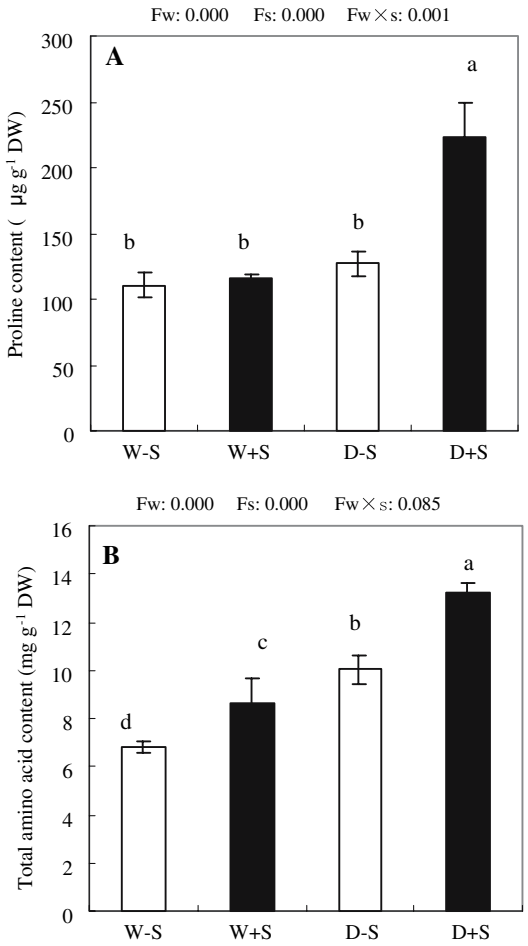

Fig. 2 Proline (**a**) and total amino acids (**b**) contents of *P. przewalskii* cuttings as affected by drought stress and exogenous SNP application. Values (five mean ± SE) followed by different letters are significantly different from each other at the $P < 0.05$ level. *Fw*, watering effect; *Fs*, SNP effect; *Fw* × *s*, watering × SNP interaction effect. *W*, well-watered treatment; *D*, drought treatment; *open square*, no SNP application; *filled square*, 0.2 mM SNP application

Antioxidant enzyme activities

Drought activated the antioxidant enzymes including SOD, GPX and APX. Under well-watered treatment, SNP application had no obvious effect on these parameters, while under drought treatment SNP application significantly increased the three enzyme activities further (Fig. 3).

Relationships between RWC and physiological and biochemical properties

RWC was negatively correlated with H_2O_2, MDA, SOD, GPX and APX activities under control and exogenous SNP application. However, RWC was negatively correlated with free proline and total amino acids contents under control, and negatively correlated with C=O under exogenous SNP application (Table 2).

Discussion

Plants usually respond to drought stress through structural modifications and growth pattern adjustment, including a reduction in total dry mass accumulation and limitation on leaf numbers and leaf areas. Our results showed that drought stress significantly decreased Sh, Bd, Tb, Ln, Tla and Ala in *P. przewalskii* cuttings, which was consistent with many previous studies (Yin et al. 2004; Zhang et al. 2004). Another consequence of drought stress in *P. przewalskii* cuttings was the decrease of leaf RWC, it could be interpreted as a mechanism that concentrates solutes in the cell sap, thereby lowering the osmotic potential and contributing to osmotic adjustment (Lissner et al. 1999). On the other hand, drought stress also induced oxidative burst as manifested by the increase of H_2O_2 content in *P. przewalskii* cuttings (Fig. 1). At low concentration, H_2O_2 and other ROS may function in cellular signaling process as secondary messengers to induce a number of genes and proteins involved in stress defenses (Jiang and Zhang 2001), while at high amounts they lead to lipid peroxidation and protein modification as indicated by the increase in MDA and C=O contents, respectively (Fig. 1).

Under drought stress, contents of total amino acids in *P. przewalskii* cuttings were significantly increased, which might contribute to osmotic adjustment and allow plant to maintain turgor pressure and adapt to limited water availability. Many higher plants have been found to accumulate proline under various stressful conditions due to its many advantages (Delauney and Verma 1993; Yin et al. 2005), while whether proline levels are reliable indicators of stress tolerance have been the subject of numerous discussions and are still vigorously debated (Hare and Cress 1997). And Kiyosue et al. (1996) also found that severe dehydration in *Arabidopsis* was associated with induction of both proline dehydrogenase and P5C synthetase, with no net accumulation of free proline, which is similar to our result that there was no significant proline accumulation under drought stress. On the other hand, H_2O_2 as secondary messenger may only occur under subtoxic condition and at higher concentration they are still harmful to plant survival. Therefore, plants have evolved an entire set of antioxidant systems to keep them under control. Drought stress caused an increase in enzyme activities such as SOD, GPX and APX in *P. przewalskii* cuttings to coordinate the AOS concentrations (Fig. 3). The role of antioxidant enzymes under stressful conditions has already been reported by many earlier studies (Schwanz et al. 1996; Kronfub et al. 1998; Duan et al. 2005; Yin et al. 2005).

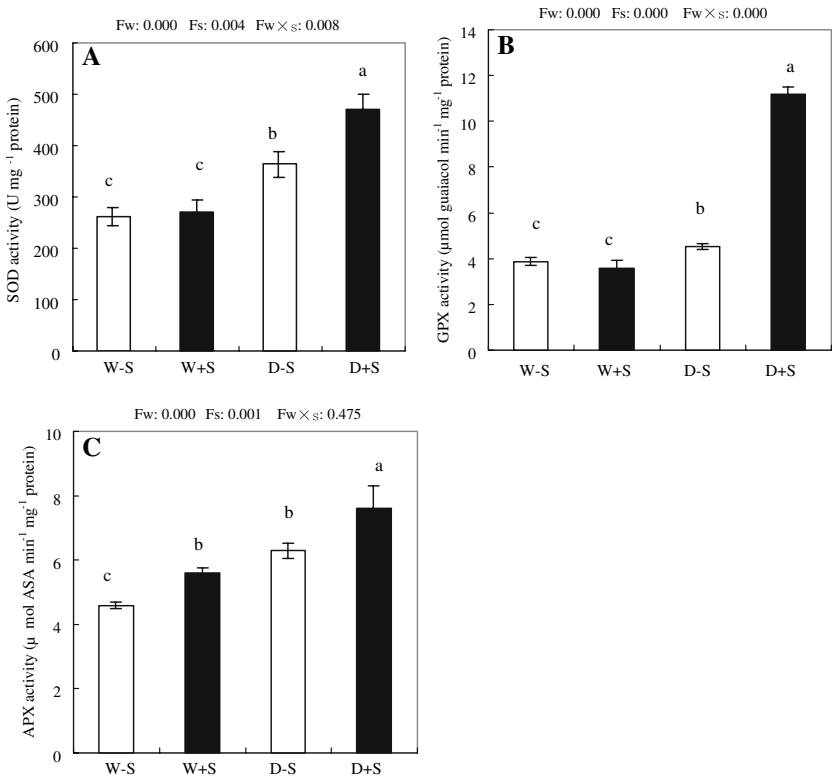

Fig. 3 SOD (**a**), GPX (**b**) and APX (**c**) activities of *P. przewalskii* cuttings as affected by drought stress and exogenous SNP application. Values (five mean ± SE) followed by different letters are significantly different from each other at the $P < 0.05$ level. *Fw*, watering effect; *Fs*, SNP effect; *Fw* × *s*, watering × SNP interaction effect. *W*, well-watered treatment; *D*, drought treatment; *open square*, no SNP application; *filled square*, 0.2 mM SNP application

Table 2 Correlation coefficients between RWC and physiological and biochemical properties of *P. przewalskii* cuttings under control and exogenous SNP application

RWC	H_2O_2	MDA	C=O	Pro	TAA	SOD	GPX	APX
Control	–0.902*	–0.960**	-0.811^{NS}	–0.951**	–0.912*	–0.920**	–0.922**	–0.913*
SNP	–0.919**	–0.944**	–0.974**	-0.646^{NS}	-0.787^{NS}	–0.917**	–0.886*	–0.908*

RWC relative water content; *H_2O_2* hydrogen peroxide; *MDA* malondialdehyde; *C=O* carbonyl content; *Pro* free proline; *TAA* total amino acids; *SOD* superoxide dimutase; *GPX* guaiacol peroxidase; *APX* ascorbate peroxidase; *NS* not significant

* $P < 0.05$; ** $P < 0.01$

In a series of studies, exogenous SNP proved to be capable of alleviating some consequences of stressful condition. For example, SNP-treated rice seedlings permitted higher survival rate under salt and heat stresses (Uchida et al. 2002), SNP-treated potato plants were resistant to oxidative stress (Beligini and Lamattina 1999) and SNP-treated wheat seedlings enhanced adaptive responses against drought stress (Garcia-Mata and Lamattina 2001). And NO was also found to stimulate seed germination and counter the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus* (Kopyra and Gwozdz 2003). In our study, under drought treatment exogenous SNP significantly increased growth and dry mass accumulation in *P. przewalskii* cuttings, while under well-watered treatment its effect was very little, which is consistent with report of Ruan et al. (2004) who found that 0.1 mM SNP hardly affected wheat seedlings grown in Hoagland's solution, while SNP application significantly enhanced its salt tolerance under 150 mM NaCl solution.

Exogenous SNP also altered some physiological and biochemical responses, including proline accumulation and ROS metabolism, which might, in part, account for its protective effect on *P. przewalskii* cuttings under drought stress. SNP increased the proline content under drought stress, which was in well agreement with Ruan et al. (2004) who found that 0.1 mM SNP effectively induced proline accumulation in wheat seedlings under salinity stress. Moreover, two NO scavengers, hemoglobin and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) inhibited the effect of SNP. Uchida et al. (2002) also found that the expression of *P5CS* (Δ^1-pyrroline-5-carboxylate synthetase), the key enzyme in proline synthesis, was increased in response to SNP in rice seedlings, which could promote proline accumulation and

confer increased tolerance to salt stress. Furthermore, previous studies reported that abscisic acid (ABA) increased significantly in many higher plants under drought stress, and it was strongly proposed to be involved in proline accumulation (Hare et al. 1999). It was showed that NO could activate the synthesis of endogenous ABA in wheat seedling leaves under salt stress, therefore, NO might induce proline accumulation via ABA transduction cascade through activation of ABA synthesis (Ruan et al. 2004). Moreover, NO may enhance the adaptive plant responses against drought stress through the induction of stomatal closure as suggested by Garcia-Mata and Lamattina (2001). They found that 150 mM SNP retained up to 15% more water than the control, which was consistent with a 20% decrease in the transpiration rate.

As is now commonly accepted NO as a second messenger in plants, it is supposed that low concentration of NO might be a signal molecule to induce/stabilize the expression of many antioxidative enzymes including SOD and CAT which are proven in many animal and plant materials (Frank et al. 2000). It was evidenced in our study that NO significantly increased the activities of SOD, GPX and APX in *P. przewalskii* cuttings under drought stress (Fig. 3). The protective effect of NO may also be related to its ability to react with some ROS, such as O_2^-. Through abrogating O_2^- mediated cytotoxic effects through the conversion of O_2^- into $ONOO^-$, NO thus act as a chain breaker and show its proposed antioxidant properties (Conner and Grisham 1996). In addition, it has also been reported that NO can react with lipid alcoxyl (LO·) and peroxyl (LOO·) radicals, leading to the expectation that NO could stop the propagation of radical-mediated lipid oxidation in a direct fashion (Lamotte et al. 2004). Therefore, NO may help plants to survive under stressful conditions through its action as signaling molecule to activate antioxidative enzymes and reaction with active oxygen and lipid radicals directly.

In conclusion, drought stress significantly decreased growth, biomass accumulation and relative water content in *P. przewalskii* cuttings; free proline was accumulated for osmotic adjustment to lower water potential for continued water uptake. Drought stress also significantly increased the H_2O_2 content and caused oxidative stress to lipids and proteins in *P. przewalskii* cuttings. The antioxidant enzymes, including SOD, GPX and APX, were activated to maintain the favorable redox state for survival under drought stress. Exogenous SNP application conferred drought tolerance by promotion of proline accumulation and activation of antioxidant enzyme activities. However, the mechanism of NO function needs to be further elucidated maybe using NO gas, other NO donors and NO scavengers.

References

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Basak M, Sharma M, Chakraborty U (2001) Biochemical responses of *Camellia sinensis* (L.) to heavy metal stress. J Environ Biol 22:37–41

Bates LS, Waldren SP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Beligni MV, Lamattina L (1999) Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. Planta 208:337–344

Bohnert HJ, Jensen RG (1996) Strategies for engineering water-stress tolerance in plants. Trends Biotechnol 14:89–97

Bradford MM (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Centritto M (2005) Photosynthetic limitations and carbon partitioning in cherry in response to water deficit and elevated [CO_2]. Agr Ecosyst Environ 106:233–242

Conner EM, Grisham MB (1996) Inflammation, free radicals and antioxidants. Nutrition 12:274–277

Correia I, Nunes A, Duarte IF, Barros A, Delfadillo I (2005) Sorghum fermentation followed by spectroscopic techniques. Food Chem 90:853–859

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J 4:215–223

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Duan B, Lu Y, Yin C, Junttila O, Li C (2005) Physiological responses to drought and shade in two contrasting *Picea asperata* populations. Physiol Plant 124:476–484

Durner J, Gow AJ, Stamler JS, Glazebrook J (1999) Ancient origins of nitric oxide signaling in biological systems. Proc Natl Acad Sci 96:14206–14207

Frank S, Kämpfer H, Podda M (2000) Identification of copper/zinc superoxide dismutase as a nitric oxide-regulated gene in human (HaCaT) keratinocytes: implications for keratinocyte proliferation. Biochem J 346:719–728

Garcia-Mata C, Lamattina L (2001) Nitric oxide induces stomatal closure and enhances the adaptive responses against drought stress. Plant Physiol 126:1196–1204

Giannopolitis CN, Ries SK (1977) Superoxide dismutase I: occurrence in higher plants. Plant Physiol 77:309–314

Haramaty E, Leshem YY (1997) Ethylene regulation by the nitric oxide ($NO^·$) free radical: a possible mode of action of endogenous NO. In: Kanellis AK, Chang C, Klee H (eds) Biology and biotechnology of the plant hormone ethylene, Kluwer, Dordrecht, pp 253–258

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 21:79–102

Hare PD, Cress WA, van Staden J (1999) Proline synthesis and degradation: a model system for elucidating stress related signal transduction. J Exp Bot 50:413–434

Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–611

Hsu YT, Kao HC (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. Plant Growth Regul 42:227–238

Jana S, Choudhuri MA (1982) Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat Bot 12:345–354

Jebara S, Jebara M, Limam F, Aouani ME (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. J Plant Physiol 162:929–936

Jiang M, Zhang J (2001) Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol 42:1265–1273

Kiyosue T, Yoshiba K, Yamaguchi-Shinozaki K, Shinozaki K (1996) A nuclear gene encoding mitochondrial proline dehedrogenase, en enzyme in proline metabolism, is upregulated by proline but downregulated by dehydration in Arabidopsis. Plant Cell 8:1323–1335

Kopyra M, Gwozdz EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of Lupinus luteus. Plant Physiol Biochem 41:1011–1017

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic, San Diego

Kronfub G, Polle A, Tausz M, Havranek WM, Wieser G (1998) Effects of ozone and mild drought stress on gas exchange, antioxidants and chloroplast pigments in current-year needles of young Norway spruce (*Picea abies* L., Karst.). Trees 12:482–489

Lamotte O, Gould K, Lecourieux D, Sequeira-Legrand A, Lebun-Garcia A, Durner J, Pugin A, Wendehenne D (2004) Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein. Plant Physiol 135:516–529

Leshem YY (1996) Nitric oxide in biological systems. Plant Growth Regul 18:155–159

Leshem YY, Wills RBH, Ku VV (1998) Evidence for the function of the free radical gas–nitric oxide (NO.)—as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol Biochem 36:825–833

Levine RL, Willians JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–363

Li C, Yin C, Liu S (2004) Different responses of two contrasting *Populus davidiana* populations to exogenous abscisic acid application. Environ Exp Bot 51:237–246

Lin J, Wang G (2002) Doubled CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat. Plant Sci 163:627–637

Lissner J, Schierup HH, Comn FA, Astorga V (1999) Effect of climate on the salt tolerance of two Phragmites australis populations.I. Growth, inorganic solutes, nitrogen relations and osmoregulation. Aquat Bot 64:317–333

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Pedroso MC, Magalhacs JR, Durzan D (2000) A nitric oxide burst precedes apoptosis in angiosperm and gymnosperm callus cells and foliar tissues. J Exp Bot 51:1027–1036

Ruan H, Shen W, Xu L (2004) Niric oxide involved in the abscisic acid induced proline accumulation. Acta Bot Sin 46:1307–1315

Schwanz P, Picon C, Vivin P, Dreyer E, Guehi JM, Polle A (1996) Response of antioxidative systems to drought stress in pendunculate oak and maritime pine as modulated by elevated CO_2. Plant Physiol 110:393–402

Sharma SS, Schat H, Vooijs R (1998) In vitro alleviation of heavy metal induced enzyme inhibition by proline. Phytochemistry 49:1531–1535

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci 163:515–523

Xing H, Tan L, Zn L, Zhao Z, Wang S, Zhang C (2004) Evidence for the involvement of nitric oxide and reactive oxygen species in osmotic stress tolerance of wheat seedlings:Inverse correlation between leaf abscisic acid accumulation and leaf water loss. Plant Growth Regul 42:61–68

Yin C, Duan B, Wang X, Li C (2004) Morphological and physiological responses of two contrasting Poplar species to drought stress and exogenous abscisic acid application. Plant Sci 167:1091–1097

Yin C, Peng Y, Zang R, Zhua Y, Li C (2005) Adaptive responses of *Populus kangdingensis* to drought stress. Physiol Plant 123:445–451

Zhang X, Zang R, Li C (2004) Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. Plant Sci 166:791–797