ISSN 1021-4437, Russian Journal of Plant Physiology, 2008, Vol. 55, No. 6, pp. 857–864. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © Y. Lei, 2008, published in Fiziologiya Rastenii, 2008, Vol. 55, No. 6, pp. 945–953.

RESERCH PAPERS

Physiological Responses of *Populus przewalskii* to Oxidative Burst Caused by Drought Stress[1]

Y. Lei

Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan, 666303 China;
e-mail: leiyb@xtbg.org.cn

Received December 18, 2007

Abstract—We measured dry matter accumulation and allocation to the roots, leaf gas exchange, chlorophyll fluorescence, antioxidant enzymes, and ABA and polyamine (PA) contents in *Populus przewalskii* under three different watering regimes (100, 50, and 25% of the field capacity) to investigate the morphological and physiological responses to water deficit in woody plants. The results showed that drought stress retarded *P. przewalskii* as evident from a decreased biomass accumulation and the reduced increment of shoot height and basal diameter. Drought stress also affected the biomass partition by higher biomass allocated to the root systems for water uptake. The contents of ABA and PAs especially were increased under stressful conditions. Drought stress caused oxidative burst indicated by the accumulation of peroxide (H_2O_2), and fluorimetric detection also confirmed the increased accumulation of H_2O_2. The antioxidant enzymes, including superoxide dimutase, peroxidase, ascorbate peroxidase, and reductase, were activated to bring the reactive oxygen species to their homeostasis; however, oxidative damages to lipids, proteins, and membranes were significantly manifested by the increase in total carbonyl (C=O) and electric conductance (EC).

DOI: 10.1134/S1021443708060186

Key words: Populus przewalskii - ABA - antioxidant enzymes - drought stress - oxidative damage - polyamines

INTRODUCTION

Water availability is an important factor affecting photosynthesis, growth, and survival of plants, mainly in arid and semi-arid regions. In general, diverse strategies including morphological, physiological, and molecular mechanisms are involved in plant responses to drought stress [1]. Stomatal control of water losses has been identified as an early event in plant responses to water deficit [2]. As drought progresses, stomatal closure occurs for longer periods of the day, reducing water loss at the time of the highest evaporative demand, leading to a near optimization of carbon assimilation in relation to water supply [3]. Nevertheless, net photosynthesis is unavoidably reduced due to decreased CO_2 availability at chloroplast level [4].

Another drawback of stomata closure is the production of reactive oxygen species (ROS) because the changes in the photochemistry of the chloroplasts in the leaves of drought-stressed plants result in dissipation of excess light energy used to form ROS [5]. ROS, such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$), are highly reactive and, in the absence of effective protective mechanism, can seriously impair the normal function of plants by lipid peroxidation, protein degradation, and DNA nicking and may even cause cell death [6]. To keep the levels of ROS under control, plants possess antioxidant systems, which are composed of metabolites, such as ascorbate, glutathione, tocopherol, etc., and enzymatic scavengers [5]. These enzymes include superoxide dismutase (SOD, EC 1.15.1.1), which reacts with superoxide radicals and converts them into O_2 and H_2O_2. H_2O_2 is then detoxified by catalase (CAT, EC 1.11.1.6) and/or ascorbate peroxidase (APX, EC 1.11.1.11). In addition, nonspecific peroxidases (POD, EC 1.11.1.7) also play an important role in the antioxidant protection. are many cases where plants growing in hostile environments exhibit increased oxy-stress enzyme activities to combat ROS deleterious effect [7, 8].

Drought stress also alters the hormone homeostasis in plants, including ABA and polyamines (PAs). ABA is thought to play an important role in the adaptation of plants to environmental stresses [9, 10]. In addition to the role of limiting transpirational losses, particularly under water deficit, ABA may be related to oxidative stress responses as cellular signal to induce a number of genes and proteins involved in stress defenses, including antioxidant enzymes and pathogenesis-related (PR)

Abbreviations: APX—ascorbate peroxidase; DAB—3,3'-diaminobenzidine; EC—electric conductance; FC—field capacity; GR—glutathione reductase; MDA—malondialdehyde; NBT–nitro blue tetrazolium; PA–polyamine; PSII—photosystem II; POD–peroxidase; Put—putrescine; ROS—reactive oxygen species; RWC—relative water content; SOD—dimutase; Spd—spermidine; Spm—spermine; WUE—water use efficiency.

[1] This text was presented by the authors in English.

proteins [11, 12]. ABA is also implicated in the process of proline accumulation for osmotic adjustment through activating the biosynthesis enzymes [13]. PAs are polybasic aliphatic amines that are ubiquitous in prokaryotic and eukaryotic organisms. It is believed that PAs play important roles in growth, key developmental processes of plants, and defense against environmental stresses [14]. However, its precise physiological relevance remains elusive.

Poplars are among the fastest growing trees under temperate latitudes and are widely used for timber, pulp, and paper [15]. They also play important roles in ecosystems by preventing soil erosion and regulating climate, thus retaining stability [2]. Unfortunately, poplar species grow in arid and sem-iarid environments and are exposed to long periods of water stress [16]. Thus, it is important to explore the basics of woody plants adaptation to water stress and maintainity growth, development, and productivity during stress periods. Therefore, in this study, we used *Populus przewalskii* as a model species to investigate the acclimation and adaptation to drought stress, especially the ROS generating and scavenging systems and their damaging effects.

MATERIALS AND METHODS

Plant materials and experiment design. Cuttings of *Populus przewalskii* were collected in March 2005 and, after sprouting and growing for about 1 month, 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 May 1 to August 1, 2005. Sixty cuttings were selected randomly to three different watering regimes as follows: one well watered treatment (100% of field capacity (FC)) and two water-stressed treatments (50 and 25% of FC). Each treatment included five replications, and 4 cuttings per replication were used.

During the experiment, the pots were rewatered to different FC by replacing the amount of water transpired every day. Following periods of rapid growth, an empirical relationship between plant fresh weight (Y, g) and plant height (x, cm), $Y = 0.975 + 0.112x$ ($R^2 = 0.968$, $P < 0.001$) [17], was used to correct pot water for changes in plant biomass. Shoot height and basal diameter were measured every ten days during the stress period.

Relative water content, ABA, and polyamines measurement. The relative water content (RWC) of leaves was calculated according to the 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. ABA was analyzed using ELISA box (purchased from Nanjing Agriculture University of China), as described by Xiong et al. [18].

PAs were extracted and measured according to Flores and Galston [19]. The standards, Put, Spm, and Spd were dissolved in 1 ml of perchloric acid and benzoylated following the same procedure as with the samples.

Gas exchange and chlorophyll fluorescence determination. During the growth season, net photosynthesis (A) and stomatal conductance (G_s) were measured with a Li-Cor 6200 portable photosynthesis system (Li-Cor, United States). Light, temperature, and humidity were 400 μmol/(m^2 s), 23 ± 1°C, and 60%, respectively. CO_2 was maintained at a constant level of 400 μmol/l using a Li-6400-01 CO_2 injector. Water use efficiency (WUE) was calculated by dividing instantaneous values of A by G_s.

Photochemical efficiency was estimated via chlorophyll *a* fluorescence of photosystem II (PSII), using a modulated PAM 2100 fluorometer (Walz, Germany) as described by Schreiber et al. [20]. The quantum yield of PSII electron transport during steady state photosynthesis (Y) was measured in light-adapted leaves under light condition, whereas the ratio of variable to maximal fluorescence (F_v/F_m) was measured in dark-adapted leaves that had been enclosed in aluminum foil for 30 min. The saturating light pulse was delivered at 260 μmol/(m^2 s) for 0.8 s.

Detection of H_2O_2 and indices of stress damage. The levels of H_2O_2 were measured by monitoring the absorbance of the titanium–peroxide complex at 415 nm, following the method of Brennan and Frenkel [21]. Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2.

The relative intactness of plasma membrane was measured as the leakage percentage of electrolytes as described by Gong et al. [22]. Leaf oxidative damage to lipids was expressed as the content of malondialdehyde (MDA) followed by the method of Hodges et al. [23]. Proteins were extracted from 0.5 g of leaf segments as described in detail [24], and oxidative damage was quantified as total protein carbonyl content by reaction with 2,4-dinitrophenylhydrazine after the removal of possible contaminating nucleic acids with 1% (w/v) streptomycin sulfate [25]. To determine changes in viability of cells affected by water stress, excised leaves were infiltrated with a 0.25% (w/v) aqueous solution of Evans blue [26]. Then the leaves were discolored in boiling ethanol to develop the blue precipitates, which were quantified by solubilization with 1% (w/v) SDS in 50% (v/v) methanol at 50°C for 30 min, and the absorbance was measured at 600 nm.

Histochemical and fluorimetric assay of H_2O_2. The detached leaves were excised and immersed in a 1% solution of 3,3'-diaminobenzidine (DAB) in 10 mM Mes buffer (pH 6.5), vacuum-infiltrated for 5 min, and then incubated at room temperature for 8 h in the absence of light. Leaves were illuminated until the appearance of brown spots characteristic of the reaction

of DAB with H_2O_2. Leaves were bleached by immersing in boiling ethanol to visualize the brown spots. H_2O_2 deposits were quantified by scanning spot using a Microtek ScanMaker 4900, and the number of pixels was quantified with Photoshop 6.0 program (Adobe Systems, United States). The results were expressed as percentage of spots area vs. total leaf area [(spot area/total leaf area) × 100] to compensate for differences in the leaf size [27].

Leaf discs (1 cm^2) were incubated into loading buffer with 50 mM Tris–KCl (pH 7.2) containing 50 μM 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Sigma) dissolved in Mops in the dark for 15 min according to Zhang et al. [28]. Then the leaf disks were washed off excess dye with 50 mM Tris–KCl (pH 7.2), transferred to slides, and illuminated using a multipurpose microscope (Carl Zeiss, AxioPlan 2 Imaging) with optical filters (488 nm excitation and 525 nm emission) to visualize the oxidized green fluorescent probe. Quantitative images were captured, and data were analyzed using the LSM program (ISIS Version 3.4.0).

Antioxidant enzymes activity assay. 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. The homogenate was centrifuged at 12000 *g* for 20 min at 4°C, and then the supernatant was used for the following enzyme assays.

(1) SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries [29]. 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) POD activity was based on the determination of guaiacol oxidation (extinction coefficient 26.6/(mM cm) at 470 nm by H_2O_2 [30]. (3) APX activity was analyzed by following the decrease in A_{290} (extinction coefficient 2.8/(mM cm) 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 sodium ascorbate, 2.5 mM H_2O_2, and 200 μl of the enzyme extract [31].

(4) GR activity was determined by following the oxidation of NADP·H at 340 nm (extinction coefficient 6.2/(mM cm) for 3 min in 3 ml of an assay mixture containing 50 mM potassium phosphate buffer pH 7.8), 2 mM Na_2 EDTA, 0.15 mM NADP·H, 0.5 mM GSSG, and 200 μl of the enzyme extract [32]. Soluble protein contents were determined by the method of Bradford [33], using BSA as a calibration standard.

Statistical analysis. The data were subjected to one-way analysis of variance using the SPSS 11.5 for windows statistical software package. Test was employed to detect significant differences between the watering regimes.

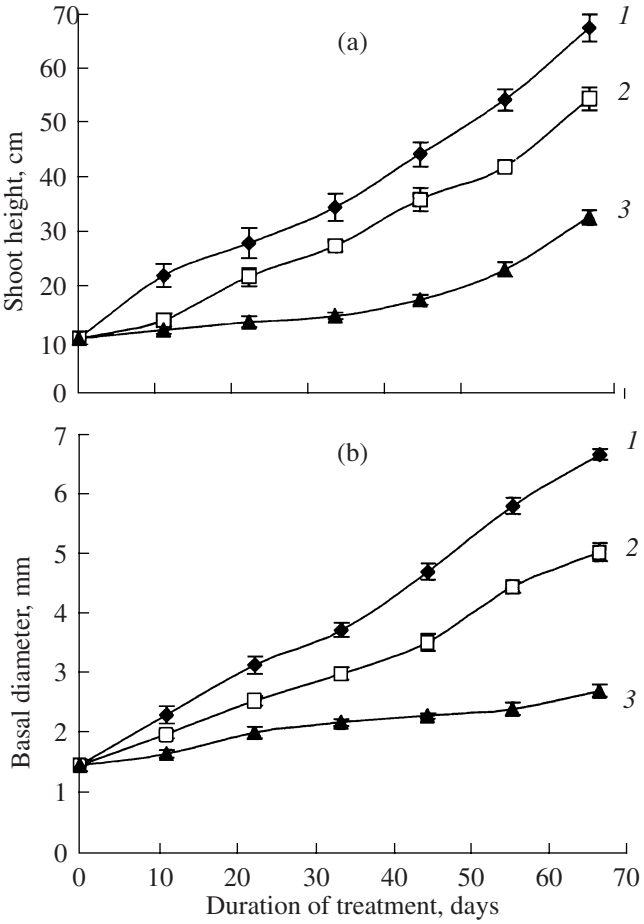

Fig. 1. Effect of drought stress on (a) shoot height and (b) basal diameter increment in *P. przewalskii.*
(*1*) 100%; (*2*) 50%; (*3*) 25% of FC.

RESULTS

Effect of Drought Stress on Biomass Accumulation and Leaf Characteristics of P. przewalskii

Drought stress significantly decreased the biomass accumulation of *P. przewalskii* and leaf characteristics including leaf number, total leaf area, and average leaf area. Drought stress also retarded the growth rate of *P. przewalskii* indicated by the reduced increment in the shoot height and basal diameter during the stress period (Fig. 1). On the other hand, *P. przewalskii* allocated more dry mass to the root systems under drought stress (Table 1).

Effect of Drought Stress on RWC, ABA, and PA Contents in P. przewalskii

Drought stress significantly decreased the leaf RWC of *P. przewalskii* ($P < 0.001$). ABA contents under 50 and 25% FC were 2.3 and 4.4 times of control (100% FC), respectively (Table 2). Drought stress also affected the PA accumulation in different pattern: Put and Spd increased significantly while, Spm showed little change.

Table 1. Effect of drought stress on biomass accumulation, leaf number, and leaf area of *P. przewalskii*

Treatment	Biomass partitioning, %			Total biomass, g	No. leaves	Total leaf area, dm^2	Average leaf area, cm^2
	roots	stems	leaves				
100% FC	17.29 ± 1.51^c	43.99 ± 0.94^a	38.72 ± 1.41^a	41.45 ± 5.02^a	69.4 ± 7.88^a	825.7 ± 84.08^a	14.04 ± 0.54^a
50% FC	26.53 ± 1.24^b	40.44 ± 0.99^a	33.03 ± 1.16^a	19.77 ± 3.31^b	39.4 ± 3.82^b	543.3 ± 39.14^b	12.69 ± 0.56^a
25% FC	32.96 ± 1.60^a	32.33 ± 2.67^b	34.71 ± 2.08^a	4.34 ± 1.44^c	16.8 ± 0.87^c	172.1 ± 17.83^c	10.15 ± 0.71^b
P	0	0.026	0.639	0	0	0	0.004

Notes: Letters refer to differences within traits between the watering regimes. Values (means of five replicates ± SE) marked by different letters are significantly different from each other at the $P < 0.05$.

Table 2. Effect of drought stress on RWC, ABA, and PA contents in *P. przewalskii*

Treatment	RWC, %	ABA, nmol/g dry wt	Putrescine, nmol/g dry wt	Spermidine, nmol/g dry wt	Spermine, nmol/g dry wt
100% FC	72.57 ± 1.56^a	0.17 ± 0.05^c	332.60 ± 11.25^c	58.40 ± 2.85^c	14.05 ± 1.75^a
50% FC	70.47 ± 0.81^b	0.39 ± 0.06^b	381.13 ± 16.90^b	74.23 ± 2.65^b	14.01 ± 2.05^a
25% FC	65.02 ± 2.31^c	0.75 ± 0.05^a	433.07 ± 7.15^a	83.91 ± 2.42^a	14.29 ± 0.73^a
P	0	0	0	0	0.974

Notes: Letters refer to differences within traits between the watering regimes. Values (means of five replicates ± SE) marked by different letters are significantly different from each other at the $P < 0.05$

Table 3. Effect of drought stress on gas exchange and chlorophyll *a* fluorescence in *P. przewalskii*

Treatment	*A*, μmol/(m^2 s);	G_s, mol/(m^2 s)	UE, μmol/mol	F_V/F_m	*Y*
100% FC	12.05 ± 0.39^a	0.28 ± 0.07^a	43.04 ± 4.04 c	0.75 ± 0.01^a	0.71 ± 0.03^a
50% FC	10.70 ± 0.23^b	0.20 ± 0.02^b	53.50 ± 4.00^b	0.73 ± 0.02^a	0.67 ± 0.01^b
25% FC	7.54 ± 0.21^c	0.12 ± 0.01 c	62.83 ± 2.65^a	0.67 ± 0.03^b	0.66 ± 0.02^b
P	0	0	0	0	0.001

Notes: Letters refer to differences within traits between the watering regimes. Values (means of five replicates ± SE) marked by different letters are significantly different from each other at the $P < 0.05$.

Effect of Drought Stress on Gas Exchange and Chlorophyll Fluorescence of P. przewalskii

Table 3 showed the changes in gas exchange and chlorophyll fluorescence parameters under different watering regimes. Compared with the control, net photosynthetic rate (*A*), and stomatal conductance (G_s) decreased significantly while WUE exhibited a significant increase under drought stress ($P < 0.001$). Drought stress also significantly decreased the chlorophyll fluorescence parameters, including maximal efficiency of PSII photochemistry (F_v/F_m) and actual quantum yield (*Y*).

Oxidative Damage and the Antioxidant Enzymes in P. przewalskii Caused by Drought Stress

Drought stress also caused oxidative burst in *P. przewalskii* as manifested by the accumulation of H_2O_2. ROS resulted in oxidative damage to membranes, lipids, proteins, and even cell viability as indicated by the increase in electric conductance, MDA, C=O, and Evans blue uptake, respectively (Table 4). Meanwhile, the system of antioxidant enzymes was activated to scavenge the ROS, as is seen from Table 5, showing a significant increase in the activities of SOD, POD, APX, and GR.

Histochemical and Fluorimetric Detection of H_2O_2 in P. przewalskii under Drought Stress

Histochemical assay of H_2O_2 showed that drought stress caused oxidative burst in *P. przewalskii.* Under 100, 50, and 25% FC, there were 0.5, 2.1, and 6.3% of the leaves colored by DAB, respectively (Fig. 2a). Fluorimetric detection also confirmed the H_2O_2 accumulation under drought stress (Fig. 2b).

Table 4. Damage to *P. przewalskiii* caused by drought stress

Treatment	H_2O_2, μmol/g dry wt	EC, %	MDA, nmol/g dry wt	C=O, nmol/g dry wt	Evans blue uptake, A_{560}/g dry wt
100% FC	4.74 ± 0.05^c	11.38 ± 0.38^b	11.84 ± 0.59^b	84.48 ± 11.53^c	0.035 ± 0.002^c
50% FC	6.13 ± 0.39^b	11.95 ± 0.99^b	12.81 ± 0.33^b	148.80 ± 11.56^b	0.047 ± 0.003^b
25% FC	7.48 ± 0.13^a	17.06 ± 2.44^a	15.50 ± 0.65^a	194.20 ± 3.42^a	0.087 ± 0.004^a
P	0	0.017	0	0	0

Notes: Letters refer to differences within traits between the watering regimes. Values (means of five replicates ± SE) marked by different letters are significantly different from each other at the $P < 0.05$.

Table 5. Effect of drought stress on antioxidant enzymes activities in *P. przewalskii*

Treatment	SOD activity, U/mg protein	POD activity, μmol guaiacol/(mg protein min)	APX activity, μmol ascorbate/(mg protein min)	GR activity, nmol NADH/(mg protein min)
100% FC	270.07 ± 11.71^b	3.52 ± 0.15^c	8.73 ± 0.55^c	0.65 ± 0.06^c
50% FC	322.35 ± 14.79^a	7.15 ± 0.24^b	17.8 ± 0.89^b	1.33 ± 0.08^b
25% FC	332.13 ± 11.07^a	8.79 ± 0.14^a	27.13 ± 1.09^a	2.08 ± 0.07^a
P	0.015	0	0	0

Notes: Letters refer to differences within traits between the watering regimes. Values (means of five replicates ± SE) marked by different letters are significantly different from each other at the $P < 0.05$.

DISCUSSION

An early morphological response of *P. przewalskii* to drought stress is growth adjustment including the reduced growth rate and the reduction in biomass accumulation, leaf number, and leaf area, which is in well agreement with earlier studies [2]. The biomass above ground was further decreased by changes in biomass partition that favored the root system; thus plants can exploit the limiting water resources [34]. Drought stress adversely affected leaf gas exchange and chlorophyll fluorescence, maybe due to the injury of the photosynthetic apparatus, dysfunction of the proteins related to photosynthesis, and enhancement of peroxidation of mesophyll cell membranes [35].

One of the characteristics of the responses of poplar to drought stress is a decrease in the leaf RWC, which may also function as a mechanism that concentrates solutes in the cell sap, thereby lowering the osmotic potential and contributing to osmotic adjustment [36]. Numerous data suggested that membranes were the primary sites of oxidative injury to cells and organelles consistent with the increase in MDA and EC in our result. Very often the MDA levels are used as a suitable marker for membrane lipid peroxidation [37]. Moreover, MDA can react with DNA bases to form adducts of deoxyguanosine and deoxyadenosine [38]. An increase of EC also suggested that drought stress caused a loss of membrane integrity in *P. przewalskii*. Oxidative damage to proteins can be assessed by the accumulation of the total carbonyl groups, and several authors have proposed the use of C=O as a reliable index of stress damage [39]. Severe drought stress may even result in cell senescence and cell death in *P. przewalskii* indicated by the increase in Evans blue uptake.

ABA was found to accumulate by 4.4 times under 25% FC as compared with control, and ABA was thought to act as a messenger in stress-perception-response pathways in environmental stresses [40]. It has been shown that ABA can cause an increased generation of H_2O_2 and O_2^- in plant tissues. And these ROS, especially H_2O_2 and O_2^- under proper concentration, are involved in cellular signaling process to induce a number of genes and proteins involved in stress defenses [41].

PAs may play a general role in coping with environmental stresses because an increase in their concentrations in plant cells is observed under a number of environmental stresses [42]. Malabika and Wu [43] demonstrated that accumulation of PAs was correlated with enhanced growth under stressful conditions maybe due to their ability to ensure the integrity of membranes, nucleic acid, and various subcellular organelles under stress conditions. Furthermore, Scaramagli et al. [32] supposed that PAs may modulate the activities of certain ion channels, especially Ca^{2+}-permeable channels, raise the cytoplasmic Ca^{2+} concentration, which was known to inactivate the K^+ inward rectifier at the plasma membrane and then stimulate stomatal closure. In our study, Put and Spd increased significantly under drought stress, while Spm showed little change. This was consistent with Bouchereau et al. [44] who suggested that Put might have more profound effects than Spd and Spm. However, in several systems, the induc-

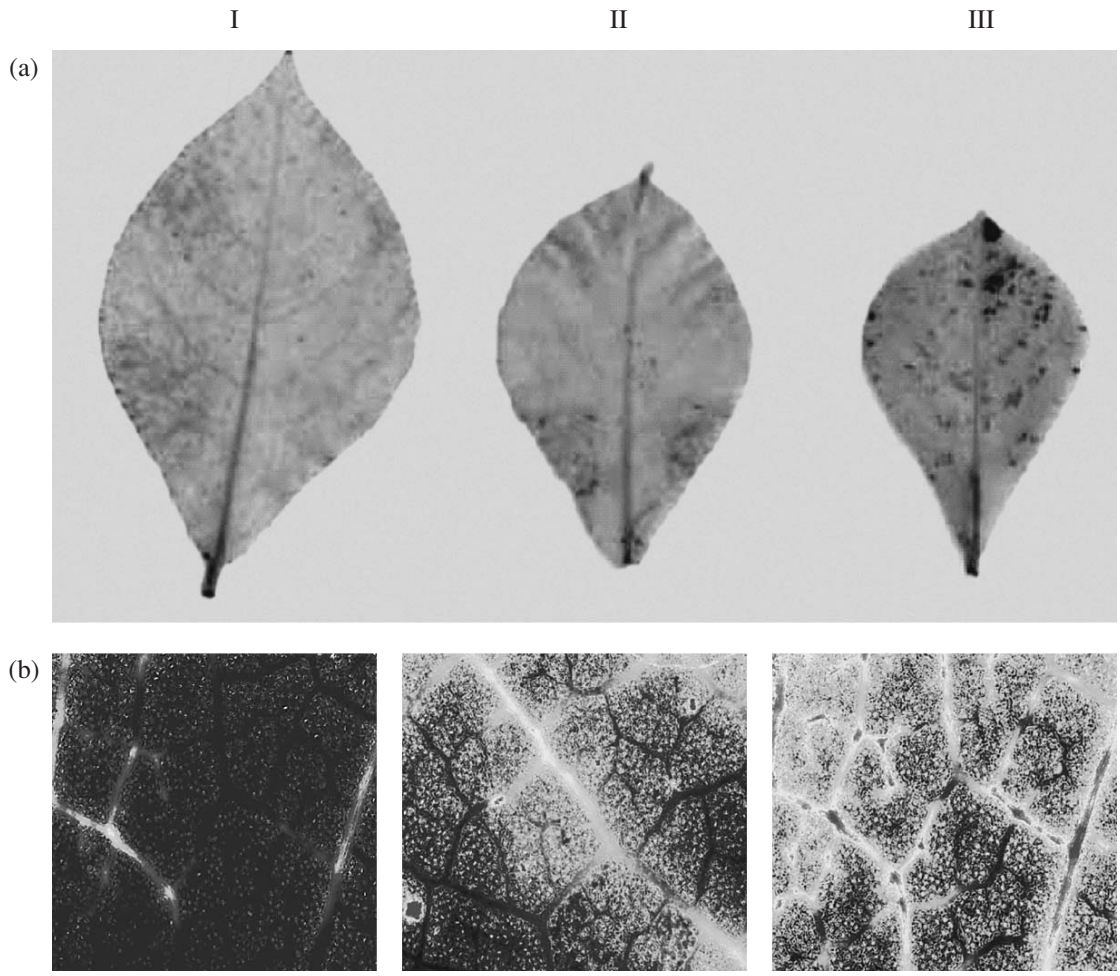

Fig. 2. (a) Histochemical and (b) fluorimetric detection of H_2O_2 in *P. przewalskii* under drought stress using DAB and H_2DCFDA, respectively.
I—100%; II—50%; III—25% of FC.

tion of Spd and Spm in the absence of Put accumulation may confer stress tolerance to plants.

ROS, as secondary messengers, may only occur under subtoxic conditions, and at the higher concentration they are still cytotoxic. Thus, plants have evolved an entire set of antioxidant systems to keep them under control. Drought stress caused an increase in the activities of SOD, GPX, APX, and GR (Table 5) to coordinate the ROS concentrations. The role of antioxidant enzymes has already been reported by many earlier studies [2, 7, 14]. However, the high contents of MDA and C=O suggested that the protective systems may not keep pace with the damaging effect in *P. przewalskii* under drought stress.

In conclusion, drought stress adversely affected the growth, leaf gas exchange, and chlorophyll fluorescence. One reason may be the oxidative burst caused by water deficit. Histochemical and fluorimetric detections also confirmed the accumulation of H_2O_2. The antioxidant enzymes, including SOD, POD, APX, and GR, were activated to scavenge the excessive ROS. However, significant damage was observed as lipid peroxidation, loss of membrane integrity, and even *P. przewalskii* cell death under drought stress.

REFERENCES

1. Kronfub, G., Polle, A., Tausz, M., Havranek, W.M., and Wieser, G., 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*, 1998, vol. 12, pp. 482–489.
2. Yin, C., Peng, Y., Zang, R., Zhu, Y., and Li, C., Adaptive Responses of *Populus kangdingensis* to Drought Stress, *Physiol. Plant.*, 2005, vol. 123, pp. 445–451.
3. Jones, H.G., *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*, Cambridge: Cambridge Univ. Press, 1992.
4. Rouhi, V., Samson, R., Lemeur, R., and Damme, P.V., Photosynthetic Gas Exchange Characteristics in Three Different Almond Species during Drought Stress and Subsequent Recovery, *Environ. Exp. Bot.*, 2007, vol. 59, pp. 117–129.

5. Asada, K., The Water–Water Cycle in Chloroplasts: Scavenging of Active Oxygens and Dissipation of Excess Photons, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, vol. 50, pp. 601–639.
6. Beligni, M.V. and Lamattina, L., Nitric Oxide Counteracts Cytotoxic Processes Mediated by Reactive Oxygen Species in Plant Tissues, *Planta*, 1999, vol. 208, pp. 337–344.
7. Guo, Z., Ou, W., Lu, S., and Zhong, Q., Differential Responses of Antioxidative System to Chilling and Drought Stress in Four Rice Cultivars, *Plant Physiol. Biochem.*, 2006, vol. 44, pp. 828–836.
8. Nayar, H. and Gupta, D., Differential Sensitivity of C_3 and C_4 Plants to Water Deficit Stress: Association with Oxidative Stress and Antioxidants, *Environ. Exp. Bot.*, 2006, vol. 58, pp. 106–113.
9. Alfredo, A., Alves, C., and Tim, L.S., Abscisic Acid Accumulation and Osmotic Adjustment in Cassava under Water Deficit, *Environ. Exp. Bot.*, 2004, vol. 51, pp. 259–271.
10. Li, C., Puhakainen, T., Welling, A., Vihera-Aarnio, A., Ernstsen, A., Junttila, O., Heino, P., and Palva, E.T., Cold Acclimation in Silver Birch (*Betula pendula*). Development of Freezing Tolerance in Different Tissues and Climatic Ecotypes, *Physiol. Plant.*, 2002, vol. 116, pp. 478–488.
11. Jiang, M. and Zhang, J., Effect of Abscisic Acid on Active Oxygen Species, Antioxidative Defence System and Oxidative Damage in Leaves of Maize Seedlings, *Plant Cell Physiol.*, 2001, vol. 42, pp. 1265–1273.
12. Rao, M.V., Paliyath, G., Ormrod, D.P., Murr, D.P., and Watkins, C.B., Influence of Salicylic Acid on H_2O_2 Production, Oxidative Stress, and H_2O_2-Metabolizing Enzymes (Salicylic Acid-Mediated Oxidative Damage Requires H_2O_2), *Plant Physiol.*, 1997, vol. 115, pp. 137–149.
13. Hare, P.D., Cress, W.A., and van Staden, J., Dissecting the Roles of Osmolyte Accumulation during Stress, *Plant Cell Environ.*, 1998, vol. 21, pp. 535–553.
14. Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Takahashi, T., Michael, A.J., and Kusano, T., A Protective Role for the Polyamine Spermine against Drought Stress in Arabidopsis, *Biochem. Biophys. Res. Commun.*, 2007, vol. 352, pp. 486–490.
15. Perry, C.H., Miller, R.C., Brooks, K.N., Brooks, R.T., and Lust, N., Impact of Short-Rotation Hybrid Poplar Plantations on Regional Water Yield, *For. Ecol. Manag.*, 2001, vol. 143, pp. 143–151.
16. Tschaplinski, T.J., Tuskan, G.A., and Gunderson, C.A., Water-Stress Tolerance of Black and Eastern Cottonwood Clones and Four Hybrid Progeny. I. Growth, Water Relations and Gas Exchange, *Can. J. For. Res.*, 1994, vol. 24, pp. 364–371.
17. Li, C., Yin, C., and Liu, S., Different Responses of Two Contrasting *Populus davidiana* Populations to Exogenous Abscisic Acid Application, *Environ. Exp. Bot.,* 2004, vol. 51, pp. 237–246.
18. Xiong, Y., Xing, G., Li, F., Wang, S., Fan, X., Li, Z., and Wang, Y., Abscisic Acid Promotes Accumulation of Toxin ODAP in Relation to Free Spermine Level in Grass Pea Seedlings (*Lathyrus sativus* L.), *Plant Physiol. Biochem.*, 2006, vol. 44, pp. 161–169.
19. Flores, H.E. and Galston, A.W., Analysis of Polyamines in Higher Plants by High Performance Liquid Chromatography, *Plant Physiol.*, 1982, vol. 69, pp. 701–706.
20. Schreiber, U., Bilger, W., and Neubauer, C., Chlorophyll Florescens as a Nonintrusive Indicator for Rapid Assessment of *In Vivo* Photosynthesis, *Ecophysiology of Photosynthesis, Ecological Studies*, Schulze, E.D. and Caldwell, M.M., Eds., Berlin: Springer-Verlag, 1994, pp. 49–70.
21. Brennan, T. and Frekel, C., Involvement of Hydrogen Peroxide in the Regulation of Senescence in Pear, *Plant Physiol.*, 1977, vol. 59, pp. 411–416.
22. Gong, M., Li, Y., and Chen, S., Abscisic Acid-Induced Thermo Tolerance in Maize Seedlings Is Mediated by Calcium and Associated with Antioxidant Systems, *J. Plant Physiol.*, 1998, vol. 153, pp. 488–496.
23. Hodges, D.M., DeLong, J.M., Forney, C.F., and Prange, R.K., Improving the Thiobarbituric Acid-Reactive Substances Assay for Estimating Lipid Peroxidation in Plant Tissues Containing Anthocyanin and Other Interfering Compounds, *Planta*, 1999, vol. 207, pp. 604–611.
24. Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechillo, S., Klucas, R.V., and Aparicio-Tejo, P., Drought Induces Oxidative Stress in Pea Plants, *Planta*, 1994, vol. 194, pp. 346–352.
25. Levine, R.L., Willians, J.A., Stadtman, E.R., and Shacter, E., Carbonyl Assays for Determination of Oxidatively Modified Proteins, *Methods Enzymol.*, 1994, vol. 233, pp. 346–363.
26. Turner, J.G. and Nobacky, A., The Quantitative Relationship between Plant and Bacterial Cells Involved in the Hypersensitive Reaction, *Phytopathology*, 1974, vol. 64, pp. 885–890.
27. Romero-Puertas, M.C., Rodriguez-Serrano, M., Corpas, F.J., Gomez, M., Del Rio, L.A., and Sandalio, L.M., Cadmium-Induced Subcellular Accumulation of O_2 and H_2O_2 in Pea Leaves, *Plant Cell Environ.*, 2004, vol. 27, pp. 1122–1134.
28. Zhang, X., Zhang, L., Dong, F., Galbraith, D.W., and Song, C., Hydrogen Peroxide Is Involved in Abscisic Acid-Induced Stomatal Closure in *Vicia faba, Plant Physiol.*, 2001, vol. 126, pp. 1438–1448.
29. Giannopolitis, C.N. and Ries, S.K., Superoxide Dismutase in Higher Plants, *Plant Physiol.*, 1977, vol. 59, pp. 309–314.
30. Lin, J. and Wang, G., Doubled CO_2 Could Improve the Drought Tolerance Better in Sensitive Cultivars than in Tolerant Cultivars in Spring Wheat, *Plant Sci.*, 2002, vol. 163, pp. 627–637.
31. Nakano, Y. and Asada, K., Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts, *Plant Cell Physiol.*, 1981, vol. 22, pp. 867–880.
32. Scaramagli, S., Biondi, S., Lenoe, A., Grub, S., and Torrigiani, P., Acclimation to Low-Water Potential in Potato Cell Suspension Cultures Leads to Changes in Putrescine Metabolism, *Plant Physiol. Biochem.*, 2000, vol. 38, pp. 345–351.
33. Bradford, M.M., A Rapid and Sensitive Method for Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein–Dye Binding, *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.

34. Sharp, R.E., Comparative Sensitivity of Root and Shoot Growth and Physiology to Low Water Potentials, *Importance of Root-to-Shoot Communication in the Response to Environmental Stress*, Davies, W.J. and Jeffcoat, B., Eds., Bristol: British Society for Plant Growth Regulation, 1990, pp. 29–44.

35. Xu, Z. and Zhou, G., Nitrogen Metabolism and Photosynthesis in *Leymus chinensis* in Response to Long-Term Soil Drought, *J. Plant Growth Regul.*, 2006, vol. 25, pp. 252–266.

36. Lissner, J., Schierup, H.H., Comn, F.A., and Astorga, V., Effect of Climate on the Salt Tolerance of Two *Phragmites australis* Populations. I. Growth, Inorganic Solutes, Nitrogen Relations and Osmoregulation, *Aquat. Bot.*, 1999, vol. 64, pp. 317–333.

37. Masia, A., Physiological Effects of Oxidative Stress in Relatian to Ethylene in Postharvest Produce, *Postharvest Oxidative Stress in Horticultural Crops*, Hodges, D.M., Ed., New York: Food Products Press, 2003, pp. 165–197.

38. Marnett, L.J., Lipid Peroxidation-DNA Damage by Malondialdehyde, *Mutat. Res.*, 1999, vol. 424, pp. 83–95.

39. Pacifici, R.E. and Davies, K.J.A., Protein Degradation as an Index of Oxidative Stress, *Methods Enzymol.*, 1990, vol. 186, pp. 485–502.

40. Zhu, J., Hasegawa, P.M., and Bressan, R.A., Molecular Aspects of Osmotic Stress in Plants, *Crit. Rev. Plant Sci.*, 1997, vol. 16, pp. 253–277.

41. Imai, R., Moses, M.S., and Bray, E.A., Expression of an ABA-Induced Gene of Tomato in Transgenic Tobacco during Periods of Water Deficit, *J. Exp. Bot.*, 1995, vol. 46, pp. 1077–1084.

42. Liu, H., Dong, B., Zhang, Y., Liu, Z., and Liu, Y., Relationship between Osmotic Stress and the Levels of Free, Conjugated and Bound Polyamines in Leaves of Wheat Seedlings, *Plant Sci.*, 2004, vol. 166, pp. 1261–1267.

43. Makabuka, R. and Wu, R., Arginine Decarboxylase Transgene Expression and Analysis of Environmental Stress Tolerance in Transgenic Rice, *Plant Sci.*, 2001, vol. 160, pp. 869–875.

44. Bouchereau, A., Aziz, A., Larther, F., and Martin-Tanguy, J., Polyamines and Environmental Challenges: Recent Development, *Plant Sci.*, 1999, vol. 140, pp. 103–125.