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The altitudinal effects on photosynthesis of *Rosa platyacantha* from the Tianshan Mountains in Northwestern China

S.H. YANG[*], J.J. WEI[*], and H. GE[*, +]

National Flowers Improvement Center of China, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China[*]

Abstract

The altitudinal effects on photosynthesis were measured on progenies of three populations of *Rosa platyacantha* Schrenk from altitudes of 1,170 (L); 1,580 (M); and 1,920 (H) m a. s. l. During the day, net photosynthetic rate (P_N) decreased in all populations due to the high air temperature in the summer. The H population showed a significantly lower P_N at noon compared to other populations. The midday depression of P_N occurred in L and M populations due to stomatal limitations, while P_N inhibition was associated with PSII activity decline in the H population. In order to avoid photodamage, the plants of H population triggered active antioxidant defenses with a higher enzyme activity and redox ratio of ascorbate at midday compared to the L and M populations. However, more oxidative injury still occurred in the H plants at noon due to higher lipid peroxidation. Our results indicated that the provenance significantly affected photosynthesis in *R. platyacantha* from northwestern China.

Additional key words: photosynthesis; gas exchange; chlorophyll fluorescence; Calvin cycle; antioxidant defense; photodamage.

Introduction

Rose is one of the most important ornamental species with over 24,000 registered cultivars in the world (Cairns 2000). The genus *Rosa* contains over 150 species in the Northern Hemisphere but only 10–15 species are used to produce cultivars (Gudin 2000). *R. platyacantha* Schrenk is an endemic species in northwestern China, widely distributed in the Tianshan Mountains from 800 to 2,000 m a.s.l. (Gu and Robertson 2003). It had been proposed as a potential wild germplasm for introgression into the cultivars, as it possesses promising ornamental traits with golden petals and resistances to cold and drought (Ma and Chen 1990).

Altitude can affect the morphological, physiological, and genetic variation patterns of a species. Plants from a higher altitude are exposed to large diurnal temperature fluctuations, low temperature, lower CO_2 pressures, and high light intensity. In general, increased leaf thickness, stomatal density, chloroplast number, higher photosynthetic capacity, carboxylation efficiency, as well as antioxidant and thermal dissipation capacity are the most common responses of high-altitude plants compared to lowland plants (Woodward and Bazzaz 1988, Sparks and Ehleringer 1997, Körner 2003, Öncel *et al.* 2004, Shi *et al.* 2006, Oh *et al.* 2013).

Some studies have shown that photosynthesis varies in response to altitude (Hiesey and Milner 1965). Wang *et al*. (2008) showed that photosynthesis of *Plantago depressa* Willd and *Setaria viridis* (L.) Beauv. grown in a common greenhouse was not significantly related to the altitude of origin. Barley, pea, and wheat collected from different altitudes in the Himalayas and then grown in a common garden showed no significant changes in the initial Rubisco activity (Kumar *et al.* 2004). In contrast, the

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[+] Correspondence author: phone & fax: +86 10 82109542. e-mail: gehong@caas.cn.
Abbreviations: APX – ascorbate peroxidase; AsA – ascorbate; CAT – catalase; C_i – intercellular CO_2 concentration; DHA – dehydroascorbate; E – transpiration rate; FBPase – fructose-1,6-bisphosphatase; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; GAPDH – NADP-glyceraldehyde-3-phosphate dehydrogenase; GPX – guaiacol peroxidase; GR – glutathione reductase; g_s – stomatal conductance; GSH – reduced glutathione; GSSG – oxidised glutathione; H – high altitude; L – low altitude; L_s – stomatal limitation value; M – middle altitude; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; PRK – phosphoribulokinase; q_P – photochemical quenching; RuBP – ribulose-1,5-bisphosphate; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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different photosynthetic responses in *Podophyllum hexandrum* Royle collected from different altitudes of the Himalayas appeared to be genetically controlled (Vats and Kumar 2006). The altitude of a provenance influenced stomatal density and carboxylation efficiency in *Nothofagus cunninghamii* (Hook.) Oerst. grown in a common garden (Hovenden and Brodribb 2000). Polle *et al*. (1999) showed that the resistance to oxidative stress of *Picea abies* (L.) H. Karst. at high altitude was associated with a high inherited level of antioxidative protection.

In this study, we investigated the gas exchange, chlorophyll (Chl) fluorescence, CO_2 assimilation, and antioxidant protection in the progenies of *R. platyacantha* originating from three different altitudes and grown in a common garden. In this way, we aimed to find understanding of the altitudinal effects on the photosynthetic adaptation in the wild rose species after the *ex situ* domestication.

Materials and methods

Plant materials: The hips of *R. platyacantha* were collected from different altitudes in the Tianshan Mountains of northwestern China in the autumn 2008. The population L (89°03'E, 43°52'N) originated from 1,170 m a.s.l., population M (87°19'E, 43°31'N) from 1,580 m a.s.l., and population H (88°08'E, 43°52'N) from 1,920 m a.s.l. The L and M populations were located at the foothills of southern slopes near the creeks at the Jimsar County and Urumqi County, respectively. The H population was located near Tianchi Lake on the northern side of the Bogda Peak in the Tianshan Mountains. The climate was characterized by a mean air temperature (T_{air}) of 7.6, 6.9, and 2.3°C for L, M, and H, respectively. Total annual rainfall was 194.7, 76.8, and 571.5 mm for L, M, and H, respectively. These data sets were obtained from the Meteorological Stations of Qitai (No. 51379), Dabancheng (No. 51466), and Tianchi (No. 51470) for L, M, and H, respectively, during the years of 1981–2001.

The achenes were released from the fruits and mixed with wet sand for cold stratification at 4°C for six months. In the spring of 2009, the achenes were sown and germinated in the experimental field of the Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences, Beijing (116°19'E, 39°57'N). The altitude of the experimental field was approximately 50 m a.s.l., and the mean annual air temperature and rainfall (1981–2010) were 12.8°C and 584.4 mm, respectively, according to the Beijing Meteorological Station (No. 54511) in Beijing, China. After almost two years of growth, the photosynthetic adaptation experiments were performed with the three populations in mid-August, 2010. The daily average T_{air} were obtained during August–September, 2010 for the three origin sites from the same meteorological stations mentioned above (Fig. 1).

Gas-exchange measurements: The leaflets of the fully-opened leaves at the top of the vigorous plants were chosen for the diurnal photosynthetic measurements, which were collected on 17 August 2010. Leaf gas exchange was analyzed every 2 h from 7:00 to 17:00 h using a portable photosynthesis system (*LI-6400XT, LiCOR Biosciences,* Lincoln, NB, USA). Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), air CO_2 concentration (C_a), transpiration rate (E), and leaf temperature (T_{leaf}) were measured. Stomatal limitation (L_s) was estimated as $1 - C_i/C_a$ (Berry and Downtown 1982). The diurnal T_{air} and PPFD were measured every 2 h during the day in the experimental field of Beijing. Moreover, the diurnal T_{air} and PPFD at the localities of L, M, and H in Xinjiang were measured with the same portable photosynthesis system on 1 September, 29 August, and 2 September 2010, respectively (Fig. 2).

Chl fluorescence parameters were measured on the same day as the measurement of photosynthesis by a portable fluorometer (*PAM-2100, Walz,* Effeltrich, Germany). The maximal efficiency of PSII photochemistry (F_v/F_m) was measured at 7:00 h on the plants kept in darkness for the night and then for 30 min at 13:00 h. Minimum fluorescence (F_o) was estimated under low modulated light of 0.6 μmol(photon) m^{-2} s^{-1}, and maximum fluorescence (F_m) was determined by a white light-saturating pulse of 8,000 μmol(photon) m^{-2} s^{-1} for 0.8 s. Variable fluorescence (F_v) was determined as $F_m - F_o$. After measurements of F_m, the following procedure was carried out every 2 h from 7:00 to 17:00 h. The leaf was exposed to actinic light similar to the ambient PPFD [250–1,400 μmol(photon) m^{-2} s^{-1}] at the measuring time and allowed to reach steady-state fluorescence (F_s). Another saturating light pulse [8,000 μmol(photon) m^{-2} s^{-1}] was used to measure the maximal fluorescence at the light-adapted state (F_m') and the minimal fluorescence at the light-adapted state (F_o') was obtained in the prescence of an infrared light [15 μmol (photon) m^{-2} s^{-1}]. The actual efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (q_P), and nonphotochemical quenching (NPQ) were calculated in 2-h intervals, from 7:00 to 17:00 h, according to the formulas: $(F_m' - F_s)/F_m'$, $(F_m' - F_s)/(F_m' - F_o')$, and $(F_m/F_m') - 1$, respectively (Genty *et al*. 1989, Demmig-Adams 2003).

Photosynthetic enzyme assays: Leaf discs (1 cm^2 in size) from the same leaflets used for photosynthetic measurements were collected at 7:00 and 13:00 h, frozen in liquid nitrogen, and stored at −80°C until being assayed. After frozen leaflets were homogenized and centrifuged, the supernatant was extracted for measurement of the initial Rubisco activity (EC 4.1.1.39), the activities of NADP-

glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12), fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11), and phosphoribulokinase (PRK, EC 2.7.1.19) according to Leegood (1990) with some modifications (Chen and Cheng 2003) using a spectrophotometer (*Model Specord 200, Analytik Jena AG,* Jena, Germany). The changes in absorbance at 340 nm corresponding to NADH or NADPH were determined. The enzyme activities were calculated per leaf area according to the formulas: Activity ($\mu mol\ m^{-2}\ s^{-1}$) = $\Delta A/\Delta t \times N / (\varepsilon \times 2 \times d)$, where $\Delta A/\Delta t$ represents the change of optical density at 340 nm per second, N represents the dilution of the reaction volume to the extraction volume, ε is the extinction coefficient of NADH or NADPH at 340 nm, and d means the optical distance of the cuvette.

Antioxidant enzyme assays: Frozen leaflets were homogenized and centrifuged with 50 mM Tris-HCl (pH 7.0) containing 20% (w/v) glycerol, 1 mM reduced glutathione (GSH), and 5 mM $MgCl_2$. The supernatant was prepared for activity determination of ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2). Catalase (CAT, EC 1.11.1.6) and guaiacol peroxidase (GPX, EC 1.11.1.7) were extracted in 0.1mM potassium phosphate buffer (pH 7.0) including 1% (w/v) polyvinylpolypyrrolidone (PVPP). To measure superoxide dismutase (SOD, EC 1.15.1.1) activity, the enzyme was extracted in 50 mM potassium phosphate buffer (pH 7.0) which contained 0.1 mM EDTA and 1% (w/v) PVPP.

The enzyme activities mentioned above were measured according to Yang *et al*. (2007) with minor modifications using a spectrophotometer (*Model Specord 200, Analytik Jena AG,* Jena, Germany). For measuring the SOD activity, the reaction mixture included the prepared enzyme extract, 13 mM L-methionine, 75 μM nitroblue tetrazolium (NBT), 10 μM EDTA and 2.0 mM riboflavin in 50 mM potassium phosphate buffer (pH 7.8). One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm. CAT activity was determined directly by the decomposition of H_2O_2 at 240 nm. GPX activity was determined by the increase in the absorbance due to the oxidation of guaiacol at 470 nm. APX activity was measured by estimating the rate of ascorbate oxidation at 290 nm. The reaction mixture for GR activity contained 2.5 mM EDTA, 0.75 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 1 mM GSSG, 0.1 mM NADPH, and enzyme extract in 100 mM potassium phosphate buffer (pH 7.5). The decrease in absorbance at 412 nm was recorded. GR activity was determined with the commercial standard GR (baker's yeast, Type III, *Sigma*, St Louis, MO, USA). Total soluble protein was determined by Bradford's method (1976), using bovine serum albumin as a standard. The enzyme activities of CAT, GPX, and APX were calculated as $\mu mol\ min^{-1}\ mg^{-1}$(protein) while the SOD and GR activities were described as unit activity mg^{-1}(protein).

Determination of ascorbate and glutathione: Frozen leaflets were homogenized and centrifuged with 5% (w/v) metaphosphoric acid for the supernatant. To determine total ascorbate, the supernatant was initially supplied with dithiothreitol (DTT), which reduces dehydroascorbate (DHA) to ascorbate (AsA). The homogenate was mixed in order with 150 mM phosphate buffer (pH 7.4), containing 5 mM EDTA and 0.5 M N-ethylmaleimide (NEM), 10% (w/v) TCA; 44% (v/v) orthophosphoric acid, 4% (w/v) 2, 2'-dipyridyl in 70% ethanol, and 3% (w/v) $FeCl_3$. The resulting solution was then incubated at 37°C for 1 h. Absorbance was read at 525 nm by the spectrophotometer mentioned above. The AsA was measured with the same procedure except DTT and NEM was substituted with H_2O. The standard curve was produced with AsA (Cakmak and Marschner 1992). Concentration of reduced (GSH) and oxidized (GSSG) glutathione were determined spectrophotometrically at 412 nm with an enzyme-recycling assay. The assay was based on sequential oxidation of glutathione by DTNB and reduction by NADPH in the presence of a known amount of glutathione reductase (GR). To quantify the GSSG content, 2-vinylpyridine was added to the extract. Standard curves were generated with reduced and oxidized glutathione (Anderson *et al*. 1992).

Measurement of hydrogen peroxide: Frozen leaflets were homogenized and centrifuged in an ice bath with 0.1% (w/v) TCA, and the supernatant was then mixed with 10 mM potassium phosphate buffer (pH 7.0) and 1 M KI. The reaction was developed within 1 h in darkness. The absorbance was measured at 390 nm by the above mentioned spectrophotometer, and hydrogen peroxide (H_2O_2) concentration was determined using a given standard curve (Alexieva *et al.* 2001).

Measurement of lipid peroxidation: Lipid peroxidation was determined by the method of Heath and Packer (1968) in terms of malondialdehyde (MDA) contents *via* the thiobarbituric acid (TBA) reaction. The amount of TBA reactive substance (TBARS) was calculated from the difference in absorbance at 532 and 600 nm using the extinction coefficient of 1.55 $mM^{-1}\ cm^{-1}$.

Statistical analysis: All data were subjected to analysis of variance (*ANOVA*) procedures using *SPSS 11.0* for *Windows* (*SPSS Inc.,* Chicago, IL, USA). Plots and tables were drawn using *SigmaPlot 10.0* (*SPSS Inc.,* Chicago, IL, USA) and *Microsoft Excel 2000* (*Microsoft Corporation,* Redmond, WA, USA). Mean and SE values represent 3–5 biological replicates of each treatment. Difference between means was analyzed by the *Duncan*'s multiple comparison tests.

Results

Climatic factors: The mean T_{air} measured by meteorological stations near the study sites during the period of August–September 2010 are shown in Fig. 1. The T_{air} decreased with the altitude. The Beijing site (50 m a. s. l.) had T_{air} that was 10°C higher than at the Tianchi site (1,920 m a. s. l.). Moreover, the diurnal T_{air} also decreased with the increased altitude among the Beijing and the provenance sites from 17 August to 2 September 2010 (Fig. 2*A*). PPFD did not change among the provenance sites. The diurnal PPFD monitored in Beijing was significantly lower than that at the provenance sites, with the maximum PPFD value of 1,358 μmol(photon) m^{-2} s^{-1} at noon (Fig. 2*B*).

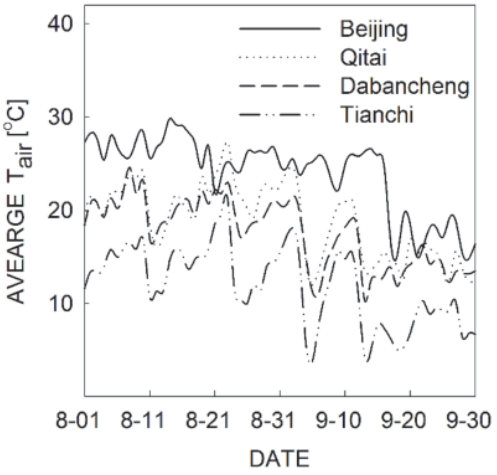

Fig. 1. The daily average air temperature (T_{air}) of the meteorological stations near the domestication and origin sites during August to September 2010. Beijing is the meteorological station for the domestication site. Qitai, Dabancheng, and Tianchi are the meteorological stations near the origin sites with altitudes of 1,170 (L), 1,580 (M), and 1,920 (H) m a. s. l., respectively.

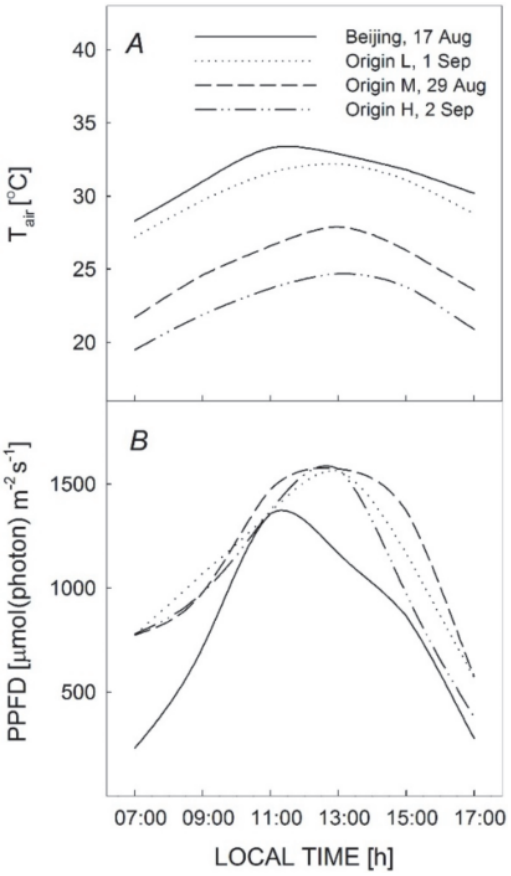

Fig. 2. Diurnal air temperatures (T_{air}) (*A*) and photosynthetic photon flux density (PPFD) (*B*) in Beijing on 17 August, and the originated sites with altitudes of 1,170 (L), 1,580 (M), and 1,920 (H) m a. s. l. on 1 September, 29 August, and 2 September, respectively.

Diurnal gas exchange: P_N of the populations from all three different altitudes peaked at 9:00 h and then decreased gradually (Fig. 3*A*). The H plants showed significantly lower P_N rates than that of L and M at 13:00 h. The g_s increased to its maximum at 9:00 h and slowly decreased until 17:00 h in the L and M populations (Fig. 3*B*). The H population exhibited slightly increased g_s values at 9:00 and 11:00 h, which decreased later to the minimum value at 17:00 h. At 9:00 h, the significantly higher g_s value was observed in the L population as compared to the H population. At 17:00 h, the L population showed the significantly lower g_s value as compared to the M and H populations. The C_i values sharply decreased below 220 μmol mol^{-1} at 13:00 and then recovered around 270 μmol mol^{-1} at 17:00 h in all populations (Fig. 3*C*). There were no significant differences in C_i between the populations. The *E* values in all populations increased from 7:00 to 9:00 h, maintaining *E* at approximately 4.5–5.6 μmol m^{-2} s^{-1} from 9:00 to 15:00 h, and then decreased at 17:00 h (Fig. 3*D*). The significantly higher *E* was observed in the L compared to H population at 9:00 h. T_{leaf} varied from 28.4 to 35.7°C during the day, but there were no significant differences during the day time between all three populations (Fig. 3*E*). The highest L_s values were reached at 13:00 h in all populations (Fig. 3*F*). However, the H plants exhibited the lower variations during the day in comparison to the L and M plants. The significantly lower L_s values were observed in the H population at 11:00 and 13:00 h as compared to the L population.

Diurnal variations in Chl fluorescence: F_v/F_m reached its maximum values (> 0.80) at 7:00 h (Table 1). There were no significant differences in F_o and F_v/F_m at 7:00 h between all three populations. However, F_o increased in the H population at 13:00 h and reached a significantly higher level than that of the L and M populations. On average, F_v/F_m at 13:00 h showed a significant decrease in all

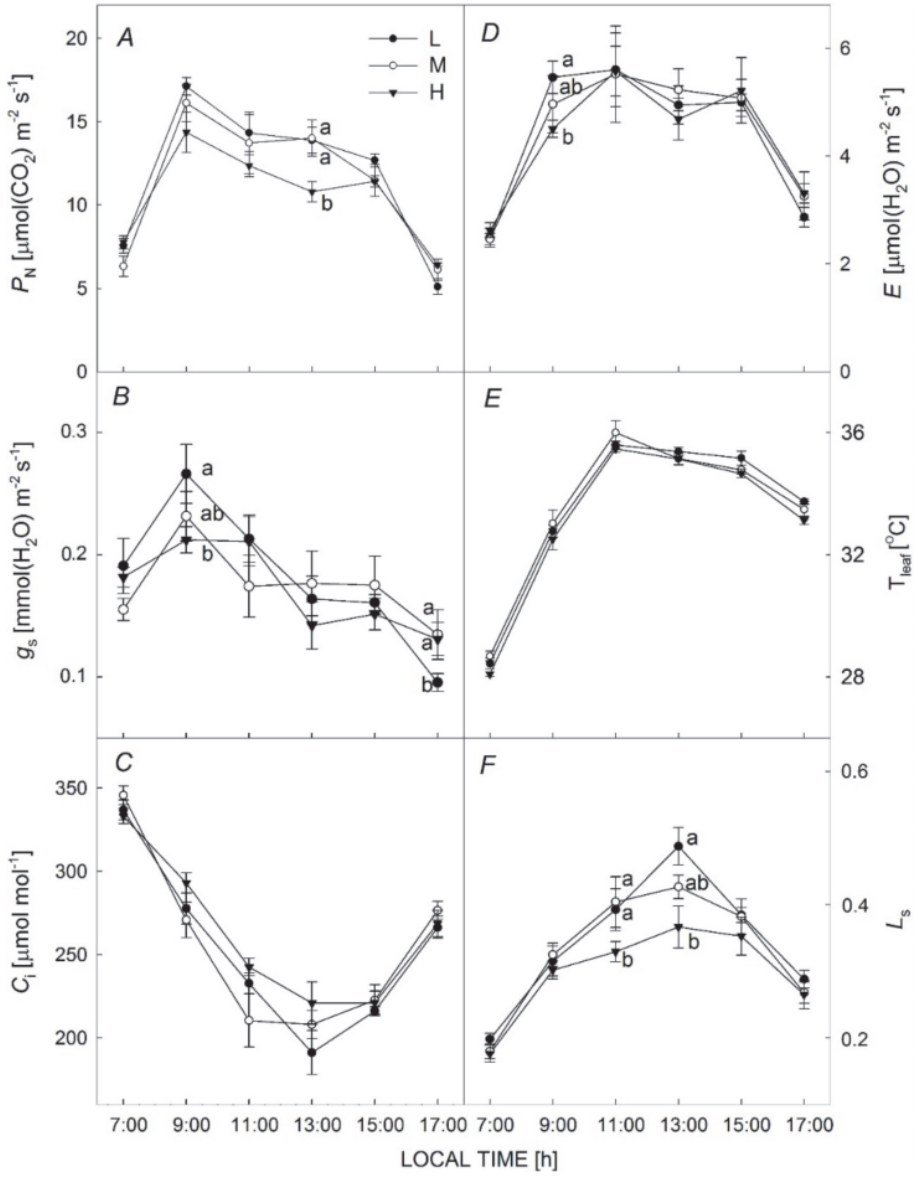

Fig. 3. The altitudinal effects on diurnal net photosynthesis (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), transpiration rate (*E*) (*D*), air temperature (T_{air}) (*E*), and stomatal limitation value (L_s) (*F*) in the progenies of *Rosa platyacantha* in the common garden. L, M, and H indicate the progenies of plants originating from sites with altitudes of 1,170, 1,580, and 1,920 m a. s. l., respectively. Values are means ± SE with five biological replicates. Means followed by *different letters* indicate significant differences among the altitudes at $p<0.05$.

Table 1. The minimal fluorescence (F_o), the maximal efficiency of PSII photochemistry (F_v/F_m), the concentrations of H_2O_2 and the thiobarbituric acid reactive substance (TBARS) contents in the progenies of *Rosa platyacantha* from different altitudes at 7:00 and 13:00 h in the common garden. Values are means ± SE with three biological replicates. Means followed by the *different letters* indicate the significant differences among the altitudes at $p<0.05$. Means followed by the asterisks indicate the significant differences between 7:00 and 13:00 h at $p<0.05$.

Altitude	F_o		F_v/F_m		H_2O_2 [μmol g^{-1}(FM)]		TBARS [μmol g^{-1}(FM)]	
[m a. s. l.]	7:00	13:00	7:00	13:00	7:00	13:00	7:00	13:00
1,170 (L)	134.7 ± 9.3^{a}	147.6 ± 4.7^{b}	$0.811 \pm 0.005^{a*}$	$0.750 \pm 0.004^{a*}$	10.7 ± 0.8^{a}	11.6 ± 0.4^{b}	17.6 ± 1.6^{a}	18.2 ± 1.0^{b}
1,580 (M)	132.8 ± 5.4^{a}	138.0 ± 4.8^{b}	$0.811 \pm 0.002^{a*}$	$0.764 \pm 0.009^{a*}$	$11.6 \pm 0.3^{a*}$	$12.6 \pm 0.1^{b*}$	21.0 ± 1.1^{a}	16.6 ± 1.8^{b}
1,920 (H)	$140.6 \pm 6.2^{a*}$	$174.8 \pm 3.0^{a*}$	$0.818 \pm 0.070^{a*}$	$0.694 \pm 0.006^{b*}$	$11.4 \pm 0.1^{a*}$	$14.0 \pm 0.4^{a*}$	$18.8 \pm 0.5^{a*}$	$24.0 \pm 0.6^{a*}$

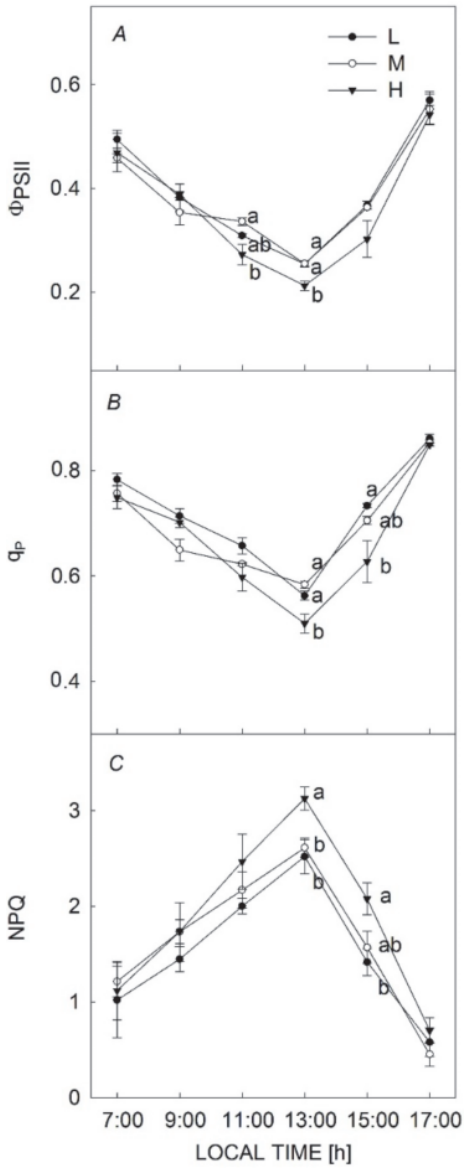

Fig. 4. The altitudinal effects on diurnal actual efficiency of PSII photochemistry (Φ_{PSII}) (*A*), photochemical quenching (q_P) (*B*), and nonphotochemical quenching (NPQ) (*C*) in the progenies of *Rosa platyacantha* in the common garden. L, M, and H indicate the progenies of plants originating from sites with altitudes of 1,170, 1,580, and 1,920 m a. s. l., respectively. Values are means ± SE with three biological replicates. Means followed by *different letters* indicate significant differences among the altitudes at $p<0.05$.

populations compared to the values at 7:00 h. The H plants exhibited a lower F_v/F_m than that of the L and M populations. Φ_{PSII} and q_P decreased in all populations up to 13:00 h and then gradually recovered at 17:00 h to the values found in the morning (Fig. 4*A*,*B*). The values of Φ_{PSII} and q_P for the H population at 13:00 h were significantly lower than those in L and M. The H plants exhibited a significantly lower Φ_{PSII} at 11:00 h and a significantly lower q_P at 15:00 h. In contrast, NPQ showed the opposite trend to q_P (Fig. 4*C*). The H plants exhibited a significantly higher NPQ than that of the L and M populations at 13:00 h and a higher NPQ compared to the L population at 15:00 h.

Photosynthetic enzyme activities: The measured photosynthetic enzymes did not exhibit the altitudinal effects at 7:00 h (Table 2), while they did show a change at noon. The increased initial Rubisco activity was observed in the M and H plants at 13:00 h. However, GAPDH activities decreased significantly in the L and M populations, while it was constant in the H plants from 7:00 to 13:00 h. No significant altitudinal or temporal effects were found for FBPase activities. PRK activities declined from 7:00 to 13:00 h in all populations. A significantly lower PRK activity was observed in the H population compared to the L and M plants at noon.

Antioxidant enzyme activities: SOD and CAT activities did not show altitudinal or temporal effects, except SOD activity in the L plants compared to the M and H populations at 7:00 h (Table 3). GPX activities decreased in the L and M plants from 7:00 to 13:00 h. APX activities did not significantly change in all populations from 7:00 to 13:00 h. A significantly higher APX activity was observed in the H population contrary to the L and M plants at noon. GR activities were not affected by the altitudes, but they increased in the L and H populations from 7:00 to 13:00 h.

Nonenzymatic antioxidants: There were no significant altitudinal or temporal effects on the total ascorbate and glutathione in all the populations (Table 4). However, the redox ratio of AsA/DHA and GSH/GSSG decreased in the populations from 7:00 to 13:00 h. In particular, AsA/DHA decreased in the M plants and GSH/GSSG decreased in the H population. Moreover, the H plants showed a significantly higher AsA/DHA ratio than that of the L and M populations.

Hydrogen peroxide and lipid peroxidation: Both H_2O_2 and TBARS contents exhibited no significant differences between the populations at 7:00 h (Table 1). However, H_2O_2 concentrations significantly increased in the M and H plants from 7:00 to 13:00 h. TBARS concentrations in the H population increased significantly from 7:00 to 13:00 h.

Discussion

Climatic factors, such as air temperature and solar irradiance, can be considerably influenced by the altitude. This research highlighted that the air temperature declined with the increased altitudes. The site of Beijing had a T_{air} that was more than 10°C higher than that of the Tianchi site. Moreover, the summer diurnal PPFD in Beijing was lower

Table 2. The photosynthetic enzyme activities of Rubisco, NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fructose-1,6-bisphosphatase (FBPase), and phosphoribulokinase (PRK) in the progenies of *Rosa platyacantha* from different altitudes at 7:00 and 13:00 h in the common garden. Values are means ± SE with three biological replicates. Means followed by the different letters indicate the significant differences among the altitudes at $p<0.05$. Means followed by the asterisks indicate the significant differences between 7:00 and 13:00 h at $p<0.05$.

Altitude [m a. s. l.]	Rubisco [μmol m^{-2} s^{-1}]		GAPDH [μmol m^{-2} s^{-1}]		FBPase [μmol m^{-2} s^{-1}]		PRK [μmol m^{-2} s^{-1}]	
	7:00	13:00	7:00	13:00	7:00	13:00	7:00	13:00
1,170 (L)	23.9 ± 8.0^{a}	34.0 ± 4.0^{b}	$26.7 \pm 2.8^{a*}$	$12.7 \pm 1.1^{b*}$	13.0 ± 2.0^{a}	11.9 ± 0.7^{a}	8.5 ± 0.8^{a}	5.7 ± 0.9^{a}
1,580 (M)	$20.8 \pm 2.0^{a*}$	$39.8 \pm 5.5^{ab*}$	$22.2 \pm 1.3^{a*}$	$16.1 \pm 1.0^{b*}$	16.5 ± 3.8^{a}	11.8 ± 1.7^{a}	7.9 ± 1.4^{a}	4.2 ± 0.3^{a}
1,920 (H)	$30.9 \pm 5.1^{a*}$	$57.8 \pm 6.5^{a*}$	22.7 ± 3.4^{a}	25.3 ± 2.5^{a}	13.4 ± 2.3^{a}	12.2 ± 1.8^{a}	$10.0 \pm 0.9^{a*}$	$2.6 \pm 0.2^{b*}$

Table 3. The antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR) in the progenies of *Rosa platyacantha* from different altitudes at 7:00 and 13:00 h in the common garden. Values are means ± SE with three biological replicates. Means followed by the different letters indicate the significant differences among the altitudes at $p<0.05$. Means followed by the asterisks indicate the significant differences between 7:00 and 13:00 h at $p<0.05$.

Enzyme activity	Altitude [m a. s. l.]					
	1,170 (L)		1,580 (M)		1,920 (H)	
	7:00	13:00	7:00	13:00	7:00	13:00
SOD [Unit mg^{-1}(protein)]	123.5 ± 14.7^{a}	90.8 ± 12.9^{a}	81.0 ± 10.7^{b}	82.6 ± 4.0^{a}	72.8 ± 9.2^{b}	60.2 ± 10.4^{a}
CAT [μmol min^{-1} mg^{-1}(protein)]	0.38 ± 0.12^{a}	0.50 ± 0.06^{a}	0.25 ± 0.07^{a}	0.42 ± 0.08^{a}	0.47 ± 0.06^{a}	0.38 ± 0.04^{a}
GPX [nmol min^{-1} mg^{-1}(protein)]	$403.2 \pm 68.8^{ab*}$	$82.9 \pm 28.3^{b*}$	$535.1 \pm 63.8^{a*}$	$74.3 \pm 4.5^{b*}$	280.5 ± 62.4^{b}	211.5 ± 32.2^{a}
APX [μmol min^{-1} mg^{-1}(protein)]	7.95 ± 1.85^{a}	4.99 ± 1.03^{b}	6.42 ± 0.98^{a}	4.55 ± 0.55^{b}	6.38 ± 1.04^{a}	7.70 ± 0.48^{a}
GR [Unit mg^{-1}(protein)]	$0.29 \pm 0.05^{a*}$	$0.75 \pm 0.10^{a*}$	0.16 ± 0.02^{a}	0.60 ± 0.16^{a}	$0.20 \pm 0.05^{a*}$	$0.76 \pm 0.09^{a*}$

Table 4. The total ascorbate and glutathione concentrations as well as the redox ratios of ascorbic acid (AsA)/dehydroascorbic acid (DHA) and reduced glutathione (GSH)/oxidized glutathione (GSSG) in the progenies of *Rosa platyacantha* from different altitudes at 7:00 and 13:00 in the common garden. Values are means ± SE with three biological replicates. Means followed by *the different letters* indicate the significant differences among the altitudes at $p<0.05$. Means followed by the asterisks indicate the significant differences between 7:00 and 13:00 h at $p<0.05$.

Altitude [m a. s. l.]	Total ascorbate [μmol g^{-1} (FM)]		AsA/DHA		Total glutathione [μmol g^{-1}(FM)]		GSH/GSSG	
	7:00	13:00	7:00	13:00	7:00	13:00	7:00	13:00
1,170 (L)	15.8 ± 5.4^{a}	26.8 ± 5.5^{a}	0.61 ± 0.33^{a}	0.35 ± 0.17^{ab}	7.1 ± 4.1^{a}	12.5 ± 5.1^{a}	2.00 ± 0.52^{a}	1.34 ± 0.47^{a}
1,580 (M)	22.9 ± 0.3^{a}	27.2 ± 2.3^{a}	$0.64 \pm 0.06^{a*}$	$0.18 \pm 0.01^{b*}$	10.5 ± 1.9^{a}	10.0 ± 1.9^{a}	1.15 ± 0.32^{a}	0.91 ± 0.20^{a}
1,920 (H)	32.1 ± 4.3^{a}	34.5 ± 5.2^{a}	0.62 ± 0.13^{a}	0.62 ± 0.06^{a}	10.6 ± 0.5^{a}	10.9 ± 1.8^{a}	$1.98 \pm 0.05^{a*}$	$1.30 \pm 0.01^{a*}$

than that in Xinjiang (Fig. 2). The significant differences of T_{air} and PPFD values between the domestication and origin sites might be vital limitation for the adaptation of progenies from the high altitudes of Xinjiang to the lowland of Beijing.

Observed P_N decreased after 9:00 h in all populations. This suggests that there was a negative effect of the altitude on photosynthesis during the summer in the progenies of *R. platyacantha* originating from different altitudes (Fig. 3*A*). According to the results of Feng *et al.* (2007), photosynthesis of *R. rugosa* decreased in response to high air temperature and high irradiance. The maximum T_{air} value at midday in Beijing was higher than that at the provenance sites of Xinjiang, while the PPFDs in Beijing were much lower than those in Xinjiang (Fig. 2). Thus, the high air temperature seems to be responsible for the P_N decrease.

The H plants exhibited a significantly lower P_N than that of the L and M populations at 13:00 h (Fig. 3*A*) which indicated a negative effect of the original altitude on photosynthesis. The values of g_s and C_i decreased in all populations at 13:00 h compared to those values at 9:00 h (Fig. *3B*,*C*). However, the L_s values significantly increased in the L and M populations, but L_s values did not vary in the H population at 13:00 compared to those before 9:00 h (Fig. 3*F*). The results indicated that the midday decline of photosynthesis in the L and M populations resulted from the stomatal limitation, while the reduction in the H population was probably due to nonstomatal limitations.

Chl fluorescence analyzes the imbalance between the absorption of light energy by Chl and the use of energy when plants are subjected to stress conditions (Maxwell

and Johnson 2000). F_v/F_m significantly decreased in all populations. F_o increased significantly only in the H population at noon compared to values of F_v/F_m and F_o at 7:00 h (Table 1), indicating the presence of photoinhibition only in the H population at noon. Moreover, significantly lower Φ_{PSII} and q_P were observed at noon in the H compared to L and M plants (Fig 4*A*,*B*), implying that the photoinhibition in the H population was associated with the impaired PSII activity. In contrast, the higher NPQ in the H plants at 13:00 and 15:00 h suggests a higher light energy dissipation as heat in the H compared to that in L and M plants (Fig. 4*C*).

Some studies have demonstrated that carboxylation efficiency was enhanced with the increasing P_N associated with rises in altitude (Hovenden and Brodribb 2000, Vats and Kumar 2006). However, the *ex situ* study on barley, pea, and wheat from the Himalayas did not show any significant change in the initial Rubisco activity with regard to altitude (Kumar *et al.* 2004). In our results, the initial Rubisco activities was enhanced, but GAPDH, FBPase, and PRK activities decreased in all populations at 13:00 h (Table 2). This suggests that enhanced carboxylation efficiency but impaired RuBP reduction and regeneration occurred in all populations under the extreme air temperature conditions at noon after domestication. Furthermore, the H plants exhibited higher initial Rubisco and GAPDH activities, but unchanged FBPase and lower PRK activities compared to other populations at 13:00 h (Table 2). The results indicated that the progenies from the highest altitude attempted to consume more energy for CO_2 fixation by enhancing the carboxylation and reduction phases to avoid photodamage, but ultimately failed to improve carbon-fixation ability in the regeneration phase during the Calvin cycle. This should be another aspect in deciphering the lower photosynthetic performance in the progenies from the highest altitude site.

The H_2O_2 content at noon in the H plants was significantly higher than that of the L and M populations, indicating more ROS produced in the progenies from the highest altitude. The higher GPX and APX activities, as well as the AsA/DHA ratio in the H population should be beneficial to remove the excess H_2O_2 compared to the populations from the lower altitudes. A similar trend was found in the seedlings of Norway spruce (*Picea abies* L.) from high altitude which exhibited higher DHA contents. This indicated a higher inherited antioxidant potential than that of those seedlings from lower altitudes (Polle *et al.* 1999). However, the TBRAS content was still significantly higher in the H plants compared to other populations at noon (Table 1), suggesting that the more oxidative injury occurred in the progenies from the highest altitude under the high air temperature stress in Beijing.

In conclusion, the progenies of *R. platyacantha*, which originated from different altitudes and were grown in a common garden in the lowland, exhibited a depression of photosynthesis during the measuring day due to the extreme air temperature conditions in the summer at the domestication site. Moreover, the effect of provenance altitude on midday photosynthetic depression was observed in all populations. The depressed photosynthesis in the populations from the two lower altitudes was due to stomatal limitation, while the photoinhibition that occurred in the population from the highest altitude was associated with the decline in PSII activity. The progenies from the highest altitude attempted to consume the excess energy by improving heat dissipation and CO_2 fixation to avoid photodamage. However, the carbon fixation was initially enhanced at the carboxylation phase but was impaired in RuBP regeneration during the Calvin cycle. To scavenge the harmful ROS generated from the excess energy, the population from the highest altitude triggered an active antioxidant defense, especially the ascorbate-glutathione cycle. However, the H population still suffered more oxidative injury, which eventually led to the more severe midday depression of photosynthesis as compared to the lower altitudes populations.

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