Comparative Study on Antioxidant Systems of Polygonum Viviparum Grown at Two Different Altitudes *

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Abstract Study on the antioxidant systems of *Polygonum viviparum* grown at two different altitudes indicated plants grown at Haibei Research Station at 3200 m altitude as compared with plants grown in Xining at 2300 m altitude had apparently higher contents of ultroviolet-absorbing compounds and ascorbic acid, and significantly higher activities of superoxide dismutase, peroxidase and ascorbic peroxidase. Higher contents of superoxide radical anions and malonadehyde were also found in plants at Haibei Research Station as compared with the plants grown in Xining which have been transplanted from Haibei Research Station for at least four years. The differences in antioxidant system reflect a long term of time of adaptation to different environments.

Key words *Polygonum viviparum*, antioxidant system, different altitudes

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1 Introduction

Plants are subjected to mild or severe photoinhibition owing to special conditions of low temperature, high radiation, especially high level of ultraviolet radiation (UV-B)[1]. In recent two to three decades, photoinhibition and photorepairation have become heated topics. Lots of investigations have been made to determine occurrence mechanisms and consequencial effects of photoinhibition. Also, a lot of documents have stated the photoprotective systems (antioxidant systems) containing in plants grown under special conditions. Investigation with alpine plants in natural conditions was still rare. Direct solar radiation intensity is strong and accounted for 70 % of total radiation at Haibei Research Station[2]. Usually total radiation intensity reaches 1000 $W \cdot m^{-2}$, and photosynthetic active radiation will reach 2300～2400 $\mu mol \cdot s^{-1} \cdot m^{-2}$, UV-B radiation intensity will reach 100 $\mu W \cdot cm^{-2}$ between 11:00 and 16:00 in sunny days in June and July[3]. In which ways can plants be adaptable to these special conditions and prevent their photosynthetic apparatus from photoinhibition? What roles do antioxidant systems play during growing season? In order to answer these questions, this study was conducted to determine differences of antioxidant systems of *Polygonum viviparum* grown at two different altitudes.

2 Material and Methods

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2.1 Plant materials and growth conditions

Polygonum viviparum in this study were collected from Haibei Research Station of Alpine Meadow Ecosystem (Latitude: 37°29′～37°45′; Longitude: 101°12′～101°33′; Elevation: 3200 m) and Northwest Institute of Plateau Biology (Xining, Elevation: 2300 m) in June 1998. *Polygonum viviparum* collected in Xining were transplanted from Haibei Research Station for at least four years ago.

2.2 UAP① determination

According to Day[4], 0.2 g plant leaf materials were put into 15 mL acidized methanol and heated up for 10 min, cooled at room temperature for 15 min then filtered and added up to 25 mL for measurement.

Relatively quantify the content of ultraviolet-absorbing compounds (UAP) as manifested by ABS (absorbance) at 300 nm wavelength.

2.3 Ascorbic acid measurement

The contents of ascorbic acid were determined as described by Hughes[5] with UV-1601 ultra-visible spectrometer made by Shidamzu Corp. Japan.

2.4 Enzyme assay

2.4.1 Extraction

Polygonum viviparum leaves (0.2 g) were homogenized with 62.5 mM PBS (pH = 7.8) containing a few quartz sand at 4 ℃. The homogenate was filtered and centrifuged at 4000 g for 10 min. The supernatant was collected and added up to 100 mL for enzyme activity assay.

2.4.2 SOD activity determination

SOD activity was determined as described by Liu[6]. The assay was performed at 20 ℃, 10 μmol·s^{-1}·m^{-2} radiation conditions in a 3 mL cuvette containing 2.4 mL 62.5 mM PBS, 0.2 mL 3.6 mM lactoflavin, 0.2mL 30 mM methionine, 0.1 mL 3 mM EDTA, 500 μL extraction solution and 0.2 mL 0.125 mM NBT (add in turn) for 25 min. Use UV-1601 ultra-visible spectrometer to detect SOD. One unit of SOD is described as the amount of enzyme that inhibited the rate of NBT reduction by 50 %, namely, μ/mg·FW·min, where $\mu = (ABS_{max} - ABS_{560nm}) / (ABS_{max}/2)$.

2.4.3 POD activity determination

According to Zhang[7], the assay was carried out in a 3 mL cuvette containing 1 mL extraction solution and 2 mL reaction mixture (to make it, add 28 μL hydroxybenzene and 19 mL H_2O_2 to 50 mL, pH = 6.0, 100 mM PBS). The activity of POD was described as the decline rate of ABS at 470 nm wavelength, which can also be expressed as ΔA_{470}/g·FW·min.

2.4.4 APX activity assay

The assay was performed in a 3 mL cuvette containing 2.5 mL 50 mM pH = 7.0 PBS, 0.3 mL 50 mM AsA, 0.1 mL 0.1 mM H_2O_2 and 100 μL enzyme extraction using UV-1601 ultra-visible spectrometer according to Nacano and Asada[8]. The APX activity was described as ΔA_{290}/mg·FW·min.

2.4.5 MDA and O_2^- measurement

The extraction was the same as for enzyme determinations above. MDA was defined as described by Liu[6], 1 mL of enzyme extraction mentioned above and trichloroacetic acid (TCA) (in which 0.5 % sulfur permued barbital acid, TBA, was contained) were mixed and covered and kept boiling for 15 min and cooled quickly. Use

① Abreviations AOS: activated oxygen species; SOD: superoxide dimutase; AsA: ascorbic acid; UAP: ultra-absorbing compounds; POD: peroxidase; MDA: malonaldehyde; UV-B: ultraviolet-B radiation; MET: methionine; ABS: absorbance; PBS: phospherate buffer solution; GR: glutathione reductase; APX: ascorbic peroxidase

trichloroacetic acid as blank to measure ABS values at 532 nm and 600 nm, then calculated the content of MDA according to the following equation: Δmol MDA $\times$(532 nm - 600 nm) = 1.55 $\times 10^{-5}$. The content of MDA was expressed as μmol/g·FW.

O_2^- determination was performed as described by Wang[9] with some modifications. The 0.9 mL of enzyme extraction mentioned above and 50 μL 0.01 mmol/L of hydrochlorinated H_2NOH were mixed, and after reacted for 20 min at room temperature, 1 mL 1 % of α-naphthalene amine and 0.33 % benzidine sulfonic acid were added and reacted for 15 min. The relative content of O_2^- was defined as ABS at 530 nm wavelength.

3 Results and discussion

3.1 UAP

The relative content of UAP was exhibited higher in *Polygonum viviparum* grown at Haibei Research Station as compared with the plants grown in Xining (Tab. 1). This higher content of UAP may be due to the induction effect of higher ultraviolet radiation intensity and its subsequent adaptive reaction in plants grown at higher altitude (3200 m). Study has indicated that ultraviolet radiation intensity at Haibei Research Station was 1.4 times stronger as the one in Xining[3]. UAP, mainly containing in epidermis cells, plays an important role in preventing plants from injury. Yi[10] and Shi[11] reported that the higher UV-B radiation plants were grown, the higher contents of UAP were contained in plants, and thus, the higher UV-B radiation would increase the content of UAP.

3.2 AsA

The content of AsA was significantly higher in *Polygonum viviparum* grown at Haibei Research Station than those plants grown in Xining (Tab. 1). AsA plays a key role as antioxidant and oxygen-scavenger in plants. Higher content of AsA was related to higher capacity for antioxidation under high radiation condition. Previous studies[12,13] indicated that AsA could react with hydroxyl radical ($\cdot OH^-$), reduce superoxide anion radical (O_2^-), quench singlet oxygen ($^1O_2$) and clean hydrogen peroxide (H_2O_2) through the ascorbate dehydroascorbate system. In addition, AsA participates in xanthophyll cycle as a reductant in which zeaxanthin plays a key role in resisting low temperature and high radiation stresses[14].

Tab. 1 Comparison of the contents of ultraviolet-absorbing compounds (UAP) and AsA of Polygonum viviparum grown at Haibei Research Station and in Xining

Location	Ultraviolet-absorbing compounds (ABS)	ASA (mmol/g·FW)
Xining (2300 m)	2.06 $\pm$0.06 [a.m.]	0.46 $\pm$0.03 [a.m.]
Haibei Station (3200 m)	3.23 $\pm$0.04 [pm]	0.56 $\pm$0.01 [pm]

a.m. Each of the data was the average of three measurements;

pm. Data in the same column with different letters are significantly different at the level of $P < 0.05$, the same below.

3.3 SOD, POD and APX

The activities of SOD, POD and AsA-POD of *Polygonum viviparum* grown at Haibei Research Station were all significantly higher than those in Xining (Tab. 2). Some studies suggested the above three enzymes can enhance anti-cold and anti-drought capacity of plants. Higher activities of SOD, POD and AsA-POD of *Polygonum viviparum* grown at Haibei Research Station were a long-term adaptive strategy. SODs, often classified into three distinct types according to their metal cofactor: Cu/Zn-SOD, Mn-SOD and Fe-SOD[15], can catalyze the dismutation of superoxide to hydrogen peroxide and molecular oxygen, and hydrogen peroxide can react with AsA through ascorbate peroxidase into water and oxygen. PODs usually occur as multiple molecular forms and have a number of potential roles in plants

growth, development, and differentiation[16]. POD metabolizes H_2O_2 to water. Enhanced POD activity suggested that high radiation intensity, especially under low temperature conditions may have enhanced the synthesis of POD. APX acts in conjunction to metabolize H_2O_2 to H_2O through a metabolic cycle, widely known as the ascorbate-glutathione cycle or Halliwell-Asada pathway.

Tab. 2　Comparison of the activities of superoxidase, peroxidase and ascorbate peroxidase and the contents of MDA and superoxide radical of Polygonum viviparum **grown at Haibei Research Station and in Xining**

Locations and altitudes	Superoxidase ($\mu g^{-1} \cdot FW$)	Peroxidase ($\Delta A_{470} g^{-1} \cdot FW \cdot min^{-1}$)	Ascorbate peroxidase ($\Delta A_{290} mg^{-1} \cdot FW \cdot min^{-1}$)	MDA ($\mu mol \cdot g^{-1} \cdot FW$)	Superoxide radical (ABS)
Xining (2300 m)	$370 \pm 6.4^{a.m.}$	$8.67 \pm 2.8^{a.m.}$	$8.01 \pm 1.05^{a.m.}$	$48.3 \pm 3.5^{a.m.}$	$0.46 \pm 0.06^{a.m.}$
Haibei Research Station (3200 m)	390 ± 8.2^{pm}	42.0 ± 13.6^{pm}	11.89 ± 2.81^{pm}	71.7 ± 4.1^{pm}	$0.47 \pm 0.04^{a.m.}$

3.4　MDA and $O_2^{\cdot-}$

Content of MDA, an important indicator of the degree of membrane damage, was about 50 % higher in *Polygonum viviparum* grown at Haibei Research Station than that in Xining shown in Tab. 2 However, the content of $O_2^{\cdot-}$ had no significant difference ($P > 0.05$) between the plants grown at two altitudes (Tab. 2). It is reasonable to proposed that membrane damage was more severe in *Polygonum viviparum* grown at Haibei Research Station though $O_2^{\cdot-}$ was efficiently cleansed by lots of oxygen-scavenging systems, which revealed that other active oxygen species (AOS), besides $O_2^{\cdot-}$, produce damage to membrane of cells.

Active oxygen species (AOS), which exhibit damages to plant cell membrane and invoke photoinhibition, can be produced during photosynthetic process in plants grown at stress conditions. Decreasing the activities of AOS can, to some extent, release the damage to plants[17]. However, plants metabolize AOS by invoking the antioxidant defence system[18,19]. This system consists of non-enzyme-catalyzing antioxidants, such as ascorbic acid, glutathione, carotenoids, α-tocopheral and flavonoid[20], as well as some enzyme-catalyzing antioxidants such as SOD, POD, APX, GR (gratathione reductase) and so on[21,22]. Although poorly containing in plants, they play important role in plants and serve as antioxidants and protect plants against the destruction caused by active oxygen species (such as singlet oxygen $^1O_2$, superoxide anion radical $O_2^{\cdot-}$, hydrogen peroxide H_2O_2 and hydroxyl radical $OH^{\cdot}$). The main photo-oxidative damage in chloroplasts is resulting from the formation of singlet oxygen by interaction of ground state triplet oxygen with triplet states of excited chlorophyll, or from the formation of superoxide radicals through oxidation of ferrodoxin by the Mehler reaction or some related oxygen species[22].

Cold-acclimated plants and sun plants always exhibit a considerably higher level of AsA, SOD, POD, and APX activities[23]. In this study, higher contents or activities of antioxidation systems exhibited in the plants grown at higher altitude areas, which revealed that alpine condition can easily induce production and assimilation of antioxidant systems, and differences of antioxidant systems between *Polygonum viviparum* grown at two different altitudes represent a long-term adaptive strategy to alpine environments.

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两个不同海拔地区珠芽蓼抗氧化系统的比较研究

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摘 要 通过对两个不同海拔地区珠芽蓼抗氧化系统的比较研究,结果表明,较高海拔地区植物叶片内抗氧化系统,如紫外线吸收色素、抗坏血酸含量以及过氧化物酶、超氧化物歧化酶和抗坏血酸过氧化酶的活性,均比低海拔地区同种植物叶片高;而丙二醛(MDA)以及超氧阴离子自由基的相对含量在较高海拔地区植物叶片内较高。两地珠芽蓼叶片内抗氧化系统的差异是植物经过长期形成的对不同地区环境的适应性反应。

关键词 珠芽蓼,抗氧化物系统,不同海拔