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Thermotolerance of the photosynthetic light reactions in two *Phaseolus* species: a comparative study

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Abstract

The common bean (*Phaseolus vulgaris* L.) is sensitive to high temperature, while an ecologically contrasting species (*Phaseolus acutifolius* A. Gray) is cultivated successfully in hot environments. In this study, the two bean species were respectively acclimated to a control temperature of 25 °C and a moderately elevated temperature of 35 °C in order to compare the thermotolerance capabilities of their photosynthetic light reactions. Growth at 35 °C appeared to have no obvious adverse effect on the photosynthetic activities of the two beans, but changed their thermotolerance. After a short period of heat shock (40 °C for up to 4 h), the photosynthetic activities of 25 °C-grown *P. vulgaris* declined more severely than those of *P. acutifolius* grown at 25 °C, implying that the basal thermotolerance of *P. vulgaris* is inferior to that of *P. acutifolius*. But after acclimating to 35 °C, the thermotolerances of the two species were both greatly enhanced to about the same level, clearly demonstrating the induction of acquired thermotolerance in their chloroplasts, and *P. vulgaris* could be as good as *P. acutifolius*. Temperature acclimation also changed plants' resistance to photoinhibition in a manner similar to those toward heat stress. In addition, acquisition of tolerance to heat and strong irradiance would reduce the dependency of the two beans on xanthophyll pigments to dissipate heat, and also seemed irrelevant to the agents with antioxidant activities such as SOD.

Additional key words: bean; chlorophyll fluorescence; heat stress; photoinhibition; thermotolerance

Introduction

Plants lack the capability of locomotion, they are thus exposed to various kinds of environmental stresses, including temperature extremes. Through adaptation and natural selection, each species of plant has an optimum range of growth temperature, and its geographical distribution is mainly determined by the temperature zone in which it can thrive. To increase the yields of crops, there has been a long history of extending the range of their distribution by plant breeding.

Phaseolus vulgaris L. (common bean) is an important food crop worldwide. However, it is very sensitive to high temperature and water deficit, the growth and productivity of which is reduced when exposed to such environments (Dale 1964). On the other hand, an ecologically contrasting species, *Phaseolus acutifolius* A. Gray (tepary bean) is cultivated successfully in places where high temperature and drought are common (Scully and Waines 1988). Compared to *P. vulgaris*, *P. acutifolius* possesses many characteristics that enable it to flourish in hot arid environments. It has a more extensive root system, better stomatal control (Markhart 1985), higher water-use efficiency (Castonguay and Markhart 1992), and more active paraheliotropism (Bielenberg *et al.* 2003). At the tissue level, *P. acutifolius* has also been characterized as generally more tolerant to high temperature than *P. vulgaris* (Lin and Markhart 1996), but no more tolerant to tissue dehydration (Castonguay and Markhart 1991). In addition, isolated mitochondria of *P. vulgaris* grown at 32 °C have reduced electron transport and are substantially uncoupled compared to 25 °C-grown plants, whereas those of *P. acutifolius* show no such effects (Lin and Markhart 1990). As for another important organelle chloroplast, study regarding its thermotolerance still has not been well documented.

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Abbreviations: A – antheraxanthin; AOS – active oxygen species; F_m – maximal fluorescence of dark-adapted leaves; F_m' – maximal fluorescence of light-adapted leaves; F_o – minimal fluorescence of dark-adapted leaves; F_o' – minimal fluorescence of light-adapted leaves; F_s – steady state fluorescence of light-adapted leaves; q_P – photochemical quenching; q_N – non-photochemical quenching; SOD – superoxide dismutase; V – violaxanthin; Z – zeaxanthin.

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It has been shown that among all cell functions, photosynthesis is one of the most heat sensitive processes (Berry and Björkman 1980, Sharkey 2005). In fact, it has been suggested that the responses of the growth of plant species to temperature are closely linked to their photosynthetic responses to temperature (Pearcy 1978, Murakami *et al*. 2000). Previous studies showed that heat can damage the oxygen-evolving complexes (Yamane *et al*. 1998), impair the electron transport systems (Sinsawat *et al*. 2004, Wise *et al*. 2004), alter the organization of thylakoid membranes (Gounaris *et al*. 1984, Mohanty *et al*. 2002), increase thylakoid ionic conductance (Bukhov *et al*. 1999, Schrader *et al*. 2004), and lower the activation level of Rubisco (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004). With regard to these findings, it would be interesting to evaluate and compare the responses of the chloroplasts of these two *Phaseolus* species to high temperature.

In this study, we found that, compared to their mitochondrial counterparts, the light reactions of the chloroplasts of *P. vulgaris* have relatively higher thermotolerance capability, and it can be as good as *P. acutifolius* after acclimating to a moderately elevated temperature.

Materials and methods

Plants: *Phaseolus acutifolius* A. Gray (tepary bean, seed source: MN258/78 = Arizona GN-76-2 race T4) and *Phaseolus vulgaris* L. (common bean, seed source: Red Cloud, California certified, 1984 crop, Lot: RK-692, Grower: 84A-816) were used in this study. They were grown in 0.8 l plastic pots (one plant per pot) containing 3:2 (v/v) soil and perlite mixture at 25 °C in a plant growth chamber with a 12-h photoperiod (400 μmol $m^{-2}s^{-1}$ light from white fluorescence lamps) under 80 % relative humidity. Plants were watered daily and fertilized weekly with *HYPONeX No. 2* (20:20:20, *Hyponex Corp.*, Maricopa, Arizona, USA) 20 days after sowing.

When first trifoliate leaves emerged, half of the plants were transferred to another growth chamber using the same environmental conditions except the temperature was raised to 35 °C, while control plants remained in the original growth chamber at 25 °C. This acclimation period lasted at least for 12 days.

F_o changes upon linear heating: The dependence of the minimal chlorophyll (Chl) fluorescence (F_o) on temperature was measured on the central leaflet of the third trifoliate leaf using a method similar to those described in other literatures (Kuropatwa *et al*. 1992, Ilik *et al*. 1997). A chlorophyll fluorometer *PAM-2000* (*Walz*, Effeltrich, Germany) was used to monitor the changes. All samples were dark adapted for at least 15 min prior to measurement. Fluorescence was excited at 655 nm by a pulsed measuring beam (600 Hz) with an intensity of about 0.5 μmol $m^{-2}s^{-1}$ and detected at wavelengths longer than 700 nm. The attached leaflets were heated in a stirred water bath at a rate of 1.5 °C min^{-1} from 25 °C up to 60 °C. The critical temperature is defined as the temperature of the intersection point between the linear portions of the fluorescence-temperature course above and below the threshold at which fluorescence raises abruptly.

Photosynthetic activity measured by Chl fluorescence: Photosynthetic activities of the two *Phaseolus* species were monitored by Chl fluorescence using *PAM-2000* fluorometer. The pulsed measuring beam provided by a light-emitting diode was modulated at 600 Hz in the absence of actinic light and 20 kHz when actinic light was turned on. The continuous actinic beam (peak wavelength 655 nm) was provided by a light-emitting diode array at an intensity of about 110 μmol m^{-2} s^{-1}. The saturating pulse light source was a 20 W halogen lamp. It provided white light pulse (0.8 s duration) at about 1500 μmol m^{-2} s^{-1}. The following fluorescence parameters were measured: the minimal fluorescence (F_o) and the maximal fluorescence (F_m) of dark-adapted samples, and the minimal fluorescence (F_o’, measured with the presence of far-red light irradiation), the maximal fluorescence (F_m’) and the steady state fluorescence (F_s) of light-adapted samples (samples illuminated with actinic light for 15 min). From these data, the maximum quantum yield of dark-adapted samples [$(F_m - F_o)/F_m$], the effective quantum yield of light-adapted samples [$(F_m' - F_s)/F_m'$], as well as the photochemical quenching [$q_P = (F_m' - F_s)/(F_m' - F_o')$] and non-photochemical quenching [$q_N = 1 - (F_m' - F_o')/(F_m - F_o)$] were calculated. All measurements were made early in the day (between 2–4 h after light on).

Heat treatment was done in the dark by placing the central leaflet of the attached third trifoliate leaves in a 40 °C water bath. Chl fluorescence was measured at a fixed time interval at room temperature. Five plants were used for each species and acclimation temperature.

Photoinhibition: The central leaflets of the attached third trifoliate leaves were exposed to irradiation of white light at 1600 μmol m^{-2} s^{-1}. The illumination was provided by a halogen lamp (250 W/ 24 V), filtered through a $CuSO_4$ solution. A J-type thermal couple attached to the leaf surface showed that there was no significant change in leaf temperature during the course of irradiation. The maximum quantum yields in the irradiated areas of the leaves were then measured at time intervals of 5, 15, 35 and 65 min each after a 15-min dark period. Four plants were used for each species and acclimation temperature.

Xanthophyll cycle analysis: Xanthophyll cycle pigments were analyzed by HPLC based on a method of Gilmore and Yamamoto (1991). Leaf discs of 1 cm^2 were ground in liquid nitrogen and extracted overnight with 1.5 ml 85 % acetone at –20 °C. Supernatants of extracts were collected by centrifugation at 12 000 × *g* for 2 min (*Centrifuge MK202, Sigma*, Osterode, Germany) and then filtered using a 0.22 μm filter. 20 μl of the extract was injected onto a reverse phase HPLC column (4.6 × 250 mm, 5 μm particle size, *Alligent Zorbax ODS*, Non-endcapped, *Agilent Technologies Inc.*, Santa Clara, CA, USA). The eluents consisted of solvent A (acetonitrile: methanol: 0.1 M Tris-HCl buffer at pH 8.0, 75:12:4, v/v) and solvent B (methanol: ethyl acetate, 68:32, v/v). The elution conditions were as follows: 100 % solvent A for 6 min, followed by a linear gradient decreasing to 0 % solvent A in 10 min, then a plateau of 100 % solvent B till the end (20 min). The flow rate was 2 ml min^{-1} and xanthophyll peaks were detected at 450 nm.

The de-epoxidation of violaxanthin (V), resulting in an accumulation of zeaxanthin (Z) *via* antheraxanthin (A) in chloroplasts, was characterized using the de-epoxidation index [=(Z+0.5×A)/(V+A+Z)]. Induction of de-epoxidation was carried out by illuminating attached leaves for 30 min with white light of 600 μmol m^{-2} s^{-1}. Five plants were used for each species and acclimation temperature.

Superoxide anion radical scavenging activity: Superoxide anion radical scavenging activities of the total soluble proteins extracted from leaves were assayed. Leaf discs of 1 g fresh mass were ground in liquid nitrogen and extracted with 0.6 ml extraction buffer containing 0.2 M NaCl, 1 mM EDTA, 2 % β-mercaptoethanol (v/v), 0.2 % Triton X-100 (v/v) and 0.1 M Tris-HCl (pH 7.8). Supernatants containing the total soluble proteins were collected by centrifugation at 10 000 × *g* for 30 min.

The superoxide anion scavenging activities were measured as the inhibition of the reduction of nitro blue tetrazolium by the superoxide anions generated in a phenazine methosulfate-NADH system based on a method modified by Lai *et al.* (2001). An aliquot of 200 μl of leaf extract was mixed with 200 μl each of 630 μM nitro blue tetrazolium, 33 μM phenazine methosulfate and 156 mM NADH. The mixture was incubated at room temperature for 5 min in the dark and the absorbance at 560 nm was measured. The percentage inhibition was calculated using the equation:

$$\% \text{ Inhibition} = [1-(A_1/A_0)]\times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the leaf extract. Heat treatment was done in the dark by placing attached leaves in a 40 °C water bath for 2 h. Four plants were used for each species and acclimation temperature.

Statistical analyses: *ANOVA* tests were performed to evaluate the significance of the differences among the means of experimental groups using *SigmaStat* (*Jandel Scientific*, San Rafael, CA, USA).

Results

Plant growth at contrasting temperatures: The leaf area of the central leaflet of the third trifoliate and the internodal length between the third and fourth trifoliates were used as indicators to plant growth. The measurements started when the fourth trifoliate leaves were visible (day 0), and all the data were normalized based on the data obtained on the 11th day. As shown in Fig. 1*A* and 1*C*, the growth of *P. vulgaris* was sensitive to temperature. The increasing rates of both leaf area and internodal length slowed down significantly when grown at 35 °C, as compared to those at 25 °C. On the other hand, the growth of the leaves of *P. acutifolius* was only affected to a limited extent (Fig. 1*B*), and the extension of internodes was similar at 25 °C and 35 °C (Fig. 1*D*).

The increase in F_o upon linear heating: A sharp rise in the minimal fluorescence (F_o) occurring over a small range of temperatures has been widely used to assess the temperatures at which plant species suffer irreversible damage in their photosynthetic systems, and used as an indicator of thermotolerance capability (Schreiber and Berry 1977, Havaux 1992). When grown at 25 °C, the critical temperature of *P. vulgaris* was lower than that of *P. acutifolius* by 2 °C (43 °C vs. 45 °C). The critical temperatures of *P. vulgaris* and *P. acutifolius* both raised by 4 °C when the growth temperature was lifted to 35 °C (47 °C vs. 49 °C).

Thermotolerance of photosynthesis: After 12 days of acclimation at 25 °C or 35 °C, the photosynthetic light reactions of the two *Phaseolus* species were probed by Chl fluorescence. It was found that the major fluorescence parameters, including the maximum quantum yield of dark-adapted samples, the effective quantum yield of light-adapted samples, the photochemical quenching (q_P) and non-photochemical quenching (q_N), were similar between *P. vulgaris* and *P. acutifolius*, and between the two treatments (25 °C vs 35 °C), suggesting that a growth temperature up to 35 °C had no apparent adverse effect on the light reactions of the two beans (data not shown).

Thermotolerance in the absence of pre-acclimation is termed basal thermotolerance, and a plant subjected to a mild heat stress resulting in improved tolerance to high temperature is known as acquired thermotolerance. To find out if the growth temperature could alter the thermotolerance of the two plant species, attached leaves were subjected to heat shock at 40 °C (a temperature that was below the critical temperatures of both beans) in the dark for up to 4 h, and their photosynthetic activities were continuously monitored by Chl fluorescence. As shown

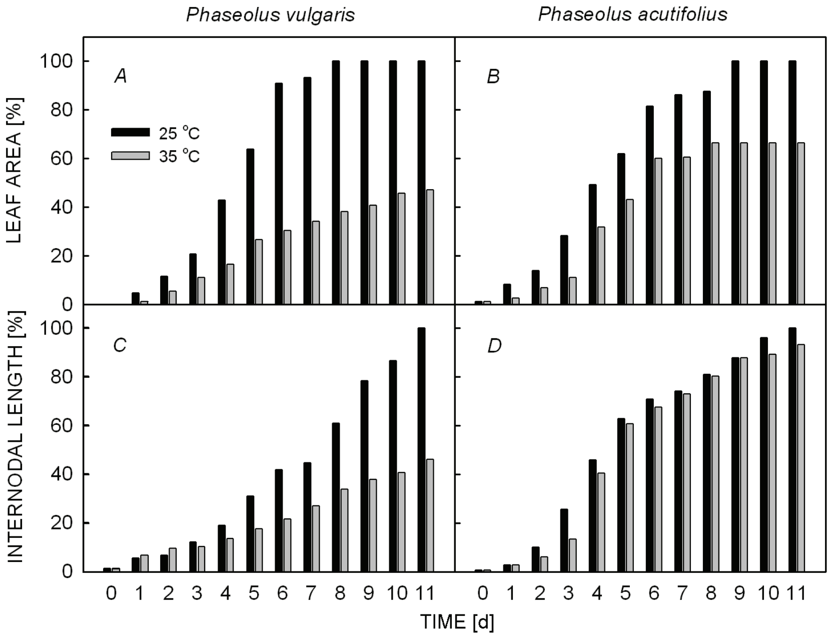

Fig. 1. The leaf areas of the central leaflet of the third trifoliates of (*A*) *P. vulgaris* and (*B*) *P. acutifolius*, and the internodal lengths between the third and fourth trifoliates of (*C*) *P. vulgaris* and (*D*) *P. acutifolius* were used as indicators to plant growth. The measurements started when the fourth trifoliate leaves were visible (day 0). All the data were normalized based on the data obtained on the 11th day. The two bean plants were respectively acclimated to 25 °C (*black bars*) and 35 °C (*grey bars*).

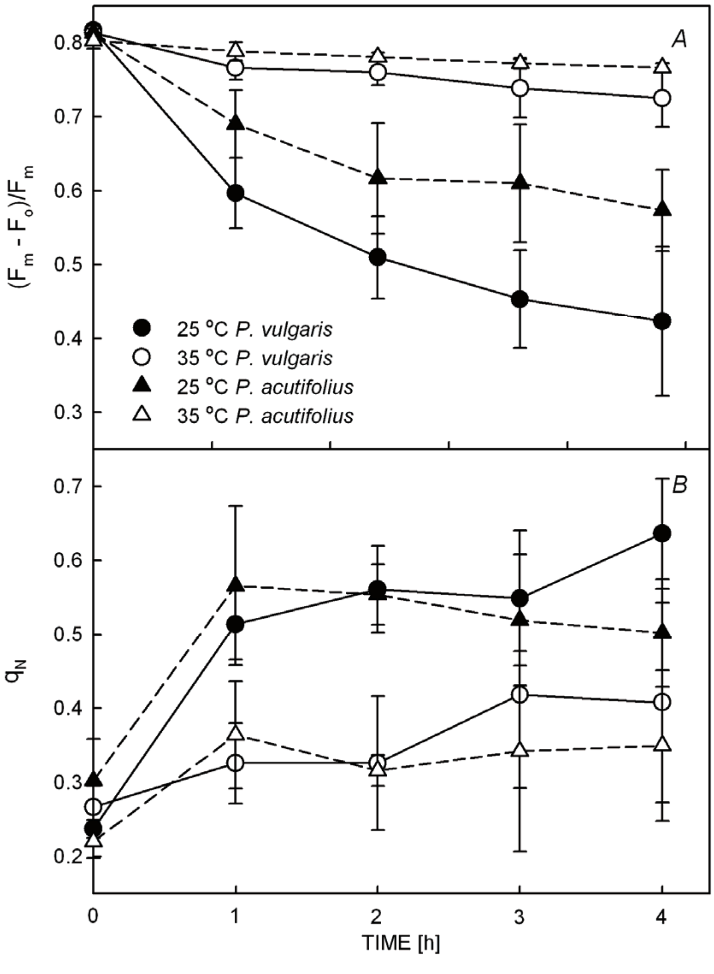

Fig. 2. (*A*) The maximum quantum yield ($(F_m – F_o)/F_m$) and (*B*) the non-photochemical quenching (q_N) as functions of the time of heat treatment at 40 °C. The data are the mean ± SD of five independent measurements. 25 °C-grown *P. vulgaris* (●); 35 °C-grown *P. vulgaris* (○); 25 °C-grown *P. acutifolius* (▲); 35 °C-grown *P. acutifolius* (△).

in Fig. 2*A*, the maximum quantum yield of *P. vulgaris* grown at 25 °C declined from 0.8 to 0.4 during the 4-hour heat shock. The extent of decrease in *P. acutifolius* grown at the same temperature was much less. The difference between *P. vulgaris* and *P. acutifolius* was significant at the end of heat treatment ($p<0.05$). As for the plants grown at 35 °C, there were hardly any decreases for the two species. The effective quantum yield and photochemical quenching of the two beans followed a very similar pattern of changes, i.e., *P. vulgaris* grown at 25 °C decreased more rapidly than *P. acutifolius* grown at the same temperature, and both species showed significant improvement in thermotolerance when the growth temperature was raised to 35 °C (data not shown).

Fig 2*B* shows the changes in the non-photochemical quenching during the course of heat shock. The q_N level of *P. vulgaris* grown at 25 °C continuously increased and reached more than two-fold after 4 h. On the other hand, *P. acutifolius* grown at 25 °C showed a significant increase only in the first hour, which was followed by a slow decline. In comparison, the increases of the two beans grown at 35 °C were moderate.

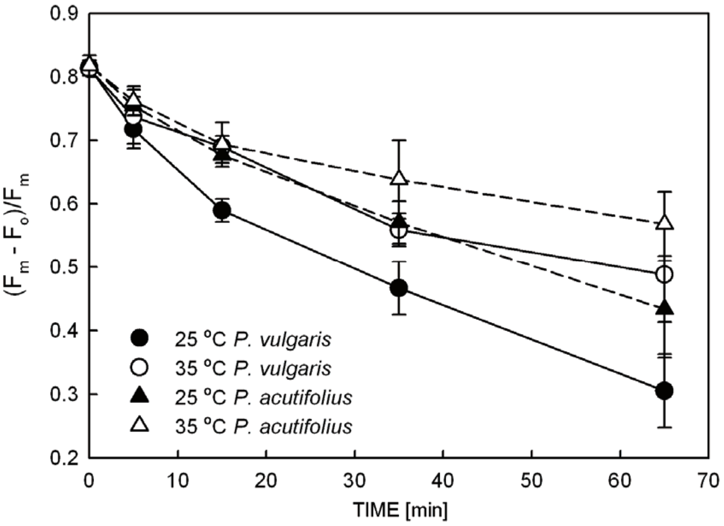

Fig. 3. The maximum quantum yield ($(F_m–F_o)/F_m$) as a function of the exposure time under white light of 1600 µmol $m^{-2}s^{-1}$. The data are the mean ± SD of four independent measurements. 25 °C-grown *P. vulgaris* (●); 35 °C-grown *P. vulgaris* (○); 25 °C-grown *P. acutifolius* (▲); 35 °C-grown *P. acutifolius* (△).

Photoinhibition: In natural environment, high temperature is most often accompanied by strong light. The attached leaves of acclimated plants were also subjected to high-intensity irradiation to find out their susceptibility to photoinhibition. As presented in Fig. 3, the maximum quantum yield of *P. vulgaris* grown at 25 °C dropped from 0.8 to 0.3 after exposure to white light of 1600 µmol $m^{-2}s^{-1}$ for 65 min. The tolerance to light stress was greatly enhanced when the growth temperature of *P. vulgaris* was shifted to 35 °C ($p < 0.05$ at 65 min). *P. acutifolius* grown at 25 °C was less susceptible to photoinhibition compared to *P. vulgaris* grown at the same temperature ($p<0.05$ at 65 min), and it also showed higher resistance to strong irradiation when grown at 35 °C ($p<0.05$ at 65 min).

Xanthophyll cycle is generally regarded as the most important photoprotective mechanism in the photosynthetic system. It helps in the heat dissipation of excess light energy. The amounts of xanthophyll cycle pigments of plants were measured using HPLC and quantitated on a Chl basis (Fig. 4*A*). They were calculated from the corresponding peak areas of HPLC chromatograms. The pool size of xanthophylls of 25 °C-grown *P. vulgaris* was larger than that of *P. acutifolius* grown at the same temperature ($p<0.05$). A shift of growth temperature to 35 °C decreased the pool in *P. vulgaris* ($p<0.05$), but no such change was observed in *P. acutifolius* ($p>0.05$).The de-epoxidation states of the xanthophyll cycle are presented in Fig. 4*B*. In dark-adapted plants, the degrees of de-epoxidation (de-epoxidation indexes) of *P. vulgaris* and *P. acutifolius* were both lowered by raising the growth temperature ($p = 0.26$ for *P. vulgaris* and $p<0.05$ for *P. acutifolius*), and the indexes were generally higher for *P. vulgaris* than for *P. acutifolius*. The de-epoxidation indexes were greatly increased by illumination with white light of 600 µmol m^{-2} s^{-1} for 30 min, with *P. vulgaris* higher than *P. acutifolius* ($p<0.05$), but the differences between the plants acclimated at 25 and 35 °C were negligible ($p>0.05$).

Superoxide anion radical scavenging activity: Heat stress can induce generation of the active oxygen species (AOS) in plants (Doke 1997), and superoxide dismutase (SOD) is one of the major enzymes that are involved in the AOS-detoxification system of higher plants. Superoxide anion scavenging activities of the total soluble proteins extracted from the leaves of the two beans were assayed. As shown in Fig. 5, a heat treatment at 40 °C for 2 h increased the SOD activity of *P. vulgaris* grown at 25 °C almost 3-fold ($p < 0.05$). A similar increase in SOD activity could also result from a shift of growth temperature to 35 °C ($p<0.05$), but 40 °C heat treatment on this high-temperature acclimated *P. vulgaris* brought about only a little further increase ($p>0.05$). As for *P. acutifolius*, the pattern of changes in SOD activities was similar, but all the values were smaller as compared with those of corresponding *P. vulgaris* plants.

Discussion

Similar to the observations in the field, a temperature rise to 35 °C markedly slowed down the growth of *P. vulgaris*, but its effects on *P. acutifolius* was relatively small (Fig. 1). The result clearly shows that a mild temperature rise, in the absence of water stress, is sufficient to affect the growth of *P. vulgaris*. This is in line with that found by Lin and Markhart (1996). Nevertheless, 35 °C had no obvious adverse effect on the light reactions of *P. vulgaris*, demonstrating that its photosynthetic system, unlike the whole plant, can tolerate moderately elevated temperature. This is also in contrast to the mitochondria of *P. vulgaris*, which have reduced electron transport and are substantially uncoupled when grown at 32 °C (Lin and Markhart 1990).

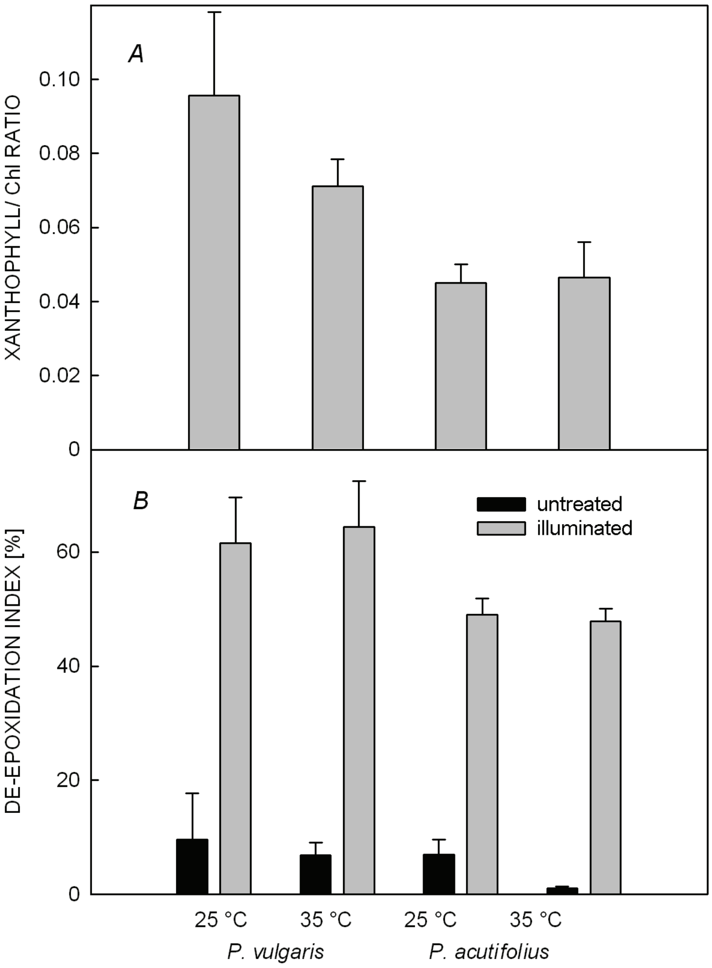

Fig. 4. (*A*) The amounts of xanthophyll cycle pigments in the leaves of *P. vulgaris* and *P. acutifolius* quantified on a chlorophyll (Chl) basis. (*B*) The de-epoxidation states of the xanthophyll cycle of *P. vulgaris* and *P. acutifolius* before (*black bars*) and after illumination with white light of 600 µmol m^{-2} s^{-1} for 30 min (*grey bars*). The two bean species were acclimated to 25 °C and 35 °C, respectively. The data are the mean ± SD of five independent measurements.

The difference between the two *Phaseolus* species in terms of the thermotolerance of photosynthetic light reactions is manifested when exposed to a short period of heat shock. As far as 25 °C-grown plants were concerned, *P. vulgaris* was less tolerant to 40 °C heat shock than *P. acutifolius* (Fig. 2*A*). However, when plants were acclimated at 35 °C, the thermotolerances of the two species were both greatly enhanced to about the same level. In other words, although *P. vulgaris* shows a lower basal thermotolerance than *P. acutifolius*, the acquired thermotolerance of *P. vulgaris* after acclimation becomes comparable with that of *P. acutifolius*. Improvement of thermotolerance through acclimation has been observed in many plant species (*e.g.* Pearcy 1978, Yamasaki *et al.* 2002, Sinsawat *et al.* 2004, Haldimann and Feller 2005). Note that the critical temperature at which F$_0$ sharply rises also gives information about the thermostability of photosynthetic systems at extreme temperatures. It was 2 °C lower for *P. vulgaris* compared to *P. acutifolius* when grown at 25 °C, but both increased by the same 4 °C when the growth temperature was raised to 35 °C, implying that the extent that the photosynthetic system of *P. vulgaris* is capable of adjusting is equivalent to that of *P. acutifolius*.

The variation pattern in the tolerance of light reactions to high light irradiation (Fig. 3) was similar to that toward heat stress, indicating that acclimation could simultaneously improve the tolerance of bean plants to both

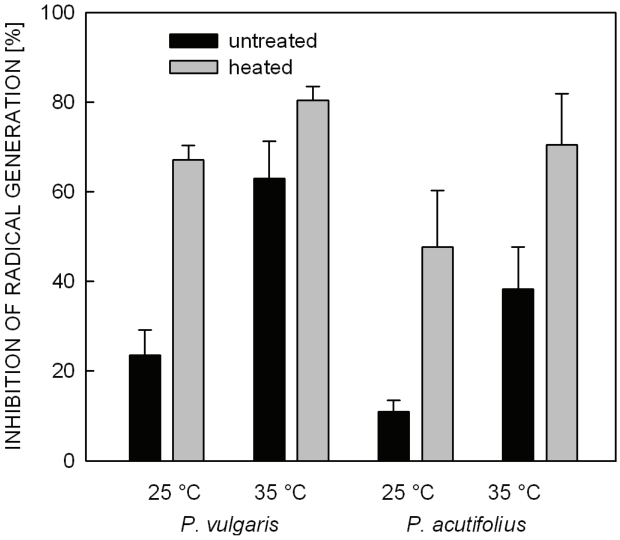

Fig. 5. Superoxide anion scavenging activities of the total soluble proteins extracted from the leaves of untreated *P. vulgaris* and *P. acutifolius* (*black bars*) and the leaves subjected to heat treatment at 40 °C for 2 h (*grey bars*). The activities were measured as the inhibition of the reduction of nitro blue tetrazolium by the superoxide anions generated in a phenazine methosulfate-NADH system as described in Materials and methods. The two bean species were acclimated to 25 °C and 35 °C, respectively. The data are the mean ± SD of four independent measurements.

types of stress. It is also noteworthy that the extents of photoinhibition of plants (Fig. 3) were directly proportional to their de-epoxidation states before illumination (Fig. 4*B*), *i.e.*, the higher the dark de-epoxidation index, the more pronounced the photoinhibition. Furthermore, 25 °C-grown *P. vulgaris*, the most vulnerable group, had the largest xanthophyll pool on a Chl basis (Fig. 4*A*). The pool size of *P. acutifolius*, which was more resistant to photoinhibition, was only half the size. In addition, the extents of decrease in quantum yield during heat treatment as shown in Fig. 2*A* were also generally in parallel with the xanthophyll pool sizes and the dark de-epoxidation indexes of plants (Fig. 4). These results seem to suggest that the plants with higher thermotolerance and also more resistant to photoinhibition are less dependent on heat dissipation *via* xanthophyll pigments. Note that the increases in the non-photochemical quenching during heat treatment were also less in acclimated plants, indicating that this heat dissipation process is less required by acclimated plants (Fig. 2*B*). A similar phenomenon has been observed in maize (Sinsawat *et al.* 2004).

Heat stress can induce active oxygen species (AOS) and turn on detoxification systems in plants (Tsang *et al.* 1991). As shown in Fig. 5, the SOD activity of *P. vulgaris* is very sensitive to temperature changes. A heat shock at 40 °C or acclimation at 35 °C all greatly enhanced the activity. But the photosynthetic activity of 25 °C-grown *P. vulgaris* was still strongly inhibited after heat shock (Fig. 2), indicating that SOD could not effectively protect the photosynthetic system of *P. vulgaris* from heat damage. The raise in SOD activity in the cells of *P. vulgaris* at elevated temperatures thus might just reflect the increasing amount of AOS. The variation pattern of SOD activity of *P. acutifolius* was similar to that of *P. vulgaris,* but the values were lower (Fig. 5). This suggests the plant species with higher thermotolerance is less dependent on SOD, and again showing the irrelevance of SOD activity to thermotolerance improvement.

Thermal dissipation through xanthophyll pigments and antioxidant systems are major protective processes of plant cells. The irrelevance of these processes to the thermotolerance of the light reactions in the two beans indicates that the modifications made during acclimation to effectively protect against both heat and light stresses are more ‘fundamental’. It has been shown that some chloroplast-targeted heat shock proteins can protect photosynthesis during both heat and photoinhibitory stresses (Schroda *et al.* 1999, Downs *et al.* 1999), and alternation of the number of unsaturated lipids in thylakoid membranes can enhance the thermotolerance of photosynthetic apparatus (Murakami *et al.* 2000). Whether these adjustments have occurred in *P. vulgaris* and *P. acutifolius* when grown at 35 °C requires further investigation.

Although thermotolerance of the light reactions of *P. vulgaris* could be greatly enhanced close to that of *P. acutifolius* if planted at 35 °C, *P. vulgaris* was still much less tolerant to high temperature than *P. acutifolius* as a whole plant (Fig. 1). It seems that different parts of plants may have different capability for acquiring tolerance to high temperature. Higher degree of protection for the photosynthetic apparatus can also be seen under water stress (Cornic *et al.* 1992, Tourneux and Peltier 1995). This is probably because chloroplasts are essential for energy production, their normal function is especially important during the period of stress. In the meantime, the photosynthetic system is the major source of free radicals in plant cells, extra protection can lower its damage and allow a faster recovery.

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