Effect of High Temperature on Photosynthesis in Beans

II. CO₂ Assimilation and Metabolite Contents

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The effect of high temperatures on CO₂ assimilation, metabolite content, and capacity for reducing power production in non-photorespiratory conditions has been assessed in two different bean (Phaseolus vulgaris L.) varieties, Blue Lake (commercially available in the United Kingdom) and Barbucho (a noncommercially bred Chilean variety), which are known to differ in their resistance to extreme high temperatures. Barbucho maintains its photosynthetic functions for a longer period of time under extreme heat compared with Blue Lake. The CO₂ assimilation rate was increased by increases in temperature, with a decrease in ratio of rates at temperatures differing by 10°C. It is suggested that limitations to CO₂ assimilation are caused by metabolic restrictions that can be differentiated between those occurring in the range of 20 to 30°C and 30 to 35°C. It is likely that changes in the capacity for Calvin cycle regeneration and starch synthesis affect photosynthesis in the range of 20 to 30°C. But following an increase in temperature from 30 to 35°C, the supply of reducing power becomes limiting. From analysis of adenylate concentration, transthylakoid energization, and, indirectly, NADPH/NADP⁺ ratio, it was concluded that the limitation in the assimilatory power was due to an oxidation of the NADPH/NADP⁺ pool. In the range of 30 to 35°C, the photosystem I quantum yield increased and photosystem II maintained its value. We conclude that the reorganization of thylakoids observed at 30 to 35°C increased the excitation of photosystem I, inducing an increase in cyclic electron transport and a decrease in the supply of NADPH, limiting carbon assimilation.

High temperatures affect photosynthesis by altering the excitation energy distribution by changing the structure of thylakoids (Berry and Bjorkman, 1980; Weis and Berry, 1988) and by changing the activity of the Calvin cycle and other metabolic processes such as photorespiration and product synthesis. Much of the attention has been focused on the former aspect, since thylakoids are highly sensitive to heat, whereas the restrictions imposed by high temperatures on carbon metabolism have been nearly exclusively interpreted as the effect on CO₂ availability. The diffusion of CO₂ and O₂ and the affinity for carboxylation of the Rubisco enzyme have been proven to be affected by increasing temperatures (Hall and Keys, 1983; Jordan and Ögren, 1984; Brooks and Farquhar, 1985), but it has been argued that the stimulation of photorespiration cannot fully explain the effect of high temperature. It has been suggested that a restricted electron transport may limit RuBP supply (Berry and Bjorkman, 1980).

It also has to be considered that changes in temperature induce variations in the activity of enzymes, and that because of their different Q₁₀ values (Pollock and Rees, 1975) such variations are not the same for all of the enzymes acting in a particular pathway. In photosynthesis, particularly in the reactions directly involved in CO₂ assimilation and Suc and starch synthesis, such a phenomenon is expected and there is evidence that changes in the properties of enzymes such as cytosolic FBPase and Suc phosphate synthase (Stitt and Grosse, 1988) caused by changes in temperature contribute to the regulation of metabolism.

We studied the effect of temperature increases on photosynthesis in two bean varieties: BL, which is commercially available in the United Kingdom, and BA, a Chilean, noncommercially bred variety. The two varieties are known to differ in their resistance to extreme high temperatures; BA maintains its photosynthetic functions for a longer period of time than BL in conditions of extreme heat (C. Pastenes and P. Horton, unpublished data). In the previous paper it was shown that increases in temperature expose a restriction in photosynthesis above 30°C in the two bean varieties, which is accompanied by changes in thylakoid properties. In this paper, the alterations in carbon metabolism are explored and it is concluded that metabolic changes are at least in part a consequence of altered thylakoid function.

Abbreviations: BA, variety Barbucho; BL, variety Blue Lake; Chl, chlorophyll; FBP, Fru-1,6-bisP; FBPase, Fru-1,6-bisphosphatase; F6P, Fru-6-P; G6P, Glc-6-P; MDH, malate dehydrogenase; PGA, glycerate-3-phosphate; Ψ₉₀, quantum yield of PSI; Ψ₁₀₀, quantum yield of PSII; Fₚₒₒ, PSI reaction center; qₑ, energy-dependent nonphotochemical quenching; qN, nonphotochemical quenching; Q₁₀, ratio of rates at temperatures differing by 10°C; RuBP, ribulose-1,5-bisphosphate; TP, triose phosphate.
MATERIALS AND METHODS

Bean plants (Phaseolus vulgaris L.) var BL and BA were grown as described in the preceding paper (Pastenes and Horton, 1996).

Gas Exchange and Freezing of Leaf Discs

CO₂ assimilation and transpiration were measured with CO₂ and water vapor gas analyzers, respectively (both model 225 mark 3, ADC, Hertshire, UK), mounted in series in an open system as described by Harris et al. (1983). Leaf discs were obtained from heated attached leaves as described before (Pastenes and Horton, 1996), cut just before the measurements, and placed in a square aluminum chamber with temperature control. The leaf discs were placed in the chamber with their borders immersed in a water-filled depression to avoid dehydration. They were illuminated with a cold lamp (model 1500, Schott KL, Walz, Effeltrich, Germany) through two fiberoptics at 45° opposite each other. The light intensity at the leaf surface was set to 850 μmol m⁻² s⁻¹. Air jets of 1000 μmol mol⁻¹ CO₂ and 2% O₂ in N₂ were directed to both leaf surfaces and after 40 min, when steady-state photosynthesis was reached, the leaf discs were freeze-clamped, which cooled the leaf below 0°C in less than 0.1 s, as described by Doncaster et al. (1989).

Metabolite and Enzyme Determination

Frozen leaf discs were divided into halves for the measurement of metabolites and NADPH-MDH and kept in aluminum foil envelopes at −70°C until processed. Separate discs were taken for adenylate determination. Each leaf disc was taken from a different plant.

Extraction was carried out in HClO₄, which has been shown to result in high recovery of metabolites (Leegood and Furbank, 1984). Metabolite determination was carried out as described by Lowry and Passonneau (1972) and Leegood (1993), measuring absorbance changes at 340 nm minus 400 nm using a dual-wavelength spectrophotometer (model DW-2000, SLM, Rochester, NY). Chl was estimated by measuring the pheophytin content of the initial pellet reserved from the leaf extraction (Vernon, 1960). For adenylate determination, no charcoal was added to the samples. NADPH-MDH activity was spectrophotometrically assayed as described by Ashton et al. (1990). NADP-MDH was fully activated by incubating the samples with 35 mmol of DTT under an atmosphere of N₂ at room temperature for 45 min.

535-nm Absorbance Changes

Measurements of 535-nm absorbance change and Chl fluorescence were carried out simultaneously in a dual-wavelength spectrophotometer (model DW-2000, SLM) and a fluorometer (Walz) on leaf pieces inserted at 45°C to the spectrophotometer light path, positioned in a glass cuvette (Ruban et al., 1993). The cuvette was filled with sufficient NaHCO₃/Na₂CO₃ buffer, pH 9, to cover the base of the leaf, and partially sealed with Parafilm (American Can, Greenwich, CT) to maintain high CO₂ and humidity.

Quantum Efficiency of PSII and PSI

The simultaneous measurement of PSII and PSI quantum yield was carried out according to the method described by Schreiber et al. (1988) using two fluorometers (both PAM 101, Walz) to record fluorescence emission and P₇₀₀ absorbance (830 nm). Measurements were made in leaves enclosed in a chamber with temperature regulation. Air containing 1000 μmol mol⁻¹ CO₂ and 2% O₂ in N₂ was passed through the chamber over both leaf surfaces. The PSII and PSI quantum yield were calculated after 20 to 25 min of illumination (steady-state photosynthesis) and recorded with a two-channel recorder. Actinic light was 800 μmol m⁻² s⁻¹ and high-intensity pulse light was 1300 μmol m⁻² s⁻¹. The maximum fluorescence and P₇₀₀ oxidation levels were recorded during the high-light pulse. One second after the pulse, a 3-s dark interval was given to allow the signal corresponding to the fully reduced level of P₇₀₀ to be recorded. One leaf was used for each individual measurement and at least four replications were carried out for each temperature.

RESULTS

Gas Exchange

The CO₂ assimilation rate measured in BA and BL (Fig. 1) increased when the temperature was raised from 20 to 35°C. The largest increase was observed from 20 to 25°C (Q₁₀ ≈ 1.96), with a smaller increase from 25 to 30°C (Q₁₀ ≈ 1.22), and an even smaller increase from 30 to 35°C (Q₁₀ ≈ 1.08); these results are the same as those recorded for O₂ evolution (Pastenes and Horton, 1996).

The transpiration rate was increased four times, from 1.8 to about 8 mmol H₂O m⁻² s⁻¹ (Fig. 1), nearly proportional to the increase in temperature from 20 to 35°C. Based on the results of calculations done according to the method of von Caemmerer and Farquhar (1981), the internal concentration of CO₂ was maintained nearly constant at about 950 μmol mol⁻¹, with an increasing conductance for CO₂ (data not shown). These parameters suggest that no limitation to photosynthesis occurs at high temperatures due to changes in resistance to CO₂; on the contrary, the internal concentration of CO₂ is maintained nearly constant throughout the thermal regime imposed on the leaves. This relationship between temperature and CO₂ conductance has not always been reported as being positively correlated (Mukohata et al., 1971), mainly because the thermal effect on stomatal conductance depends on the water status of the leaf compared with the air. Therefore, if the vapor pressure difference is allowed to increase with temperature, the stomatal conductance will become limiting to photosynthesis, mostly in C₃ plants in normal air (Monson et al., 1982). Obviously, only a significant negative effect of temperature on stomatal conductance would have been limiting to photosynthesis under the experimental conditions used, since the atmosphere was enriched in CO₂.

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Figure 1. CO₂ assimilation (A) and transpiration (B) rates upon increases in temperature from 20 to 35°C in BL (●) and BA (○). Each point represents the average and SE of at least eight samples.

to a level saturating for photosynthesis, accompanied by a low O₂ concentration.

Metabolite Content

The concentration of the major hexose phosphate, G6P, decreased with the increase in temperature from 20 to 35°C in both varieties, from 200 to 110 nmol mg⁻¹ Chl in BL, and from 170 to 110 nmol mg⁻¹ Chl in BA (Fig. 2A). It is important to note that even though there appears to be a difference in shape of the temperature response between the varieties, the important feature is the consistent decrease in the hexose phosphate concentration with temperature increase. Similarly, a decrease in the F6P content (Fig. 2A) from 120 to 80 nmol mg⁻¹ Chl in BL and from 95 to 55 nmol mg⁻¹ Chl in BA was observed between 20 and 35°C.

PGA, the first product after CO₂ incorporation into the Calvin cycle, appears to maintain its concentration with increasing leaf temperature in both varieties (Fig. 2B). In the TPs (i.e. glyceraldehyde phosphate and dihydroxyacetone phosphate) the changes are less clear between 20 and 30°C, but as temperature was increased to 35°C, a sharp decrease in the concentration was observed (Fig. 2C). FBP, on the other hand, showed a strong and continuous decline in both varieties (Fig. 2D), from about 31 nmol mg⁻¹ Chl at 20°C to about 10 nmol mg⁻¹ Chl at 35°C. RuBP decreased when temperature was increased from 30 to 35°C in BA, but in BL its concentration changed less markedly, resembling the changes observed in TP (Fig. 2E).

The relative changes in level of each metabolite depended on the temperature range examined and in some cases were insensitive to changes in temperature, and CO₂ assimilation increased. Such changes in metabolite pools upon increases in temperature have been interpreted as resulting from specific temperature-dependent regulation of the enzymes of the Calvin cycle and of starch or Suc synthesis (Leegood, 1993). This regulation can be investigated by examining ratios between certain metabolites.

The G6P/F6P ratio has been reported to be three times higher in the cytosol than in the stroma (Dietz and Heber, 1984; Gerhardt et al., 1987). G6P/F6P calculated from the metabolite data (Fig. 2F) was markedly decreased in BL;
the value was nearly 75% lower at 35°C than at 20°C. In BA, however, this ratio appeared to be constant from 20 to 35°C, although there is an indication of a decrease at 30°C. The decline in the G6P/F6P ratio in BL suggests that there was a trend toward stromal compartmentation of the hexose phosphates induced by temperature (Gerhardt et al., 1987), suggesting a possible limitation in the pathway to starch synthesis. The difference in the extent to which the ratio declined between the varieties could be due to the fact that Suc and starch accumulate in different proportions, depending on the time the plant has been exposed to light (Gerhardt et al., 1987; Neuhaus and Stitt, 1990). However, the leaves were always darkened for at least 1 h and taken randomly from the growth room during the day.

A well-known effect of temperature on photosynthesis is the increase of photospiration due to a change in gas solubility, affecting the actual concentration of CO₂ reaching the acceptor compared with O₂ (Ku and Edwards, 1977; Hall and Keys, 1983; Jordan and Ögren, 1984), but also because of a decline in the CO₂/O₂ specificity of Rubisco, as demonstrated by in vitro and in vivo experiments (Jordan and Ögren, 1984; Brooks and Farquhar, 1985). Even though high CO₂ concentration greatly reduced the probability for photospiration, changes in Rubisco activity could still affect the rate of CO₂ assimilation at a given temperature. However, the data shown in Figure 2G suggest that any change in Rubisco activity did not limit photosynthesis in either variety; on the contrary, the observed decrease in the RuBP/PGA ratio is evidence of an increase in the flux through Rubisco.

The regeneration sequence in the Calvin cycle comprises the metabolic reactions in the stroma, from TP to RuBP. Hence, the ratio TP/RuBP is an indicator of the capacity of the cycle to regenerate the RuBP (Neuhaus and Stitt, 1989), and as shown in Figure 2H, this ratio tended to increase in both varieties when temperature was increased from 25 to 30°C. This suggests that a possible limitation in photosynthesis appears in the regeneration phase of the Calvin cycle, simultaneously with the threshold at which the Q₁₀ for CO₂ assimilation rate declines. The TP/RuBP ratio reached the highest level at 30°C, and declined markedly at 35°C, suggesting a new constraint in the assimilation rate when the temperature was raised to 35°C. The ratio FBP/F6P (Fig. 2I) was maintained nearly constant in both bean varieties when temperature was increased from 20 to 25°C, and decreased from 25 to 35°C. These data suggest that the FBPase-catalyzed reaction does not limit the response of photosynthesis to temperature.

The two-step reaction for the reduction of CO₂ is driven by the ATP and NADPH produced by the electron transport in thylakoids. Therefore, the PGA/TP ratio serves to estimate the assimilatory power (Heber et al., 1986), the product between the redox ratio (NADPH/NADP), and the phosphorylation potential (ATP/ADP). The stromal pH must also be known, but it has been determined to saturate at rather low light intensities, varying between 7.5 and 8.2 (Heldt et al., 1973).

The PGA/TP ratio in both bean varieties (Fig. 2J) was approximately constant between 20 and 30°C, but increased dramatically when the leaf temperature was increased from 30 to 35°C, revealing a significant restriction of ATP and/or NADPH supply. The possibility that a restriction on ATP supply was responsible for the effect of high temperature on photosynthesis was tested directly by measuring the adenylate content of freeze-clamped leaf discs. The ATP/ADP ratio decreased approximately 15% in both varieties when the temperature increased from 20 to 30°C (Fig. 3), and was maintained as temperature was further increased from 30 to 35°C. The ATP/ADP ratio coincides with the lowest value reported in vivo experiments (Dietz and Heber, 1984; Dietz and Heber, 1986; Neuhaus and Stitt, 1989; Rao et al., 1990), and this ratio is known to be low in conditions of increasing light and CO₂, as used here (Dietz and Heber, 1984; Dietz and Heber, 1986). The slight decrease in the ATP/ADP ratio is accompanied by a stimulation in CO₂ assimilation and is likely to be the effect of more efficient catalytic reactions in the Calvin cycle (Heber et al., 1986). Rather than a failure in ATP synthesis, there is an increase in the rate of ATP consumption with the concomitant relative increase in ADP.

535-nm Absorbance

Light scattering at 535 nm has been chosen as an indirect indication of the energization capacity of thylakoids, since such a signal has been related to the formation of qE by increasing the pH in the chloroplasts (Krause, 1973; Ruban et al., 1992, 1993). The extent of the difference between the light/dark steady-state absorbance change upon illumination decreased with the increase in temperature from 20 to 30°C (data not shown). This is consistent with the decrease in qN over this temperature range (Pastenes and Horton, 1996). As temperature was increased from 30 to 35°C, the extent of the difference between steady-state light/dark levels was maintained or slightly increased, suggesting that at 35°C thylakoids are able to accumulate protons in the lumen at least up to a level sufficient to cause A535 comparable to that observed at 30°C (data not shown).

Figure 3. Effect of increasing leaf temperature on ATP/ADP ratio in BL (○) and BA (●). Each point represents the average and se of at least four samples.
NADP-MDH Activity

The reversible light-dark activation-inactivation of the enzyme NADP-MDH is strongly influenced by the NADPH/NADP ratio; a high ratio favors a more active enzyme (Scheibe and Jacquot, 1983; Ashton et al., 1990). Figure 4 shows that the in vivo activity of NADP-MDH expressed as a percentage of the total activity measured is temperature-insensitive in the range of 20 to 30°C, with an average value above 30%. This is similar to results previously reported by Holaday et al., (1992) in bean leaves at 27°C. However, at 35°C the activation state decreased to lower than 20% in both varieties, indicating a decrease in the NADPH/NADP ratio.

Quantum Efficiencies of PSII and PSI

As expected, $\Phi_{\text{PSII}}$ increased with temperature in both varieties, reaching a maximal value at 30°C and maintained at 35°C (Fig. 5). Similar data were obtained when $\Phi_{\text{PSII}}$ was measured simultaneously with $O_2$ evolution (Pastenes and Horton, 1996). $\Phi_{\text{PSII}}$ follows a similar pattern, but there is a continued increase in this parameter from 30 to 35°C, translating into a decrease in the ratio $\Phi_{\text{PSII}}/\Phi_{\text{PSI}}$ over this thermal range. A nonlinear relationship between $\Phi_{\text{PSII}}$ and $\Phi_{\text{PSI}}$ has been reported when an imbalance is created between the capacity of electron transport and the sink for reducing equivalents in the stroma (Harbinson et al., 1990a, 1990b). Under such circumstances, this is interpreted as the occurrence of photosynthetic control over electron transport involving cyclic electron flux through PSI (Harbinson et al., 1990b). These observations, however, were later suggested to be an artifact, due to the incapacity of the method to detect those PSI centers that are reduced and incapable of being oxidized due to acceptor-side limitations. This deviation is likely to occur in conditions of decreasing CO$_2$ under constant light intensity and during photosynthetic induction (Klughammer and Schreiber, 1994). A similar deviation from linearity is observed in Figure 5 when temperature was increased from 30 to 35°C in both bean varieties. $\Phi_{\text{PSII}}$ tends to continue increasing, compared with $\Phi_{\text{PSII}}$, which seems to maintain a constant value. To make sure that such a response was not due to an incapacity of the light pulse for oxidizing P$_{700}$ centers the maximal signal amplitude corresponding to the absorbance difference between the dark-adapted state (all P$_{700}$ in a reduced state) and the high-intensity light pulse after far-red light (all P$_{700}$ in an oxidized state) was measured according to Klughammer and Schreiber (1994) at 30 and 35°C. This amplitude was not different from that obtained in steady-state photosynthesis, according to the procedure described in “Materials and Methods” (data not shown). More specifically, no further increase in the signal was recorded when applying the light pulse after far-red light, illumination, or at steady state, suggesting that no acceptor-side limitation is significant at high temperatures. Such a limitation is in fact not expected from the NADP-MDH data that indicated decreased NADPH/NADP with temperature increases.

DISCUSSION

The rate of increase in CO$_2$ assimilation with increasing temperatures progressively decreases until reaching a level beyond which only a small increase in gas exchange is observed. The maximum change in rate is observed in the range of 20 to 25°C, reaching nearly a maximum assimilation rate at 30°C. A further temperature increase to 35°C results in only a minor change in the gas-exchange rate.

It has been reported previously that temperature limits CO$_2$ availability, either because of physiological responses of leaves, which results in increases in resistance to the gas diffusion (Mukohata et al., 1971; Monson et al., 1982), or because of the relative solubility of CO$_2$ and O$_2$ which compete for the same acceptor (Ku and Edwards, 1977; Hall and Keys, 1983; Jordan and Ogren, 1984). It has also been reported that heat alters the functioning of Rubisco by varying the substrate specificity of the enzyme (Jordan and
Ogren, 1984; Brooks and Farquhar, 1985) and its activity (Weis, 1981; Santarius et al., 1991). It is clear from the data presented here, however, that the restrictions in CO₂ assimilation are not linked to the availability of CO₂ and, very importantly, are not related to the activity of Rubisco (Fig. 2). That Rubisco activity did not limit photosynthesis, however, does not mean that its activity was not affected. For example, Kobza and Edwards (1987) found that mild increases in temperature resulted in reduced Rubisco activity without limiting the rate of photosynthesis, and maintained a low RuBP/PGA ratio.

The observed response of CO₂ assimilation to temperature seems to be related to the capacity for final product synthesis and to the capacity of the Calvin cycle to regenerate RuBP, as well as to the failure of the system to maintain a necessary assimilatory power. These restrictions became evident at different temperatures, as shown by the different temperature responses of various metabolites. In general, the changes occurring from 20 to 30°C can be distinguished from those observed from 30 to 35°C, although some changes were continuous throughout the temperature range.

The activity of Suc phosphate synthase and cytosolic FBPase do not appear to restrict Suc synthesis, since there was no accumulation of hexose phosphates (Fig. 2A). In fact, studies with partially purified Suc phosphate synthase have shown that this enzyme has a relatively high Q₁₀ and that the cytosolic FBPase becomes less sensitive to inhibition by Fru-2,6-bisphosphate and AMP (Stitt and Grosse, 1988). The decrease in the hexose phosphate level indicates that Suc synthesis is favored by high temperatures (Stitt and Grosse, 1988).

G6P and F6P are present in the chloroplast and cytosol within the pathways for starch and Suc formation, respectively, and are interconverted by the enzyme phosphoglucomerase isomerase. This reaction is in equilibrium in the cytosol, but displaced from equilibrium in the stroma toward G6P, where it has been proposed to have a rate-limiting function in starch synthesis (Dietz, 1985; Gerhardt et al., 1987). The stromal compartmentation of G6P and F6P in BL, demonstrated by the decrease in G6P/F6P ratio (Fig. 2F), suggests that starch synthesis does not match the increase in the capacity for Suc synthesis as the temperature increases from 20 to 30°C.

In the regeneration phase of the Calvin cycle, the carbon flux is mainly controlled by stromal FBPase, sedoheptulose-1,7-bisphosphatase, and ribulose-5-phosphate kinase (Buchanan, 1980). The increase in the TP/RuBP ratio (Fig. 2H) suggests that a possible limitation is induced in the regeneration pathway by temperature when it is increased from 25 to 30°C. If a limitation does exist in this pathway, it is unlikely that it is at the stromal FBPase, since the ratio FBP/F6P (Fig. 2I) indicates that the FBPase-catalyzed reaction is favored by increases in temperature, particularly in the range of 25 to 30°C. In addition, there is no accumulation of FBP.

Beyond 30°C the assimilation of CO₂ does not respond to temperature and a new limitation to photosynthesis appears, this time related to the capacity to reduce PGA to TPs (Fig. 2J). The increase in the ratio of PGA/TP means that high temperature affects the capacity to generate the assimilatory power, i.e. the synthesis of ATP and NADPH needed to sustain further increases in CO₂ assimilation. It is unlikely that such a restriction originated from a decrease in the Pi pool in the chloroplast, since no hexose accumulation was observed in the current study. On the contrary, hexose tended to decrease (Fig. 2A). Therefore, the restriction in the photosynthetic process at 30 to 35°C resulted from a more subtle change in thylakoid function.

The capacity for phosphorylation in chloroplasts is not negatively affected by heat and, more specifically, the transition from 30 to 35°C does not show any substantial decrease in either the 535-nm absorbance change (data not shown) or in the ATP/ADP ratio (Fig. 3). This suggests that protons accumulate in the chloroplast lumen and also that membrane-bound ATPases respond to that energization according to substrate availability. On the contrary, the NADPH/NADP ratio, indirectly measured through the activation state of NADP-MDH, decreases during the transition from 30 to 35°C. This effect is accompanied by an imbalance between the quantum yields of PSI and PSII (Figs. 4 and 5), suggesting that it is the supply of NADPH that limits carbon assimilation.

The limitation of NADPH supply could arise from inactivation of electron transport, perhaps at PSI. However, this would lead also to an unaltered ratio of PSII/PSI efficiency. Alternatively, the restriction in supply of NADPH could arise from the changes in thylakoid function and organization that occur at this temperature range (Pastenes and Horton, 1996). The transition to state 2 and the increase in PSI could limit the supply of electrons from PSII while stimulating PSI excitation. Although this situation tends to increased P₇₀₀ oxidation, an increase in P₇₀₀ reduction without a concomitant increase in PSI efficiency could be explained by an increase in the rate of cyclic electron flow around PSI. This cyclic flow could be stimulated by the lateral redistribution of Cyt b₅₆₅ (Vallon et al., 1991).

The putative increase in PSI cyclic electron flow could have a physiological role. Even though the ATP/ADP does not change, an increase in permeability of the thylakoids due to high temperatures (Emmett and Walker, 1973; Santarius et al., 1991) could still occur if cyclic electron transport is simultaneously enhanced, hence counteracting proton leakage. This would be consistent with the limiting factor in PGA reduction being NADPH. If this is the case, the structural changes leading to a favoring reduction of the Cyt b₅ complex, like those associated with state transitions, would help to prevent a decrease in ATP, but at the expense of NADPH. Therefore, such structural changes to thylakoids caused by increases in temperature, namely state transitions and PSI centers, would be significant in enabling plants to carry out photosynthesis in conditions of proton leakage. This is perhaps in addition to a role in the prevention of PSII from overexcitation in conditions of high light intensities associated with high temperatures in the field (Havaux et al., 1991).
Differences between the varieties were observed in the various metabolite ratios (e.g., G6P/F6P) and suggest some variation in the temperature response of carbohydrate metabolism. Similarly, differences in thylakoid function were apparent from the difference in qN profiles (Pastenes and Horton, 1996). However, neither of these are strongly reflected in the steady-state rate of photosynthesis, which responded similarly to temperature in BL and BA.

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LITERATURE CITED


