

BRIEF COMMUNICATION

Thermal sensitivity of the pool size of electrons available to P700⁺ reduction in intact maize leaves

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Abstract

The relative size of the pool of electrons accumulated in stroma reductants during actinic irradiation, which can be donated to P700⁺ via the intersystem chain, was estimated after short-term exposure of intact *Zea mays* leaves to elevated temperatures. When the temperature increased from 25 to 50 °C by 5 °C steps, the relative size of the stroma electron pool went through a maximum at around 30 °C, and decreased gradually thereafter.

Additional key words: heat stress; stroma reductants; *Zea mays*.

In eukaryotic photosynthetic organisms, the stroma matrix of chloroplasts contains a large number of redox reactants, among which Fd_{ox}/Fd_{red} and NADP⁺/NADPH function as the central molecules for distributing electrons and juncture enzymatically numerous redox processes. Fd, an electron carrier shared by both linear and cyclic electron transport chains and dissociating in stroma matrix, also interacts with a number of Fd-dependent enzymes such as ferredoxin-NADP⁺ oxidoreductase, nitrite reductase, glutamate synthase, sulfite reductase, Fd-thiorodoxin reductase, etc. NADPH, as the end product of linear photosynthetic electron transport in thylakoid membranes and the reducing power for carbon assimilation, is also involved in many other redox reactions in stroma such as the scavenging of superoxide and hydrogen peroxide (Więckowski and Bojko 1997, Asada *et al.* 1998). In C₄ plants, the largest sink of stromal NADPH is the reduction of PGA as well as C₄ acid, where triose phosphates account for a large portion of stroma reductants (Stitt and Heldt 1985). These reductants in stroma constitute together an electron pool from which, under certain conditions, electrons can be donated to the intersystem chain (Leegood *et al.* 1983, Aristarkhov *et al.* 1987), and through it, to P700⁺. In

comparison with the abundance of literature concerning heat-induced structural and functional changes in photosynthetic apparatuses in thylakoid membranes and the soluble enzymes associated with CO₂-fixation, investigations on the thermal sensitivity of the stroma electron pool are rare. We demonstrate temperature-dependence during actinic irradiation of the relative size of the pool of electrons accumulated in stroma reductants that can be donated in intact maize leaves to P700⁺ via the intersystem chain. We interpret this in terms of the thermal sensitivity of photosynthetic linear electron transport and carbon metabolism.

Plants of maize (*Zea mays* L.) were grown in Hoagland solution on a windowsill at temperatures ranging from 14 to 26 °C. The third fully unfolded leaves attached to seedlings were used for measurements. The relative size of the pool of stroma electrons that can be donated to P700⁺ via the intersystem chain was estimated in intact leaves by measuring the redox change in P700 with actinic and 50-ms multiple-turnover irradiations under a background of far-red radiation (Fig. 1), as initially described by Asada *et al.* (1992). Redox state of P700 was monitored by absorbance difference between 810 and 830 nm, using a dual wavelength emitter detector

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Abbreviations: AR – actinic radiation; Fd, Fd_{ox}, and Fd_{red} – ferredoxin, oxidised ferredoxin, and reduced ferredoxin, respectively; FR – far-red radiation; PGA – 3-phosphoglycerate; PS – photosystem; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.

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unit *ED-P700DW-E* combined with *PAM-101* (Walz, Effelrich, Germany), as described previously (Jin *et al.* 2001). The complementary area between the stationary level of $P700^+$ attained by far-red irradiation, the oxidation curve of $P700$ after a 50-ms multiple-turnover light (S_{MT}), and that between the stationary $P700^+$ level and the oxidation curve of $P700$ after actinic irradiation (S_{AL}) were calculated by integration of digitised outputs using home-designed software. They represent the amount of electrons in the intersystem chain and the stroma reductants, respectively. The ratio of S_{AL} to S_{MT} is taken as a measure of the relative size (in terms of molar ratio relative to that in the intersystem chain) of the pool of electrons accumulated in stroma reductants during actinic irradiation that can be donated to $P700^+$ via the intersystem chain (Asada *et al.* 1992, 1993, Mi *et al.* 1994). The far-red radiation (>720 nm, $7.3 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$) was provided by a *GR720* filter (Schott, Mainz, Germany). The saturating 50-ms multiple-turnover radiation [$660 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] and actinic radiation [$250 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] were provided by a LED-array cone with peak emission at 655 nm (*High-Power-LED-Lamp*, Walz) (Mi *et al.* 2000). The abaxial sides of intact attached leaves were pressed on a thermostatted glass platform covered by a piece of black cotton cloth, and the adaxial sides of the leaves were placed facing the detector encircled by a piece of plastic foam, as described in Jin *et al.* (2001).

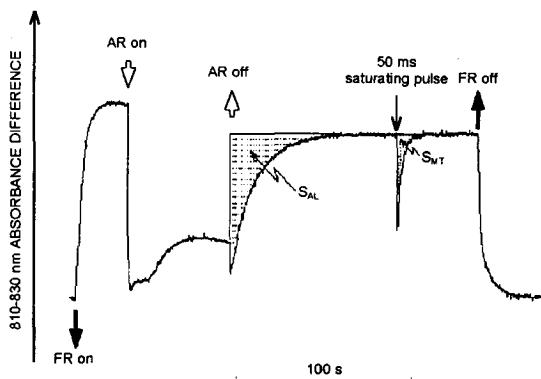


Fig. 1. A cycle of irradiation of an intact maize leaf for determination of the relative size of the pool of stromal electrons that can be donated to $P700^+$ via the intersystem chain after actinic irradiation. The leaf had been dark-adapted for 2 h at room temperature (23°C) and kept at 30°C for 20 min before measurement, which was carried out at the same temperature. Irradiances by FR (>720 nm), red actinic radiation (AR, with a peak at 655 nm), and the 50 ms saturating pulse (MT, with a peak at 655 nm) were 7.3 , 250 , and $660 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, respectively. The complementary areas between the stationary level of $P700^+$ and the oxidation curves of $P700$ under the background FR after the irradiation with AR and MT are referred to as S_{AL} and S_{MT} , respectively. The relative size of the stroma electron pool is represented as the ratio of S_{AL} to S_{MT} .

Fig. 2 shows the effects of 20 min exposure of intact attached maize leaves to elevated temperatures on the relative size of the pool of electrons available to $P700^+$ reduction. The value increased with the temperature rise from 25 to 30°C , and declined gradually beyond 30°C . Although this behaviour is in general agreement with the temperature-dependence manners of both the capacity and the quantum yield of CO_2 assimilation in most plants (Berry and Björkman 1980), the optical temperature of photosynthesis in maize is always beyond 30°C .

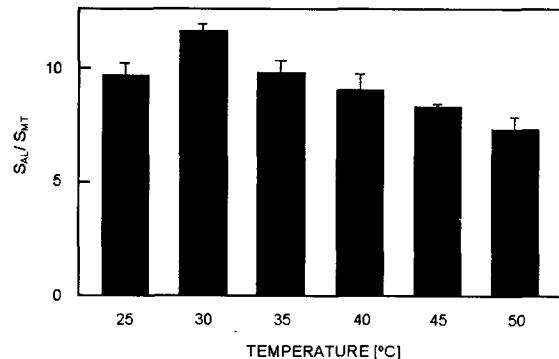


Fig. 2. Temperature-dependence of the relative size of the pool of electrons in stroma reductants in intact maize leaves that can be donated to $P700^+$ via the intersystem chain after actinic irradiation. The relative size of the stroma electron pool was estimated by the ratio of S_{AL} to S_{MT} . The leaves had been dark-adapted for 2 h at room temperature (23°C) and kept at indicated temperatures for 20 min before measurement, which was carried out at the same temperature. Means of three replicates, with standard errors.

Despite the fact that the light-driven NADPH production and the operation of the Calvin cycle are spatially separated in C_4 plants, chloroplasts in mesophyll cells are still important locations for PGA reduction, wherein one half of the PGA are reduced into triose phosphates by NADPH (Stitt and Heldt 1985). The intercellular diffusion of PGA and triose phosphates is maintained by high concentration gradient between the bundle sheath and mesophyll, and the triose phosphates in maize leaves are located mainly in mesophyll rather than bundle sheath during photosynthesis (Stitt and Heldt 1985, Leegood and Osmond 1990). Thermal enhancement of the CO_2 assimilation rate and the increase in pool size of triose phosphates in the range of 5 - 30°C has been reported in maize leaves (Labate *et al.* 1990). The mild heating-induced increase in the relative size of the stroma electron pool shown in Fig. 2 can be attributed to the accumulation of triose phosphates, the potential candidates that may donate electrons to $P700^+$.

Water-splitting complexes in thylakoid membranes are very heat-susceptible (Katoh and San Pietro 1967, Yamashita and Butler 1968, Santarius 1975, Havaux *et al.* 1991). Heat-induced inactivation of the water-splitting

complexes may suppress linear electron transport and reduce the supply of Fd_{red} and NADPH. Recently, some investigations (Law and Crafts-Brandner 1999, Crafts-Brandner and Salvucci 2000, Salvucci *et al.* 2001) on C_3 plants suggest the exceptionally high sensitivity of RuBPCO activase to thermal de-naturation, which, under high temperatures, causes inactivation of RuBPCO and a decrease in PGA. This mechanism, if existing in maize, may also cause a heat-depression of the stroma electron pool. Since triose-phosphates constitute the bulk of stroma reductants in maize leaves (Stitt and Heldt 1985), the decline in the supply of PGA, the precursor of triose-phosphates, may also lead to a diminishing of the size of

electron pool in stroma. Thus, a decline beyond 30 °C shown in Fig. 2 might be attributed to the thermal inhibition of both linear electron transport and RuBPCO activity.

In summary, the integration of the thermal enhancement of photosynthetic carbon metabolism under mild heating and the inactivation of linear electron transport with increasing temperature might result in the temperature-dependence of the relative size of stromal electron pool that goes through a maximum at around 30 °C. Due to the complexity of composition of stroma reductants and their relationship with numerous metabolic processes, this explanation is tentative.

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