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Electron flux in photosystem II and photon/electron sinks in wild and domesticated watermelon plants during the progress of drought stress

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Introduction

Plants have several mechanisms to weaken oxidative stresses brought about by the excess photon energy under drought. In addition to such morphological responses to drought as curling of leaves, plants have biochemical protection mechanisms against oxidative stresses under drought. To weaken the damages driven by the excess photon energy not utilized by the photosynthetic carbon reduction (PCR) cycle, photosystem II (PSII) dissipates the energy as heat (Deming-Adams and Adams 1992). This process is driven by accumulation of protons in the thylakoid lumen and can be detected as non-photochemical quenching (NPQ). The cyclic electron transport system within PSII and the xanthophyll cycle participate in the dissipation of the excess photons with and without the involvement of the reaction center of PSII, respectively (Miyake and Yokota, 2001). In this context, the biochemical events for the excess photon energy dissipation may be referred to as "photon sink". Exposed to drought, furthermore, plants increase the activities of superoxide dismutatse (SOD) and ascorbate peroxidase (APX) (Smirnoff 1993) and the amount of antioxidants, glutathione (GSH) and α-tocopherol. These increases in the antioxidative activities contribute to lower the steady-state concentration of O_2^- and H_2O_2, both of which are produced at PSI, and to prevent the PCR enzymes and thylakoidal lipids from oxidative inactivation and peroxidation, respectively (Asada 1999).

If the photon energy utilization in the Rubisco-related reactions in the stroma, the activity of PSII and the antioxidative activities are tuned in a coupled manner under drought, the efficiency of plants for protecting themselves against the oxidative stresses will be further strengthened. For example, if a decrease in the relative quantum yield of PSII [ϕ(PSII)] by NPQ under the limitation of the turnover of the PCR cycle lowers the flux in photosynthetic electron transport, Je(PSII), the rate of photoreduction of O_2 at PSI decreases. Furthermore, if the activities of enzymes for scavenging active oxygens increase, the steady state concentration of active oxygen will be kept at a lower value. However, simultaneous analyses of Je(PSII), the rate of the utilization of electrons by Rubisco-related reactions, the rate of photoreduction of O_2, and the activities of active oxygen-scavenging enzymes have not been tried so far, but are expected to gives us important information on how plants regulate these factors not to be damaged oxidatively under drought.

This paper describes common and different responses of the wild and domesticated watermelon plants to drought stress in water deficit.

Materials and methods

Seeds of the wild species of watermelon plants (*Citrullus lanatus* sp. #101117-1 from the International Watermelon Seed Bank of the Tottori Horticultural Center) and the domesticated watermelon plants (*Citrullus lanatus* L. cv. Sanki) were grown under the standard air-equilibrated conditions in a daily cycle consisted of 35˚C and 40% relative humidity in the light at 1,000 μmol m^{-2} s^{-1} of photon flux density for 16 h/25°C and 60% relative humidity in the dark for 8 h. Drought was induced in plants by withholding irrigation without changing other growth conditions. All measurements were made using the fourth fully expanded leaves three weeks after sowing. The rates of CO_2 exchange and transpiration and of electron transport through PSII [Je(PSII)] of leaves attached to plants were determined with an open gas-exchange system and PAM, respectively, that were equipped with a temperature-controlled chamber at 30˚C, as described by Miyake and Yokota (2000). Enzymes were assayed as described in the literature. The flux of the alternative electron flow, Ja, is that of electrons that are not used by the PCR and photosynthetic carbon oxidation (PCO) cycles in the total electron flux driven by PSII can be calculated as [Je(PCII) – Je(PCR + PCO)] , where Je(PCR + PCO) is the electron flux for the PCR and PCO cycles (Miyake and Yokota 2000, 2001).

Results and discussion

Effects of drought on the stomatal aparture and the A/Ci relationship of both species of watermelon

We compared the rates of transpiration and the net CO_2 assimilation rate between both species during the progress of drought stress in order to analyze the effect of drought on their gas exchange rates. The rates of transpiration and the CO_2 assimilation of the wild species decreased similarly to those of the domesticated species. On the third day after stopping irrigation, the rates decreased to about 1% of their initial values in both species. These results indicate that the difference in the water content between both species does not affect the rate and the magnitude of the decrease in the stomata conductance and that the decrease in the net CO_2 assimilation during the progress of drought is simply due to the stomatal closure.

Effects of drought on the activities of Rubisco and chloroplast

After withholding watering, Rubisco of both species maintained its original levels of initial and total activities for initial two days; 75 and 82 μmol CO_2 m^{-2} s^{-1}, respectively, for the wild species and 55 and 63 μmol CO_2 m^{-2} s^{-1}, respectively, for the domesticated species. After then, the activities of Rubisco of both wild and domesticated species decreased to about 20% and 30%, respectively, of their original levels. The activation state of Rubisco of both species was more than 90% for the experimental period, irrespective of a strong difference in desiccation between both species. The activities of chloroplast fructose bisphosphatase (FBPase) of both species of watermelon gradually decreased from 30 to about 20 μmol FBP m^{-2} s^{-1} five days after the stop of irrigation. Chloroplast FBPase of both species was more than 80% activated under drought stress used in this study.

Effects of drought on the activities of enzymes involved in scavenging of active oxygens

The effects of drought on the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) were examined with both species of watermelon(Fig. 1A).

In the fourth leaves of wild watermelon, the activity of chloroplast APX increased by a factor of 1.6 from 160 to 250 µmol ascorbate m^{-2} s^{-1} in the initial two days (Fig. 1A). Thereafter, the activities decreased gradually to 100 µmol ascorbate m^{-2} s^{-1}. On the other hand, the activity of cytosolic APX remained constant for five days under drought stress. The activity of MDAR also increased from 190 to 240 µmol NADH m^{-2} s^{-1}, similarly to that of chloroplast APX. The overall behavior of MDAR resembled that of chloroplast APX. Contrary to chloroplast APX and MDAR, the activity of DHAR were kept constant for the initial two days after stopping irrigation and then its activity decreased from 65 to 20 µmol DHA m^{-2} s^{-1} in the subsequent three days. Furthermore, the activity of GR was constant at 45 µmol NADPH m^{-2} s^{-1} for five days. The responses of these enzyme to drought were also similar in the domesticated species (Fig. 1B).

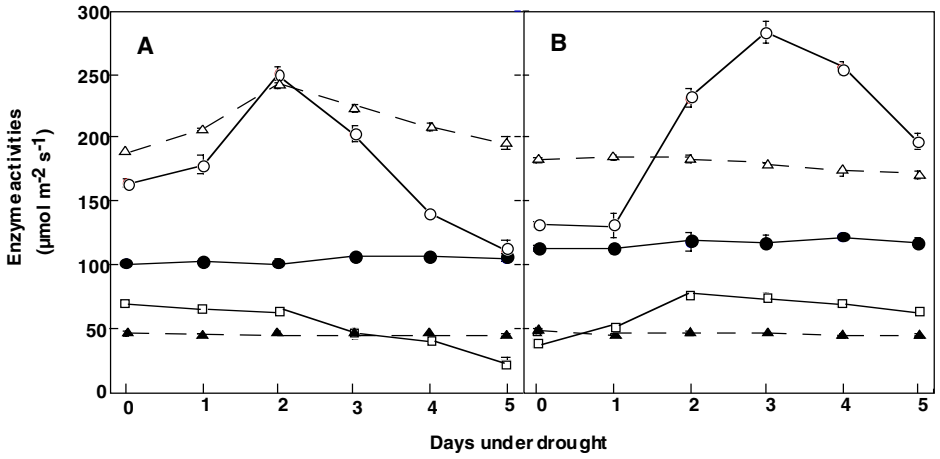

Fig. 1. Changes in activities of chloroplast and cytosolic APXs and of the enzymes involved in the regeneration of ascorbate in the wild (**A**) and domesticated species (**B**). Symbols are as follows: Chloroplast APX, open circles; cytosolic APX, closed circles; MDAR, open triangles; DHAR, open squares; GR, closed triangles. The data were the means of three independent experiments and SD is shown by vertical bar.

Responses of photochemical efficiency on PSII, Je(PSII) and Je(PCR + PCO) to drought

We examined the relationship between the rates of the release of electrons from PSII [Je(PSII)] and of the utilization of electrons by Rubisco-related reactions [Je(PCR + PCO)] at several Ci's under drought. After stopping irrigation, the responses of Je(PCR + PCO) to various Ci were almost the same in both species of watermelon stressed for one day. Although Je(PSII) decreased with decreasing Ci in both wild and domesticated watermelon, their numerical values of Je(PSII) decreased in individual ways. The values of Je(PSII) were almost equal to those of Je(PCR + PCO) and Ja almost completely disappeared in the wild species (Fig. 2A). On the other hand, Je(PSII) still largely exceeded Je(PCR + PCO) in the domesticated species, indicating the existence of Ja (Fig. 2B). This reveals that in the wild species of watermelon, alternative electron flows including photoreduction of O_2 to O_2^- at PSI of thylakoids almost disappeared in the initial step in its drought response.

Interestingly, NPQ of the wild plants stressed for one day was much higher than that of the well-watered plants, even if the leaves with and without one-day stress showed the same CO_2 assimilation rate. Furthermore, NPQ increased from 1.9 to 2.1 with decreasing CO_2 assimilation rates from 18 to 3 µmol m^{-2} s^{-1}. The drought treatment for

one more day still caused a further increase in NPQ to 2.5 to 2.6. On the contrary, the domesticated plants showed a similar level of the increase in NPQ before stress when the CO_2 assimilation rate decreased. However, further progress of the drought stress did not induce a further increase of NPQ. The observed large NPQ in the wild species may be the cause of disappearance of Ja.

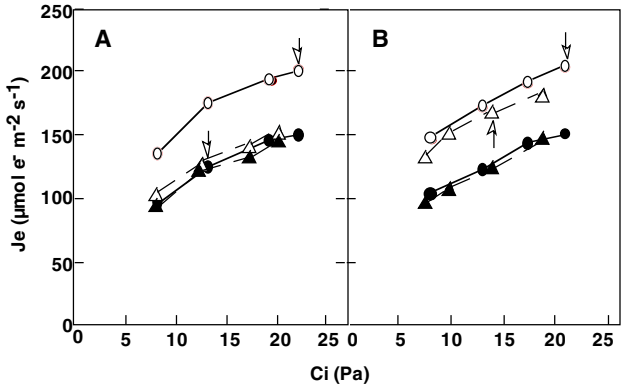

Fig. 2. Changes in the relationship between Je (PSII) and Je (PCR + PCO) of wild (**A**) and domesticated watermelon leaves (**B**) . Open and closed symbols are for Je (PSII) and Je (PCR + PCO), respectively. Circles are data points of the leaves before stress and triangles are for the leaves stressed for one day. Arrows point to the data obtained with the ordinary CO_2 concentration (36 Pa).

Thus, the responses of the leaves in wild watermelon to drought progressed consecutively as folows; closing the stomata and down-regulation of PSII to match the rate of the release of electrons from PSII to the demand for electrons by the PCR and PCO cycles, the curtailment of the electron flux in PSII and dissipation of absorbed photons as heat, the up-regulation of the activity of chloroplast APX and maintenance of high activities of ascorbate-regeneration system, and the degradation of Rubisco proteins. These progressive responses of wild watermelon to drought are quite new.

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