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Dynamic response of O_2-dependent alternative electron flow to the growth irradiance in pea leaves

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Introduction

Plants require light and CO_2 for photosynthesis controlling their growth. However, one of the two substrates light can be the threatening factor damaging the photosynthesis and suppresses their growth. For example, on exposed to the environmental stress, drought, chilling or salinity stress, plants suffer from remarkable inhibition of growth. Under these conditions where the fixation of CO_2 by photosynthesis is suppressed, the excess photonenergy against its usage by photosynthesis causes photoinhibition at PSII of thylakoids in chloroplasts and gives destructive damages to the photosynthesis (Asada 1999).

The extent to which plants suffer from photoinhibition depends on their growth conditions. For example, plants grown under high light have more tolerance against photoinhibition than those under low light. This suggests that the high light-grown plants have some mechanisms to escape from the excess photon energy. The candidates for the mechanisms contain both heat dissipation- and electron sink-systems. Non-photochemical quenching by LHCII-aggregation and xanthophylls cycle dissipates the excess photon energy (Horton and Ruban 1992). Photorespiration functions as an electron sink (Cornic and Briantais 1991, Kozaki and Takeba 1996).

Asada (1999) suggests that O_2-dependent alternative electron flow (AEF) the water-water cycle functions as an electron sink. In the leaves of wild watermelon, the activity of the water-water cycle increases at high light and/or low CO_2. Its activity reaches the activities of both photosynthesis and photorespiration. That is, the water-water cycle has a large electron flux and can be an electron sink (Miyake and Yokota 2000).

In the present work, we investigated the responses of the O_2-dependent AEF in pea to the growth irradiance. If the water-water cycle function in dissipating of the excess photon energy as an electron sink, plants grown under high light should have both high activity of O_2-dependent alternative electron flow and high activities of the enzymes consisting of the water-water cycle to back up the enhanced O_2-dependent AEF. In the followings, the results are shown and the physiological function of the water-water cycle is discussed.

Materials and Methods

Seeds of pea were sown in 500-ml paper pot containing commercial peat-based compost. Until 4^{th} leaves fully expanded, the plants were grown under the standard air-equilibrated conditions in a daily cycle consisted of 30_ and 60% relative humidity in the three irradiance-conditions [50 (LL), 400 (ML) and 1,000 (HL) μmol photon m^{-2} s^{-1}] for 16h and 25_ in the dark for 8h.

The rates of CO_2 exchange and transpiration of leaves attached to plants were determined by an open gas-exchange system that was equipped with a temperature-controlled chamber at 30_, simultaneously with the measurement of Chl fluorescence, as described by Miyake and Yokota (2000). Gas-exchange and Chl fluorescence parameters were calculated as described by von Caemmerer and Farquhar (1981) and Oxborough and Baker (1997). The electron fluxes of O_2-dependent [Ja(O_2-depend)] and O_2-independent [Ja(O_2-independ)] AEF were estimated by the method of Miyake and Yokota (2000).

Intact chloroplasts from pea were isolated as described by Asada et al. (1990). The isolated chloroplasts were subjected to osmotic shock to separate thylakoids and stroma fractions. Using the stroma, the activities of SOD, APX, MDAR, DHAR and GR were assayed and thylakoid-bound APX were also assayed using the thylakoids, and the amount of ferredoxin (Fd) and the activity of Fd-$NADP^+$ oxidoreductase (FNR) were assayed using both the thylakoids and stroma, as described by Miyake and Asada (1992).

Results

Characteristics of photosynthesis

Both the rate of net CO_2-assimilation and the total electron flux of PSII [Je(PSII)] of pea leaves at the atmospheric CO_2 and O_2 and the saturated light intensity increased as the growth irradiance increased from LL to HL. The electron flux to both photosynthesis and photorespiration [Je(Rubisco)] also increased. The magnitude of AEF, Ja, increased, where O_2-dependent AEF was main electron flux and O_2-independent AEF was negligible. The enhancement of these electron fluxes lowered the values of 1-q_p, showing the oxidation of photosynthetic electron transport system, with the increase in the growth irradiances. Furthermore, the ratio of the occupation of AEF in Je(PSII) increased. The ratio of Ja to Je(Rubisco) also increased.

The activities of the enzymes in the water-water cycle and the amount of Fd

Both the activities of chloroplastic SOD, APX, MDAR, DHAR, GR and FNR and the amount of Fd on the basis of leaf area increased with the increase in the growth irradiance. The activities of these enzymes showed the positive linear relation with the amount of Fd, and that of Fd had a positive linear correlation with Ja(O_2-depend).

Discussion

We demonstrated that the pea plants grown at high irradiance have a larger Ja, a higher activity of the water-water cycle, than the plants grown at lower irradiance do. These results support our hypothesis and suggest that the water-water cycle functions as an electron sink under the limited photosynthesis. The enhancement of the activities of the enzymes in the water-water cycle and the amount of Fd back up the physiological function of the cycle.

O_2-dependent AEF also responds to the leaf age. Young leaves have higher activity of O_2-dependent AEF, the water-water cycle, compared to older ones. High content and activity of both Fd and chloroplastic APX in young leaves support the functioning of the water-water cycle (data not shown). These results suggest that the requirement of the water-water cycle as electron sink changes in the stage of growth. We need further research for the elucidation of the physiological meaning of the water-water cycle.

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