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Role of photorespiration in dissipation of absorbed photon excess in *Spinacia oleracea* L. plants exposed to low temperature and high irradiance

N D'Ambrosio**,** C Arena, A Virzo De Santo

Dipartimento di Biologia Vegetale, Università di Napoli Federico II
Via Foria 223, 80139 Napoli, Italy
Fax: +39081450165; e- mail: dambrosi@unina.it

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Introduction

An excess of absorbed light energy by light harvesting complexes over the amount dissipated through photosynthetic electron transport is a phenomenon widely experienced by plants in nature. Several environmental constraints (high irradiance, low or high temperature, low CO_2 concentration, water limitation, nutrient availability) can determine a light absorption excess in plants. This excess overexcites the photosynthetic reaction centres and induces photoinhibition (Powles 1984; Björkman and Demming-Adams 1994). Several studies have demonstrated that photorespiration, by consuming ATP and NADPH, drives a non-assimilatory electron transport preventing photoinhibition in different environmental conditions (Wu *et al.* 1991; Osmond and Grace 1995; Kozaki and Takeba 1996; Foyer and Noctor 2000). However, the contribute of photorespiration to protect against photoinhibition is still controversial. In an alpine species it was observed that photorespiration protects from photoinhibition also at low temperature (Streb *et al.* 1998). Moreover recently, a minimal role of photorespiration to prevent photoinhibition has been proposed in cold-acclimated plants (Savitch *et al*. 2000). The aim of this work was to study the role of photorespiration in dissipation of absorbed photon excess in winter-hardened and dehardened plants of *Spinacia oleracea* L. grown outdoors.

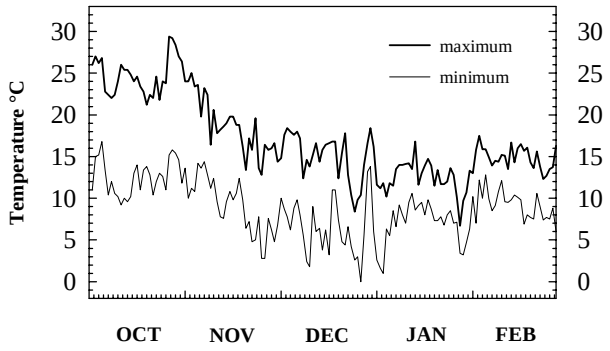

Fig. 1. Maximum and minimum air temperatures in Naples during the period October 1999-February 2000.

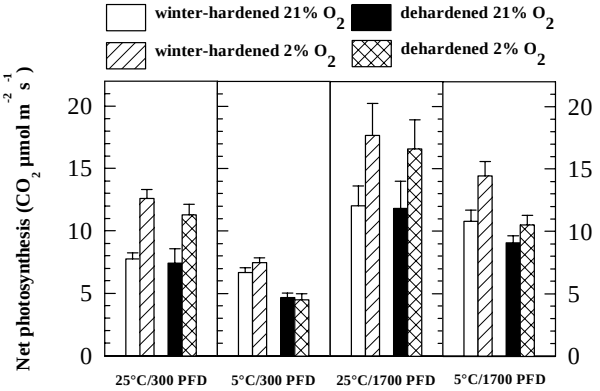

Fig. 2. Net photosynthetic rates of spinach leaves exposed to different experimental conditions. Data are the means ± SE, n=4.

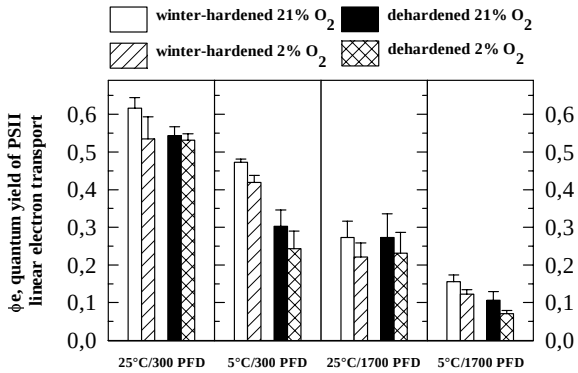

Fig. 3. Values of quantum yield of PSII linear eletcron transport in WH and DH spinach plants. Data are the means ± SE, n=4.

Materials and methods

Plants of *S. oleracea* L. were grown outdoors at the Department of Plant Biology of Naples University from October 1999 to February 2000. The daily maximum and minimum temperatures experienced by the spinach plants during this period are shown in Figure 1. In February the loss of hardening was induced by moving winter-hardened plants to a climate chamber for 20 days at controlled environmental conditions (temperature: 25/15°C day/night; PFD: 200 µmol m^{-2} s^{-1}; photoperiod: 15/9h light/dark; RH 65/85% day/night). Simultaneous measurements of gas exchange and chlorophyll *a* fluorescence emission were performed in the same plant group, before and after the induced loss of hardening. All measurements were made at photorespiratory (21% O_2) and non photorespiratory (2% O_2) conditions after exposing the spinach plants for 3h at 65% RH and different combinations of temperature and PFD (25°C/300 PFD; 5°C/300 PFD; 25°C/1700 PFD; 5°C/1700 PFD). Gas exchange measurements were performed by an open system (Minicuvette System, Walz, Germany) equipped with an infrared gas analyzer (Unor, Maihak, Germany). A special climatized cuvette (GK-022, Walz, Germany) was used to fit the fiberoptics of the fluorometer at 60° to the leaf plane. Chlorophyll *a* fluorescence was measured by a pulse amplitude modulated fluorometer (PAM-2000, Walz, Germany). The nomenclature and calculation of the chlorophyll fluorescence were according to van Kooten and Snel (1990).

Results

CO_2 assimilation

In all the experimental conditions no statistically significant difference was found between the CO_2 assimilation rates measured at ambient O_2 concentration of winter-hardened (WH) and dehardened (DH) spinach plants (Fig. 2). The values of the net photosynthetic rates measured at 2% O_2 concentration were always significantly ($P < 0.05$) higher than at ambient O_2 in WH as well as in DH plants; however at 5°C/300 PFD the CO_2 assimilation rates did not change significantly with O_2 concentration. The percentage inhibition of photosynthesis by O_2 (IPO) changed according to the different experimental conditions: it was the highest at 25°C/300 PFD (WH: 38.5%, DH: 31.5%) and the lowest at 5°C/300 PFD (WH: 10.3%, DH: 4.4%). Moreover no significant difference in IPO was found between WH and DH plants except at 5°C/1700 PFD where a significantly lower IPO was found in DH than in WH plants.

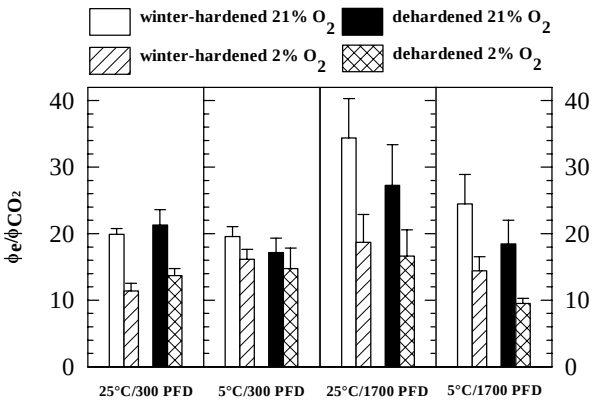

Fig. 4. Effect of different experimental conditions on $\phi_e/\phi CO_2$ ratio of spinach leaves. All data are means ± SE, n=4.

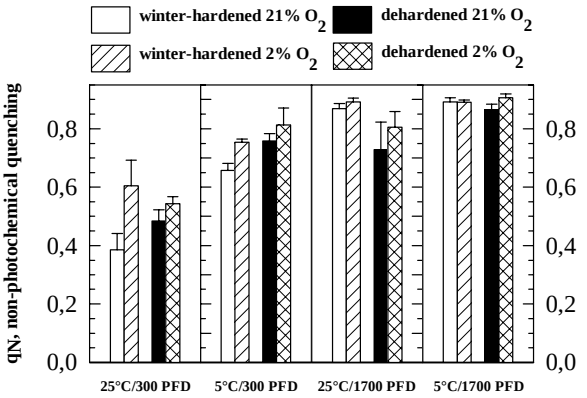

Fig. 5. Responses of thermal dissipation expressed by non-photochemical quenching (q_N) in WH and DH spinach plant. Data are the means ± SE, n=4.

Chlorophyll a fluorescence emission

Significant differences ($P < 0.05$) in the quantum yield of PSII linear electron transport (ϕ_e) between the WH and DH plants were observed at 5°C/300 PFD and 5°/1700 PFD at the two O_2 concentrations (Fig. 3). Significant differences ($P < 0.05$) in the ϕ_e values between photorespiratory (21% O_2) and non photorespiratory (2% O_2) conditions were found at 5°C/300 PFD in WH and in DH plants and at 25°C/1700 PFD in WH plants. It was also measured the ratio between the quantum yield of PSII linear electron transport and that of CO_2 assimilation (ϕ_e/ϕ_{CO2}) that it is an estimate of the partitioning of reductive power between assimilatory and non-assimilatory sinks. At 5°C/1700 PFD the ϕ_e/ϕ_{CO2} ratio measured at 21% O_2 concentration was significantly higher ($P < 0.05$) in WH than in DH plants (Fig. 4). Significant decrements at 2% O_2 of the ϕ_e/ϕ_{CO2} ratios ($P < 0.05$) measured at 2% O_2 were evident in WH plants at high light conditions (25°C/1700 PFD; 5°C/1700 PFD) and in both types of plants at 25°C/300 PFD.

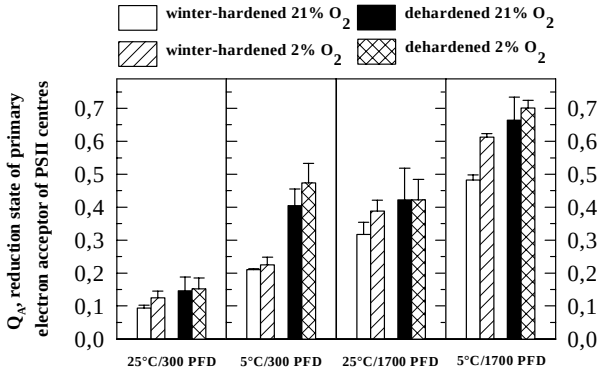

Fig. 6. Values of the redox state of Q_A in WH and DH spinach plants exposed to different conditions. Data are the means ± SE, n=4.

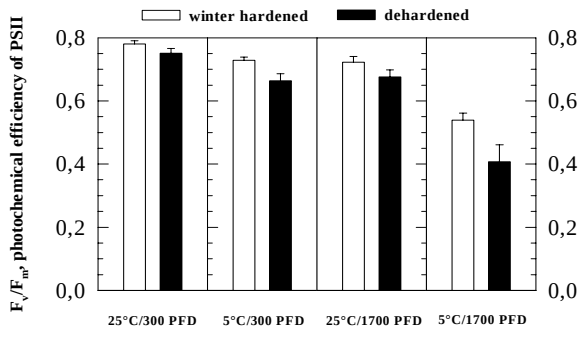

Fig. 7. Photochemical efficiency of PSII, expressed by F_v/F_m ratio, in spinach plants after 3h exposure to different temperature and light regimes. Data are the means ± SE, n=4.

The thermal dissipation was estimated by the non-photochemical quenching coefficient of fluorescence q_N. At low temperatures and high light conditions (combined or not) the q_N coefficients increased significantly ($P < 0.01$) with respect to 25°C/300 PFD in both types of plants (Fig. 5). In all spinach plants exposed to 5°C/300 PFD the q_N values measured at 2% O_2 were significantly ($P < 0.05$) higher than at 21% O_2, while at 25°C/300 PFD the q_N values were significantly higher ($P < 0.05$) at 2% O_2 only in WH plants. In both plant groups exposed to 5°C/300 PDF it was observed a significant increase of the Q_A values ($P < 0.05$) compared to 25°C/300 PFD and the values were significantly higher ($P < 0.05$) in DH than in WH plants at both O_2 concentrations (Fig. 6). By exposing the spinach plants to 5°C/1700 PFD the highest values of the redox state of Q_A were measured; the Q_A values of DH plants measured at 21% were significantly higher ($P < 0.05$) than those of the WH ones and the difference at the two O_2 concentrations were significant only in the WH plants ($P < 0.01$). The photochemical efficiency of PSII, F_v/F_m, decreased significantly ($P < 0.05$) at a similar extent in WH and in DH plants exposed to 5°C/300 PFD (WH: 7.5%; DH: 8.7%) and 25°C/1700 PFD (WH: 7.4 %; DH: 9.1%) with respect to 25°C/300 PFD (Fig. 7). When both plants were exposed to 5°C/1700 PFD significantly higher decrements of F_v/F_m values (WH: 30.9%; DH: 45.2%) ($P < 0.05$) were measured compared to 25°C/300 and only in this conditions the F_v/F_m values of WH plants were significantly higher ($P < 0.05$) compared to those of DH ones.

Discussion

Although Huner *et al.* (1993) suggested an increase of the photosynthetic activity in cold acclimated plants, we did not found any significant difference in CO_2 assimilation rates measured at ambient O_2 concentration between WH and DH plants of spinach (Fig. 2). Other authors (Boese and Huner 1990; Martindale and Leegood 1997) did not observe any change in light saturated rates of photosynthesis measured at ambient CO_2 between cold acclimated and non-acclimated spinach plants. In contrast with the other experimental conditions, at 5°C/300 PFD the CO_2 assimilation rate did not change significantly by suppressing photorespiration both in WH and in DH plants. It was evident that at low temperature the photorespiratory metabolism was reduced due to the increase of the ratio of carboxylation to oxygenation of ribulose 1,5 bisphosphate (Chen and Spreitzer 1993). However it may be possible that a decrease of photosynthetic rate at 5°C/300 PFD in relation to 25°C/300 PFD was masked by a reduced photorespiratory rate. When the spinach plants were exposed to 25°C/300 PFD the suppression of photorespiration induced a greater partitioning of the reductive power to assimilatory sinks (Fig. 4) and a q_N increase (Fig. 5): these responses were sufficient to keep low the redox state of Q_A (Fig. 6) in WH and DH plants. At 5°C/300 PFD since the photorespiration was very low its contribute to photochemical dissipation of energy excess was negligible in both types of plants. However, at this condition when plants were exposed at 2% O_2 a further decrease of ϕ_e was observed compared to 21% O_2 (Fig. 3). This would suggest that other processes consuming O_2 (f.e. Mehler reaction) could sustain a non-assimilatory electron transport. Nevertheless, at 5°C/300 PFD and at 2% O_2 it was also observed a significant increase of q_N that could compensate the reduced photochemical activity. At 25°C/1700 PFD the suppression of photorespiration induced only in WH plants a significant decrease of ϕ_e and a major partitioning of reductant power to assimilatory sinks. No relevant role was evident in mitigating the sensitivity to photoinhibition in WH and DH plants. When exposed to 5°C/1700 PFD the spinach plants experienced a severe stress, as evidenced by the lowest ϕ_e values attained, and exhibited a great sensitivity to photoinhibition expressed by high decrements in F_v/F_m (Fig. 7), probably due to a lower photorespiratory rate. In fact the susceptibility to photoinhibition in DH plants is associated to a high redox state of Q_A. Furthermore, the WH plants exposed to 5°C/1700 PFD, that showed higher

photorespiratory rate, were able to keep the redox state of Q_A at a degree significantly lower than that of DH plants. That photorespiration had an important role in keep at a low level the redox state of Q_A was also confirmed by exposing the WH plants at 2% O_2: in this case the redox state of Q_A was not significantly different from that of DH plants regardless of O_2 concentration. The ϕ_e/ϕ_{CO2} ratio was significantly lower in DH than in WH plants indicating that the reductive power was less utilized in non-assimilatory sinks when photorespiration decreased. The contribute of photorespiration to the photochemical dissipation of absorbed photon excess was remarkable at 5°C/1700 PFD because the thermal dissipation had reached the maximum intensity and no difference was detectable between DH and WH plants also at different O_2 concentrations.

Acknowledgments

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