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BRIEF COMMUNICATION

Influence of short-term osmotic stress on the photosynthetic activity of barley seedlings

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Abstract

Oxygen evolution and chlorophyll *a* fluorescence transients of two barley (*Hordeum vulgare* L) cultivars subjected to polyethylene glycol induced osmotic stress was examined. The relative water content of the plants was used as a measure of their water status. The results suggested that although dehydration was considerable, photosystem 2 was weakly affected by the osmotic treatment.

Additional key words: chlorophyll fluorescence, *Hordeum vulgare*, oxygen evolution, polyethylene glycol 8000, relative water content.

Water availability is one of the main environmental parameters, which affects plant growth and productivity. Excessive drought and salinity may result in the development of osmotic stress (Zhu *et al*. 1997, Nayyar 2003/4). Polyethylene glycol (PEG) is a neutral osmotically active polymer, which is most frequently used in plant water deficit studies to induce dehydration by decreasing the water potential of the nutrient solution (Krizek 1985, Jacomini *et al.* 1988, Hsu *et al.* 2003a).

Chlorophyll fluorescence from intact leaves, especially fluorescence induction patterns, proved to be a reliable, non-invasive method for monitoring photosynthetic events and for judging the physiological status of the plant (Van Rensburg *et al*. 1996, Strasser *et al.* 2000, Rizza *et al.* 2001). The ratio of variable (F_v) to maximal (F_m) fluorescence is an important parameter used to assess the physiological state of the photosynthetic apparatus. It represents the maximum quantum yield of primary photochemical reaction of photosystem 2 (PS 2). Environmental stresses that affect PS 2 efficiency are known to provoke a characteristic decrease in the F_v/F_m ratio (Krause and Weis 1991). However, some authors (Kaiser 1987, Chaves 1991) point to the remarkable resistance of the photosynthetic apparatus to water shortage. It has been estimated that 30 % leaf water deficit is the limit above which the photosynthetic biochemistry is significantly affected (Cornic *et al.* 1992). According to Kaiser (1987) water potential is not a decisive factor for explaining the effects of desiccation. Instead, the change in water content and thus in cell volume, has to be taken into account. These considerations emphasize on the importance of the osmotic adjustment as a mechanism to maintain cell volume during drought in natural conditions.

The present study was undertaken in order to assess the impact of short-term osmotic treatment on the photosynthetic activity of two contrasting barley (*Hordeum vulgare* L.) cultivars, drought-susceptible Houters and drought-tolerant Odesskii.

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Abbreviations: PEG - polyethylene glycol, PS 2 - photosystem 2, RWC - relative water content.
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Seeds were germinated in Petri dishes on wet filter paper in a thermostat in the dark for 2 d. The seedlings were grown hydroponically on full strength Knop nutrient solution for 7 d in a climatic chamber at irradiance of 120 μmol $m^{-2}s^{-1}$ (400 - 700 nm) with a 12-h photoperiod and air temperature 23 - 25 °C. Nine-day-old seedlings were subjected to osmotic stress by immersing their roots in 25 % PEG 8000 (Fluka, Germany) dissolved in nutrient solution for 6 to 48 h. The roots of the control plants were left in nutrient solution. After the imposition of stress the leaves of the plants were used for analysis of the relative water content (RWC). Water content was estimated according to Turner (1981) and was calculated from the equation:

RWC = (FM - DM)/(SM - DM),

where FM is the fresh mass of the leaves, SM is the mass at full water saturation, measured after floating the leaves for 24 h in water at room temperature, and DM is the mass estimated after drying the leaves for 4 h at 80 °C or until a constant mass is achieved.

Oxygen evolution was measured on leaf segments (10 cm^2) of the central part of the first fully developed leaf with a leaf disc oxygen electrode (*Hansatech, King's Lynn*, Norfolk, UK) as described by Walker (1987) at saturating irradiance of 1 500 μmol m^{-2} s^{-1} PAR.

Chlorophyll content in the leaves was determined in 80 % acetone extracts according to Lichtenthaler (1987). The absorbance was measured at 663 and 645 nm, using spectrophotometer *Specol 10* (*VEB Carl Zeiss*, Jena, Germany). Chlorophyll fluorescence was measured *in situ* with a portable fluorometer *HandyPEA* (*Hansatech*, King's Lynn, Norfolk, UK). The actinic light (λ = 650 nm) is supplied by three light-emitting diodes. Irradiance at the leaf surface was *ca.* 3 000 μmol m^{-2} s^{-1}. The fluorescence signal is separated from the actinic light by a high-pass filter (λ > 700 nm) and sampled every 10 μs. Fluorescence induction transients were determined within 1 s. The recordings were performed on the middle part of the abaxial side of the first fully developed leaf, after dark-adaptation for 60 min. F_0 is the initial fluorescence emission by antenna Chl *a* molecules and corresponds to the digitized fluorescence value at 20 μs (Strasser *et al.* 2000). F_m is the maximum total fluorescence value. Variable fluorescence (F_v) = F_m - F_0. The F_v/F_m ratio measures the efficiency of excitation energy capture by open PS 2 reaction centres representing the maximum capacity of light-dependent charge separation (Krause and Weis 1991).

The relative variable fluorescence at the intermediate J step (2 ms), $V_J = (F_J - F_0)/(F_m - F_0)$, is used to characterize the efficiency of the electron transfer between Q_A and Q_B (Strasser *et al.* 2000). It is generally accepted that the J step in the fluorescence induction curve arises from photoinduced reduction of the primary quinone acceptor Q_A.

The water status of the plants was significantly affected by the osmotic treatment. It caused a decrease in the RWC of both cultivars studied (Table 1). Of the two cultivars Odesskii lost water slowly but after 48 h of stress exhibited a greater water deficit. Houters dehydrated faster to a certain level without losing additional water.

The photosynthetic activity of the two cultivars was assayed from oxygen production and from chlorophyll *a* fluorescence transients. The substantial decline in oxygen evolution after 48 h of PEG treatment (57 % decrease of O_2 evolution rate for cultivar Odesskii and 55 % decrease for cultivar Houters) could be connected with effect of osmotic stress on stomatal conductance (Pandey *et al.* 2003/4). Such possibility has been proved earlier by other authors for various plant species (Chaves 1991, Cornic *et al.* 1992). Furthermore, as it is known that high

Table 1. Relative water content (RWC), contents of chlorophyll (Chl) *a* and *b*, and Chl *a*/*b* ratio in the leaves of PEG-treated and control plants of two barley cultivars. Means ± SE, *n* = 5 for RWC, *n* = 3 for Chl.

Cultivar	PEG treatment [h]	RWC [%]	Chl *a* [mg g^{-1}(d.m.)]	Chl *b* [mg g^{-1}(d.m.)]	Chl *a*/*b*
Odesskii	0	95.8 ± 0.4	7.13 ± 0.52	3.02 ± 0.29	2.36 ± 0.40
	6	86.2 ± 3.1	9.45 ± 0.30	3.78 ± 0.02	2.50 ± 0.09
	24	79.8 ± 2.0	8.28 ± 0.04	3.57 ± 0.07	2.32 ± 0.05
	30	76.2 ± 2.0	7.80 ± 0.10	3.52 ± 0.04	2.22 ± 0.05
	48	70.5 ± 2.6	6.88 ± 0.65	2.72 ± 0.34	2.53 ± 0.55
Houters	0	97.3 ± 0.4	10.57 ± 1.14	4.37 ± 0.33	2.42 ± 0.44
	6	88.6 ± 3.6	8.40 ± 0.74	3.38 ± 0.30	2.48 ± 0.44
	24	74.3 ± 2.6	7.96 ± 0.33	3.42 ± 0.19	2.32 ± 0.23
	30	74.3 ± 2.5	10.03 ± 0.44	4.49 ± 0.11	2.23 ± 0.15
	48	74.1 ± 2.3	9.18 ± 0.43	3.43 ± 0.25	2.68 ± 0.32

molecular mass PEG is scarcely transported into the leaves (Janes 1974, Lawlor 1970), direct effect of PEG molecules on stomata seems quite unlikely.

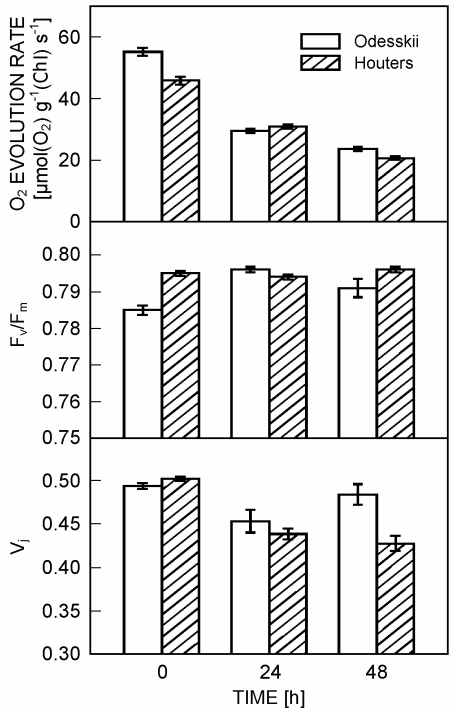

Fig. 1. Effect of PEG treatment of two barley cultivars on the rate of oxygen evolution, measured on leaf discs with a Clark-type electrode, and on the chlorophyll *a* fluorescence parameters F_v/F_m and V_J, registered from dark-adapted leaves using a *HandyPEA* fluorometer. The *vertical bars* represent SE, $n = 5$ for O_2 evolution, $n = 16$ for fluorescence parameters.

Upon the duration of the PEG treatment a gradual decrease in the fluorescence intensity was observed. This is evidenced by changes in the values of the initial (F_0) and maximum (F_m) fluorescence (data not shown). However, no significant variation in the F_v/F_m ratio was registered, suggesting that the efficiency of the quantum yield of PS 2 was not lowered (Fig. 1). The unaffected F_v/F_m confirms the observation that the photosynthetic machinery is resistant to water deficit as discussed by other authors (Chaves *et al.* 2002, Cornic and Fresneau 2002). Water stress has been shown to reduce chlorophyll content in some cases (Hsu *et al.* 2003b). In our study, the fluorescence behaviour could not be attributed to a decrease in chlorophyll content (Table 1). Instead, it can be speculated that the lower fluorescence is either due to a smaller effective antennae cross-section or to processes that lead to increased non-radiative energy dissipation. It is known that drought may lead to an increase in non-photochemical quenching (Sheuermann *et al.* 1991, Biehler *et al.* 1997).

The decrease in the relative variable fluorescence V_J at the J step of the induction curve (Fig. 1) represents an interesting consequence of the PEG treatment. V_J is a measure of the fraction of reduced Q_A^- (Havaux and Strasser 1992). It is controlled by the ratio of the rate of reduction of Q_A to Q_A^- and reoxidation of Q_A^- by Q_B. The observed decline of V_J in PEG-treated samples could be a result of lowered excitation pressure on PS 2 and consecutively a slower rate of Q_A reduction.

The chlorophyll fluorescence induction parameters suggest that the PS 2 reaction centres are essentially unaffected by PEG treatment, but the rate of light absorption or excitation energy transfer towards the reaction centre is lowered. The PS 2 antenna complexes might undergo some structural rearrangements leading to increased heat dissipation, *e.g.* as a consequence of changes in grana packing due to the altered ionic strength of the surrounding medium.

In conclusion, the experimental data show that the two contrasting cultivars respond in a rather similar way to the imposed stress thus suggesting that the observation reflects a general feature rather than specificity in the stress tolerance. The PS 2 activity of both cultivars is weakly affected under the considered osmotic conditions. Although PEG obviously causes rapid dehydration PS 2 retains its efficiency. This is in accordance with the mentioned concepts considering the 30 % limit of leaf water deficit for certain disturbances in the functioning of the photosynthetic machinery (Kaiser 1987, Chaves 1991, Cornic *et al.* 1992).

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