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Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress

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Abstract

Two physiological tests for screening drought tolerance of barley (*Hordeum vulgare*, L.) plants are compared in this work. Water deficit is induced by treating the plants' roots with polyethylene glycol (PEG 8000). The relative water content (RWC) of the plants is used as a measure of the water status. Conductometrically determined electrolyte leakage from the leaf tissue demonstrates the membrane injury caused by dehydration. It is shown that the injury index increases with the decrease of the RWC of the leaves. The F_v/F_m ratio is employed to assess changes in the primary photochemical reactions of the photosynthetic apparatus after dehydration. The results suggest that PSII is weakly affected by the imposed osmotic stress. The fluorescence behaviour of the examined cultivars is related to their RWC.

Keywords: Chlorophyll fluorescence; PSII; Electrolyte leakage; Barley; PEG 8000

1. Introduction

The term osmotic stress is commonly used to refer to situations where insufficient water availability limits plant growth and development. It can result from drought as well as from excessive salinity. Due to reduced water absorption and cellular dehydration, chilling and freezing may also lead to osmotic stress [1].

Polyethylene glycols or PEGs are a group of neutral osmotically active polymers with a certain molecular weight. PEG 8000 (the number signifying molecular mass) is most frequently used in plant water deficit studies to induce dehydration by decreasing the water potential of the nutrient solution [2,3].

The use of chlorophyll fluorescence from intact, attached leaves proved to be a reliable, nonintrusive method for monitoring photosynthetic events and for judging the physiological status of the plant [4,5]. Fluorescence induction patterns and derived indices have been used as empirical diagnostic tools in stress physiology [6]. Thus, PSII fluorescence can be regarded as a biosensing device for stress detection in plants. The F_v/F_m ratio represents the maximum quantum yield of the primary photochemical reaction of PSII. It is an important parameter of the physiological state of the photosynthetic apparatus. Environmental stresses that affect PSII efficiency lead to a characteristic decrease in the F_v/F_m ratio [7].

The measurement of solute leakage from plant tissue is a long-standing method for estimating membrane integrity in relation to environmental stresses, growth and development, and genotypic variation [8–11]. In this regard, the degree of cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance.

2. Experimental

Two barley (*Hordeum vulgare*, L.) cultivars, Houters and Odeski, differing in their field performance, were germinat-

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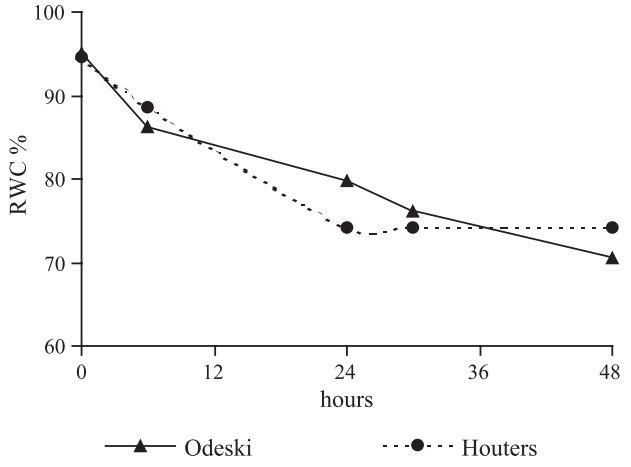

Fig. 1. Effect of the duration of the treatment with 25% PEG on the relative water content (RWC %) of two barley cultivars. The values at 0 h represent the untreated controls.

ed in Petri dishes on wet filter paper in a thermostat in the dark for 2 days. The seedlings were grown hydroponically on full strength Knopp nutrient solution for 7 days in a climatic chamber at 120 μmol/m^2 s PFD (400–700 nm) with a photoperiod 12/12 h day/night 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 6–48 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 RWC and membrane injury index [cell membrane stability (CMS)]. The water content was estimated according to Turner [12] and was evaluated from the equation:

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}),$$

where FW is the fresh weight of the leaves, TW is the weight at full turgor, measured after floating the leaves for 24 h in water in the light at room temperature and DW is the weight estimated after drying the leaves for 4 h at 80 °C or until a constant weight is achieved.

For determination of cell membrane stability (CMS), 20 leaf pieces (2 cm each) of stressed and unstressed plants were washed with distilled water to remove the solution from the injured cells and tissue particles after which the samples were immersed in 20 ml distilled water at room temperature. After 24 h, the conductivity of the solutions was determined. The samples were autoclaved for 15 min, cooled to room temperature and the conductivity of the solutions was read again. The electrolyte leakage was measured with a conductometer Elwro No5721, Poland. CMS or the so-called injury index was estimated from the formula:

$$I = 1 - (1 - T_1/T_2)/(1 - C_1/C_2) \times 100,$$

where T_1 and T_2 are the first and second (after autoclaving) measurements of the conductivity of the solutions in which the treated samples are immersed and C_1 and C_2 are the respective values for the controls.

Chlorophyll fluorescence was measured in situ with a portable fluorometer Handy PEA (Hansatech, UK) with a maximal time resolution of 10 μs. The initial fluorescence values F_o correspond (with a deviation of less than 1%) to the digitized fluorescence value of 20 μs [6]. The fluorescence transients were measured within 1 s. The recordings were performed on the first fully developed leaf, after dark adaptation period of 60 min. All measurements were from the middle part of the abaxial side of the leaves. F_o is the initial fluorescence emission by antenna Chl *a* molecules. F_m is the maximum total fluorescence value. $F_v = F_m - F_o$ is the variable fluorescence. The F_v/F_m ratio measures the efficiency of excitation energy capture by open PSII reaction centres representing the maximum capacity of light-dependent charge separation [7].

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 relative variable fluorescence at the intermediate J step (2 ms), $V_j = (F_j - F_o)/(F_m - F_o)$, is used to characterize the efficiency of the electron transfer between Q_A and Q_B [6].

3. Results and discussion

The imposed osmotic stress significantly affected the water status of the plants. It caused a decrease in the RWC of both cultivars studied (Fig. 1). Odeski 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.

Membrane injury in the leaves of the two genotypes increased with the duration of the stress (Fig. 2). Cultivar

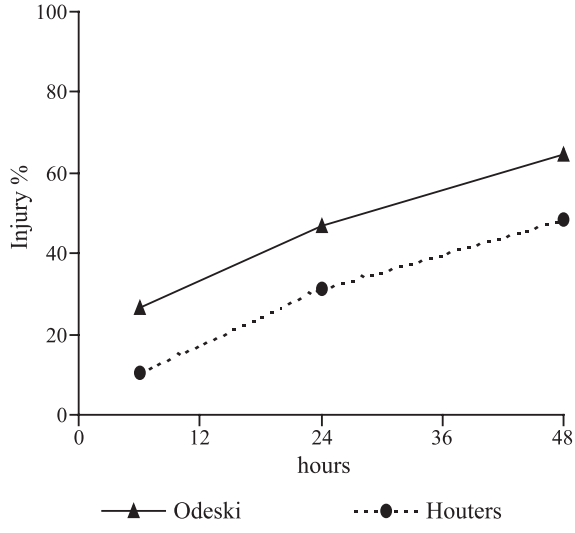

Fig. 2. Membrane injury estimated as ion release (%) from leaf tissue of two barley cultivars as a function of the duration of the osmotic stress caused by 25% PEG.

Table 1
Effect of the duration of osmotic stress with 25% PEG on the fluorescence transients

Variant	F_o	F_m	F_v	F_v/F_m	V_j	ΔV_j (%)
Cultivar Odeski						
Control	333.5 ± 9.3	1625.4 ± 54.8	1291.9 ± 48.4	0.795 ± 0.005	0.4934 ± 0.012	
6-h stress	330.9 ± 9.9	1568.9 ± 59.3	1238.0 ± 51.4	0.789 ± 0.005	0.4641 ± 0.048	5.94
12-h stress	293.4 ± 16.9	1427.0 ± 94.9	1133.6 ± 79.9	0.794 ± 0.006	0.4778 ± 0.015	3.16
24-h stress	271.4 ± 10.8	1315.0 ± 45.4	1058.1 ± 44.9	0.796 ± 0.003	0.4530 ± 0.052	8.19
48-h stress	326.3 ± 18.4	1563.3 ± 119	1237.0 ± 104.3	0.791 ± 0.010	0.4839 ± 0.047	1.93
Cultivar Houters						
Control	326.6 ± 9.7	1595.0 ± 52.2	1268.4 ± 43.6	0.795 ± 0.003	0.5020 ± 0.009	
6-h stress	291.8 ± 19.0	1461.1 ± 74.9	1169.4 ± 56.9	0.800 ± 0.004	0.4643 ± 0.014	7.51
12-h stress	279.0 ± 12.1	1359.7 ± 54.6	1080.7 ± 43.1	0.795 ± 0.002	0.4696 ± 0.023	6.45
24-h stress	280.2 ± 12.6	1348.3 ± 56.4	1068.2 ± 44.1	0.794 ± 0.003	0.4383 ± 0.024	12.69
48-h stress	280.8 ± 12.2	1378.1 ± 48.5	1097.4 ± 37.0	0.796 ± 0.003	0.4273 ± 0.035	14.88

Initial fluorescence F_o, maximum fluorescence F_m, variable fluorescence $F_v = F_m$-F_o, F_v/F_m ratio and relative variable fluorescence at the J step, $V_j = (F_j$-$F_o)/(F_m$-$F_o)$ in two barley cultivars, $\Delta V_j = [V_j(0)$-$V_j(h)]/V_j(0)$, where $V_j(0)$ is V_j of the control and $V_j(h)$ is V_j of the respective PEG treated sample.

Houters underwent lower membrane injury than cultivar Odeski. This correlated with the water status of the two cultivars, i.e., greater water loss corresponded to greater membrane damage. Similar dependence for other barley cultivars is discussed in Ref. [11].

A gradual decrease in the fluorescence intensity was observed upon the duration of the PEG treatment. This is evidenced by changes in the values of the initial and maximum fluorescence, F_o and F_m (Table 1). No significant variation in the F_v/F_m ratio was registered, suggesting that the efficiency of the quantum yield of PSII was not lowered. The unaffected F_v/F_m means that there is no loss in the yield of PSII photochemistry and confirms the resistance of the photosynthetic machinery to water deficit [13,14]. This fluorescence behaviour could not be attributed to a decrease in chlorophyll content, which was not observed in our case (data not shown). It can be speculated that the lower fluorescence is either due to a smaller antenna cross-section or to a process increasing the nonradiative energy dissipation. It is known that drought may lead to an increase in nonphotochemical quenching [15,16]. These processes, however, are expected to relax during the 1-h period of dark adaptation of the samples [17]. We suppose that the changes in fluorescence intensity result from long-term structural/conformational changes, presumably in the PSII antennae, which lead to increased energy dissipation.

An intriguing effect of PEG treatment was the decrease in the relative variable fluorescence V_j at the J step of the induction curve. V_j is a measure of the fraction of reduced Q_A^- [18]. Rise in the J step is usually interpreted as a decreased efficiency of Q_A^- reoxidation [6]. It is possible that the lowered excitation pressure on PSII in PEG-treated samples leads to a slower rate of reduction of Q_A thus decreasing the fraction of closed reaction centres at the J step.

In their work, Ögren and Oquist [19] have established that primary events in electron transport appeared to be less affected as judged from the analysis of the early phases of fluorescence kinetics. They concluded that the photochemistry of the studied material (willow leaves) was not affected until extreme drought. The effect of rapid dehydration appears to be different from that of drought at the mechanistic level. The characteristics of fluorescence induction are clearly different in leaves suffering rapid dehydration and those experiencing drought at the same degree of inhibition of the photosynthetic capacity. The extent of desiccation required to induce a given depression in photosynthetic capacity is much larger during rapid dehydration than during drought [20].

Our results suggest that although dehydration is high, the inhibition of the electron transport between Q_A and Q_B is not prominent under these conditions of osmotic stress. The imposition of severe osmotic stress causes rapid dehydration but PSII retains its efficiency. Nevertheless, the stress considerably affects the plants causing injury to their cell membranes. This is not surprising, bearing in mind that cell membranes are among the first targets of attack under stress conditions [21].

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