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Drought stress induced in barley cultivars (Hordeum vulgare L.) by polyethylene glycol, probed by germination, root length and chlorophyll a fluorescence rise (OJIP)

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Abstract

Seed germination and seedling establishment of six varieties of barley plants (Hordeum vulgare L.) were tested for drought tolerance using polyethylene glycol-6000 solutions (PEG) with different osmotic potentials (0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa). Seeds of three varieties obtained from the National Institute of Agricultural Research (INRA) of Morocco and three landrace populations collected at three localities in the south of Morocco were used in the present study. In addition, seed germination, emergence and root length were measured. The performance index (PI) and the maximum quantum yield of primary photochemistry (φ_{Po}) extracted from the polyphasic fluorescence transient (OJIP) were used to evaluate drought tolerance. The sensitivity to the osmotic stress of all measured parameters was cultivar dependent. The different varieties showed a gradual decrease in the performance index (PI), it varied between 86% and 73% of the control under severe osmotic stress (-2 MPa). Therefore, the osmotic stress has little effect on the maximum quantum yield of photosystem II ($\varphi_{Po} = F_V/F_M$). The studied varieties can be split into three groups that varied in the reduction of their PI at low and high osmotic stress. We show that a positive correlation exist between change in performance index and root length measured after the different PEG-6000 treatments. These results suggest that chlorophyll a fluorescence, and especially the performance index, could be used for the screening of barley varieties for drought tolerance.

Keywords: Hordeum vulgare, *drought tolerance, polyethylene glycol, polyphasic fluorescence transient (OJIP), performance index*

Résumé

Tolérance au déficit hydrique d'orge (*Hordeum vulgare* L.) induit par le polyéthylène glycol, examinée par la germination, la longueur racinaire et la fluorescence chlorophyllienne (OJIP). – *La germination et l'établissement de jeune plantes de six variétés d'orge (Hordeum vulgare L.) ont été examinés pour leur tolérance au déficit hydrique dans des solutions du polyéthylène glycol-6000 avec différents potentiels osmotiques (0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa et -2 MPa). Des graines de trois variétés obtenues de l'Institut National de la Recherche Agricole (INRA) du Maroc et de trois populations collectées à trois localités dans le sud du Maroc ont été employées dans la présente étude. La germination, l'émergence et la longueur de racine ont été étudiées. L'index de performance (PI) et le rendement quantique maximale de la photochimie primaire (φ_{Po}) extraites à partir de la courbe de fluorescence OJIP ont été employés pour évaluer la tolérance au déficit hydrique. La sensibilité au déficit hydrique a varié selon les variétés. Les différentes variétés ont montré une diminution progressive de l'index de performance (PI), il a varié entre 86% et 73% relativement au contrôle sous un stress osmotique sévère*

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(-2 MPa). Le déficit hydrique a montré peu d'effet sur le rendement quantique maximale de la photochimie primaire de photosystème II ($\varphi_{Po} = F_V/F_M$). Une corrélation entre l'index de performance et la longueur de racine a été observée après les traitements de PEG-6000. Ces résultats suggèrent que l'analyse des paramètres de la fluorescence chlorophyllienne et particulièrement l'index de performance, pourrait être employée pour élucider la tolérance au déficit hydrique des variétés d'orge.
Mots-clés: Hordeum Vulgare, *tolérance au déficit hydrique, polyéthylène glycol, fluorescence chlorophyllienne (OJIP), index de performance.*

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crops cultivated in Morocco and is grown in a wide geographic range under varied agro-climatic conditions. During all or part of growth and development, the barley plants are subjected to various environmental stresses such as drought stress. In many parts of Morocco the availability of water is limited by inadequate and unpredictable rainfall and drought is a serious agronomical problem and the major factor limiting crop production.

Selection for drought tolerant varieties is one of the best ways to confront the water scarcity in hostile arid and semi-arid environments and it is presumed that barley landraces survive the fluctuations of biotic and abiotic stresses because of their high level of heterogeneity (Czembor 2000). For this reason, fast and non-invasive methods to assess drought tolerance in the early stages of plant development are necessary.

The analysis of changes in chlorophyll *a* fluorescence kinetics provides detailed information on the structure and function of the photosynthetic apparatus, especially photosystem II (Strasser et al. 2004). Chlorophyll *a* fluorescence analysis has become one of the most powerful and widely used biophysical techniques available to plant physiologists and ecophysiologists (Maxwell and Johnson 2000; Strasser et al. 2004). Many abiotic stresses can directly or indirectly, affect the photosynthetic activity of the leaves and consequently alter the chlorophyll *a* fluorescence kinetics (Belkhodja et al. 1994; Christensen et al. 2003). In earlier studies, Belkhodja et al. (1994) have shown the potential use of chlorophyll *a* fluorescence as a tool for screening salt tolerance in barley and it was used also as a selection criterion for grain yield in durum wheat under Mediterranean conditions (Araus et al. 1998).

In this work we used polyethylene glycol (PEG-6000) solutions to induce drought stress in higher plants (Ranjbarfordoei et al. 2000). PEG-6000 simulates low water potentials associated with dry soils. Three tested varieties with known response (Arig 8, Lannaceur and Rabat 071) and three landraces (Aït Baha, Ighrem and Tarodant) collected from different localities of south Morocco were exposed to this type of drought stress. We investigated the polyphasic chlorophyll *a* fluorescence transient (OJIP), germination and root length to describe the response of the different varieties of barley plants. The studied varieties were sorted for their drought tolerance by agro-morphological their characteristics under near optimal and drought conditions (El Madidi et al. 2005). They were arranged in three groups that varied in their tolerance to drought stress namely group I including Aït Baha, group II including Tarodant and Ighrem and group III including Arig 8, Lannaceur and Rabat 071. According to El Madidi et al. (2005) Aït baha performed better during drought conditions than the varieties of groups II and III.

Materials and Methods

Plant material

The following barley varieties were used: Arig 8, Lannaceur, and Rabat 071 obtained from the National Institute of Agricultural Research (INRA) of Morocco and three landrace populations collected by the Provincial Direction of Agriculture (DPA) originating from three localities in south Morocco: Aït Baha (Altitude 550m, Latitude 30°05 N, Longitude 9°33 W), Ighrem (Altitude 1800m, Latitude 30°06 N, Longitude 8°27 W) and Tarodant (Altitude 235m, Latitude 30°28 N, Longitude 8°52 W).

Germination, emergence and root length

Drought stress was induced by polyethylene glycol (PEG-6000) treatments. A range of osmotic potentials (0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa) was produced using aqueous solutions of PEG-6000 prepared according to Michel and Kaufmann (1973). Grains were sterilised by a 10 min treatment with a 5% calcium hypochlorite solution and then rinsed several times with distilled water. Thirty seeds were allowed to germinate at 24±1 °C on a sheet of Watman filter paper placed in Petri dishes for each experimental treatment. The experimental design was hierarchical in a completely randomised design with four replications. Five ml of solution of PEG-6000 was added to Petri dishes every 48 h. In the case of the control treatment

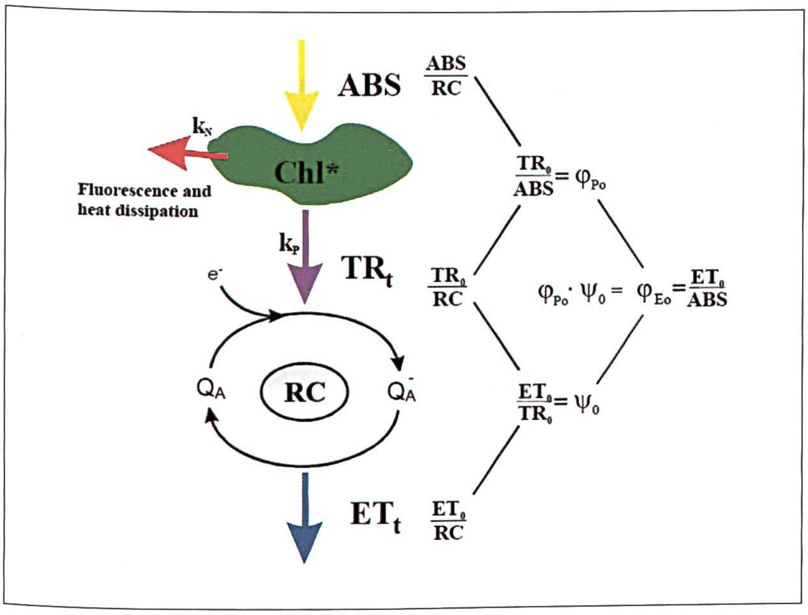

Fig. 1. A highly simplified working model of the energy fluxes in the photosynthetic apparatus (from Calantzis 2002). ABS refers to the photon flux absorbed by the chlorophyll antenna pigments Chl of the photosystem II. Part of this excitation energy is dissipated, mainly as heat and less as fluorescence emission. Another part is channelled to the reaction centre RC, as trapping flux TR_o. In the reaction centre, the excitation energy is converted into redox energy by reducing the electron acceptor Q_A to Q_A^-, which is then reoxidised to Q_A, creating an electron transport ET_o. The three yields φ_{Po}, ψ_o and φ_{Eo} are directly related to the three energetic fluctuations, as the ratio of any two of them.*

distilled water (0 MPa) was added. For each treatment, the final germination and emerged seedling percentages as well as root length were recorded after 7 days of germination. Data for root length were obtained from 10 seedlings in each replication.

Plant growth conditions

For all treatments, the seeds were soaked for 24 h in the solutions of PEG-6000 in the dark and germinated. The seedlings were grown hydroponically on Hoagland's nutrition solution for seven days and placed in a growth chamber at an irradiance level of 120 µmol $m^{-2}s^{-1}$. On the seventh day, drought stress was induced by immersing the roots of the seedlings in PEG-6000 solutions for 48 h and in distilled water for the control plants and immediately afterwards the chlorophyll *a* fluorescence was measured in each adapted state.

Chlorophyll *a* fluorescence measurements

The chlorophyll *a* fluorescence of the first leaves was measured at room temperature with a portable fluorimeter (Plant Efficiency Analyser, built by Hansatech Instruments Ltd. King's Lynn Norfolk, UK). After 1 h dark-adaptation, the leaves were exposed to a strong 1 s light pulse (600 Wm^{-2}), which was provided by an array of six light-emitting diodes (peak 650 nm). The chlorophyll *a* fluorescence emission kinetics induced by the strong light pulse was measured and digitised from 10 µs to 1 s by the instrument. A highly simplified working model of the energy fluxes in a photosynthetic apparatus is shown in Fig. 1. Based on the measurement of the OJIP fluorescence transient, the JIP-test (For review see Strasser et al. 2004) uses the theory of energy fluxes in biomembranes to calculate several phenomenological and biophysical expressions for a given physiological state (Strasser 1986). It translates shape changes of the OJIP transient to quantitative changes of a set of parameters such as specific fluxes, phenomenological fluxes and vitality indices (Strasser et al. 2004). It can be used to compare the physiological states of a treated versus a non-treated sample.

Results

Seed germination, emergence and root length

The percentage of final seed germination and emergence decreased as the osmotic potential increased, but the extent of this decrease was cultivar-dependent (Tab. I). The PEG-induced decrease of final germination and emergence has been reported in the literature (Almansouri et al. 2001; Murillo-Amador et al. 2002). Aït Baha and Lannaceur showed the highest germination potential under drought stress conditions (Tab. I). The percentage of final seed germination in Aït Baha was 100, 93.3, 92.5, 70.5 and 66.2% respectively at 0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa. In Lannaceur, it was 100, 95.6, 89.2, 66.1 and 54.9%. The inhibition of the final seed emergence by PEG-6000 was more pronounced in Ighrem and Rabat 071. Aït Baha and Lannaceur showed the highest emergence (Tab. I). Like final seed germination and emergence, the root length declined with increased osmotic stress in all varieties. Severe osmotic stress (-2 MPa) decreases the root length by more than 50% relatively to the control in all varieties except in Lannaceur. Root length development is most affected in Rabat 071 by PEG treatments at -2 MPa with a reduction of 62% relative to the control (Tab. I).

Table I. Percentage of the relative germination, emergence and root length relative to the control (0 MPa) of the different varieties of barley plants at different polyethylene glycol solutions (PEG-6000).

	Germination %					Emergence %					Root length %				
PEG (MPa)	0	-0.5	-1	-1.5	-2	0	-0.5	-1	-1.5	-2	0	-0.5	-1	-1.5	-2
Aït Baha	100	93.3	92.5	70.5	66.2	100	83.2	78.8	70.5	55.7	100	71.6	68.6	58.6	47.1
Ighrem	100	56.7	49.5	45.4	42.3	100	49.4	48	46.7	41.3	100	92.1	78.9	65.8	47.4
Tarodant	100	90.1	82.3	60.2	55.2	100	97.9	82.3	55.7	38.9	100	70.0	68.0	56.4	42.1
Arig 8	100	95.3	82.4	65.8	55.2	100	95.9	75.6	51.5	41.0	100	81.8	60.6	59.2	43.1
Lannaceur	100	95.6	89.2	66.1	54.9	100	91.3	78.1	50.8	44.9	100	86.6	84.0	66.2	53.5
Rabat 071	100	95.6	75.3	50.3	34.6	100	71.7	63.3	42.7	30.2	100	85.3	71.6	54.8	38.0

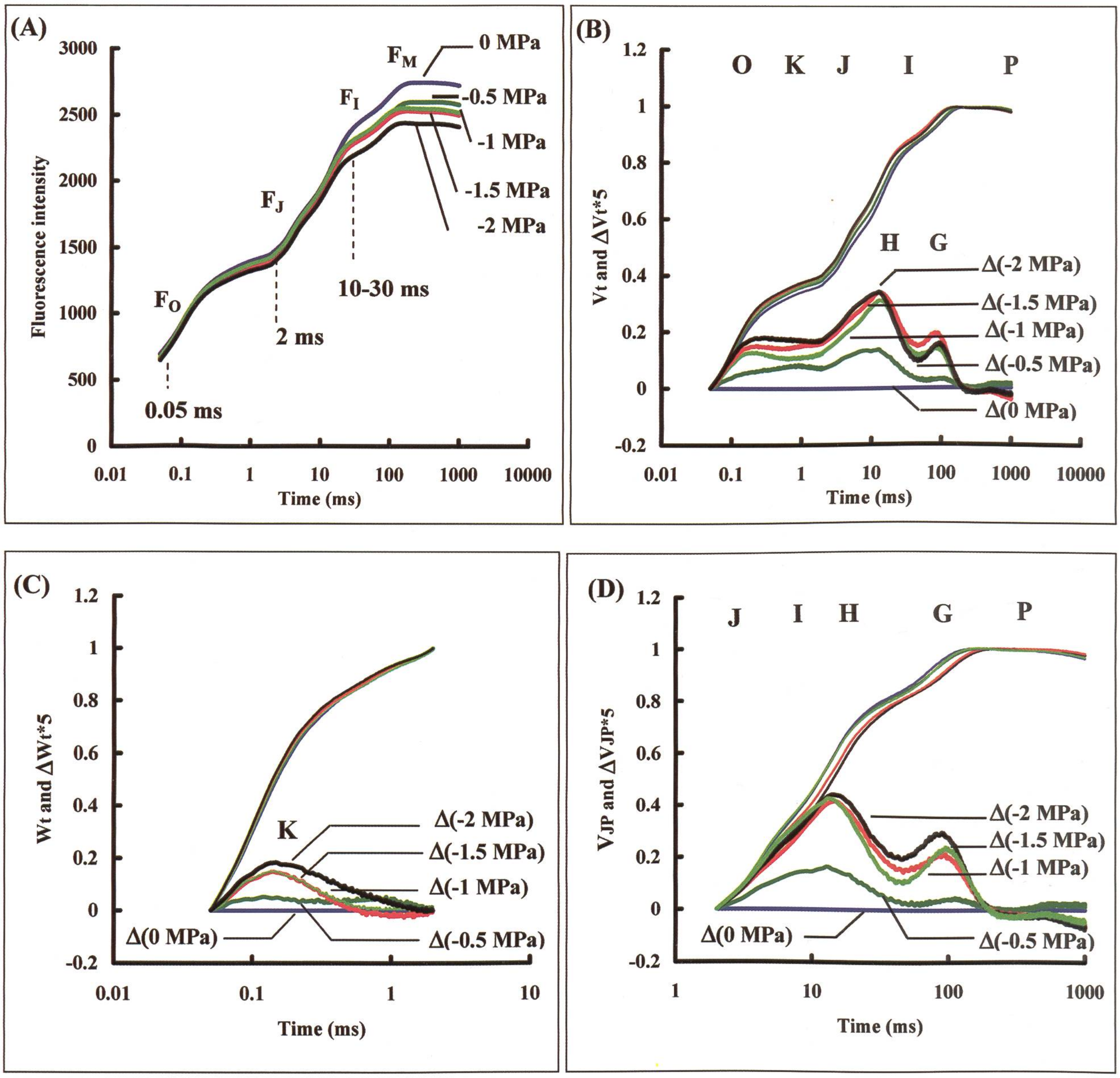

Fig. 2. An example of chlorophyll a polyphasic fluorescence rise O-J-I-P, exhibited by Aït Baha under different PEG-6000 solutions (0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa). The transients were plotted on a logarithmic time scale from 50 µs to 1 s (Fig. 2A). The marks refer to the selected fluorescence data used by the JIP-test for the calculation of structural and functional parameters. Fig. 2B shows the relative variable fluorescence (V_t) and (ΔV_t) of the different fluorescence transients. Fig. 2C shows the relative variable fluorescence (W_t) and (ΔW_t) in single turn-over phase (O-J). Fig. 2D shows the relative variable fluorescence (V_t^{MT}) and (ΔV_t^{MT}) in multiple turn-over phase (J-P).

Chlorophyll (Chl) a fluorescence rise OJIP

The polyphasic Chl *a* fluorescence transient in different varieties was recorded and plotted on a logarithmic time scale (see for example Fig. 2A).

It was assumed that leaves were in the dark-adapted state after 1 h of dark-adaptation (all Q_A in the oxidised state). Upon illumination of the sample, an increase of Chl *a* fluorescence emission was observed from an initial minimal value F_o at 50 µs (when all reaction centers were open and all Q_A oxidised) to a maximal level F_M (when all reaction centers were closed and all Q_A reduced). The PS II fluorescence yield increases following tri-phasic kinetics O-J, J-I and I-P (Strasser et al. 1995). The plateau around 2 ms is called the J-step and provides information on single turnover (Q_A reduction). During the J-I and I-P phases multiple charge separations occur and the redox components of the electron transport chain become reduced. The I-step around 20-30 ms is suggested to be related to heterogeneity of components such as Q_A and Q_B during the filling up of the plastoquinone pool. The P level is reached when all the accessible electron carriers between Q_A and ferredoxin are reduced.

Chl *a* fluorescence transients of plants exposed to different concentrations of PEG were normalised between F_o (50 µs) and F_M (reached after about 200 ms) and expressed as relative variable fluorescence V_t (Fig. 2B). V_t is defined as $(F_t - F_o) / (F_M - F_o)$ and this expression can be taken as a measure of the fraction of the primary quinone electron acceptor of PS II in its reduced state $[Q_A^- / Q_{A\,(total)}]$ (Strasser et al. 1995). Differences of the relative variable fluorescence ΔV_t of -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa transients minus the 0 MPa transient showed two peaks around the I step at ~10 ms labelled as H band and at ~100 ms labelled as G band. These two bands were clearly distinguished when the Chl *a* fluorescence transients were normalised in multiple turn-over phase between F_J (2 ms) and F_M expressed as V_t^{MT}, where $V_t^{MT} = (F_t - F_J)/(F_M - F_J)$ and by calculation of differences between fluorescence transients of stressed and control samples ΔV_t^{MT} (Fig. 2D).

The Fig. 2C shows Chl *a* fluorescence transients during the single turnover phase, double normalised between F_o and F_J and which can be expressed as $W_t = (F_t - F_o)/(F_J - F_o)$. Taking the difference of the different transients in Fig. 2C (ΔW_t) between fluorescence transients in stressed to control samples a peak is observed, the so-called K band. The K-band can be observed by eye in the fluorescence rise of heat-stressed samples (Guissé et al. 1995; Srivastava et al. 1997; Lazár et al. 1997).

Performance index (PI) and the maximum quantum yield of primary photochemistry (φ_{Po})

The performance index (PI) is one of the chlorophyll fluorescence parameters that provides useful and quantitative information about the state of plants and their vitality. The expression for the performance index is derived in analogy to the Nernst equation that is used to determine the redox potential of a system. The expression for the performance index is:

$$PI = [\gamma_o/(1-\gamma_o)].[\varphi_{Po}/(1-\varphi_{Po})].[\psi_o/(1-\psi_o)]$$

The expression $\gamma_o/(1-\gamma_o)$ is derived by the JIP-Test as equal to the ratio of reaction centers and the absorbance (RC/ABS). Therefore

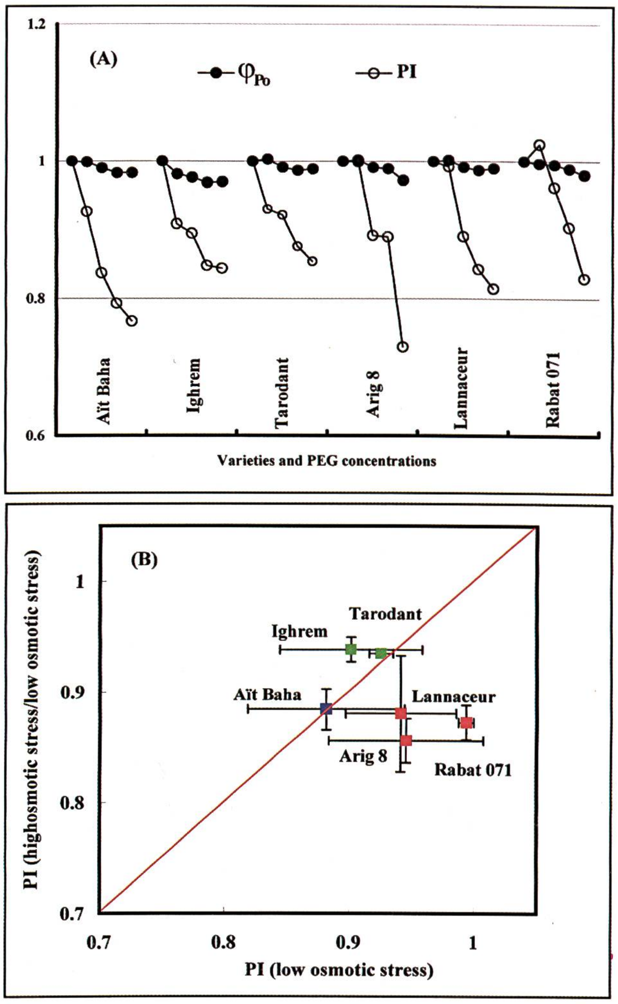

Fig. 3. Effect of PEG-6000 on performance index (PI) and maximum quantum yield of primary photochemistry φ_{Po} (= 1-(F_o/F_M)) relatively to the control (0 MPa) of the studied varieties of barley plants (A). φ_{Po} shows a little difference to the control and PI relatively to the control decrease as osmotic potential increased. B shows the response of the different varieties by PI at low osmotic stress (average of -0.5 MPa and -1 MPa) relative to the control and at high osmotic stress (average of -1.5 MPa and -2 MPa) relative to the low osmotic stress. Means ± SE.

Table II. Effect of PEG (MPa) on the relative variable fluorescence at K step ($W_k = (F_K\text{-}F_o)/(F_J\text{-}F_o) = TR_o/RC$) and the slope of the relative variable fluorescence (dVt/dto) of the studied varieties of barley plants. Values are means (SD) calculated from five separate measurements.

	dV_t/dt_o					W_K				
PEG (MPa)	0	-0.5	-1	-1.5	-2	0	-0.5	-1	-1.5	-2
Aït Baha	1.09 (0.04)	1.14 (0.05)	1.18 (0.05)	1.20 (0.03)	1.23 (0.02)	2.94 (0.06)	2.97 (0.07)	3.00 (0.06)	3.03 (0.07)	3.05 (0.04)
Ighrem	1.16 (0.08)	1.14 (0.04)	1.18 (0.02)	1.22 (0.05)	1.25 (0.04)	2.98 (0.18)	3.07 (0.09)	3.04 (0.07)	3.12 (0.07)	3.12 (0.06)
Tarodant	1.18 (0.05)	1.24 (0.08)	1.21 (0.05)	1.23 (0.07)	1.27 (0.15)	3.00 (0.21)	3.00 (0.07)	3.05 (0.09)	3.02 (0.16)	3.10 (0.16)
Arig 8	1.23 (0.07)	1.25 (0.06)	1.30 (0.08)	1.29 (0.08)	1.41 (0.03)	3.03 (0.14)	3.08 (0.10)	3.11 (0.08)	3.11 (0.09)	3.23 (0.06)
Lannaceur	1.13 (0.03)	1.14 (0.02)	1.20 (0.05)	1.22 (0.04)	1.25 (0.02)	3.02 (0.08)	3.00 (0.11)	3.05 (0.11)	3.08 (0.09)	3.12 (0.03)
Rabat 071	1.14 (0.04)	1.20 (0.06)	1.20 (0.03)	1.22 (0.05)	1.23 (0.03)	2.89 (0.07)	3.24 (0.11)	3.30 (0.05)	3.37 (0.17)	3.36 (0.03)

$RC/ABS = [(F_{2ms}\text{-}F_{50\mu s})/4.(F_{300\mu s}\text{-}F_{50\mu s})].\ F_V/F_M$

$\varphi_{Po}=1\text{-}(F_o/F_M)$ is the fraction of excitons trapped per photon absorbed. It corresponds to the maximum quantum yield of primary photochemistry. $\psi_o=1\text{-}V_J$ is the fraction of electrons transported (ET_o) beyond Q_A- per exciton trapped (TR_o) by the reaction centers of PS II. It is the probability that the energy of a trapped exciton is used for electron transport beyond Q_A.

The performance index (PI) is a fluorescence parameter used in revealing differences in response of PS II to stress (Clark et al. 2000; Van Heerden et al. 2004). Under drought stress, the different varieties showed a gradual decrease in the PI relative to the control sample except in Rabat 071 at -0.5 MPa where PI was higher than the control (Fig. 3A). Under severe osmotic stress (-2 MPa), the decrease in PI varied between 86% and 73% relative to the control. Among barley varieties, Aït Baha exhibited a higher performance index. The decrease of PI relative to control was more pronounced in Arig 8, it was 1, 0.99, 0.89, 0.88 and 0.73 respectively at 0 MPa, -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa. In this study, the PEG-6000 effect showed little difference in φ_{Po} (F_V/F_M) to the control. Therefore, there is no loss in the yield of PS II primary photochemistry (Fig. 3A).

With the aim to screen for the tolerance to drought stress induced by PEG-6000 of the studied varieties, the relative PI in low osmotic stress (average of $PI_{-0.5\ MPa}$ and $PI_{-1\ MPa}$) and the relative PI in high osmotic stress (average of $PI_{-1.5\ MPa}$ and $PI_{-2\ MPa}$) were calculated. Fig. 3B, presents the response of the six varieties according to the relative PI at low osmotic stress and the percentage of reduction of PI in high osmotic stress relative to PI in low osmotic stress. The variety Aït Baha, has the same percentage of reduction of PI in low and high osmotic stress (the value were on the line = 1). The reduction percentage was 12%. The varieties Ighrem and Tarodant showed a high reduction of PI relative to the control at low osmotic stress, it was 10 and 7% respectively in Ighrem and Tarodant. In the other hand, Arig 8, Lannaceur and Rabat 071 had a high reduction of PI at high osmotic stress compared to the reduction of PI at low osmotic stress. It was 15, 12 and 13% respectively in Arig 8, Lannaceur and Rabat 071.

Fluorescence parameter dV_t/dt_o

Tab. II shows the variation in the initial slope of the relative variable fluorescence (dV_t/dt_o) for the different varieties of barley under the increase of PEG concentrations. dV_t/dt_o expresses the difference between the maximal rate of reduction of Q_A (TR_o/RC) and that of Q_A- re-oxidation (Strasser et al. 2004). Therefore, dV_t/dt_o indicates the accumulation of Q_A-.

$dQ_A\text{-}/dt_o = (dV_t/dt)_o \cong (\Delta V/\Delta t)_o$
$= \{[\Delta F / (F_M - F_o)] / \Delta t_o\}$
$\cong 4.(F_{300\,\mu s} - F_{50\,\mu s}) / (F_M - F_{50\,\mu s})$

The initial slope of the relative variable fluorescence $(dV/dt)_o$ was also described as

$(dV_t/dt)_o = [d(Q_A\text{-}/ Q_{A\,(total)}) / dt]_o$

Under drought stress induced by PEG, dV_t/dt_o increases and as a consequence the fraction Q_A-$/Q_{A\,(total)}$ increases (Tab. II). Therefore, the maximum

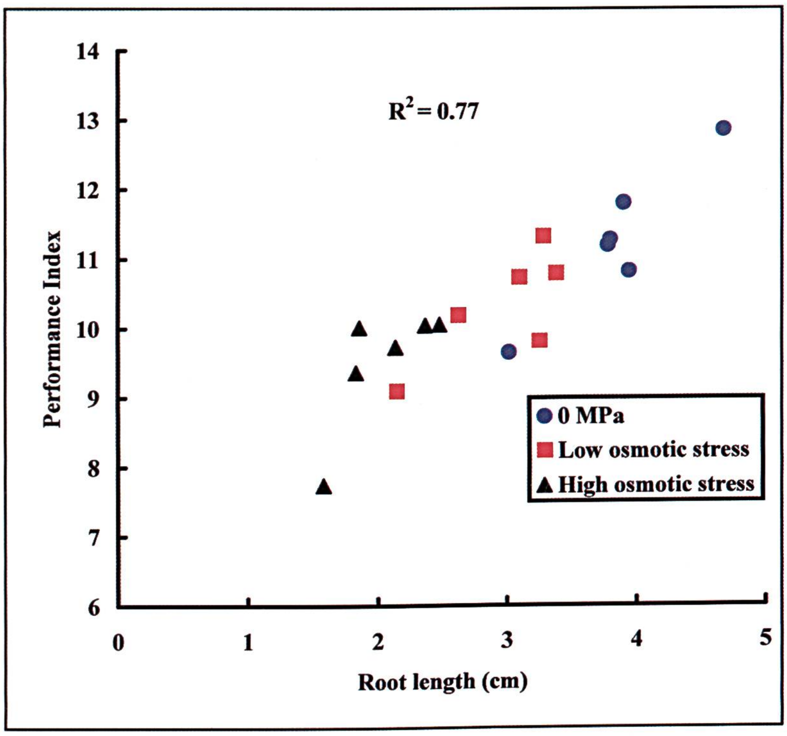

Fig. 4. Relationship between the root length (cm) with performance index of the studied varieties of barley plants under drought stress induced by PEG-6000. The control conditions (0 MPa) designed by circle, low osmotic stress (average of -0.5 and -1 MPa) designed by square and the high osmotic stress (average of -1.5 and -2 MPa) designed by triangle.

rate of the accumulation of the fraction of closed reaction centers increases. Arig 8 has the highest increase of dV_t/dt_0 at different PEG concentrations relative to the control.

Fluorescence parameter W_K

As the PEG-concentration increases, the value of W_K at 300 µs calculated as TR_o/RC by JIP-test increases in all varieties (Tab. II). It can be explained by an imbalance between the electron flow leaving the reaction centers on the acceptor side and the electron flow coming to the reaction centers from the donor side (Strasser 1997). The highest value of W_k was observed in Rabat 071 at different PEG concentrations. In Aït Baha, Ighrem and Tarodant, the PEG treatments had a little effect on W_k.

In order to know the degree of relationship between the fluorescence parameters and root length, Fig. 4 shows the correlation between performance index and root length under control conditions, low osmotic stress (average of the parameters in -0.5 MPa and -1 MPa treatments) and high osmotic stress (average of the parameters in -1.5 MPa and -2 MPa treatments). We could show a high correlation between root length with performance index ($R^2 = 0.77$).

Discussion

The varieties of barley plants were compared with respect to their tolerance to drought stress under controlled conditions during germination, seedling emergence and the early seedling growth stage. The germination process can be defined in terms of three successive steps: water uptake by the seed known as imbibition, followed by the elongation of the embryo, leading to radicle emergence (Bewley 1997). This study shows that polyethylene glycol (PEG-6000) caused a decrease of the germination potential and root elongation. This decrease, as reported by Almansouri et al. (2001), was due to an inhibition of water uptake by the seeds. The final germination decreased on average by 12.2, 21.5, 40.3, and 49% respectively at -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa. The final emergence decreased on average by 18.4, 29, 47, and 58% respectively at -0.5 MPa, -1 MPa, -1.5 MPa and -2 MPa and the root length decreased by 18.8, 28.1, 39.8, and 54.8%. In addition, the results revealed that the studied varieties had very clear differences in germination, emergence and root length in each treatment. Among the studied varieties, Aït Baha showed a higher germination and emergence potential and root prolongation under conditions of osmotic stress.

The phase (J-P) in the shape of the polyphasic fluorescence transients proved to be sensitive to drought stress (appearance of H and G bands). The bands H and G were found by Tsimilli-Michael et al. (1998) in the fluorescence rise of foraminifers at high light intensity. The origin of these two bands is not known. The sequence OKJIHGP expresses the sequence of redox states in the heterogeneous reaction centers of PS II in leaves of barley plants. The different steps of the polyphasic fluorescence transient were labeled in alphabetical order from the slower to the faster part of the transient. The bands K, H and G can often only be distinguished by calculation of derivatives or calculation of differences between fluorescence transients. The appearance of the K band coincides with limitations on the donor side of PS II (Guissé et al. 1995; Srivastava et al. 1997).

Our data show that drought stress induced by PEG-6000 has little effect on the F_V/F_M value as also shown by Lu and Zhang (1999) in wheat plants. The unaffected F_V/F_M confirms the high stability of the quantum yield of primary photochemistry of PS II under drought stress as reported by other authors (Cornic and Fresneau 2002; Tezara et al. 2003). In contrast; as shown in the Fig. 3A the performance index (PI) is a sensitive biophysical parameter to stress whereas the maximum quantum yield of primary photochemistry (F_V/F_M) is not sensitive to drought stress. Similar results reported elsewhere show that PI is a much more sensitive indicator of stress. PI was used to describe the reduction of dark-chilling stress in N_2-fixing soybean genotypes (Van Heerden et al. 2004); to study the effect of chromate during several stages of vegetative growth of *Spirodela polyrhiza* (Susplugas et al. 2000) or the response of Beech to ozone exposure (Clark et al. 2000). The plant performance under drought stress expressed by PI is the result of a combination of three independent expressions, the concentration of reaction centers per chlorophyll, the performance of the light reactions $\varphi_{Po}/(1-\varphi_{Po})$ and the performance of the dark reactions $\psi_o/(1-\psi_o)$. This means that the PI is affected by changes in either antenna properties, trapping efficiency or electron transport beyond Q_A.

The data in Fig. 3B show that the studied varieties can be split in three groups on the basis of the response of their PI to low and high osmotic stress respectively. El Madidi et al. (2005) obtained the same three groups studying the drought stress tolerance of these varieties in field. In Aït Baha the reduction of the PI in response to osmotic stress was independent of the severity of the osmotic stress. The PI of the members of group II: Tarodant and Ighrem were slightly more affected by a low osmotic stress than by a high osmotic stress. Only the varieties of group III showed a reduction of their PI that was dependent on the severity of the osmotic stress. Aït Baha, Tarodant and Ighrem are landraces that were collected in the South of Morocco in a semi-arid environment. Their tolerance to PEG-induced drought stress is in agreement with their ability to grow in an environment where exposure to long periods of drought is common.

The fluorescence parameters provide information about the fundamental biophysical properties of photosynthetic energy conversion and the photosynthetic electron transport under stress conditions. The increase of dV_t/dt_0 could be due to an inhibition of the rate of Q_A- re-oxidation. The appearance of the K band was predominant after a strong heat treatment and it was demonstrated under water stress (De Ronde et al. 2004). We suggest that the appearance of the K band was a criterion for sensitivity of plants to stress and could be considered as indicator for physiological disturbances before appearance of visible stress signs. The high correlation observed between PI and root length suggests the existence of an association between the fluorescence parameters and root length. In a previous study, a high correlation was found between the reduction in PI of beech subjected to ozone exposure and the visual symptoms development and biomass loss (Clark et al. 2000). Sarker et al. (2004) revealed that an imposed periodic drought reduced the root hydraulic conductance and restricted the ability of water translocation through roots to the leaves. The essential function of roots is to supply the shoot with water from the soil. However, the inhibition of water uptake by roots immersed in PEG solutions was followed by a variation in fluorescence parameters. Root length and performance index (PI) could be used as criteria in screening the tolerance of varieties to drought stress in the early plant stages.

The different responses to drought stress of the studied varieties can be due to different capacities for water acquisition and it might be the result of adaptive responses to the different environments. El Madidi et al. (2005) reported the existence of variability in tolerance to drought stress among Moroccan barley landraces collected in south Morocco based on agro-morphological characteristics. The landraces varieties e.g. Aït Baha offer a tool for the selection of tolerant varieties as confirmed by the previouse study (El Madidi et al. 2005) and may be required in breeding programs. However, barley landraces in Morocco are disappearing due to severe drought and desertification. Missions to collect barley varieties are highly recommended.

Drought tolerance in the early seedling stage is not correlated with drought tolerance in later developmental stages. However, it is necessary to measure and analyse the tolerance to drought stress during different stages of plant growth to have an efficient screening.

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