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Chlorophyll Fluorescence as a Possible Tool for Salinity Tolerance Screening in Barley (*Hordeum vulgare* L.)[1]

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The application of chlorophyll fluorescence measurements to screening barley (*Hordeum vulgare* L.) genotypes for salinity tolerance has been investigated. Excised barley leaves were cut under water and incubated with the cut end immersed in water or in a 100-mM NaCl solution, either in the dark or in high light. Changes in rapid fluorescence kinetics occurred in excised barley leaves exposed to the saline solution only when the incubation was carried out in the presence of high light. Fluorescence changes consisted of decreases in the variable to maximum fluorescence ratio and in increases in the relative proportion of variable fluorescence leading to point I in the Kautsky fluorescence induction curve. These relative increases in fluorescence at point I appeared to arise from a delayed plastoquinone reoxidation in the dark, since they disappeared after short, far-red illumination, which is known to excite photosystem I preferentially. We show that a significant correlation existed between some fluorescence parameters, measured after a combined salt and high-light treatment, and other independent measurements of salinity tolerance. These results suggest that chlorophyll fluorescence, and especially the relative fluorescence at point I in the Kautsky fluorescence induction curve, could be used for the screening of barley genotypes for salinity tolerance.

Salinity limits the production of agricultural soils in large areas of the world. In saline areas or areas irrigated with saline waters most crop plant species exhibit marked reductions in yield. For comparative purposes the conductivity leading to 50% decreases in yield (EC_{50}) is often used as an index of salinity tolerance. One of the few commercial crops relatively tolerant to salinity is barley (*Hordeum vulgare* L.); barley genotypes may exhibit EC_{50} of 15 dS m^{-1} or higher (Maas and Hoffman, 1977). Since genetic variability is known to occur in barley (as well as in other species) with respect to salinity tolerance, research programs are being carried out to screen germplasm for salt tolerance and breed more tolerant lines. The classical screening method for salt tolerance is based on the yield responses to salt. However, since screening on the basis of yield is very expensive, breeders are in search of indirect parameters based on the physiological responses of plants to salinity that could be useful for screening. Such parameters should be easy to measure and possible to apply to a large number of plants in a relatively short time.

Salinity causes decreases both in growth and in the net photosynthesis of higher plants (Long and Baker, 1986). This may open the possibility of using photosynthetic parameters in salt-tolerance screening. The rationale for the view that changes in leaf photosynthetic parameters may be used to carry out screening of stress-resistant cultivars is that these parameters would reflect any constraint acting on the photosynthetic processes. Therefore, more stress-tolerant cultivars are expected to exhibit photosynthetic parameters less disturbed in the presence of the stress. Changes in the composition and function of the photosynthetic apparatus of plants in response to salinity have been described in the recent literature. For instance, some changes in the photosynthetic pigment composition, such as decreases in neoxanthin and increases in zeaxanthin at the expense of violaxanthin, have been reported in salt-stressed sorghum (Sharma and Hall, 1992). The kinetics of Chl fluorescence, a tool that monitors the function of the photosynthetic apparatus, has been shown to change in response to water stress and salinity (Havaux et al., 1988; Bongi and Loreto, 1989; Mekkaoui et al., 1989; Monneveux et al., 1990). Chl fluorescence could be an excellent tool for screening since it is easy to measure and may allow for the screening of large numbers of genotypes in a short time. This approach has been used with some apparent success in the last few years in screening several cereal crops for salinity tolerance. For instance, a good correlation between some parameters derived from the Chl

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Abbreviations: dS m^{-1}, deciSiemens per meter, electrical conductivity unit; $EC_{50}Y$ and $EC_{50}GE$, electrical conductivity leading to 50% decreases in yield and germination-emergence, respectively; I, P, S, M, and T, Kautsky notation of the different points in the fluorescence induction curve; F_o, initial Chl fluorescence; F_p, Chl fluorescence intensity at the peak of the fluorescence induction curve; F_v, variable part of Chl fluorescence ($F_p - F_o$); F_i, F_s, and F_t, Chl fluorescence intensity at the I, S, and T points in the fluorescence induction curve, respectively; Ψ_S, osmotic potential; Ψ_W, water potential; Q_A and Q_B, primary and secondary quinone acceptors in PSII, respectively.

fluorescence induction curve from very young plants and salinity tolerance has been reported for at least two cereals, *Triticum durum* and *Triticum aestivum* (Mekkaoui et al., 1989; Monneveux et al., 1990). These experiments were carried out with (a) intact plants exposed to saline solution and (b) leaves from plants not exposed to salinity during growth that were excised and incubated in saline solutions for a short time.

The kinetics of Chl fluorescence from barley plants grown in saline nutrient solutions under relatively low light (approximately one-fifth of full sunlight) showed little change when compared with that of controls (Morales et al., 1992). This supports the view that any possible changes in fluorescence may not result from salinity itself, but from the interaction of salinity and other stressing factors such as high light, which are poorly defined in previous papers reporting fluorescence changes. The aim of this work was to examine in detail the possible changes in Chl fluorescence parameters induced in excised barley leaves by incubation in water or in saline solutions under different illumination conditions. Furthermore, we have investigated the possible causes of these changes in fluorescence. Finally, 15 cultivars of barley, known to differ in their salinity tolerance, have been studied with Chl fluorescence techniques after a combined salt and high-light treatment, and the relationships between fluorescence parameters and some independent measurements of salinity tolerance have been analyzed.

MATERIALS AND METHODS

Plant Culture

Barley (*Hordeum vulgare* L.) seeds were germinated and grown in vermiculite moistened with half-strength Hoagland solution in a growth chamber with a PPFD of 300 μmol m^{-2} s^{-1} at a temperature of 25°C, 80% RH, and a photoperiod of 16/8 h light/dark. For most experiments two barley cultivars were used: Albacete (salt tolerant) and Igri (salt sensitive). In the experiment using 15 cultivars, plants were germinated and grown on vermiculite in a glasshouse. Genotypes used were Albacete, Viva, Pane-1, Pallas, Welam, Mogador, Ibon 3–95, Klaxon, Berta, Tecla, Ibon 3–56, Priver, Flavia, Igri, and Steptoe. Plants were used at the third or fourth leaf stage.

Light and Salt Treatments

Barley leaves were excised under water and placed in vials containing deionized water or NaCl solutions of different concentrations. Leaves were either left in the dark or illuminated with white light (1000 or 2000 μmol m^{-2} s^{-1} through a fiber optic equipped with 1 KG1 and 3 KG3 Schott heat-absorbing filters) for different times. Results similar to those presented here were obtained with halogen lamps fitted with water filters to avoid heating (not shown). This latter setup permitted the illumination of a larger number of leaves simultaneously. For comparison, some barley leaves were left to dehydrate in the air either in the dark or in high light.

Water and Osmotic Potential

The leaf Ψ_W was measured according to Turner (1988) with a pressure chamber (Soil Moisture Co., Santa Barbara, CA). The sap was extracted by pressure from liquid nitrogen-frozen leaf tissue (Higgs and Jones, 1990), and the Ψ_S was measured with a 5500 Wescor vapor pressure osmometer (Wescor, Logan, UT).

Chl Fluorescence Measurements

Chl fluorescence at room temperature was measured as described previously (Morales et al., 1991). Leaves were kept in the dark for 30 min before measurement of Chl fluorescence. A leaf area of 0.18 cm^2 was delimited by a leaf clip (Hansatech, Kings Lynn, UK). Blue light (light from a 150-W tungsten lamp powered with a stabilized power supply and passing through 1 KG1 and 3 KG3 Schott infrared filters plus a 620-nm cut-off filter) was passed through a Copal photographic shutter (opening time 2 ms) and a Schölly fiber-optic guide. Light intensity was 150 μmol photons m^{-2} s^{-1} at the leaf level. Typical signals were similar to those described in a previous paper (Morales et al., 1991). Fluorescence was detected through a 3-mm Schott RG-665 filter and a 680-nm interference filter (10-nm bandpass) with a photodiode (Hansatech) and the signal was fed to a digital storage oscilloscope. For the rapid and slow kinetics, PSII fluorescence was monitored for 2 s and 15 min, respectively. Peak I in the Chl fluorescence induction curve is thought to reflect the balance between the light-induced reduction of Q_A and the simultaneous Q_A reoxidation by oxidized Q_B. The magnitude of peak I depends on the light intensity used in fluorescence measurements, which was kept constant at 150 μmol photons m^{-2} s^{-1} throughout this work. Far-red illumination was obtained from a 150-W tungsten lamp equipped with 1 KG1 and 1 KG3 Schott filters plus an RG-9 or an RG-715 Schott filter. PPFDs transmitted by the RG-9 and the RG-715 filters were estimated to be 3 and 15 μmol photons m^{-2} s^{-1}, respectively (as measured with a quantum sensor from Skye, Powys, UK).

RESULTS

Chl Fluorescence and Water Status of Barley Leaves Growing on Vermiculite

Intact (nonexcised) barley leaves exhibited similar fluorescence transients whether they were illuminated for 2 h (and then dark adapted for 30 min) or dark adapted for several hours. These leaves had F_v/F_p ratios of 0.83 to 0.85, and $(F_i - F_o)/F_v$ ratios of 0.17 to 0.18 (Table I). These values are similar to that found in barley grown in hydroponics (Morales et al., 1992) or in the field (our unpublished data). In the conditions used in this work the $(F_p - F_s)/F_s$ ratio was quite different for the two barley cultivars, 0.17 for Albacete and 0.34 for Igri. The $(F_p - F_t)/F_t$ ratio was approximately 3 for both cultivars. The Ψ_W of these untreated leaves ranged between −0.3 and −0.4 MPa, whereas the Ψ_S was approximately −1.2 and −1.3 MPa for Albacete and Igri, respectively (Table I). Turgor values approached 0.90 MPa.

Chl Fluorescence and Water Status of Excised Barley Leaves Incubated in the Dark

The incubation of excised barley leaves in water in the dark did not change the F_v/F_p or $(F_i - F_o)/F_v$ ratios when

compared with the controls (Table I). The $(F_p - F_s)/F_s$ ratio decreased in both barley cultivars, whereas the $(F_p - F_t)/F_t$ ratio increased in Albacete and decreased in Igri. The Ψ_W of these leaves was close to −0.3 MPa, whereas the Ψ_S was approximately −1.3 MPa (Table I). Turgor values approached 1.0 MPa. When compared with those of controls, these values indicate that leaves may take some additional water during the incubation, thus slightly increasing their turgor, although fluorescence parameters were unchanged.

The incubation of excised barley leaves in a saline solution in the dark did not cause significant changes on the ratio F_v/F_p, which remained close to 0.83 (Table I, Fig. 1). The $(F_i - F_o)/F_v$ ratios were also quite similar to those found in controls (Table I, Fig. 1). The $(F_p - F_s)/F_s$ and $(F_p - F_t)/F_t$ ratios decreased in both barley varieties when compared with the controls. The Ψ_W and Ψ_S of these leaves were in the ranges −0.1 to −0.2 MPa and −1.5 to −1.8 MPa, respectively (Table I). Apparent turgor values reached values from 1.3 to 1.7 MPa. Therefore, incubating the barley leaves in the dark under saline solutions had little effect on the water status of the leaves, as indicated by the Ψ_W values, and did not appear to cause significant changes in the fluorescence parameters examined.

Barley leaves partially dehydrated in air in the dark did not show significant changes in the ratio F_v/F_p, which remained close to 0.83 (Table I). The $(F_i - F_o)/F_v$ ratios increased somewhat, to reach values of 0.19 to 0.21 (Table I). These leaves exhibited Ψ_W and Ψ_S values in the ranges −1.3 to −1.8 MPa and −1.4 to −2.3 MPa, respectively. Turgor was estimated to be approximately 0.2 and 0.5 MPa in Albacete and Igri, respectively. Evidently, considerable losses in turgor did not cause significant changes per se in the fluorescence induction curve of barley leaves.

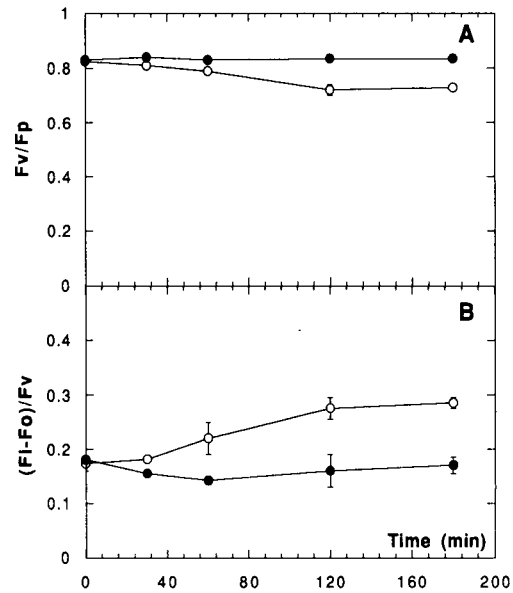

Figure 1. Changes in fluorescence with time of treatment in excised Igri barley leaves with the cut end immersed in 100 mM NaCl. Measurements were made after 30 min of dark adaptation time. ●, Incubation made in the dark; ○, incubation made under a PPFD of 1000 μmol quanta m^{-2} s^{-1}. A, F_v/F_p; B, $(F_i - F_o)/F_v$. Values are the mean ± SE (n = 4).

Chl Fluorescence and Water Status of Excised Barley Leaves Incubated in the Light

When excised barley leaves were incubated in water under an illumination of 1000 μmol m^{-2} s^{-1}, a small decrease in the

Table I. *Changes in fluorescence and water relations caused by different treatments in excised barley leaves*

Intact leaves from plants growing on vermiculite and kept in the light for 2 h were used as controls. Intact leaves kept in the dark for longer times gave similar results. Light treatment was 1000 μmol quanta m^{-2} s^{-1} for 2 h. All leaves were dark adapted for 30 min before measurements were made. Values are the mean ± SE (n = 4).

	Intact Leaves	Excised Leaves					
		Incubation 2 h dark			Incubation 2 h light		
		Water	100 mM NaCl	Air	Water	100 mM NaCl	Air
Albacete							
F_v/F_p	0.85 ± 0.01	0.83 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.80 ± 0.03	0.76 ± 0.02	0.62 ± 0.01
$(F_i - F_o)/F_v$	0.17 ± 0.02	0.15 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.24 ± 0.05	0.19 ± 0.02
$(F_p - F_s)/F_s$	0.17 ± 0.02	0.08 ± 0.02	0.13 ± 0.01	0.35 ± 0.01	0.19 ± 0.01	0.55 ± 0.11	0.08 ± 0.01
$(F_p - F_t)/F_t$	2.89 ± 0.26	3.90 ± 0.10	2.30 ± 0.10	2.60 ± 0.01	2.45 ± 0.01	2.70 ± 0.30	2.80 ± 0.15
Ψ_W (MPa)	−0.28 ± 0.01	−0.31 ± 0.03	−0.13 ± 0.06	−1.28 ± 0.60	−0.25 ± 0.05	−0.21 ± 0.02	−1.55 ± 0.10
Ψ_S (MPa)	−1.20 ± 0.02	−1.34 ± 0.02	−1.80 ± 0.01	−1.43 ± 0.01	−1.47 ± 0.01	−2.07 ± 0.01	−1.29 ± 0.03
Turgor (MPa)	0.92	1.03	1.67	0.15	1.22	1.86	−0.26
Igri							
F_v/F_p	0.83 ± 0.02	0.83 ± 0.01	0.83 ± 0.01	0.81 ± 0.01	0.77 ± 0.03	0.72 ± 0.02	0.62 ± 0.02
$(F_i - F_o)/F_v$	0.18 ± 0.02	0.16 ± 0.01	0.16 ± 0.03	0.21 ± 0.01	0.16 ± 0.02	0.28 ± 0.02	0.27 ± 0.04
$(F_p - F_s)/F_s$	0.34 ± 0.06	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.40 ± 0.01	0.45 ± 0.06	0.06 ± 0.01
$(F_p - F_t)/F_t$	3.06 ± 0.10	2.52 ± 0.30	2.50 ± 0.20	3.35 ± 0.01	2.30 ± 0.01	2.38 ± 0.02	2.45 ± 0.20
Ψ_W (MPa)	−0.42 ± 0.02	−0.26 ± 0.04	−0.23 ± 0.03	−1.75 ± 0.05	−0.30 ± 0.04	−0.26 ± 0.02	−1.86 ± 0.04
Ψ_S (MPa)	−1.27 ± 0.03	−1.28 ± 0.15	−1.54 ± 0.02	−2.27 ± 0.02	−1.52 ± 0.04	−2.63 ± 0.08	−1.69 ± 0.03
Turgor (MPa)	0.85	1.02	1.31	0.52	1.22	2.37	−0.15

F_v/F_p ratio was apparent (Table I). However, the $(F_i - F_o)/F_v$ and $(F_p - F_s)/F_s$ ratios were unchanged when compared with the controls. The $(F_p - F_t)/F_t$ ratio decreased with this treatment in both cultivars. This treatment led to Ψ_W values of −0.3 MPa and Ψ_S values of −1.5 MPa, giving a turgor of 1.2 for both barley cultivars. These values indicate that small changes in fluorescence may arise from incubation of excised leaves in water in the light, although the water status was similar to that of controls.

However, when barley leaves were incubated in 100 mM NaCl and illuminated at 1000 μmol m^{-2} s^{-1}, changes in the rapid Chl fluorescence kinetics were apparent. The ratio F_v/F_p decreased from 0.84 to approximately 0.76 to 0.72 after 2 h of incubation, whereas the ratio $(F_i - F_o)/F_v$ exhibited increases from the control value of approximately 0.18 to values of 0.24 and 0.28 in Albacete and Igri, respectively (Table I, Fig. 1). The changes in these parameters were proportional to the duration of the treatment (Fig. 1) and became larger under higher light intensities (results not shown). These changes are similar to changes reported to occur in other cereals when subjected to salt stress (Mekkaoui et al., 1989; Monneveux et al., 1990). This treatment led to Ψ_W values of −0.2 and −0.3 MPa, Ψ_S values of −2.1 and −2.6 MPa, and turgor estimates of 1.9 and 2.4 MPa for Albacete and Igri, respectively (Table I). The $(F_p - F_s)/F_s$ increased in both cultivars, whereas the $(F_p - F_t)/F_t$ ratio decreased in Igri and did not change in Albacete. From the Ψ_W measurements, these leaves appeared to maintain a good water status. Lower Ψ_S values (leading to increases in estimated turgor) may likely arise from salt uptake into the apoplast that would in turn decrease the Ψ_S in the sap.

For comparison we examined the changes that occurred in barley leaves partially dehydrated in air in the light. This treatment caused major decreases in the ratio F_v/F_p, down to 0.62 (Table I). The $(F_i - F_o)/F_v$ ratios increased to reach values of 0.19 to 0.27 (Table I). The $(F_p - F_s)/F_s$ ratios decreased to almost 0, reflecting the very slow quenching of fluorescence in these leaves during the first seconds of illumination. Leaves were markedly dehydrated, exhibiting low Ψ_W values of −1.6 to −1.9 MPa and Ψ_S values of −1.3 to −1.7 MPa. Turgor was estimated to be between −0.1 and −0.3 MPa, indicating that plasmolysis had occurred.

Origin of Changes in Fluorescence in Excised Barley Leaves Incubated in 100 mM NaCl and High Light

We have investigated the causes of the changes in fluorescence parameters described above by following the changes in the values F_o, F_p, F_v/F_p, and $(F_i - F_o)/F_v$ in a barley leaf during dark adaptation. Fluorescence was measured at different times during dark adaptation with 2-s light pulses (PPFD of 150 μmol m^{-2} s^{-1} of blue light, as described in "Materials and Methods"). The results shown in Figure 2 indicate that fluorescence relaxed in a complex way, most of the decrease in $(F_i - F_o)/F_v$ (and part of the decrease in F_v/F_p) relaxing in approximately 1 h. Therefore, a large part of the change in $(F_i - F_o)/F_v$ is not likely to arise from photoinhibitory damage to the Q_B binding protein. It should be noted that small additional increases in F_v/F_p are also apparent after 1 h, which may be due to protein synthesis.

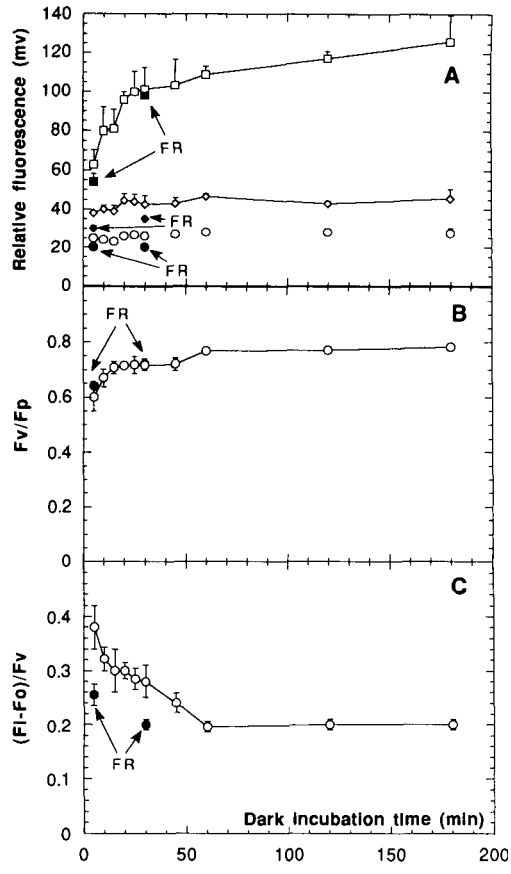

Figure 2. Changes in fluorescence with the time of dark adaptation in Igri barley leaves pretreated with 100 mM NaCl under a PPFD of 1000 μmol quanta m^{-2} s^{-1} for 2 h (open symbols). Arrows and closed symbols indicate the effect of 30 s of far-red light as indicated in "Materials and Methods." A, F_o, F_i, and F_p; B, F_v/F_p; C, $(F_i - F_o)/F_v$. Values are the mean ± SE (n = 4).

Barley leaves treated with salinity and high light were illuminated for 30 s with far-red light (RG-9 or RG-715 Schott filters) prior to the fluorescence measurements. Far-red light preferentially excites PSI and should remove any remaining electrons accumulated in the plastoquinone pool. We found that short illumination with far-red light decreased significantly the fluorescence at point I (Fig. 2A). Far-red illumination induced little change in the F_v/F_p ratio (Fig. 2B). However, far-red light induced significant decreases of the $(F_i - F_o)/F_v$ ratio even after 5 min of dark relaxation; after 30 min of dark relaxation far-red illumination induced values for the $(F_i - F_o)/F_v$ ratio close to those of controls (Fig. 2C). From these data we conclude that the high $(F_i - F_o)/F_v$ ratios in leaves treated with salt and high light appear to be the indirect result of an incomplete reoxidation of the plastoquinone pool in the dark. An incomplete reoxidation of plastoquinone will in turn affect the redox state of Q_A, the first quinone acceptor in the intersystem transport chain, to which fluorescence intensity is directly linked. An incomplete reoxidation of the intersystem electron carrier pool in the dark may arise from effects of the treatment on chlororespiration

(Gibbs et al., 1990) or other oxidative processes; the nature of such processes deserves further investigation.

Correlations between Fluorescence Tests and Tolerance-Related Parameters

Our data show that changes in Chl fluorescence occur in excised barley leaves subjected to specific treatments. However, for these changes to be applicable to practical screening some correlation with independent measures of salinity tolerance must be demonstrated. Some cultivars of barley have been evaluated recently for two different parameters that estimate salinity tolerance, EC_{50}GE and EC_{50}Y (Martínez-Cob et al., 1987; Royo and Aragüés, 1993). The EC_{50}GE is the concentration of salt necessary for 50% reduction in the rate of plants surviving germination and emergence (Martínez-Cob et al., 1987). The EC_{50}Y is the concentration of salt necessary for 50% reduction in yield in field experiments in an artificial salt gradient (Aragüés et al., 1992; Royo and Aragüés, 1993). In these field experiments the component of germination-emergence was removed by germinating the plants with no salt load in the soil.

The fourth leaf of 15 barley cultivars differing in salt tolerance was excised, incubated in 100 mM NaCl for 2 h under an illumination of 1000 μmol m^{-2} s^{-1} for 2 h, and dark-adapted for 30 min before measurement of fluorescence. The tolerance of the same cultivars to salt was estimated elsewhere by the two different methods described above (Martínez-Cob et al., 1987; Royo and Aragüés, 1993). The EC_{50}GE and EC_{50}Y of these 15 cultivars were in the range 19 to 46 dS m^{-1} and 12 to 23 dS m^{-1}, respectively. We found some correlation between the EC_{50}GE and some fluorescence parameters such as the F_v/F_p ratio ($r = 0.52$, $P < 0.05$), and especially with the parameters derived from point I in the fluorescence induction curve, the $(F_p - F_i)/F_i$ ratio ($r = 0.66$, $P < 0.01$) and the $(F_i - F_o)/F_v$ ratio ($r = 0.75$, $P < 0.01$) (Table II, Fig. 3). Only one parameter was correlated with the EC_{50}Y, the $(F_i - F_o)/F_v$ ratio ($r = 0.45$, $P < 0.10$) (Table II).

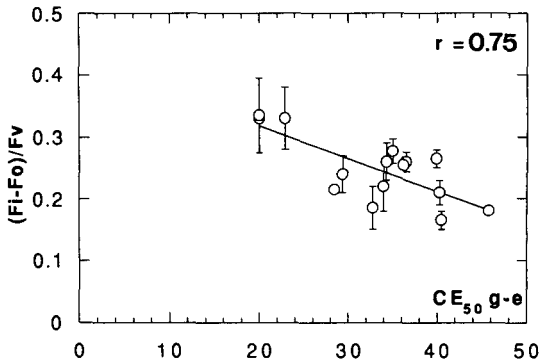

Figure 3. The ratio $(F_i - F_o)/F_v$ after a combined salt and high-light treatment versus the EC_{50}GE in 15 barley genotypes differing in salt tolerance. Fluorescence values are the mean ± SE ($n = 3$).

DISCUSSION

Chl fluorescence is one of the few physiological parameters that have been shown to correlate with salinity tolerance (Mekkaoui et al., 1989; Monneveux et al., 1990). The main objective of this work was to analyze in detail the possible changes in the Chl fluorescence from excised barley leaves caused by incubation in saline solutions. Our data indicate that excised barley leaves incubated in darkness, either in water or in saline solutions, exhibited no significant changes in their Chl fluorescence induction curves. Incubation in water under high light caused only very small decreases in the ratio F_v/F_p. However, incubation in saline solutions under high light led to significant changes in the rapid kinetics of the Chl fluorescence induction curve from barley leaves. The changes observed were small but significant decreases in the F_v/F_p ratio and significant increases in the $(F_i - F_o)/F_v$ ratio. These results suggest that fluorescence changes recently reported for other cereal species (Mekkaoui et al., 1989; Monneveux et al., 1990) may arise not from salinity itself but rather from the interaction of salinity and high light.

Our data indicate that the changes in the rapid Chl fluorescence arise specifically from the interaction between high light and salinity and are not a consequence of an imbalance in the water relations caused by salinity. Leaves subjected to the combined effects of salinity and light maintained a good water status. On the other hand, changes in fluorescence after severe dehydration in the dark shown here were quite different from those caused by salinity. The changes in fluorescence found in barley leaves dehydrated in the light are in good agreement with data found for other species, indicating that when plant leaves are subjected to sudden water stress the slow Chl fluorescence kinetics are markedly affected (Havaux et al., 1988; Ogren, 1990).

The apparent decrease in photochemical efficiency and the increase of fluorescence at point I in fluorescence induction

Table II. *Correlation coefficients between fluorescence parameters and other independent estimates of salinity tolerance in barley*

Excised barley leaves were incubated in 100 mM NaCl with a PPFD of 1000 μmol quanta m^{-2} s^{-1} for 2 h, and fluorescence measurements were made after 30 min of dark adaptation. Values for EC_{50}GE and EC_{50}Y of 15 barley cultivars were taken from Martínez-Cob et al. (1987) and Royo and Aragüés (1993), respectively.

	F_v/F_p	$(F_p - F_i)/F_i$	$(F_i - F_o)/F_v$	$(F_p - F_s)/F_s$	$(F_p - F_t)/F_t$
EC_{50}GE	0.52[a]	0.66[b]	0.75[b]	0.20 ns[c]	0.37 ns
EC_{50}Y	0.22 ns	0.20 ns	0.45 ns	0.36 ns	0.20 ns

[a] $P < 0.05$. [b] $P < 0.01$. [c] ns, Not significant at the $P = 0.05$ probability level.

curve observed in excised barley leaves under combined salt and high light treatment could be caused by different factors. The increase in fluorescence at point I has been suggested to arise from the presence of PSII centers unable to reduce Q_B (Cao and Govindjee, 1990; Krause and Weis, 1991). However, an incomplete reoxidation of the plastoquinone pool during the 30-min dark period before measurements were made could have led to an impairment of the electron transport after Q_A during the first milliseconds of illumination, therefore inducing increases in peak I. In excised leaves treated with salt and high light the relative increases in fluorescence at point I disappeared after a 1-h dark incubation time. Furthermore, we have tested the effect of far-red light on the $(F_i - F_o)/F_v$ ratio and found that short illuminations with far-red light (which excites PSI preferentially) considerably decreased the fluorescence at point I. From these data we conclude that the high $(F_i - F_o)/F_v$ ratios in salt-treated leaves were due to an incomplete reoxidation of the plastoquinone pool in the dark. An incomplete plastoquinone reoxidation in the dark has been reported previously for other plant species (Groom et al., 1992).

The changes in the fluorescence induction curve caused by the combination of high light and salinity were larger in a salt-sensitive than in a salt-tolerant cultivar, suggesting that they could be used to test for salinity tolerance. We have studied the possible usefulness of such a fluorescence test by comparing the fluorescence results with two independent tolerance tests using 15 barley cultivars differing in salinity tolerance. Our results show that there was a significant correlation between the fluorescence parameters related to point I, such as the $(F_i - F_o)/F_v$ ratio, and EC_{50}GE. A weaker correlation was found between the same parameter and EC_{50}Y. These results suggest that tests similar to the one proposed here may be useful in screening programs for salinity tolerance. Changes in Chl fluorescence in response to salinity have been proposed elsewhere for screening genotypes for salinity tolerance in other cereal species (Mekkaoui et al., 1989; Monneveux et al., 1990). However, in these previous reports no information on the light conditions during the salinity treatment was given. Therefore, in view of our results we suggest that these tests should be reexamined to investigate the possible interaction between light and salinity.

A major strength of this type of screening method is that genotypes to screen may be germinated and grown under nonsaline conditions in the greenhouse, salt being applied to excised leaves only at the time of light treatment. The fluorescence measurements can be carried out in approximately 2 s after dark adaptation, making this method applicable to a large number of genotypes. It is obvious that some changes could be made in the screening test proposed here. For instance, one may use different times of incubation, different salt concentrations, and different light intensities than those used here, provided the association between salinity and high light is maintained. In barley we have tested times of incubation of up to 3 h, salinities from 50 to 250 mM NaCl, and PPFDs of 1000 and 2000 μmol m^{-2} s^{-1}, and found results similar to those presented in this work (Belkhodja, 1993). Other possible changes include the light intensity used to measure fluorescence, because the relative intensity of peak I depends on this parameter.

At present we can only speculate on the underlying reason for the empirical correlation found between photosynthetic parameters after a treatment including sudden exposure to salinity and high light and the EC_{50}GE. The reason for this correlation is not apparent since the EC_{50}GE is estimated under a dark-only treatment, and changes in fluorescence occur only after a combined salt and high-light treatment. However, common points between these two experimental protocols are that in both cases plant material is used at very early developmental stages and that plant material is suddenly exposed to the stress. When older leaves are exposed to similar treatments the differences between genotypes disappear (results not shown). It is possible that older leaves may develop different mechanisms of tolerance than those exhibited by less-developed plant tissue.

In conclusion, we have shown that changes in fluorescence occur in excised barley leaves exposed to saline solutions only in the presence of high light. These changes in fluorescence seem to arise from a delayed plastoquinone reoxidation in the dark. Also, we have shown that a significant correlation exists between some fluorescence parameters and independent measurements of salinity tolerance. These results suggest that Chl fluorescence could be used as a tool for the screening of cultivars for salinity tolerance.

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