Effects of salinity on chlorophyll fluorescence and photosynthesis of barley (*Hordeum vulgare* L.) grown under a triple-line-source sprinkler system in the field

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Abstract

In flag leaves of four cultivars of barley (*Hordeum vulgare* L.) grown in the field under a triple-line-source sprinkler system, that produces a linear soil salinity gradient, a decrease in net carbon dioxide assimilation rate (P_N) and stomatal conductance for water vapour (g_s) was found. These changes were related to salinity tolerance at moderate salinity. With increasing salinity, P_N was saturated at low irradiances and stomatal frequencies increased. A decrease in photosystem 2 (PS2) efficiency was not found in the field after dark adaptation even at high salinity. Salinity induced only small decreases in the actual PS2 efficiency at midday steady-state photosynthesis, indicating that the photosynthetic electron transport was little

affected by salinity. Therefore, using PS2 efficiency estimates in attached leaves is probably not a useful tool to screen barley genotypes grown under saline conditions in the field for salinity tolerance. In contrast, excised flag leaves from high salinity plots, once in the laboratory, exhibited a decrease in the variable to maximum chlorophyll fluorescence ratio as compared to excised leaves from control plants. On the other hand, the P_N rate might allow for a good discrimination between tolerant and non-tolerant cultivars.

Additional key words: chlorophyll fluorescence; cultivar differences; photosystem 2 efficiency; stomatal conductance and frequency; transpiration rate.

Introduction

Salinity is a major problem in today's irrigated agriculture, since millions of tons of salt, dissolved in the irrigation water, are added annually to cultivated soils (Kingsbury *et al.* 1984). Modern agriculture management practices often worsen the extent of salinity by remobilising salts from deep soil layers.

The decline in productivity observed for many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity (Long and Baker 1986). The reduction in photosynthetic capacity with salt stress is associated with a decreased stomatal conductance (Downton 1977, Walker *et al.* 1981, Seemann and Critchley 1985, Brugnoli and Lauteri 1991, Rajasekaran *et al.* 1997, Sreenivasulu Reddy *et al.* 1998). However, salinity inhibits non-stomatal processes (Gale *et al.* 1967, Seemann and Critchley 1985, Bongi and Loreto 1989, Ziska *et al.* 1990, Sreenivasulu Reddy *et al.* 1998). Heterogeneity in stomatal aperture may complicate, in some cases, the interpretation of gas exchange data (Brugnoli and Lauteri 1991). A decrease in photosynthesis could otherwise be an indirect consequence of the impaired physiology of the plant growing under salt stress. For instance, Munns (1993) suggested that salts taken up by plants may not directly control their growth by affecting turgor, photosynthesis, or enzyme activities; rather, the build-up of salt in old leaves may hasten leaf death. This may in turn affect the supply of assimilates or hormones to the growing regions, thereby affecting growth.

In any case, changes in photosynthetic parameters could potentially be used as a screening method for salinity tolerance in plants, because the more tolerant cultivars are expected to exhibit fewer disturbances in the photosynthetic processes when growing under salinity. In this context, it may be important to conduct experiments under field conditions, because there is some indication that salt stress may be higher in the presence of high photosynthetic photon flux densities (PPFDs). For instance, the decreases in P_N and g_s with salinity in cowpea were more evident at high than low PPFD (Plaut *et al.* 1990).

A simple, non-intrusive way to monitor the performance of the photosynthetic apparatus is to measure Chl fluorescence. These measurements have been proposed for screening salt tolerance in different crop species (Smillie and Nott 1982, Havaux *et al.* 1988, Mekkaoui *et al.* 1989, Monneveux *et al.* 1990). A significant correlation between Chl fluorescence parameters from excised leaves fed with saline solution at high PPFD and the resistance to salinity at the germination-emergency stage has been

found in several barley genotypes (Belkhodja *et al.* 1994) and also in rice (Tiwari *et al.* 1997).

Barley is one of the few economically important crops relatively tolerant to salinity (Maas and Hoffman 1977). Some authors have measured the effects of salinity on several aspects of barley photosynthesis (Rawson 1986, Rawson *et al.* 1988, Sharma and Hall 1991, Dunn and Neales 1993, Maslenkova *et al.* 1993, Shen *et al.* 1994). Since genetic variability occurs in barley with respect to salinity tolerance (Royo and Aragüés 1991, 1993), research programs are being developed to screen germplasm for salt tolerance and to breed more tolerant lines. The aim of this work was to investigate whether cultivars of barley known to differ in salinity tolerance show differences in photosynthetic parameters when grown under salinity. We have used a salinity gradient, induced and maintained by a triple-line-source sprinkler system in field conditions. Four genotypes were tested: Albacete, one of the most tolerant cultivars under field conditions, and Igri, Mogador, and Dacil, considered as having less tolerance to salinity (Royo and Aragüés 1991, 1993).

Materials and methods

Plants: Barley (*Hordeum vulgare* L. cvs. Albacete, Igri, Dacil, and Mogador) was grown in the field on a Typic xerofluvent soil with a silty-clay-loam texture, under a triple-line-source (TLS) sprinkler system. The TLS consists of three parallel sprinkler lines, with a lateral spacing equal to the sprinkler's wetted radius (Aragüés *et al.* 1992), and was designed and run by the Soils and Irrigation Unit of the Servicio de Investigación Agroalimentaria of the Diputación General de Aragón. The TLS was located in the central part of the Ebro river basin (0°49'W, 41°44'N).

A stock saline solution was made by adding NaCl and $CaCl_2 \times 2\ H_2O$ (1:1, m:m) to water from the irrigation supply [2 dS m^{-1}, where Siemens (S) is the international electromagnetic unit for conductance] until complete dissolution, resulting in a final electrical conductivity (EC) of approximately 19 dS m^{-1} (Aragüés *et al.* 1992). Plants in the TLS received irrigation water from the saline stock (central sprinkler line) and also from non-saline water (two lateral sprinkler lines). This system results in an uniform water distribution and a linear soil salinity gradient from the central part of the TLS (highest salinity) to both laterals (lower salinity). Irrigation water salinity was measured by using section cups, and soil salinity at different locations and depths was monitored with salinity sensors (*Soil Moisture*, Santa Barbara, CA, USA).

Ten individual salinity treatments (plots 1.25×1.25 m) were made. Pre-wetting and post-washing were made with non-saline water to minimise foliar damage by salts (Aragüés *et al.* 1994, Benes *et al.* 1996). Experiments were done in four crop seasons, from 1990-1991 through 1993-1994. The salinity of the irrigation water received by plants with this system ranged between 2 and 18 dS m^{-1} (Isla *et al.* 1997). Most of the values presented in this work are from treatments corresponding to irrigation water salinity of approximately 3, 8, and 15 dS m^{-1}. These treatments led to soil salinity (electromagnetic sensor readings) of approximately 1.0, 1.4, and 2.0 dS m^{-1}, respectively (treatments 1, 4, and 8 in Isla *et al.* 1997). The cvs. Albacete,

Igri, and Dacil were used, except for the season 1992-1993, where the cv. Dacil was replaced by the cv. Mogador.

Gas exchange measurements were made on attached flag leaves in the field with a portable gas exchange system (*Li-Cor LI-6200*, Lincoln, NE, USA), using a 250 cm^3 chamber in the closed circuit mode. Leaf and chamber air temperature, humidity, PPFD, transpiration rate (E), and P_N were recorded during the measurements. g_s was calculated by using a boundary layer resistance value obtained previously with wet filter paper pieces of a similar size and shape to the leaves used. Leaf area was measured with a *Delta T* meter (Powys, UK). All measurements were taken on fully expanded attached flag leaves (at the beginning of April) on sunny days with a PPFD (300-800 nm) of 1600-2000 $\mu mol(photon)$ m^{-2} s^{-1}. The instantaneous transpiration efficiency was calculated as the ratio P_N/E. Photosynthesis PPFD response curves were obtained from attached leaves at atmospheric CO_2 partial pressure with PPFDs from 200 to 1800 $\mu mol(photon)$ m^{-2} s^{-1}, using neutral mesh filters. For each irradiance, leaves were allowed to adapt for 30 min prior to measurements.

Stomatal frequencies were counted in replicas of the leaf surfaces by using *Porvil L* (*Bayer*, Germany), obtained by mixing the base material and the catalyzer (1:1, v:v) for 30 s. The *Porvil* replica was then coated with a 10 % collodion solution, and after drying, the coating was peeled off and the number of stomata counted with a light microscope. Stomatal frequencies per area presented are the averages of three or four counts per treatment.

Chlorophyll (Chl) fluorescence was measured at anthesis in flag leaves. The PS2 Chl fluorescence induction kinetics were measured in attached, 30 min dark-adapted leaves, with a portable chlorophyll fluorometer (*PEA*, Plant Efficiency Analyser, *Hansatech*, Kings Lynn, UK). These measurements were made at dawn and midday. This apparatus uses modulated red radiation from a photodiode emitter unit. F_0, F_m, and F_v were measured (for nomenclature see van Kooten and Snel 1990). PPFD used was 1000 $\mu mol(photon)$ m^{-2} s^{-1}. This irradiance produced maximum F_v/F_m ratios.

Room temperature continuous Chl fluorescence was measured in excised leaves in a darkroom as described in Belkhodja *et al.* (1994). Measurements were made on flag leaves collected at midday, when irradiated by full sunlight. Barley leaves were cut under water, transported rapidly under a dark cloth to the laboratory, and kept in a darkroom for 30 min at approximately 25 °C before Chl fluorescence measurements were made. A leaf area of 0.18 cm^2 was delimited by a leaf clip (*Hansatech*, Kings Lynn, UK). Blue radiation (from a 150-W tungsten lamp powered with a stabilised power supply and passing through one *KG1* and three *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* fibre-optic guide. Irradiance was 150 $\mu mol(photon)$ m^{-2} s^{-1} at the leaf surface. This intensity produced maximum F_v/F_p ratios in salt-stressed and control barley (Morales *et al.* 1992). Chl fluorescence was detected through a 3 mm *Schott RG-665* filter and a 680 nm interference filter (10 nm bandpass) with a photodiode (*Hansatech*, Kings Lynn, UK), and the signal was fed to a digital storage oscilloscope. Fluorescence was monitored for 2 s, and parameters measured were F_0, F_i, F_p, and F_v.

Modulated Chl fluorescence measurements were made in attached leaves in the field in 1994 with a *PAM 2000* fluorometer (*Walz*, Effeltrich, Germany). F_0 was measured by switching on the modulated radiation at 600 Hz; PPFD was less than 0.1 μmol(photon) m^{-2} s^{-1} at the leaf surface. F_m was measured at 20 kHz with a 1 s pulse of 6000 μmol(photon) m^{-2} s^{-1} of "white light". The experimental protocol for the analysis of the Chl fluorescence quenching was essentially as described by Genty *et al.* (1989). F_0 and F_m were measured before dawn. Fluorescence quenchings were measured at the same time than the photosynthesis:PPFD response curves and with the same protocol. The F_s and F_m' values were measured first at full sunlight. Then, irradiance was decreased stepwise. After 30 min of adaptation to the new irradiance, the F_s and F_m' values were measured again in the same leaf area. The actual PS2 efficiency (quantum yield of PS2 electron transport, Φ_{PS2}; Schreiber *et al.* 1995) was calculated as $(F_m'-F_s)/F_m'$ (Genty *et al.* 1989, Harbinson *et al.* 1989). The non-photochemical quenching (NPQ) was calculated as $(F_m/F_m') - 1$, according to Bilger and Björkman (1990).

Results

Salinity effects on barley gas exchange: P_N of barley flag leaves was lower in plants grown under salinity than in the controls (Fig. 1*A*). The decrease in P_N from control (3 dS m^{-1}) to the highest irrigation water salinity (15 dS m^{-1}) ranged from 34 to 41 % in the three cultivars of barley. Genotypic variation was apparent in the moderate water salinity treatment (8 dS m^{-1}). At this salinity, P_N was not affected when compared to the controls in cv. Albacete, showed a small, non-significant reduction in cv. Dacil (10 %), and decreased significantly ($p<0.01$) by 33 % in cv. Igri. This degree of irrigation water salinity (8 dS m^{-1}) is commonly found in field conditions in the area.

The low P_N values in leaves of plants grown under salinity was accompanied by low g_s (Fig. 1*B*). The reduction in g_s from the control to the highest irrigation water salinity was 63, 67, and 70 %, for cvs. Albacete, Dacil, and Igri, respectively. A linear relationship between P_N and g_s was found for the three genotypes ($r = 0.99$, $p<0.05$, for all cvs., Fig. 2). However, P_N for a similar g_s value was always higher in Albacete than in Igri.

E decreased in response to increasing salinity in the irrigation water (Table 1). From 3 to 15 dS m^{-1} the decreases in E were 50, 43, and 55 % in cvs. Albacete, Dacil, and Igri, respectively. The instantaneous transpiration efficiency, P_N/E, was generally higher in response to salinity (Table 1). The cv. Albacete showed the maximum P_N/E values, both for moderate and high salinity.

The P_N *versus* PPFD curves were markedly different in control and salinity-affected Albacete barley (Fig. 3). The maximum P_N was markedly lower in plants grown under salinity than in the controls. Control leaves showed an almost two-fold increase in P_N, from 14 to 27 μmol(CO$_2$) m^{-2} s^{-1}, when PPFD changed from 300 to 1700 μmol(photon) m^{-2} s^{-1}. However, in the highest salinity treatment the maximum P_N, 12 μmol(CO$_2$) m^{-2} s^{-1}, was only 33 % higher than the values obtained at low PPFD. Values in Fig. 3 suggest that saturation irradiance was reduced by salinity.
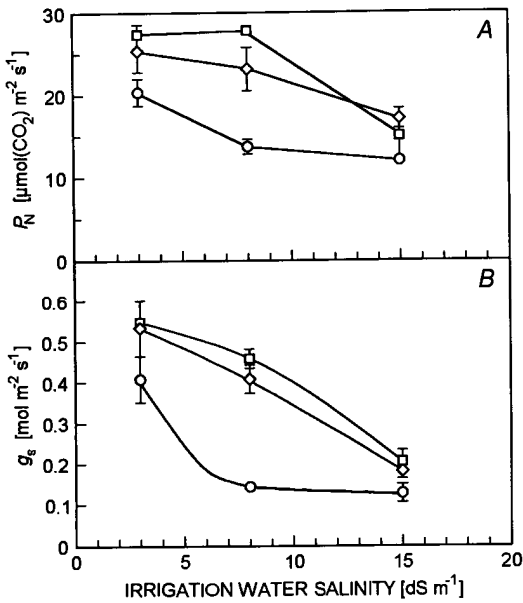
Fig. 1. Net photosynthetic rate, P_N (A) and stomatal conductance, g_s (B) in attached flag leaves of barley cultivars Albacete (□), Igri (○), and Dacil (◊) in the field *vs.* irrigation water salinity in 1994. Means ± SE of three replications. In 1994 the cv. Mogador was not grown.

Table 1. Gas exchange parameters (*E*, transpiration rates, and P_N/E, instantaneous transpiration efficiency, where P_N is net photosynthetic rate) in attached barley flag leaves in the field *vs.* irrigation water salinity. Values are the means ± SD of three replications.

| Genotype | Parameter | Irrigation water salinity [dS m⁻¹] | | |
		3	8	15
Albacete	E	6.38 ± 0.16	6.13 ± 0.36	3.24 ± 0.31
	P_N/E	4.29 ± 0.13	4.53 ± 0.32	5.33 ± 0.52
Dacil	E	5.72 ± 0.65	5.99 ± 0.53	3.29 ± 0.19
	P_N/E	4.48 ± 0.29	3.81 ± 0.24	5.12 ± 0.32
Igri	E	6.21 ± 0.72	3.26 ± 0.14	2.81 ± 0.44
	P_N/E	3.30 ± 0.19	4.14 ± 0.14	4.26 ± 0.62

Salinity effects on stomatal frequency: The three cultivars showed an increase in stomatal frequency in response to salinity (Fig. 4). Flag leaf size was significantly lower in plants grown under salinity than in the controls (values not shown). At high salinity the cv. Dacil had a higher stomatal frequency than cvs. Igri and Albacete. Controls of the three cultivars had approximately 60-70 stomata mm⁻², while salinity-affected leaves (15 dS m⁻¹) of Dacil had 110 stomata mm⁻². A negative relationship between flag leaf area and stomatal frequency was found (values not shown).
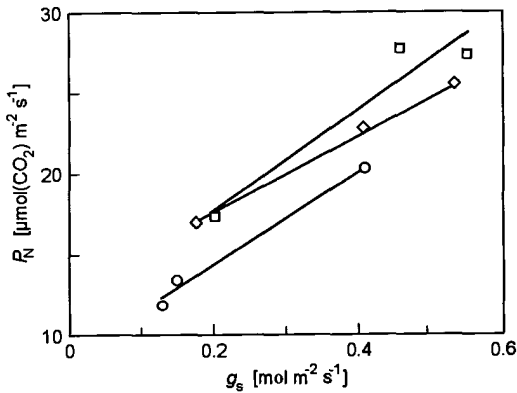
Fig. 2. Relationship between net photosynthetic rate, P_N, and stomatal conductance, g_s, in the three barley cultivars in 1994. Means ± SE of three replications. Symbols as in Fig. 1.
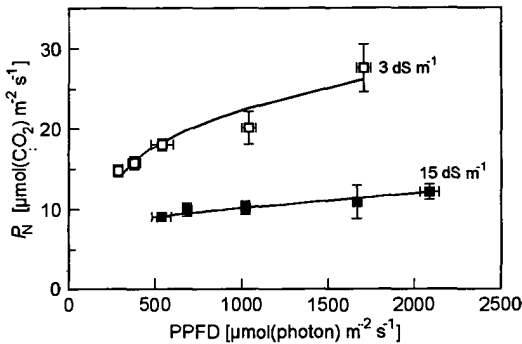

Fig. 3. Changes in leaf net photosynthetic rate (P_N) in attached Albacete barley flag leaves in the field in response to photosynthetic photon flux density (PPFD) at two different salinity levels in 1994. Means ± SE of three replications.

Salinity effects on the Chl fluorescence induction curve: In attached flag leaves the F_v/F_m ratio, obtained with the *PEA* apparatus in the field in 1992, was unaffected by salinity (Fig. 5A). This occurred in the three genotypes tested, both when measurements were made early in the morning (06:00 h solar time) and at midday. However, the F_v/F_m values were significantly lower $(p<0.01)$ at midday (*open symbols*) than in the early morning (*solid symbols*). These slightly lower midday F_v/F_m values were also found in 1993 and 1994 (values not shown).

In contrast, when excised barley leaves sampled at midday were analysed in the laboratory they showed, in most cases, significant $(p<0.01)$ decreases in the F_v/F_p ratios with salinity (Fig. 5B). These leaves also showed increases in the $(F_i-F_0)/F_v$ ratio at high salinity, with all cultivars behaving similarly (not shown). The F_v/F_p values decreased from control values of 0.8 to approximately 0.6-0.7 in the salinity-treated plants. The $(F_i-F_0)/F_v$ ratios were 0.2 at low salinity and increased significantly to approximately 0.3-0.4 in all cultivars with high salinity (not shown).

Salinity effects on the actual PS2 efficiency and non-photochemical quenching: The actual efficiency of PS2 (Φ_{PS2}) at 300-550 μmol(photon) m^{-2} s^{-1} was significantly lower ($p<0.05$) in leaves from Albacete barley grown under salinity than in the controls (Fig. 6*A*). At higher irradiances there was no difference in Φ_{PS2} between salt-stressed and control leaves. At PPFDs close to those found under natural conditions for growth [1600 μmol(photon) m^{-2} s^{-1}], Φ_{PS2} values were only slightly lower at high than at low salinity. The NPQ values increased in response to salinity and to PPFD (Fig. 6*B*). At low PPFDs [500 μmol(photon) m^{-2} s^{-1}] NPQ was very low in the controls but was significantly larger in the high salinity treatment. This agrees with the low PPFD needed for saturation in the salinity affected leaves (Fig. 3). As in the case of Φ_{PS2} the changes in NPQ with salinity were relatively larger at low than at high PPFDs.
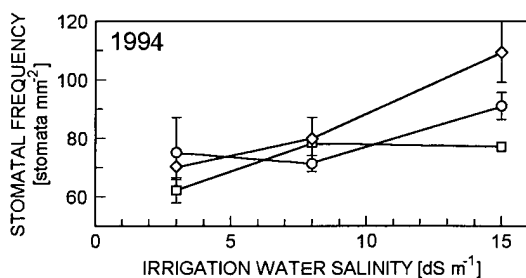

Fig. 4. Changes in the stomatal frequency in attached barley flag leaves in the field *vs*. irrigation water salinity in 1994. Means ± SE of three replications. Symbols as in Fig. 1.

Discussion

When barley was grown under salinity in a TLS sprinkler system there was a marked decrease in crop yield (Royo and Aragüés 1993) and in the size and fresh and dry mass of the flag leaves (unpublished). Flag leaf P_N was markedly decreased by salinity. This was accompanied by decreases in g_s. These general trends were similar for all barley cultivars, although at moderate irrigation water salinity (8 dS m^{-1}) the photosynthetic parameters were less affected in Albacete than in the other cultivars. The highly significant correlation between P_N and g_s may suggest that reductions in CO_2 assimilation rate were largely accounted by stomatal closure, and therefore stomatal effects could be the most important ones to justify photosynthesis depression. Although stomatal closure in response to salinity occurs in barley (Sharma and Hall 1991, Shen *et al.* 1994), stomatal control of P_N may become progressively less effective as stress intensifies (see Tezara and Lawlor 1995 for water stress). For instance, the down-regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity could cause an important depression in P_N under water stress (Medrano *et al.* 1998). RuBPCO activity decreases in salt-treated barley (Shen *et al.* 1994).
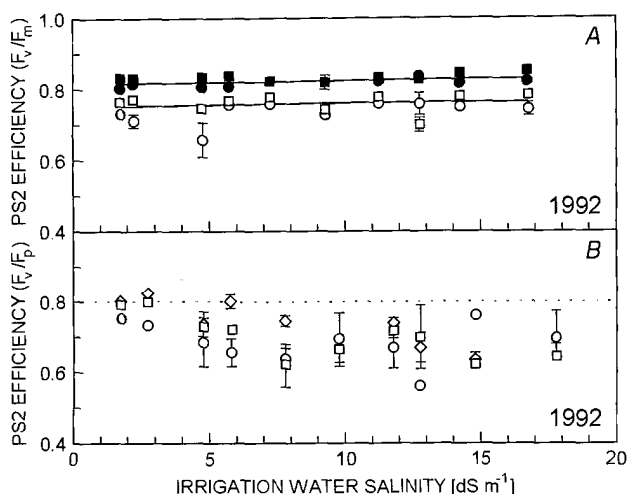
Fig. 5. Photosystem 2 efficiency (A: F_v/F_m; B: F_v/F_p) in attached (A) or excised (B) flag leaves of barley cultivars Albacete (□), Igri (○), and Dacil (◊) in the field (A) or in the laboratory (B), at predawn and midday, vs. irrigation water salinity in 1992. Means ± SE of three replications. In A, predawn data = *solid symbols*; midday data = *open symbols*. Irradiance was 1000 (A) or 150 (B) μmol(photon) m^{-2} s^{-1}. Similar results (not shown) were found in 1993 for Albacete, Igri, and Mogador, and in 1994 for Albacete, Igri, and Dacil.

Salinity also resulted in increased stomatal frequency and a reduction in leaf size. This was possibly due to a greater effect of salinity on cell expansion than on cell division. Bhagwat and Bhatia (1993) found both an increased stomatal frequency and a reduction in leaf size in response to water stress in bread wheat.

Control leaves had P_N *versus* PPFD curves similar to those found in other C_3 species, saturation occurring at high PPFDs. However, in plants grown at high salinity the saturation of P_N occurred in the flag leaves at low PPFDs. Similar results were obtained by Rawson (1986). This cannot be explained by photoinhibition (see below), but could be related to stomatal and non-stomatal factors that may reduce the photosynthetic performance of the leaves in the plants growing under salinity.

High soil salinity induced by the TLS sprinkler system under field conditions did not induce sustained photoinhibition in barley. The pre-dawn PS2 photochemical efficiency (estimated by F_v/F_m) was unaffected by salinity in attached barley leaves growing at high PPFD in the field. These results agree with those found in low PPFD-grown cowpea, wheat, and barley by Larcher et al. (1990), Mishra et al. (1991), and Morales et al. (1992), respectively. Although salinity stress may enhance susceptibility to photoinhibition (Sharma and Hall 1991), in our case the combination of full sunlight and high salinity did not cause photoinhibition in barley. A small decrease in PS2 efficiency was found in the flag leaves at midday, but its extent was similar in salinized and control plots. This may be caused by photosynthesis down-regulation mechanisms that occur under field conditions and do not relax completely after the 30-min dark adaptation protocol used in fluorescence measurements.
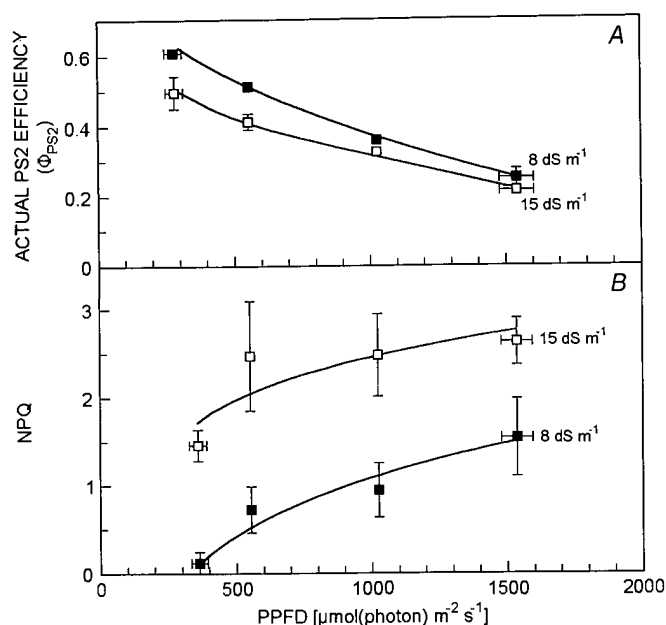
Fig. 6. Actual photosystem 2 efficiency (Φ_{PS2}) and changes in non-photochemical quenching (NPQ), in response to photosynthetic photon flux density (PPFD), in attached Albacete barley flag leaves grown in 1994 at two different salinity levels in the field.

Our results indicate that high salinity induced by the TLS sprinkler system under field conditions caused only slight decreases in the actual efficiency of PS2 (Φ_{PS2}) and increases of NPQ. Since the parameter Φ_{PS2} is equivalent to the fraction of radiant energy absorbed by PS2 that is utilised in PS2 photochemistry, salinity appears to have little effect on PS2 photosynthetic electron transport at steady-state photosynthesis. Since the same leaves had marked reductions in their P_N, there would be a surplus of chemical energy and reducing power, obtained from photosynthetic electron transport, that must be dissipated in processes other than carbon fixation. That barley affected by salinity can dissipate efficiently the excess of energy not used in photosynthesis is shown by the absence of signs of damage, as indicated by the unimpaired photochemical efficiency (Fig. 5) and the practically unchanged photosynthetic pigment composition (Abadía *et al.* 1998). One possibility is that this dissipation could be mediated by Mehler-type reactions, as proposed by Biehler and Fock (1996) for water-stressed wheat. Also, energy could be dissipated in photorespiratory processes or through the malate valve (Fridlyand *et al.* 1998). The increase in NPQ measured in these leaves could be due to the reduction in P_N as well as in saturation PPFD.

Our results indicated that there was a similar response pattern to salinity in the four barley cultivars used, which differ in salinity tolerance. However, the cv. Albacete performed photosynthetically better than the other cultivars at moderate

salinity, in good agreement with the known salt tolerance of this genotype both in the field and in the laboratory (Royo and Aragüés 1991).

One of our aims was to investigate the behaviour of selected photosynthetic parameters in response to salinity, in the search for traits which may be useful to screen germplasm for salt tolerance. We found that the PS2 efficiency estimated by Chl fluorescence in attached barley leaves in the field does not change significantly with salinity. Therefore, using PS2 efficiency estimates in attached leaves is not a useful tool to screen barley genotypes for salinity tolerance. On the other hand, the P_N might allow for a good discrimination between tolerant and non-tolerant cultivars, especially for moderate irrigation water salinities in the field (8 dS m^{-1}).

However, when Chl fluorescence was measured with the laboratory apparatus 30 min after leaf excision, decreases in F_v/F_p and increases in $(F_i-F_0)/F_v$ were found in salt-stressed leaves when compared to the controls. These changes were not found with the *PEA* device in 30 min dark-adapted, attached leaves in the field. We believe that the differences found between attached and excised leaves did not arise from the use of different Chl fluorescence measuring devices, because with both devices irradiances were chosen to obtain maximum F_v/F_p or F_v/F_m ratios. The decreases in F_v/F_p and increases in $(F_i-F_0)/F_v$ found here in excised leaves are similar to those found in barley leaves fed with saline solutions and submitted to high irradiance (Belkhodja *et al.* 1994). Salinity decreases the photochemical efficiency of wheat grown at low PPFDs and later submitted to photoinhibitory PPFDs (Mishra *et al.* 1991). These changes in Chl fluorescence in excised, salt-stressed leaves could be explained by changes in fluorescence quenching. The nature of this quenching deserves further investigation. Decreases in photochemical quenching upon dark adaptation were found in excised leaves fed with saline solutions (Belkhodja *et al.* 1994) and also in iron-deficient leaves (Belkhodja *et al.* 1998). This may be caused by chlororespiratory processes (Corneille *et al.* 1998, Feild *et al.* 1998).

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