FIELD SCREENING OF BARLEY CULTIVARS TO SOIL SALINITY USING A SPRINKLER AND A DRIP IRRIGATION SYSTEM

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Key words: Barley, Drip irrigation, Leaf ion concentration, Salinity tolerance, Triple line source sprinkler irrigation

Abstract

The establishment of proper agronomical practices and plant breeding programs for saline environments is limited by the lack of adequate field screening methods. We assessed the relationships between leaf ion concentration and grain yield in a set of barley cultivars and compared their ranking for salinity tolerance established with a triple-line-source (TLS) sprinkler system, where the absorption of salts is through the leaves and the roots, with that obtained with a drip-irrigation (DI) system, where the absorption of salts is only through the roots. The saline solution in both systems was made up of sodium and hydrated calcium chloride (1:1 w/w). Except for the highest saline treatments, direct leaf absorption of toxic Na^+ and Cl^- was minor or negligible, but it was substantial for Ca^{2+}. Irrespective of barley cultivar and leaf age, the accumulation of Cl^- in the TLS was 1.5 to 2.5 times greater than

Na^+. There was no significant correlation between grain yield and leaf sap ion concentration among eighteen barley cultivars. Thus, leaf ion concentrations should not be used as screening tools in breeding programs for increasing salinity tolerance in barley. The highest-yielding cultivars under non-saline conditions were also most productive under moderately saline conditions, though not under high-saline conditions. Although grain yields of the eighteen barley cultivars in the TLS were substantially lower than in the DI, the salinity tolerances estimated in both systems were significantly correlated ($P<0.05$), indicating that the simple and inexpensive TLS irrigation system could be successfully used in screening for salinity tolerance in barley.

Introduction

Soil salinity affects around 1000 million ha of the world´s cultivated land (Szabolcs, 1989). This area is expected to increase in the future and more effort and economic resources will be needed to cope with the problem, which is especially important in developing countries where yield stability is critical for subsistence of population (Flowers and Yeo, 1995).

One of the principal limitations for the successful screening of crops and cultivars for characterising their salinity tolerance under field conditions is the inherent spatial and temporal variability of soil salinity (Blum, 1988; Noble and Rogers, 1992). Since the extrapolation of results obtained through screening under highly controlled conditions (i.e., greenhouse and hydroponic cultures) to real field conditions has had limited success (Shannon, 1985), new and simpler field screening methods are needed to enable more effective breeding for salinity tolerance (Blum, 1988). These field methods should allow for the evaluation of crops up to the adult stage, since the salinity tolerance at germination and early developmental stages is not necessarily correlated with tolerance at the adult stage (Royo and Aragüés, 1991; Rumbaugh et al., 1993).

Recently, Aragüés at al. (1992) proposed a new field screening method, the Triple Line Source sprinkler system (TLS), for the study of the salinity tolerance of crops that fulfilled most of the requirements (Royo and Aragüés, 1993). However, the system may not be suitable for crops which can absorbs ions easily through their leaves, since wetting by the saline waters may facilitate the absorption of ions through the leaves, as shown by Aragüés et al. (1992), Grattan et al. (1994) and Benes et al. (1996a). Although Aragüés et al. (1994) and Benes et al. (1996b) have demonstrated that short pre- and post irrigations with fresh water minimise this problem in barley and maize, more information is needed to validate the TLS system.

Information concerning the intra-specific variability of the foliar uptake of salts in barley is lacking. Gorham et al. (1994) showed that the mechanisms of foliar and root absorption of salts were not closely related in barley. Two barley cultivars, grown in hydroponics for 14 days, showed different rankings in leaf ion concentrations when absorption took place through the leaves or through the roots. If this result is confirmed under field conditions using a larger set of barley cultivars, grown until maturity, it would imply that the ranking in salinity tolerance established with the TLS system is likely to differ from that obtained in other systems where the foliage is not wetted.

Grattan et al. (1994) studied the chloride accumulation and partitioning in barley as affected by differential root and foliar salt absorption under the TLS system, concluding that most of the Cl^- in young barley leaves (i.e., flag leaves) originated from foliar absorption, whereas the Cl^- in leaves older than the flag leaf-2 originated from root absorption. These findings need to be substantiated in studies where foliar plus root absorption of salts (i.e., the TLS) are compared with studies with root absorption only (i.e., as in a drip irrigation system).

Ion exclusion by roots is generally accepted as a mechanism for salt tolerance in non-halophytes (Greenway and Munns, 1980). However, information concerning the mechanisms

responsible for the greater salt tolerance of barley is still limited (Gorham, 1992) and more work is needed to establish the importance of this mechanism relative to others.

The aim of our work was to elucidate some of these questions by comparing the results obtained with a set of barley cultivars using the TLS system, where the absorption of salts is through the roots and the leaves, with results obtained using a drip irrigation (DI) system, where the absorption of salts is only through the roots. Here, we report the variability in Cl^-, Na^+ and Ca^{2+} concentrations in leaves of six barley cultivars sampled at different ages and grown in ten salinity treatments of the TLS system, and the yield and leaf Cl^-, Na^+, Ca^{2+} and K^+ concentrations of eighteen barley cultivars grown in three salinity treatments of the TLS and the DI systems. On the basis of the results, we assessed possible relationships among leaf ion concentration and grain yield in these barley cultivars and compared the ranking in salinity tolerance of eighteen barley cultivars established with the TLS system with that determined with the DI system.

Materials and Methods

We present results from three field experiments carried out in 1992/93 and 1993/94. Two of these experiments were conducted using a triple line source sprinkler irrigation system (TLS93 and TLS94), and the third using a drip irrigation system (DI94). In the three experiments the usual agricultural practices of the area were used. Before sowing, the field was fertilized with 75, 75 and 75 kg. ha^{-1} of N, P_2O_5 and K_2O respectively. At tillering, 66 kg ha^{-1} of N was given as ammonium nitrate.

Triple Line Source sprinkler irrigation experiments (TLS93 and TLS94)

A TLS system, described in detail by Aragüés et al. (1992), was installed in 1993 and 1994 at the field experimental station of the Agronomic Research Service for Aragón

(Servicio Investigación Agroalimentaria, Zaragoza, Spain) located in the central part of the Ebro river basin (0º49` W, 41º44` N). The soil is a *Typic xerofluvent* with a silty-clay-loam texture. Briefly, the TLS system consists of three parallel sprinkler lines spaced 15 m apart, a distance equivalent to the wetted radius of a sprinkler. Sprinklers along the lateral are spaced 4.5 m apart. A saline solution made up of NaCl and hydrated $CaCl_2$ (1:1 w/w) is injected in the centre line while the outer lines discharge fresh water (EC < 2 dS m^{-1}). This relatively high Ca:Na ratio is consistent with the composition of the saline soils in the area and is needed to minimise the deleterious effect of sodium on the infiltration rate of the soil. The TLS arrangement produces a linear salinity gradient between the centre and the outer laterals while providing an even distribution of water (irrigation uniformity > 90%). Ten individual salinity treatments (as plots 1.25 x 1.25 m) arranged in a strip perpendicular to the laterals are delimited between each lateral pair. In order to minimise salt absorption by leaves, we give short fresh water irrigations before and after each saline irrigation.

Six commercial barley cultivars (Albacete, Barbarrosa, CM-67, Igri, Mogador and Begoña) were grown in the TLS93 experiment to evaluate differences in leaf ion content (Cl^-, Na^+, and Ca^{2+}) measured at various sampling dates in leaves of different ages. Plots of three rows, 1.25 m long, were sown in one strip in each of the ten salinity treatments (S0= non saline, S9= highly saline). In the TLS94 experiment twelve cultivars (Albacete, Alpha, Barbarrosa, Cameo, Criter, Dpche-18, Igri, Kvl-468, Malta, Mogador, Pen and Reinette) were sown in two strips, each one with ten salinity treatments (i.e., each cultivar was sown in twenty salinity treatments as plots of six rows, 1.25 m long), to evaluate differences in leaf ion content (Cl^-, Na^+, Ca^{2+} and K^+) and to establish their salinity-yield response functions. In addition, six Moroccan barley cultivars (Acsad-60, Acsad-176, Aglou, Annoceur, Asni and Merzaga) were sown in six saline treatments (S0, S1, S3, S5, S7, S9) in the TLS94 experiment. Table 1 gives the general characteristics of these field trials. At the end of the

growing season all the experimental plots were mechanically harvested and the grain was collected and weighed. For comparison purposes with the three saline treatments (Control, Medium and High) of the DI system, we obtained the grain yields in the corresponding Control, Medium and High saline treatments of the TLS by averaging the grain yields measured in treatments S0 and S1, S4 and S5, and S8 and S9, respectively.

Drip Irrigation System experiment (DI94)

A drip irrigation system was installed in the 1993/94 growing season close to the TLS94 experiment, but on a sandy-loam soil. The layout of the system consisted of three 3000 L PVC tanks, a pumping system, three 75 mm-diameter polyethylene lines (one for each saline treatment) and 25 mm-diameter laterals with emitters located 0.2 m apart. Each plot was 1.45 m long x 1.25 m wide, and consisted of six rows of plants and three irrigation laterals located 0.42 m apart. Each plot had 23 evenly spaced emitters which delivered a flow of 12 l h^{-1}, giving uniform and complete wetting of the soil. The same eighteen barley cultivars used in the TLS94 were sown in DI94 with three saline treatments (C-control: EC = 1.5 dS m^{-1}; M-medium salinity: EC = 10.1 dS m^{-1}; H-high salinity: EC = 16.7 dS m^{-1}) made up of NaCl and hydrated $CaCl_2$ (1:1 w/w)) and three replications, except for the Moroccan cultivars which only had two replications. The experiment was of a split-plot design with the salinity treatments as the main plots. Table 1 gives the general characteristics of this field trial. At the end of the growing season all the experimental plots were mechanically harvested and the grain was collected and weighed.

Leaf sampling and ion analysis

In the TLS93 experiment two samplings were carried out at the vegetative stage (sampling of the last fully expanded leaf (L6) of the principal tiller) and at the heading stage

(sampling of the flag leaf (FL) as representative of younger tissue, and of the second leaf below the flag leaf (FL-2) as representative of older tissue). In this experiment all the saline treatments (S0 to S9) were sampled from the first replication. In the TLS94 and the DI94 experiments the cultivars were sampled only at heading, taking the first leaf below the flag leaf (FL-1) when the flag leaf was fully expanded. In TLS94 the leaf samples from control (S0), medium (S4) and high saline treatments (S8) were taken from the first replication. In DI94 the leaf samples of the three saline treatments were taken from the first replication.

Three samples of about ten leaves were taken from the principal tillers of each experimental plot in all sampling dates. The leaf samples were collected early in the morning and brought to the laboratory, where they were rinsed for 5 to 10 s in each of 3 trays of distilled water to remove surface salts, blotted dry, placed in 5 ml plastic syringes, and frozen. After thawing, the leaf sap (extracted by applying pressure to the plunger of the syringe) was diluted, added to a dilution of Schinkel buffer solution (10 g l^{-1} CsCl and 100 g l^{-1} $LaCl_3$) and the cations Na^+, Ca^{2+} and K^+ analysed in a Perkin-Elmer model 3030 atomic absorption spectrophotometer. Chloride was measured in a Buchler chloridometer by adding 10 μL of the leaf sap to a dilute acid solution (10% acetic and 0.64% concentrated HNO_3) according to the procedure of Cotlove (1963).

Water and soil analysis

After each irrigation, the volume of water applied by the TLS system was measured in pluviometers installed in each salinity treatment, and its EC determined. Figure 1 shows the time-averaged irrigation water EC measured in TLS 93 and 94. In DI94 the EC of the solutions prepared in each tank were measured. Table 2 presents the average EC values and ionic concentrations of the irrigation waters measured in the saline treatments S0, S4 and S8 of TLS94 and in the C, M and H treatments of DI94.

Soil salinity in the TLS was periodically measured (seven times in 1993 and six times in 1994) in each saline treatment by means of an electromagnetic sensor (model EM-38, Geonics Ltd., Ontario, Canada) placed on the ground in its horizontal-dipole position. The results given in Figure 1 show that the imposed soil salinity gradients were linear. The slopes of the regressions of both soil and irrigation water EC on saline treatment number were not significantly different ($P>0.05$) between years.

Soil samples were taken at various depths of the C, M and H saline treatments in the three experiments to measure the gravimetric soil water content (SWC) and the soil saturation percentage (SP). The mean SWC values were similar and their standard deviations were low (data not shown) due to the high irrigation frequencies used in these experiments. Soil samples were also taken in the three experiments for analysis of EC, Cl^-, Na^+ and Ca^{2+} in the soil saturation extract (data not shown).

Statistical analysis

Analyses of linear regression were performed using the SAS statistical package (SAS Institute Inc., 1988). Comparisons among the regression parameters were made using a F test, taking the root mean error (RME) of the overall regression (all cultivars) as an error term of all pairwise comparisons. The salinity tolerance of the barley cultivars was obtained from the EC_{50} values estimated by fitting the grain yield and ECa observations to the van Genuchten (1983) curvilinear model (TLS experiments), or by calculating the ratios of grain yields measured in the H and the C treatments (DI experiment).

Results and discussion

Leaf age and leaf ion concentrations of six barley cultivars (TLS93 experiment)

The effect of increasing Cl^-, Na^+ and Ca^{2+} concentrations of the irrigation water on the leaf sap Cl^-, Na^+ and Ca^{2+} concentrations of the L6, FL and FL-2 leaves is shown in Figure 2 (linear regressions of the average values of the six barley cultivars) and in Table 3 (linear regressions for each barley cultivar). Leaf ion concentrations increased linearly with an increase in the corresponding irrigation water ion concentrations, as shown by the high and significant ($P<0.01$, except for Igri-FL-Cl^- in Table 3, significant at $P<0.05$) linear correlation coefficients. For a given and similar Cl^- and Na^+ irrigation water concentration, leaf Cl^- was, irrespective of the leaf age, 1.5 to 2.5 times greater than leaf Na^+ (Fig. 2). Similar results were obtained by Maas et al. (1982) and Aragüés et al. (1994), indicating the preferential absorption of Cl^- over Na^+ by barley plants.

The slopes and the intercepts of the Cl^-, Na^+ and Ca^{2+} linear regressions obtained for the flag leaf (FL) of each cultivar were lower than those obtained for the L6 and FL-2 leaves (except in the Ca-intercepts), indicating that increases in leaf ion concentration per unit increase in irrigation water ion concentration were lower in the FL. This lower FL salt accumulation was significant for Cl^- and Na^+ since their average concentrations in the FL were around 2.5 to 3 times lower than the average L6 and FL-2 concentrations at any given irrigation water salinity level (Figure 2). Differences among the FL and FL-2 ion concentrations may be due to the fact that flag leaves have been exposed to salinity for less time than the older FL-2 leaves. However, this argument is not valid when the flag leaves are compared to the similarly young L6 leaves, indicating that the FL is better protected against ion accumulation than other young leaves, similarly to results obtained by Aloy (1995). Grattan et al. (1994) showed ears and peduncles, whose photosynthesis contributes substantially to grain filling in barley, were also able to exclude ions under saline conditions. In contrast, the L6 and FL-2 leaves showed a relatively similar behaviour in their ion accumulation patterns (Figure 2 and Table 3).

For a given leaf, differences in the regression coefficients among the six barley cultivars were in general not significant (P>0.05), indicating that the pattern of leaf salt accumulation was similar among cultivars (Table 3). The only consistent differences were found in the regression coefficients of the FL Cl^-, Na^+ and Ca^{2+}, which were always significantly lower in CM-67 than in Begoña (Table 3). In addition, the CV's of the average intercept and regression coefficient were highest for the FL, indicating that this young tissue is the one that better discriminates differences in ion concentration among the six barley cultivars.

Finally, K^+ measurements on the three leaves indicated that increases in soil salinity did not decrease K^+ in L6 and FL-2, whereas salinity slightly increased K^+ in FL (data not presented). Our results do not corroborate those of Cramer et al. (1991) who found that leaf K^+ concentrations decreased with increases in soil salinity.

Leaf ion concentrations in eighteen barley cultivars (TLS94 and DI94 experiments)

Figure 3 shows the mean leaf sap Cl^-, Na^+, Ca^{2+} and K^+ concentrations of the eighteen barley cultivars grown in three saline treatments of the TLS and DI. We choose the saline treatments 0 (Control), 4 (3 for the Moroccan cultivars) (Medium), and 8 (7 for the Moroccan cultivars) (High) of the TLS because their irrigation water EC values were the closest to those imposed in the C, M and H treatments of the DI (Table 2).

Average leaf sap Cl^- concentrations were significantly lower in the C and M treatments and significantly higher in the H treatment of the TLS. No differences in leaf sap Na^+ were found between the two systems, though the Ca^{2+} concentrations were always higher in the TLS. If the differences obtained between the TLS (root + leaf absorption of ions) and the DI (root absorption of ions) are attributed to direct absorption of salts by leaves wetted in the TLS, these results indicate that leaf salt absorption is minor or negligible for the toxic Na^+

and Cl^- (except in the H treatment) ions but substantial for the Ca^{2+} ion. Based on the leaf Cl^- and Na^+ concentrations, our conclusion is that foliar ion uptake in the TLS is less than might be anticipated. However, this conclusion should not be extrapolated to usual management practices used in sprinkler irrigation systems, since we applied short (3-min) pre- and post-irrigations with fresh water in order to minimise salt absorption by leaves (Aragüés et al., 1994; Grattan et al., 1994; Benes et al, 1996b).

Differences in leaf K^+ concentrations between the two systems were only significant in the C treatment, where they were 19 % lower in the TLS than in the DI. This may be associated with the higher soil K^+ concentrations measured in the DI soil (data not shown). Leaf K^+ concentrations did not decrease with an increase in soil salinity, a result which differs from that obtained by Cramer et al. (1991) in NaCl treated barley and from the conclusion generally accepted in the literature in that leaf K^+ decreases with salinity and induces a K^+ deficiency. Cramer et al. (1991) and Huang and Redmann (1995) found that the addition of 5-10 meq Ca^{2+} l^{-1} maintained adequate ion relations, thus minimising the negative effects of salinity in barley plants. In our experiments, the Ca^{2+} concentrations measured in the saline irrigation waters were large enough (Table 2) to prevent the nutritional disorders associated with high Na^+ concentrations.

For any given saline treatment in the TLS or the DI, no significant ($P>0.05$) correlations were found between leaf Cl^- and Na^+ concentrations measured in the eighteen barley cultivars, indicating that the accumulation of these two ions differed among the cultivars tested. On the other hand, positive correlations were found between leaf Cl^- and leaf Ca^{2+} concentrations among the eighteen barley cultivars grown both in the medium (correlations significant at $P<0.05$) and in the high saline treatments (correlations significant at $P<0.01$), suggesting that the foliar accumulation of these two ions is similar among the cultivars tested.

To elucidate the variability in foliar absorption of Cl^-, Na^+ and Ca^{2+} ions among the eighteen barley cultivars grown in the TLS and DI, we normalised the leaf ion concentrations measured in the M treatments by dividing them by the corresponding soil solution ion concentrations estimated in the TLS and DI. We choose the M treatments because their EC soil water (EC_{sw}) values were the closest in both irrigation systems (EC_{sw} = 17.1 dS m^{-1} in the TLS and 20.1 dS m^{-1} in the DI). Figure 4 shows the normalised TLS leaf ion concentrations plotted against the normalised DI leaf ion concentrations. For cultivars falling above the 1:1 line the direct absorption of salts by the leaves wetted in the TLS is considerable, whereas for cultivars close to or below the line the foliar salt absorption in the TLS is negligible.

The variability of the normalised Cl^- values among cultivars was high, and eight of the eighteen cultivars were above the 1:1 line, indicating that absorption of Cl^- by leaves was taking place. The cultivar Cameo (9) exhibited the largest leaf Cl^- uptake, followed by cultivars Dpche-18 (11), Mogador (16), Reinette (18) and Barbarrosa (8). The other cultivars did not show Cl^- uptake by leaves. The variability of the normalised Na^+ values among cultivars was also high, but fifteen of the eighteen cultivars fell below the 1:1 line, suggesting that leaf Na^+ uptake was only important in Barbarrosa (8), Criter (10) and Dpche-18 (11). Finally, the variability of the normalised Ca^{2+} values was much lower and all the cultivars exhibited moderate to large leaf Ca^{2+} uptake in the TLS, as indicated by their position above the 1:1 line. These results are in agreement with those obtained by Maas et al. (1982), Gorham et al. (1994) and Aragüés et al. (1994), where leaf uptake of Cl^- exceeded that of Na^+ in plants sprinkler-irrigated with saline waters, but differ from those obtained by Benes et al. (1996a) with the spring barley cultivar Kym, where Na^+ was greater than Cl^- leaf uptake.

Grain yield and leaf ion concentrations in eighteen barley cultivars (TLS94 and DI94 experiments)

Grain yields were not significantly correlated with leaf sap ion concentrations of the eighteen barley cultivars in the C, M and H saline treatments of the TLS94 and DI94 experiments, except for Cl^- and Ca^{2+} in the M treatment (TLS94) and for Cl^- in the H treatment (DI94) (Table 4). However, these exceptions were only apparent, since the observations were not normally distributed and the significance of the correlations was due to the extreme values found in one or two barley cultivars. In addition, the K^+/Na^+ ratios were not correlated with grain yield. Thus, bulk leaf ion concentrations of Cl^-, Na^+, Ca^{2+}, K^+ and the ratio K^+/Na^+ are not the cause of the differences in grain yield observed in these barley cultivars, suggesting that they cannot be used in screening for increasing salinity tolerance in barley. Similar results were found by Rawson et al. (1988) using a set of 20 barley, wheat and triticale cultivars grown under glasshouse conditions. Recently, Fricke et al. (1996) found a differential accumulation of Cl^- and Na^+ among epidermal and mesophyll barley cells, suggesting that differences in leaf compartmentation could play a relevant role in barley salt tolerance, and that studies using the bulk leaf values may be misleading as they do not detect potential ion exclusion mechanisms by the cytoplasm.

Salinity tolerance of eighteen barley cultivars established in the TLS94 and DI94 experiments

Figure 5 shows the grain yield of eighteen barley cultivars measured in the C, M and H saline treatments of the TLS94 and DI94 experiments. The average yield of the eighteen barley cultivars measured in the C treatment, was 1.14 times greater in the DI than in the TLS. The C.V. of the average grain yield was 21% in TLS and 14% in DI. There was a significant ($P< 0.01$) and positive linear correlation between the grain yields measured in the C treatments of the two systems, with a regression coefficient not significantly different ($P> 0.05$) from one, indicating that the yields and ranking of these cultivars in the absence of salinity were similar in both irrigation systems.

The average yield of the eighteen barley cultivars measured at intermediate salinity levels (M treatment) was 1.49 times greater in the DI than in the TLS. The C.V. of the average grain yields were 23% in the TLS and 13% in the DI (i. e., similar to the corresponding C.V. found in the C treatment) indicating that the genetic variability in grain yield did not decrease in a moderately saline environment. The lower yields in the TLS were attributed to the toxic effect of ions absorbed by the wetted leaves. The results given in Fig. 3 suggest that, of the ions analysed, this toxic effect should be attributed to the Ca^{2+}, since it is the only ion with greater leaf sap concentrations in the TLS than in the DI. In any case, this apparent toxic effect did not significantly affect the ranking of cultivars based on grain yields measured in the M treatment of both irrigation systems since the correlation coefficient, although low (Fig. 5), was significant at $P<0.05$, and the regression coefficient was not significantly different from one ($P>0.05$). For each experiment, there was a significant linear correlation between the grain yield in the C treatment and in the M treatment ($r=0.73^{***}$ in the TLS and $r=0.51^{*}$ in the DI). This result is in agreement with those of Rawson et al. (1988) and suggests that the highest-yielding cultivars under non-saline conditions would also be the most productive under moderately saline conditions.

The average yield of the eighteen barley cultivars at high salinity levels (H treatment) was 3.3 times greater in the DI than in the TLS, probably due to the toxic effect of the Cl^{-} and Ca^{2+} ions in the TLS (Fig. 3). Although the genetic variability of grain yields measured in the H treatment was similar to that in the C and M treatments (C.V. of the average grain yields in the H treatment = 34% and 14% in the TLS and DI, respectively), the grain yields were not significantly correlated (Fig. 5) indicating that the ranking of cultivars established in the two irrigation systems at this high salinity level was different. For each experiment, the linear correlations between the grain yield in C treatment and the grain yield in H treatment were not significant ($r= 0.07^{ns}$ in the TLS and $r= 0.43^{ns}$ in the DI). The generalisation of Richards

(1983) that “higher yields in salinized fields may be more easily obtained by breeding in non-saline than saline conditions” may not always be applicable, since it depends on the severity of the salinity stress. According to our results, Richard´s conclusion is valid for moderate but not for high salinity stresses.

Although absolute grain yield is more important than relative yield from an economic point of view, the latter (i.e., yield at a given salinity relative to the yield measured under non-saline conditions) is considered to be a more appropriate index for comparing the salinity tolerance of crops or cultivars in breeding programs for increasing salinity tolerance. In the TLS, with ten salinity treatments, we estimated the EC_{50} (the EC at which yield is reduced 50% over the control) from the curvilinear response model of van Genuchten (1983) as the most suitable salinity tolerance parameter. In the DI, with only three saline treatments, the tolerance parameter was obtained from the ratio grain yield in the high saline (H) treatment: grain yield in the control (C) treatment (Y_H/Y_C)**.** The ratio grain yield in the medium (M) treatment: grain yield in the control (C) treatment (Y_M/Y_C) was not calculated because the decrease in grain yield in the M treatment relative to that in the C treatment was not significant.

A positive and significant ($P< 0.05$) linear correlation coefficient was found between the EC_{50} (TLS) and the Y_H/Y_C (DI) values obtained for the eighteen barley cultivars (Fig. 6). If the cv. Alpha (5, in Fig. 6) is deleted from the regression analysis (due to the inconsistent EC_{50} values obtained in the first (1.49 dS m^{-1}) and second (0.97 dS m^{-1}) replications of the TLS), the correlation coefficient increases to 0.54 (significant at $P< 0.02$). In addition, Fig. 6 shows that the three barley cultivars with major deviations from the general linear trend (i.e., Criter-10, Malta-14, and Merzaga-15) have EC_{50} values higher than expected, suggesting that these deviations are not caused by toxic ion effects from the absorption of salts by the leaves in the TLS.

Although the established regressions have relatively low correlation coefficients, our results indicate that, both in absolute (up to ECe values of around 7-10 dS m^{-1}) and relative terms, the ranking in salinity tolerance of the eighteen cultivars obtained in the TLS and in the DI irrigation systems is significantly correlated ($P<0.05$), even though the direct absorption of salts by the leaves occurring in the TLS may have a deleterious effect on grain yield for ECiw values above 12 dS m^{-1}. The simple and inexpensive TLS irrigation system may then be successfully used in screening for salinity tolerance in barley, although this conclusion should not be extrapolated to other crops before their ion absorption through leaves has been ascertained.

Conclusions

Leaf sap Cl^-, Na^+ and Ca^{2+} concentrations measured in six barley cultivars grown in ten salinity treatments of the TLS (triple line source irrigation system) increased linearly with an increase in the corresponding irrigation water ion concentrations. The average Cl^- and Na^+ concentrations measured in the flag leaf (FL) were 2.5 to 3 times less than those measured in the fully expanded 6^{th} leaf (L6) of the principal tiller and in the second leaf below the flag leaf (FL-2). The pattern of leaf salt accumulation was similar among the six barley cultivars, so that leaf sap Cl^- was, irrespective of the leaf age, 1.5 to 2.5 times greater than leaf sap Na^+, indicating the preferential absorption of Cl^- over Na^+ in sprinkler irrigated barley plants.

The comparison of average leaf ion concentrations measured in 18 barley cultivars grown in three saline treatments (control-C, medium-M and high-H) of the TLS and the drip irrigation (DI) systems shows that direct leaf salt absorption in the TLS was minor or negligible for the Na^+ and Cl^- ions (except in the H treatment), but substantial for Ca^{2+}. Ion uptake by the wetted leaves in the TLS was important in five (Cl^-), three (Na^+) and all (Ca^{2+}) the cultivars. Averaged leaf K^+ concentrations were similar in the two irrigation systems

(except in the control or non-saline treatment) and did not decrease with an increase in soil salinity. The lack of significant correlations between grain yields and leaf sap ion concentrations (Cl^-, Na^+, Ca^{2+} and K^+/Na^+) indicates that ion concentration should not be used in screening for increased salinity tolerance in barley breeding programs.

The significant correlation between grain yield of the 18 barley cultivars measured in non-saline and moderately-saline (ECe < 10 dS m^{-1}) conditions indicates that the highest-yielding cultivars under non-saline conditions were also most productive under moderately saline conditions. However, this conclusion is not valid for high-saline conditions, since their yields were not correlated with those obtained under non-saline conditions. The generalisation of Richards (1983) that "higher yields in salinized fields may be more easily obtained by breeding in non-saline than saline conditions" should be regarded with caution, since it depends on the severity of the salinity stress.

The salinity tolerance of the 18 cultivars established in the TLS and DI was significantly correlated ($P<0.05$), both in absolute (up to ECe values of around 7-10 dS m^{-1}) and relative terms, even though the direct absorption of salts by the leaves occurring in the TLS system has an important deleterious effect on grain yield for ECiw values above 12 dS m^{-1}. We conclude that, with some limitations, the simple and inexpensive TLS irrigation system may be successfully used in screening for salinity tolerance in barley.

Acknowledgements

The authors acknowledge the assistance of J. Gaudo, M. Izquierdo, T. Molina, M. A. Monesma and L. Naval. This work was financed by the European Union STD program and by a grant to R. Isla given by Diputación General de Aragón (Spain).

References

Aloy M 1995 Leaf ion concentrations and salt tolerance in barley. Ph.D. Thesis. University of Wales, Bangor, UK. 229 pp.

Aragüés R, Royo A, and Faci J 1992 Evaluation of a triple line source sprinkler system for salinity crop production studies. Soil Sci. Soc. Am. J. 56, 377-383.

Aragüés R, Royo A and Grattan SR 1994 Foliar uptake of sodium and chloride in barley sprinkler-irrigated with saline water: effect of pre-irrigation with fresh water. Eur. J. Agron. 3(1), 9-16.

Benes S E, Aragüés R, Austin R B and Grattan S R 1996a Brief pre- and post-irrigation with freshwater reduces foliar salt uptake in maize and barley irrigated with saline water. Plant and Soil 180, 87-95.

Benes S E, Aragüés R, Grattan S R and Austin R B 1996b Foliar and root absorption of Na^+ and Cl^- in maize and barley: implications for salt tolerance screening and the use of saline sprinkler irrigation. Plant and Soil 180, 75-86.

Blum A 1988 Plant breeding for stress environments. CRC Press. Florida. USA. 223 p.

Cotlove E 1963 Determination of true chloride content of biological fluids and tissues. II. Analysis by simple nonisotopic methods. Anal. Chem. 35, 101.

Cramer G R, Epstein E and Lauchli A 1991 Effects of sodium, potassium and calcium on salt stressed barley. II.- Elemental analysis. Physiol. Plant. 81, 197-202.

Flowers T J and Yeo A R 1995 Breeding for salinity resistance in crops plants : Where next ?. Aust. J. Plant Physiol. 22, 875-884.

Fricke W, Leigh L A and Tomos A D 1996 The intercellular distribution of vacuolar solutes in the epidermis and mesophyll of barley leaves changes in response to Nacl." Journal of Experimental Botany 47(302), 1413-1426.

Genuchten M Th van 1983 Analyzing crop salt tolerance data: Model description and user's manual. Washington: USDA-ARS-USSL Research Report Nº120.

Gorham J 1992 Stress tolerance and mechanisms behind tolerance in barley. Barley Genetics VI, 1035-1049.

Gorham J, Papa R and AlloyLeonart M 1994 Varietal differences in sodium uptake in barley cultivars exposed to soil salinity or salt spray. J. Exp. Bot. 45, 895-901.

Grattan S R, Royo A and Aragüés R 1994 Chloride accumulation and partitioning in barley as affected by differential root and foliar salt absorption under saline sprinkler irrigation. Irr. Sci. 14, 147-155.

Greenway H and Munns R 1980 Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Of Plant Physiol. 31, 149-190.

Huang J and Redmann R E 1995 Solute adjustment and calcium supply in cultivated and wild barley. J. Plant Nutrition 18(7), 1371-1389.

Maas E V, Grattan S R and Ogata G 1982 Foliar salt acumulation and injury in crops sprinkled with saline water. Irrig. Sci. 3, 157-168.

Noble C L and Rogers M E 1992 Arguments for the use of physiological criteria for improving the salt tolerance in crops. Plant and Soil 146, 99-107.

Rawson H M, Richads R A and Munns R 1988 An examination of selection criteria for salt tolerance in wheat, barley and triticale genotypes. Aust. J. Agric Res. 39, 759-772.

Richards R A 1983 Should selection for yield in saline regions be made on saline or in non saline soils ?. Euphytica 32, 431-438.

Royo A and Aragüés R 1993 Validation of salinity crop production functions obtained with the triple line source sprinkler system. Agron. J. 85, 795-800.

Royo A y Aragüés R 1991 Tolerancia a la salinidad de 48 cultivares de cebada en la fase de emergencia. Invest. Agraria, Prod. Protec Vegetal 6(1), 17-26.

Rumbaugh M D, Pendery B M and James D W 1993 Variation in the salinity tolerance of strawberry clover (*Trifolium fragiferum* L). Plant and Soil 153, 265-271.

SAS Institute Inc 1988 SAS/STAT™ Guide for personal computers, Versión 6 Edition. Cary, NC : SAS Institute Inc., 378 pp.

Szabolcs I 1989 Salt-affected soils. CRC Press, Boca Ratón, FL., 274 p.

Table 1. Principal features of the 1993 and 1994 Triple Line Source (TLS) and 1994 Drip Irrigation (DI) experiments.

	TLS93	TLS94	DI94
Number of cultivars	6	18	18
Sowing date	27/11/92	19/11/93	18/11/93
Harvest date	2/07/93	22/06/94	20/06/94
Number of plants m^{-2} ± std. dev.	220 ± 17	206 ± 25	228 ± 23
Length of plot, m	1.25	1.25	1.45
Rows $plot^{-1}$	3	6	6
Area of each plot (m^2)	0.8	1.6	1.8
First saline irrigation	3/02/93	31/01/94	26/01/94
Last saline irrigation	31/05/93	31/05/94	21/05/94
Number of irrigations	31	28	40
Seasonal saline irrigation, mm	399	351	479
Seasonal rain plus non-saline irrigation, mm	128	118	81
EC irrigation water interval, dS m^{-1} [a]	3.2 - 19.9	1.9 - 17.3	1.5 - 16.7
Seasonal evapotranspiration, mm [b]	241	271	271

[a] Temporal average electrical conductivity of irrigation water applied

[b] Measured in a lysimeter adjacent to the TLS plot

Table 2. EC and concentrations of Cl^-, Na^+ and Ca^{2+} in the water applied in three saline treatments (control-C, medium-M and high saline-H) of the TLS94 and DI94 experiments.

	Saline treatment	EC[a] water applied ± SD dS m^{-1}	Cl^- [b]	Na^+ [b]	Ca^{2+} [b]
			mmol L^{-1}		
TLS94	C (#0)	1.9 ± 0.6	4.5	9.2	3.7
	M (#4)	8.0 ± 2.0	83.2	55.9	22.7
	H (#8)	15.1 ± 2.8	160.3	96.9	44.6
DIS94	C	1.5 ± 0.5	16.0	4.8	3.7
	M	10.1 ± 1.4	103.2	37.1	24.9
	H	16.7 ± 3.6	190.0	91.6	58.8

[a] Average values of 28 (TLS) and 40 (DI) irrigations

[b] Values measured in one irrigation

Table 3. TLS93 experiment: intercepts (a), slopes (b) and correlation coefficients (r) of linear regression of leaf sap Cl^-, Na^+ and Ca^{2+} concentrations on the corresponding Cl^-, Na^+ and Ca^{2+} concentrations in the irrigation water. Analysis performed on three leaf ages (L6, FL and FL-2) sampled in six barley cultivars grown in ten salinity treatments.

Cultivar [a]	Leaf [b]	Chloride mmol l^{-1}			Sodium mmol l^{-1}			Calcium mmol l^{-1}		
		a	**b**	**r**	**a**	**b**	**r**	**a**	**b**	**r**
Albacete	L6	159b	1.38a	0.95	111c	0.85b	0.88	36a	0.94a	0.84
Barbarrosa	L6	122b	1.24a	0.97	71ab	0.70ab	0.92	25a	0.95a	0.94
Begoña	L6	134b	1.27a	0.93	59ab	0.84b	0.94	26a	1.15a	0.91
CM-67	L6	68a	1.20a	0.99	55a	0.44a	0.83	26a	1.12a	0.96
Igri	L6	172b	1.35a	0.95	113c	0.93b	0.86	33a	1.02a	0.93
Mogador	L6	139b	1.47a	0.98	81b	0.87b	0.91	34a	1.10a	0.97
Albacete	FL	35a	0.56ab	0.85	20ab	0.63b	0.96	26ab	0.62a	0.93
Barbarrosa	FL	20a	0.57ab	0.83	6a	0.52b	0.87	26ab	0.58a	0.86
Begoña	FL	16a	0.69b	0.93	21ab	0.59b	0.84	30b	1.13b	0.93
CM-67	FL	30a	0.30a	0.82	9a	0.19a	0.94	29b	0.53a	0.89
Igri	FL	112b	0.47ab	0.71	38b	0.42ab	0.78	16a	0.52a	0.89
Mogador	FL	98b	0.63ab	0.87	36b	0.44ab	0.81	18ab	0.53a	0.90
Albacete	FL-2	172a	1.06a	0.87	73bc	1.08 b	0.88	21a	0.95a	0.90
Barbarrosa	FL-2	184a	1.04a	0.92	52ab	1.05 b	0.89	17a	0.81a	0.93
Begoña	FL-2	152a	1.06a	0.90	45a	0.65a	0.88	19a	1.06a	0.90
CM-67	FL-2	142a	1.02a	0.90	55ab	0.86ab	0.98	17a	1.19a	0.96
Igri	FL-2	165a	1.41a	0.92	58ab	1.12b	0.98	17a	1.17a	0.94
Mogador	FL-2	187a	1.32a	0.95	83c	0.84ab	0.95	23a	1.11a	0.92

[a] Comparisons among cultivars performed within each leaf age. For each column, values with the same letter are not significantly different (P>0.05).

[b] Leaves sampled in different dates due to differences in phenology

Table 4. Coefficients of linear correlation (r) between leaf (FL-1) sap Cl^-, Na^+, Ca^{2+}, K^+ concentrations and K^+/Na^+ ratios and grain yields of eighteen barley cultivars measured in the C (control), M (medium) and H (high) saline treatments of the TLS94 and DI94 experiments.

System	Saline treatment	Cl^-	Na^+	Ca^{2+}	K^+	K^+/Na^+
TLS94	C	-0.15	0.15	0.33	0.12	0.14
	M	-0.73**	-0.31	-0.59*	-0.16	0.26
	H	-0.45	-0.12	-0.45	-0.48	0.14
DI94	C	-0.14	0.13	0.18	-0.03	0.05
	M	-0.16	0.05	-0.36	-0.34	-0.22
	H	-0.51*	-0.04	-0.18	-0.33	0.23

**,*: significant at $P<0.01$ and $P<0.05$, respectively

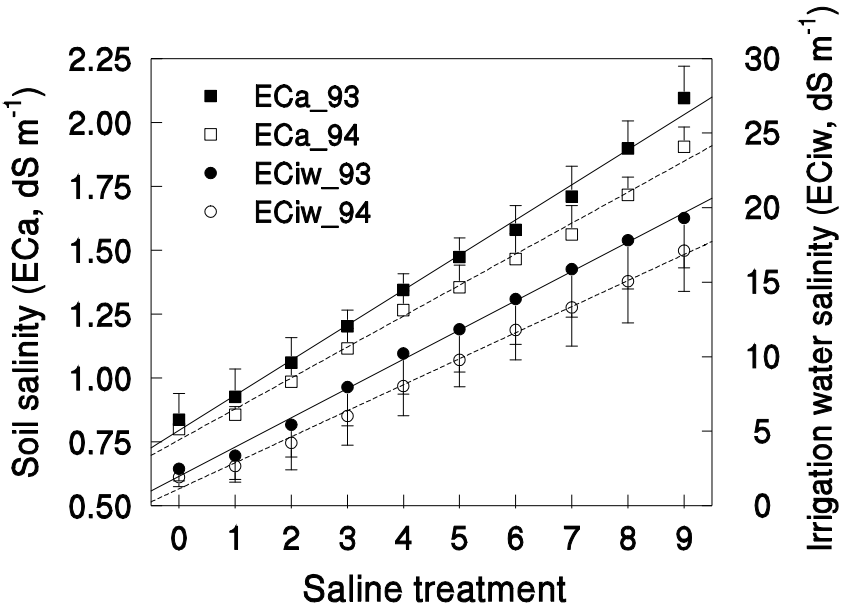

Figure 1. Time-averaged values of soil salinity (ECa, electromagnetic sensor readings) and irrigation water salinity (ECiw) measured in each saline treatment of the TLS93 and TLS94 experiments. The bars are one standard deviation of the mean. The "EC-saline treatment" regression lines are also shown.

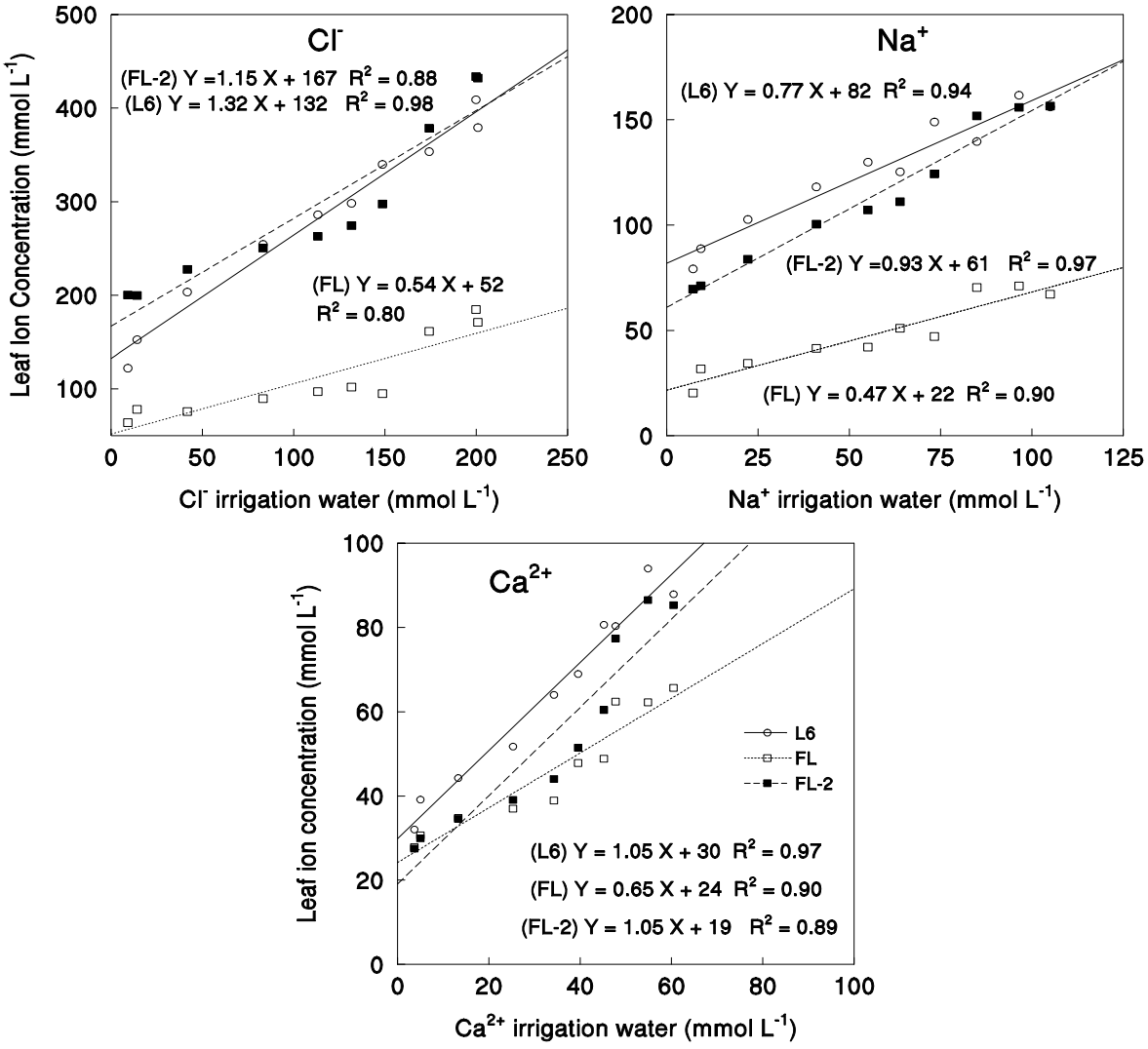

Figure 2. Average leaf sap Cl^-, Na^+ and Ca^{2+} concentrations of six barley cultivars grown in the TLS93 experiment relative to the irrigation water Cl^-, Na^+ and Ca^{2+} concentrations. The linear regression curves for each sampled leaf (L6: leaf number six; FL: flag leaf; FL-2: second leaf below the flag leaf) are also shown

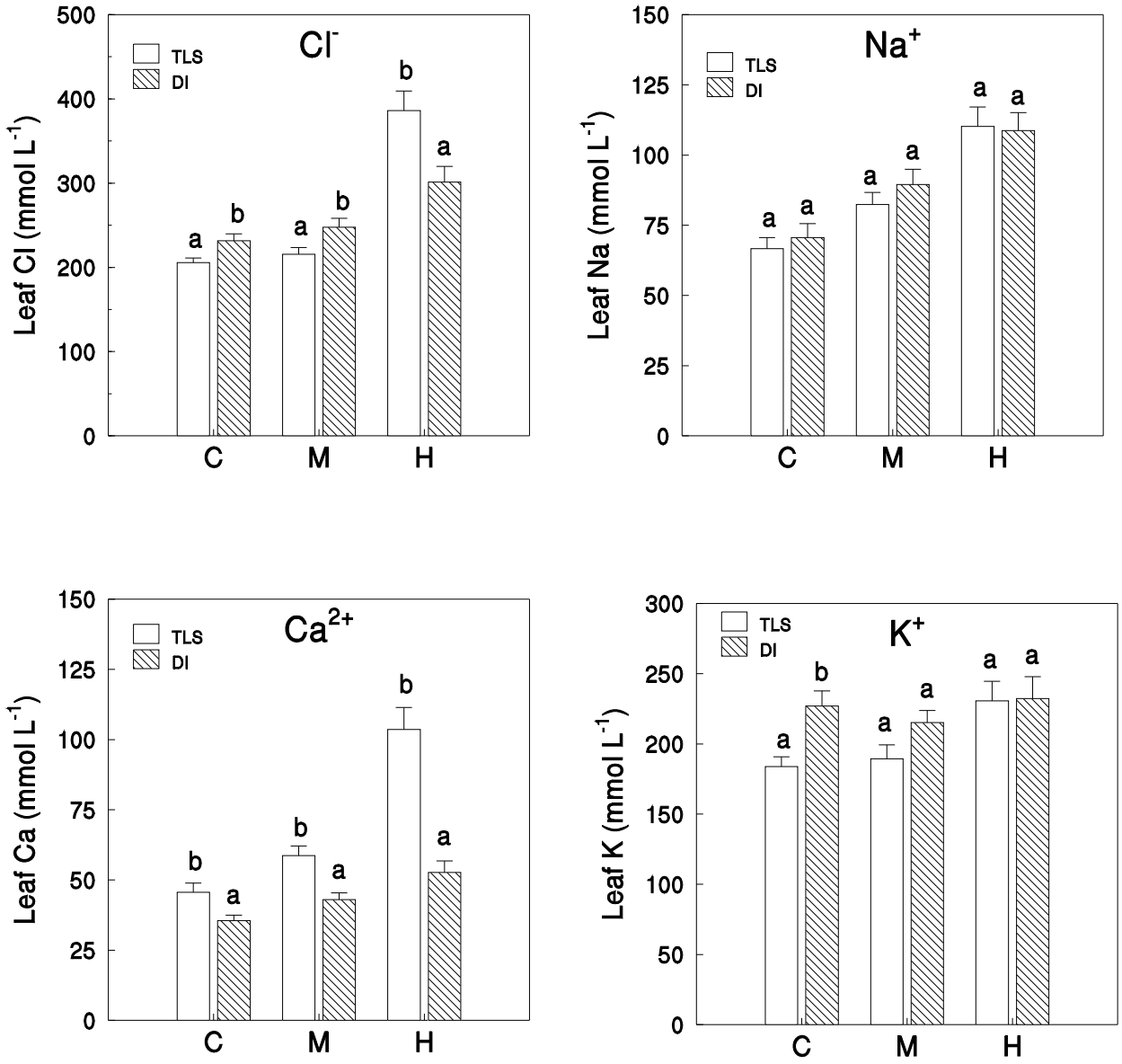

Figure 3. Leaf sap Cl^-, Na^+, Ca^{2+} and K^+ concentrations measured in three saline treatments (C-control, M- medium, and H-high salinity) of the TLS94 and DI94 experiments. Each value is the average of 18 barley cultivars. The bars indicate the standard error of the mean. Within each saline treatment, values with the same letter are not significantly different (P>0.05).

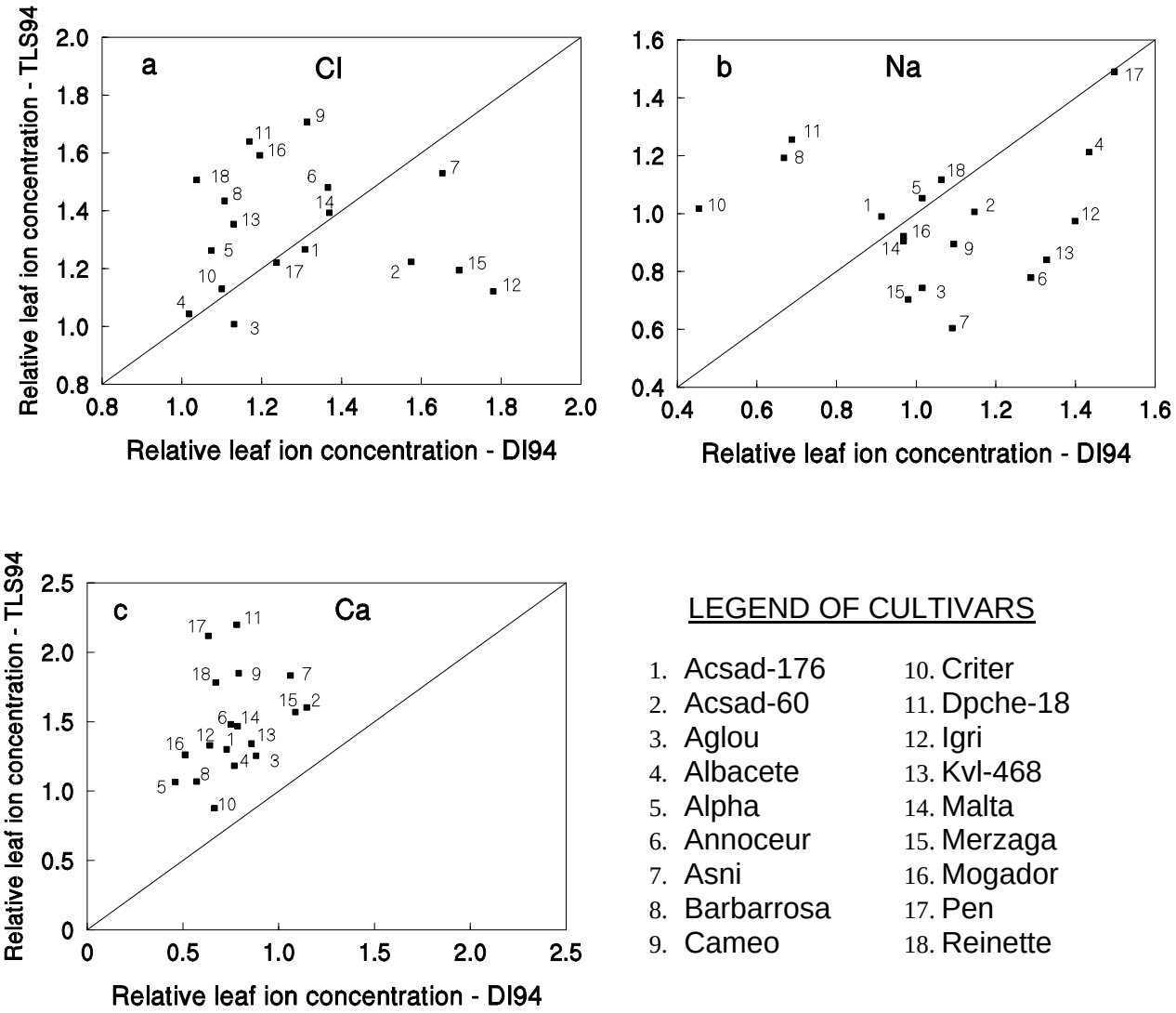

Figure 4. Relationships between relative leaf Cl^-, Na^+ and Ca^{2+} concentrations measured in eighteen cultivars grown in the medium (M) saline treatment of the TLS and DI experiments. Relative leaf ion concentration is the ratio "(flag leaf-1) ion concentration/soil solution ion concentration".

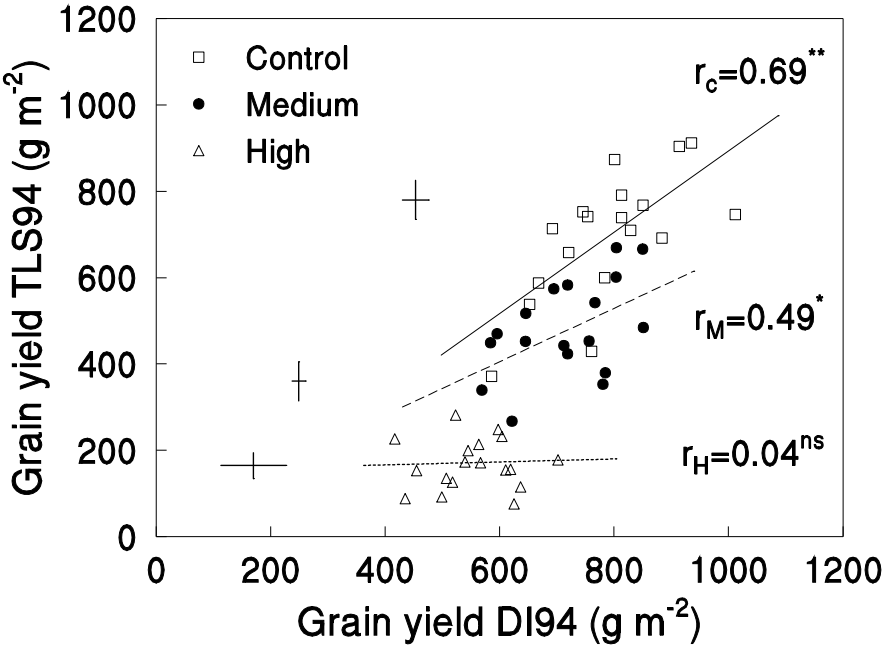

Figure 5. Grain yields of eighteen barley cultivars measured in the C, M and H saline treatments of the TLS94 and DI94 experiments. For each treatment, the values of the linear correlation coefficient are indicated. The bars show the averaged standard deviation of the mean values obtained in the TLS (vertical bars) and the DI (horizontal bars).

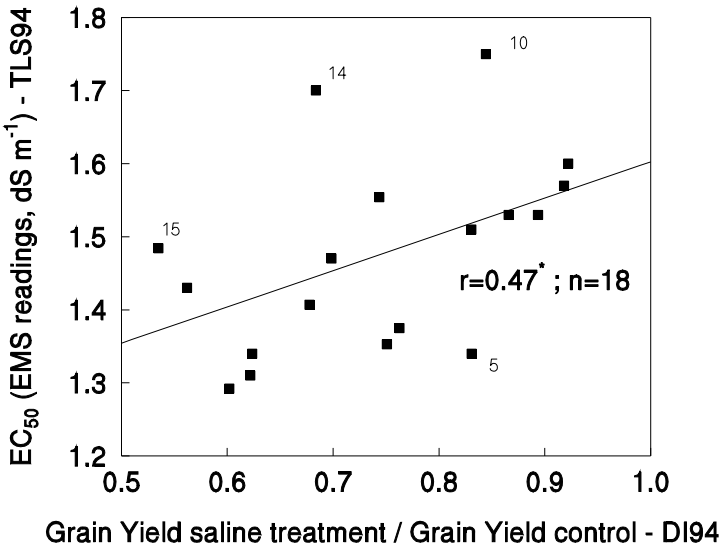

Figure 6. Relationship between relative salt tolerances of the eighteen barley cultivars established in the TLS94 and the DI94 experiments. The linear correlation coefficient is also shown. Observations 5, 10, 14, 15 corresponds to cultivars Alpha, Criter, Malta and Merzaga respectively.