

**Assessment of sub-soil salinity and sodicity constraints to barley and faba bean production****23.02.11**

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Introduction

Soil salinity and sodicity are two of the principal limitations to growth and yield of crop in arid and semi-arid areas of Australia (Rengasamy 2010). While saline-sodic soils have a number of nutritional limitations, breeding for salt tolerance has focussed largely on Na⁺ exclusion. The importance of high Cl⁻ is poorly defined for soils in southern Australia, despite some soils containing high concentrations of Cl⁻. Despite numerous reports showing variability in ion exclusion for many crops, few salt-tolerant genotypes have been released (Flowers and Yeo 1995). This lack of success may be due in part to physiologists conducting experiments under ideal, controlled conditions (e.g. hydroponics, sand cultures and greenhouse or growth chamber environments). However, genotypic differences measured under controlled conditions may not correspond to those observed under actual field conditions (Tavakkoli et al. 2010a; Tavakkoli et al. 2010b). Therefore, the objective of our study was to assess the value of Na⁺ and Cl⁻ exclusion to yield in saline soils as a basis of developing more salt tolerant varieties.

Methods**Study one: Relationships between soil salinity and yield in barley**

Field experiments were conducted at four sites in Victoria (Birchip, Walpeup, Manangatang and Werrimull) and one in South Australia (Georgetown). The Victorian sites were part of a larger program on the effects of tillage and stubble management on yield, while the SA site was part of a variety evaluation program. The barley variety SloopA was grown at the four sites in 2001-2004. The sites at Walpeup, Werrimull and Manangatang were grown on a dunes-swale system. These experiments were designed in blocks, corresponding approximately to landscape units (e.g. face of dune, top of dune, swale) with two blocks making up a replicate. Plots in these experiments were 12m wide and between 40 and 150m long. The Birchip site was on a level site with Gilgai features, and was laid out the same way as the other experiments, but with each block of identical length (55m) and wider plots (18m) to better encompass the Gilgai variation. At Georgetown, 10 genotypes of barley (Barque73, Clipper, FlagshipA, FleetA, HindmarshA, Keel Mundah, Schooner, Skiff and SloopA) were grown in 2008. A randomized, complete block design was used at all locations with three replications.

At the four Victorian sites soil samples were collected from each plot to a depth of 1.2m. Cores were divided into 0-10, 10-25, 25-50, 50-75, 75-100 and 100-150 cm layers. Measurements of pH (H₂O), Na⁺, Cl⁻ and electrical conductivity (saturated paste extract; ECe) were made on each sample. At Georgetown, soil samples were taken from 0-15, 15-30, 30-60, 60-90, 90-110 cm depths with an auger to the depth of 110 cm and analysed for pH, EC, Cl⁻ and exchangeable Na⁺.

Whole shoot samples at anthesis (Zadoks growth stage (ZGS) 65) were taken from the four Victorian sites, dried to a constant weight and ground to a fine powder. At Georgetown two randomly-selected plants from each plot were sampled at ZGS 45, 65 and 92. The plants were washed and separated into the upper and lower two leaves of the main stem for dry weight measurements and ionic analysis. All the samples were digested in 4% w/v nitric acid. The concentrations of Na⁺ and K⁺ in the digested samples were determined using a flame photometer. Chloride concentrations of the digested extracts were determined using a chloride analyser. Grain yield was determined by hand harvesting from each plot.

Study Two: Genotypic variation of barley genotypes in response to soil salinity

A field trial was conducted to assess the genotypic variation among 12 barley genotypes (selected from experiment 2) in response to salinity stress at Hart, South Australia. The site received 404 mm of rainfall in 2009, compared to the long term average of 460 mm. The soil at Hart is a calcareous gradational clay loam, and is the most extensive soil of the region (Hall et al. 2009). The topsoil is alkaline, non-saline and non-sodic but the subsoil is strongly alkaline (pH ≥ 9) and the ECe and exchangeable Na⁺ percentage (ESP), soluble Na⁺ and Cl⁻ concentrations increased with depth.

A randomized, complete block design with four replications was used. Basal fertiliser was applied with the seed as 12 kg P/ha of triple superphosphate (N:P:K:S = 0:17:0:0). Granular urea (46:0:0:0) was applied by hand immediately prior to sowing and as a post emergent application. The target plant population was 180 plants m². The plots were 6 rows x 20 m with an inter-row spacing of 225 mm. Weeds and disease, when present, were controlled by a range of herbicides and fungicides.

At ZGS 45, 65 and 92, five randomly-selected plants from each plot were sampled, washed and separated into the upper and lower leaves of the main stem to measure the distribution of Na⁺, K⁺ and Cl⁻. At ZGS65, ten soil cores were randomly taken from a soil depth of 0-100 cm and the pH, ECe soluble Na⁺, Ca²⁺ and Mg²⁺ and Cl⁻ were determined. The plots were machine harvested using a Wintersteiger plot harvester to determine grain yield.

Study three: salt tolerance in faba bean

Field experiments were conducted on a farmer's field at Pinery, South Australia (34° 18' 0" South, 138° 27' 0" East) in 2008 and 2009. The climate of this region is Mediterranean with an annual rainfall of 351 mm in 2008 and 405 mm in 2009 respectively. The soil at Pinery site is a calcareous loam with loamy surface grading to clay loamy subsoil. The topsoil is alkaline and the subsoil is strongly alkaline (pH ≥ 9.5). The topsoil is non-saline and non-sodic but the soil ECe and ESP increased with depth as did Na⁺ and Cl⁻ concentration too.

The trials were planted in plots 6 m long x 1.5 m wide at a plant density of 25 seeds/ m² on the 14 May 2008 and 11 May 2009. The trial was a randomised block design with four replications. Fertiliser was applied at the time of sowing as 150 kg/ha 5:14:0:13 (N, P, K and S) + 2% Zn in 2008 and as 150kg/ha 5:14:0:7 +2.1% Zn in 2009. Weeds were controlled with a range of commercially-available herbicides and the plots were sprayed with insecticides to control insects. All pesticides

were applied at the recommended rates and times and growth and yield of the faba beans were not affected by weed competition or insect damage. Measurements were made on six genotypes (NuraA, FarahA, Flord, Fiesta, CairoA and Manafest) in 2008 and on all 11 genotypes in 2009. At growth stages 51 (first flower buds visible outside leaves), 65 (full flowering) and 75 (50% of pods have reached final length) (Meier 2001) ten randomly-selected leaflets from each plot were sampled.

Ten soil cores were randomly taken from a soil depth of 10–75 cm. Electrical conductivity (ECe), pH, soluble Na⁺, Ca²⁺ and Mg²⁺ were determined in a saturated paste extract. Chloride concentration was determined using a chloride analyser. Grain yield was measured by harvesting each plot with a small plot harvester. The harvest dates were 11 November 2008 for the first year and 16 November 2009 for the second year.

Statistical analysis

All data were analysed by ANOVA. The relationship between soil chemical properties, plant ion composition and yield were investigated by using simple correlations and regressions.

Results

Study One

The pH and ECe increased with depth in all soils but there was marked variation among the five sites in the extent of these changes. The concentrations of Na⁺, Cl⁻, and the ECe and ESP of the soil at Georgetown and Birchip were higher than those at other locations (Fig 1). Within each of the Victorian sites, variation in grain yield of Sloop barley was significantly correlated to soil Na⁺ and Cl⁻ concentrations except at Walpeup where soil Na⁺ concentration was not correlated with grain yield (Fig 2).

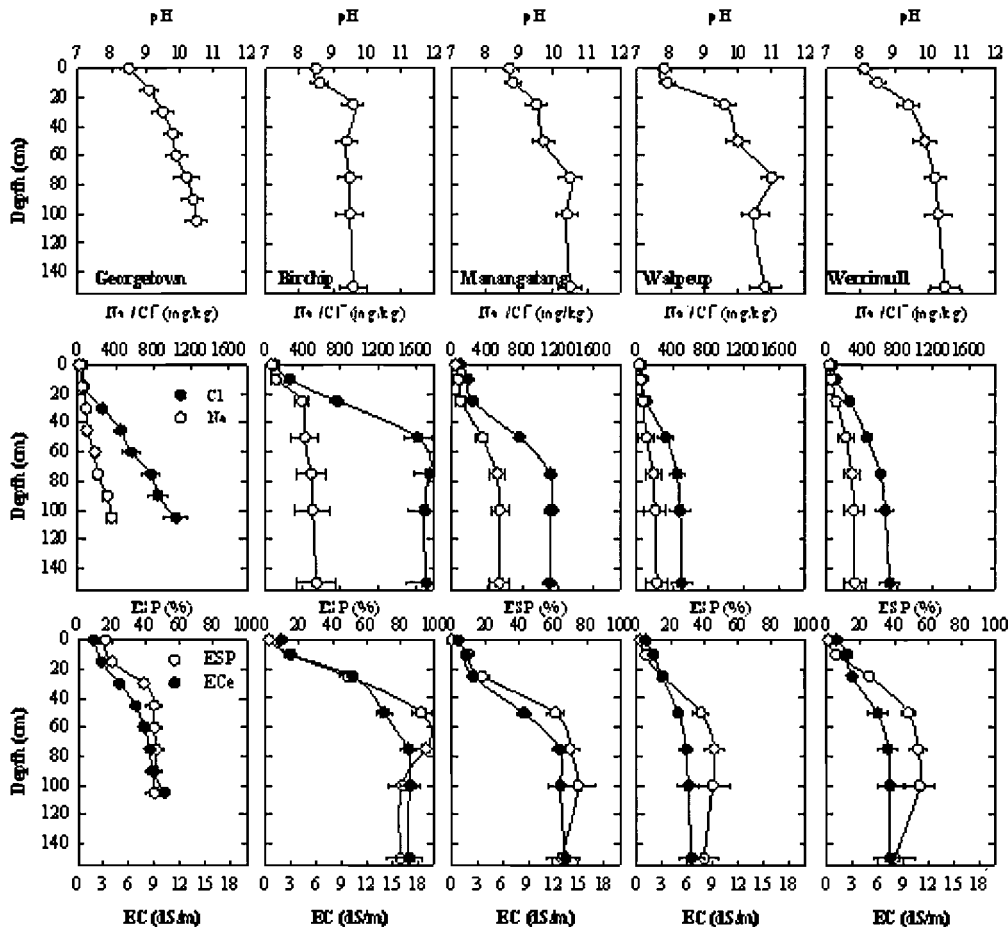


Figure 1. Selected characteristics of saline-sodic soils used in this study. Georgetown is the South Australian site and the rest are in Victoria. Error bars are standard errors of the means (n=3).

There were significant differences in yield among the 10 genotypes at Georgetown and these were correlated with the ion composition of the leaves, but the strength of the association differed with growth stage (Fig 3). The highest correlation between grain yield and the concentrations of Na⁺, Cl⁻ and K⁺ in the leaves was found at ZGS 65 whereas earlier or later sampling time did not indicate a significant relationship. Although the upper leaves had lower concentrations of Na⁺ and Cl⁻ and higher K⁺ than the lower leaves, these were more strongly correlated with yield compared with the concentrations in the lower leaves (Fig 4). The ratios of K⁺/Na⁺ in the upper and lower leaves of the main stem showed significant genotypic differences under field conditions.

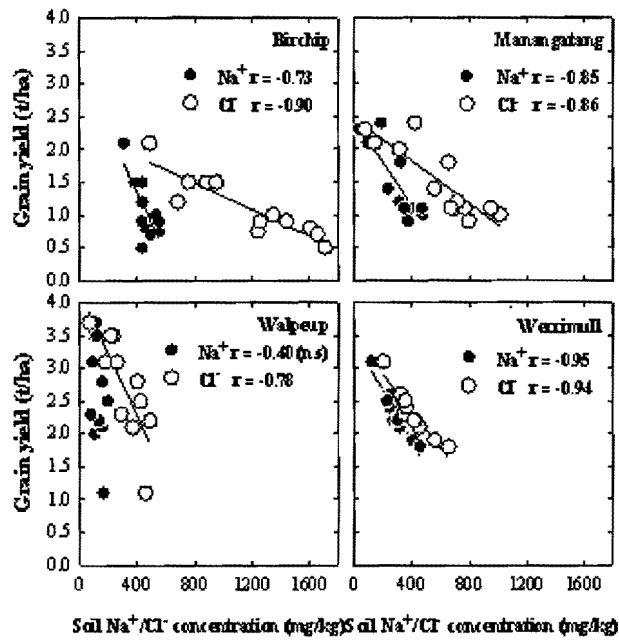


Figure 2. The relationships between variations in grain yield of barley variety Sloop, and variations in soil Na⁺ and Cl⁻ concentrations in four saline-sodic sites in southern Australia. The correlation with grain yield is shown at each site.

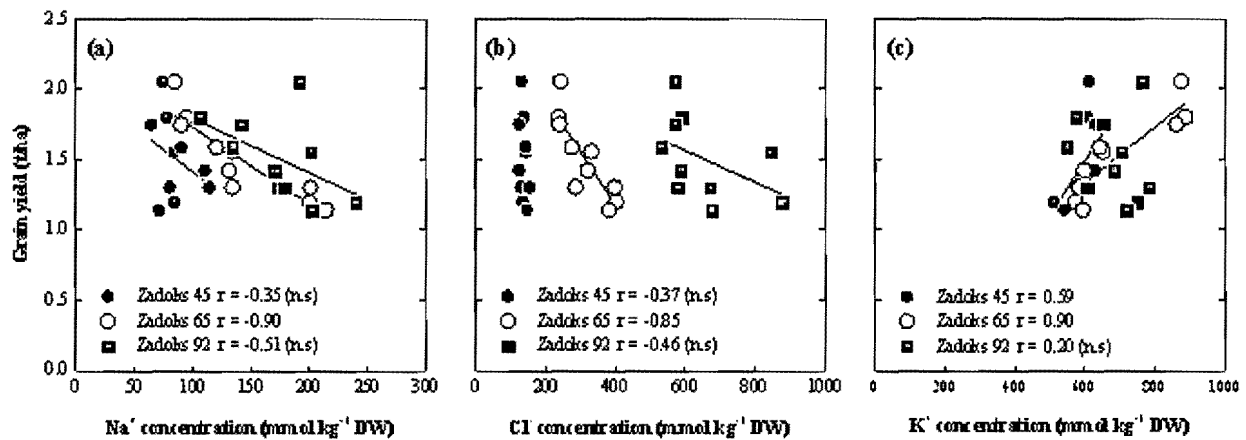


Figure 3. The relationships between grain yield and a) Na⁺, b) Cl⁻ and c) K⁺ concentration of the youngest fully expanded leaf of 10 genotypes of barley at three different growth stages grown at Georgetown. The correlation with yield at each sampling time is shown.

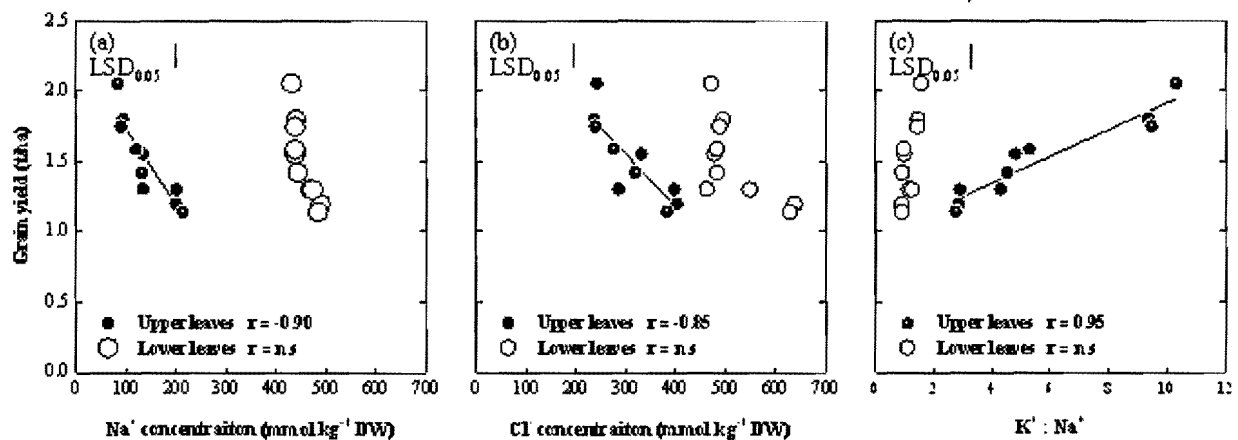


Figure 4. The relationships between grain yield and (a) Na⁺, (b) Cl⁻ concentration and (c) K⁺:Na⁺ concentration of the lower leaves (○) and upper fully expanded leaf (●) of 10 genotypes of barley at ZGS 65 grown at Georgetown.

Study Two

Grain yield production ranged from 3320 kg ha⁻¹ in MaritimeA to 5538 kg ha⁻¹ in CapstanA. Variation in yield was significantly associated with the uptake of Na⁺ and Cl⁻ and with the leaf osmotic potential. Those varieties that were best at

excluding Na⁺ and Cl⁻ generally produced the highest yields.

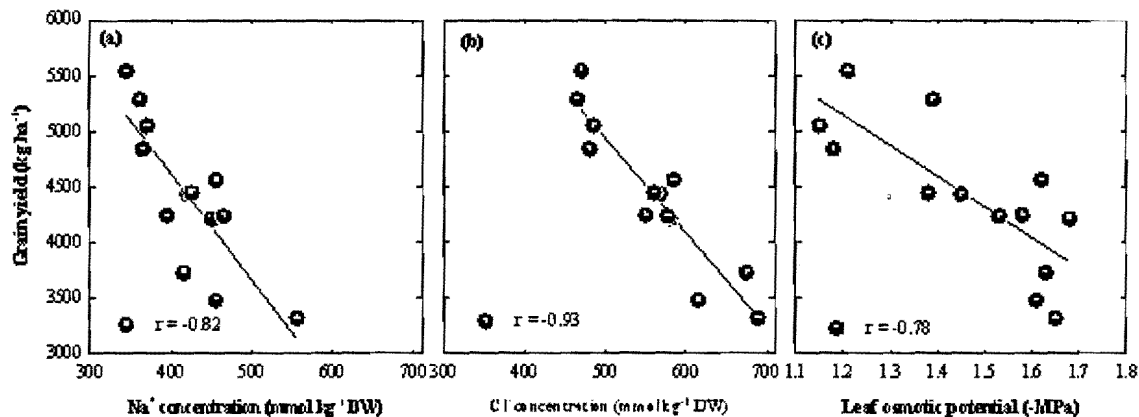


Figure 5. The relationship between grain yield and leaf concentration of (a) Na⁺ concentration (mmol kg⁻¹ DW), (b) Cl⁻ concentration (mmol kg⁻¹ DW), and (c) leaf osmotic potential (-MPa) of 13 barley genotypes grown at Hart site in 2009. The results are from youngest emerged leaves at ZGS 65. Fitted curves are derived from linear regression. The vertical bars are LSD at 95%. Values are averages (n=4).

Study Three

In 2008, grain yield of the 6 genotypes ranged from 843 kg ha⁻¹ in Manafest to 1370 kg ha⁻¹ in Fiord. Concentrations of Na⁺ and Cl⁻ varied by between 2 and 3 fold and the highest yielding varieties were those that were best at excluding Na⁺ and Cl⁻. As well, K⁺ concentration varied about 2-fold and high concentrations of K⁺ were associated with high yields (Fig 6a-c).

Similar results were found among a wider range of varieties in 2009 (Fig 6d-f). Grain yield of the 11 genotypes ranged from 2923 kg ha⁻¹ in the breeding line Acc 1477/4 to 3650 kg ha⁻¹ in Nura and there was a negative association between the uptake of Na⁺ and Cl⁻ and grain yield. The varieties Cairo and Manafest accumulated the highest concentrations of Na⁺ and Cl⁻, while a number of breeding lines had less than half the concentrations of Na⁺ and Cl⁻ of these varieties. As well, K⁺ concentration varied about 2-fold and the observed variations among the genotypes in K⁺ concentrations were negatively related to the Na⁺ concentration and positively related to the grain yield.

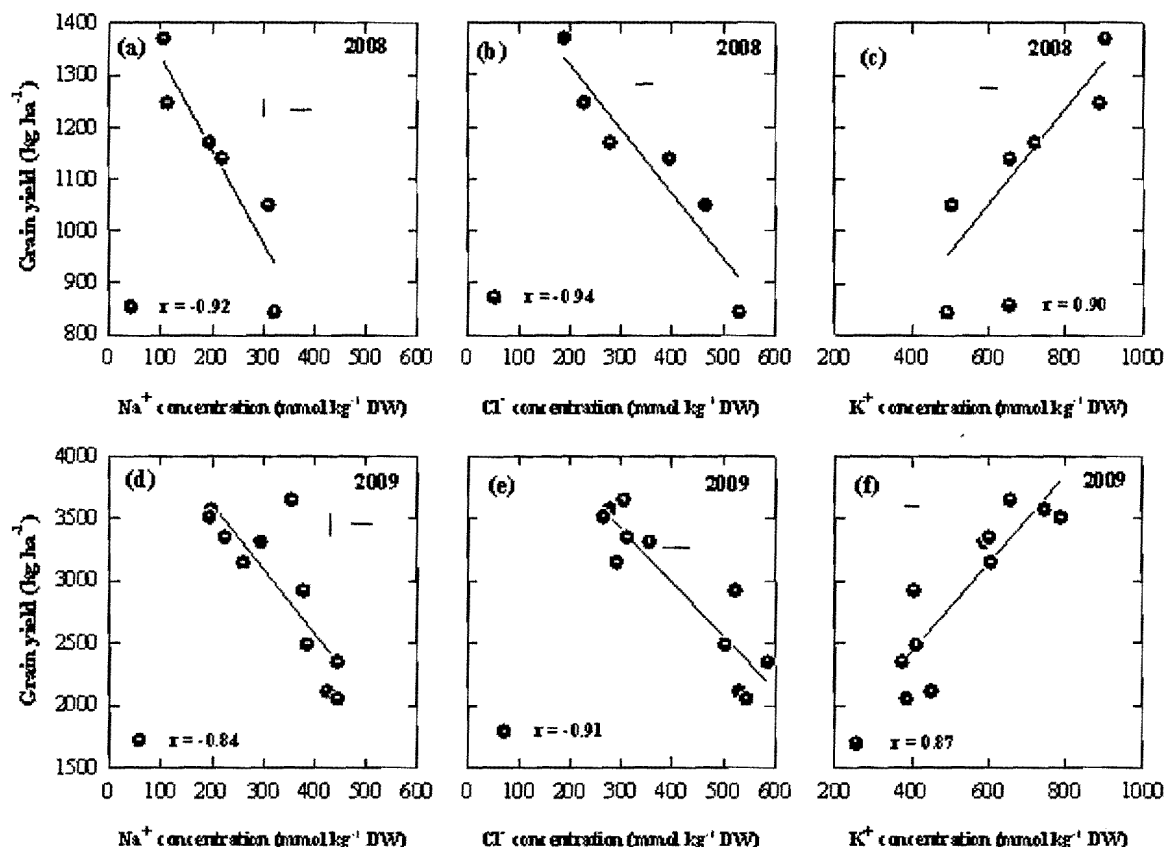


Figure 6. The relationship between grain yield and leaf concentration of (a) Na⁺ (mmol kg⁻¹ DW), (b) Cl⁻ (mmol kg⁻¹ DW), (c) K⁺ (mmol kg⁻¹ DW) of faba bean genotypes grown at Pinery site in 2008 and 2009. The results are from youngest emerged leaves at full flowering. The vertical bars are LSD at 95%. Values are means (n=4).

Discussion

The level of subsoil salinity affected the variation in yield at each of the sites. The spatial variation in yield within each of the

Victorian sites was related to the concentrations of Na⁺ and Cl⁻ in the subsoil and this influenced the concentrations of Na⁺ and Cl⁻ in the plant. This provides evidence of the importance of ion exclusion as a potential means of improving yield on the saline-sodic soils of the region. This is supported by the results from Georgetown (Fig 3) and Hart (Fig 5) that showed that differences in yield among adapted varieties of barley was strongly associated with the ability to exclude Na⁺ and Cl⁻. After anthesis, the upper leaves, and especially the flag leaf, make a major contribution in terms of the photosynthetic supply towards the grain yield. By contrast, the salt taken up by the plant tends to concentrate in the older, lower leaves; where over an extended period of time this produces high concentrations of Na⁺ and Cl⁻, causing the leaves to die (Munns et al. 2006). The maintenance of low Na⁺ and Cl⁻ in actively growing tissues such as young leaf blades and sheaths could be an important mechanism contributing to the enhanced salt tolerance of some genotypes (Hasegawa et al. 2000). According to Boursier et al. (1987) the salt tolerance of different barley genotypes has been associated with their respective abilities to selectively partition Na⁺ and Cl⁻ into old leaves and sheaths and K⁺ into growing tissues. In the present work, the order of genotypes for Na⁺ and Cl⁻ concentrations in the upper two leaves of the main stem were closer with their rankings in terms of their salt tolerance (on the basis of grain yield) than were those derived using the concentrations of these ions in the lower two leaves. Furthermore, the high-yielding genotypes accumulated less Na⁺ and Cl⁻ in the upper two leaves than did the low-yielding or moderate genotypes. Consequently, grain yield from salt-affected soils was highly significantly correlated with Na⁺ and Cl⁻ concentrations in the upper two leaves (Fig 3), but generally not in the lower, two leaves under field conditions. Thus, measurement of Na⁺ and Cl⁻ in young leaves under field conditions might be an effective selection criterion for salinity tolerance, more so than the concentration of Na⁺ and Cl⁻ in the entire plant, which does not correlate well with the salt tolerance in some genotypes.

In faba bean, the tested physiological traits showed significant genotypic variation, indicating that the traits that have a significant genotypic variation may possibly be used as screening criteria (Fig 6). The increased production of faba bean under rainfed conditions on saline-sodic soils highlights the importance of improving salinity tolerance through breeding. The availability of large and useful genotypic variation as shown in this study, and the high association of Na⁺ and Cl⁻ exclusion and K⁺/Na⁺ ratio with biomass indicates that the introduction of low Na⁺ and Cl⁻ accumulation into modern cultivars should be possible as part of a faba bean breeding program.

Conclusion

For selecting barley cultivars with salinity tolerance, measurement of the upper two leaves of the main stem at ZGS 65, including a simple measurement of dry weight, Cl⁻, Na⁺ and K⁺ concentrations appears to provide a reliable criterion to evaluate the tolerance of genotypes under field conditions.

The reduction in plant growth was correlated with both Na⁺ and Cl⁻ concentration in plant tissues, which indicates not only toxic concentrations of Na⁺, but Cl⁻ may also be contributing to growth reduction under saline conditions.

This study also clearly shows that a number of processes are involved in salt tolerance and that the relative importance of these traits may differ with the severity of the salt stress. If the importance of different mechanisms to salinity tolerance differs by the severity of stress, robust levels of salt tolerance may depend on more than one mechanism. Selection for improved salt tolerance therefore needs to be able to identify these.

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