RESEARCH PAPER

Salt tolerance in wild Hordeum species is associated with restricted entry of Na\(^+\) and Cl\(^-\) into the shoots

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Abstract

Eight wild Hordeum species: H. bogdani, H. intercedens, H. jubatum, H. lechleri, H. marinum, H. patagonicum, and H. secalinus, and cultivated barley (H. vulgare) were grown in nutrient solution containing 0.2 (control), 150, 300, or 450 mol m\(^{-3}\) NaCl. In saline conditions, the wild Hordeum species (except H. marinum) had better Na\(^+\) and Cl\(^-\) ‘exclusion’, and maintained higher leaf K\(^+\), compared with H. vulgare. For example, at 150 mol m\(^{-3}\) NaCl, the K\(^+\):Na\(^+\) in the youngest, fully expanded leaf blades of the wild Hordeum species was, on average, 5.2 compared with 0.8 in H. vulgare. In H. marinum grown in 300 mol m\(^{-3}\) NaCl, K\(^+\) contributed 35% to leaf ψ\(_{\text{m}}\), whereas Na\(^+\) and Cl\(^-\) accounted for only 6% and 10%, respectively. By comparison, in H. vulgare grown at 300 mol m\(^{-3}\) NaCl, K\(^+\) accounted for 19% and Na\(^+\) and Cl\(^-\) made up 21% and 25% of leaf ψ\(_{\text{m}}\), respectively. At 300 mol m\(^{-3}\) NaCl, glycinebetaine and proline together contributed almost 15% to ψ\(_{\text{m}}\) in the expanding leaf blades of H. marinum, compared with 8% in H. vulgare. Decreased tissue water content under saline conditions made a substantial contribution to declines in leaf ψ\(_{\text{m}}\) in the wild Hordeum species, but not in H. vulgare. A number of the wild Hordeum species were markedly more salt tolerant than H. vulgare. H. marinum and H. intercedens, as examples, had relative growth rates 30% higher than H. vulgare in 450 mol m\(^{-3}\) NaCl. Hordeum vulgare also suffered up to 6-fold more dead leaf material (as a proportion of shoot dry mass) than the wild Hordeum species. Thus, several salt-tolerant wild Hordeum species were identified, and these showed an exceptional capacity to ‘exclude’ Na\(^+\) and Cl\(^-\) from their shoots.

Key words: Asparagine, barley (Hordeum vulgare), Cl\(^-\), glycinebetaine, K\(^+\), Na\(^+\), proline, osmotic potential, salt tolerance, Triticeae, wild relatives.

Introduction

Saline environments affect plant growth in two ways: (i) salts in the soil solution lower the external water potential, reducing cell turgor; and (ii) salts taken up by plants in the transpiration stream accumulate in leaves and become toxic (Greenway and Munns, 1980; Munns and Termaat, 1986). Munns et al. (1995) have described the effect of these dual stresses on the growth of wheat and barley as a ‘two-phase growth response to salinity’. In the first ‘osmotic’ phase, salts in the external solution induce water stress which causes the large, almost immediate decrease in plant growth. In the second ‘salt-specific’ phase, ions accumulating in the leaves reach toxic levels, causing necrosis and reducing the photosynthetic area, and subsequently further declines in growth (Munns, 1993; Munns et al., 1995). The time between the first and second phases can be anything from hours to days to weeks, depending on the sensitivity of the plant to salt and its ability to ‘exclude’ Na\(^+\) and Cl\(^-\) from the shoot (i.e. restrict rates of entry of these ions). Munns et al. (1995) suggest that the reduction in growth during the first phase is similar amongst genotypes of...
wheat or barley, and it is often not until the second phase that differences in salt tolerance become apparent. This variation amongst genotypes in tolerance of prolonged salinity was attributed to differences in ability to avoid ion toxicity in the shoot (Munns et al., 1995).

Tolerant members of the Triticeae avoid ion toxicity in the shoot by restricting the rate of entry of Na⁺ and Cl⁻ and by minimizing the adverse effects of these ions when in shoot tissues. Bread wheat (Triticum aestivum), for example, restricts Na⁺ transport to leaf tissues (i.e. through Na⁺ ‘exclusion’) and maintains high selectivity of K⁺ over Na⁺ (Gorham et al., 1986; Gorham, 1993). Na⁺ ‘exclusion’ and K⁺/Na⁺ selectivity in barley (Hordeum vulgare ssp. vulgare) are poor by comparison (Gorham et al., 1990a; Munns et al., 2002); however, the adverse affects of Na⁺ within leaves of barley are minimized by its compartmentalization into vacuoles (with Cl⁻) and the production of organic solutes to osmotically ‘balance’ the cytosol (Greenway and Munns, 1980; Munns, 2002; Tester and Davenport, 2003). The importance to salt tolerance of restricting entry of Cl⁻, in addition to entry of Na⁺, is recognized (Munns, 2002), and barley genotypes differ in rates of Cl⁻ uptake (Greenway, 1962); however, amongst Triticeae species there appears to be much less variation in Cl⁻ ‘exclusion’, as compared with Na⁺ ‘exclusion’ (Husain et al., 2004; Shah et al., 1987).

The difference in Na⁺ ‘exclusion’ and K⁺/Na⁺ selectivity between bread wheat and barley has been partially attributed to the presence of the ‘enhanced K⁺/Na⁺ discrimination’ character in bread wheat, but not in barley (Gorham et al., 1990a, b). This character has also been identified in some wild Triticeae species (Gorham, 1993). The genetics and physiology of this trait have been described for wheat and its ancestors (Gorham et al., 1990b; Gorham, 1993; Dvorák et al., 1994), leading to significant advances in the understanding of variation in salt tolerance amongst members of the genus Triticum. In the case of the genus Hordeum, Na⁺ ‘exclusion’ and K⁺ uptake have largely been investigated in species with the I genome (barley and H. vulgare ssp. spontaneum) (Greenway, 1962; Storey and Wyn Jones, 1978a; Gorham et al., 1990a; Forster et al., 1994), despite cytogenetical studies indicating that the genus Hordeum contains four distinct genomes; H, I, X, and Y (Jacobsen and von Bothmer, 1992; Svitasev et al., 1994). However, the limited data available on K⁺/Na⁺ discrimination in Hordeum species with the H, X, or Y genomes indicates there may be significant variation in this trait within the genus (Suhayda et al., 1992; Gorham, 1993; Huang and Redmann, 1995; Howes Keiffer and Ungar, 1997b). For example, Suhayda et al. (1992) reported that shoot Na⁺ concentrations in H. jubatum (H genome) were three to four times lower than those in H. vulgare, when plants were grown at 150 mol m⁻³ NaCl. The present study therefore evaluated a range of wild Hordeum species for salt tolerance and leaf ion concentrations and compared these with H. vulgare, under a range of external NaCl treatments.

Although H. vulgare is regarded as salt tolerant compared with bread wheat and other cultivated Triticeae, barley cultivars still experienced a 55–58% decline in biomass at 150 mol m⁻³ NaCl (i.e. less than one-third the NaCl concentration in sea water) (Greenway, 1962). However, an ecogeographical study of the genus Hordeum (von Bothmer et al., 1995) estimated that more than half of the wild, or non-cultivated, Hordeum species occupy habitats that are saline; ranging from mildly saline pastures occupied by the progenitor of H. vulgare, to salt lake environments habituated, for example, by species of H. patagonicum (von Bothmer et al., 1995). However, data are lacking to confirm the putative salt tolerance of these wild Hordeum species. The few studies that have been conducted on salt tolerance in wild Hordeum species have generally been ecological in nature, or focused upon germination or survival of seedlings (Nevo et al., 1993; Howes Keiffer and Ungar, 1997a; Mano and Takeda, 1998). However, the ability to germinate or survive in saline nutrient solution is a poor indicator of salt tolerance (Richards et al., 1987; Rawson et al., 1988), particularly for annual species where biomass production is a better predictor of yield (Munns, 2002). In addition, with the exception of work on H. jubatum (see above) (Suhayda et al., 1992; Huang and Redmann, 1995; Howes Keiffer and Ungar, 1997b), and a report summarizing shoot K⁺:Na⁺ ratios in some wild Hordeum species (Gorham, 1993), traits contributing to salt tolerance in wild Hordeum species are largely unexplored.

This study evaluated the tolerance of eight wild Hordeum species, and cultivated barley, to growth in nutrient solution containing 0.2 (control), 150, 300, or 450 mol m⁻³ NaCl. Osmotic potential (Ψₛ) of expressed leaf sap, and Na⁺, Cl⁻, and K⁺ concentrations, in variously aged leaf tissues were compared for the various species. Concentrations of asparagine (Asn), glycinin, and proline (Pro) were compared for leaf tissues of H. vulgare and H. marinum. The species originated from saline, intermediate, and non-saline habitats (R von Bothmer, unpublished field notes). Hordeum vulgare ssp. vulgare and H. jubatum were included since the responses of these species to saline conditions had been documented (Greenway, 1962; Gorham et al., 1990a; Suhayda et al., 1992). The study identified a number of Hordeum species that were markedly more salt tolerant than H. vulgare, and which demonstrated highly efficient Na⁺ and Cl⁻ ‘exclusion’ and better maintenance of leaf K⁺ concentrations, at high external NaCl. The present findings are discussed in the context of understanding of salt tolerance in the Triticeae.

Materials and methods

Plant materials and culture

Salt tolerance was evaluated in nine species from the genus Hordeum (Table 1). The species studied, represent: (i) the four taxonomic sections of the genus; Hordeum, Stenostachys, Anisolepis, and
Table 1. Hordeum species used in the present study; listed in alphabetical order with their ploidy level, proposed genomic constitution (von Bothmer et al., 1986; Taketa et al., 1999), their taxonomic section, and accession numbers (or cultivars) from the Nordic Gene Bank (Alnarp, Sweden)

<table>
<thead>
<tr>
<th>Species</th>
<th>Ploidy</th>
<th>Genome</th>
<th>Section</th>
<th>Accession</th>
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<tr>
<td>H. bogdanii</td>
<td>2x</td>
<td>H</td>
<td>Stenostachys</td>
<td>H4014</td>
</tr>
<tr>
<td>H. intercedens</td>
<td>2x</td>
<td>H</td>
<td>Anisoplepis</td>
<td>H1940</td>
</tr>
<tr>
<td>H. jubatum</td>
<td>4x</td>
<td>HH</td>
<td>Criteion</td>
<td>H4215</td>
</tr>
<tr>
<td>H. lechleri</td>
<td>6x</td>
<td>HHH</td>
<td>Criteion</td>
<td>H6074</td>
</tr>
<tr>
<td>H. marium ssp.</td>
<td>4x</td>
<td>XX</td>
<td>Stenostachys</td>
<td>H819</td>
</tr>
<tr>
<td>gassoneanum</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. marium ssp.</td>
<td>2x</td>
<td>Y</td>
<td>Hordeum</td>
<td>H10260</td>
</tr>
<tr>
<td>glaucum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. patagonicum ssp.</td>
<td>2x</td>
<td>H</td>
<td>Stenostachys</td>
<td>H480</td>
</tr>
<tr>
<td>sanacruense</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. secalinum</td>
<td>4x</td>
<td>XH</td>
<td>Stenostachys</td>
<td>H296</td>
</tr>
<tr>
<td>H. vulgare ssp.</td>
<td>2x</td>
<td>I</td>
<td>Hordeum cv.</td>
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</tr>
<tr>
<td>vulgare</td>
<td></td>
<td></td>
<td>'Golf'</td>
<td></td>
</tr>
</tbody>
</table>

* Obtained from Svalöf-Weibull AB.

**Experimental design and NaCl treatments**

The plants were exposed to one of four NaCl concentrations: 0.2 (control), 150, 300, or 450 mol m\(^{-3}\). This range of concentrations was chosen to reflect the expected wide variation in salt tolerance amongst the diverse Hordeum species.

Each species x NaCl treatment combination was represented by three replicate pots. Pots were arranged in a randomized complete block design with three blocks. At the time treatments were imposed, an initial harvest was taken (i.e. leaving three plants per pot); shoot and root dry mass were measured and plant developmental stages recorded. NaCl treatments were initiated 7 d after the seedlings were transplanted into 4.5 dm\(^3\) plastic pots. NaCl concentrations were increased by 50 mol m\(^{-3}\) d\(^{-1}\) until the prescribed treatment concentrations were reached. The NaCl was added towards the end of each artificial day. After reaching the final NaCl concentrations, treatments were maintained for 16–21 d (plants were harvested as replicate blocks over the final 5 d). All nutrient solutions were renewed every 7 d throughout the experiment.

**Harvest procedure**

All pots in block one (i.e. the first replicate of each species x NaCl treatment combination) were harvested first (pots selected randomly), then block two and finally block three. The roots and stem base of each plant were rinsed three times, for 10 s each time, in deionized water. The roots were separated from the shoot, blotted dry, and set aside. All main tillers were divided into five leaf blade/tissue classes: (i) expanding leaf blades (the sheath was slit open to allow the entire leaf blade to be retrieved); (ii) youngest fully expanded leaf blades (no sheath); (iii) second youngest fully expanded leaf blades (no sheath); (iv) dead leaf tissues; (v) other, including stems, sheaths and all other ‘green’ and turgid leaf blades other than the three youngest (see above).

Fresh masses were recorded for the expanding and youngest fully expanded leaf blades to enable assessment of water content after the dry mass data were also taken. A subsample of each of these two tissues was taken immediately, sealed into an air-tight cryo-vial and frozen at -70 °C for subsequent measurement of sap $\Psi_s$ (see below). The expanding leaf blades, the youngest fully expanded, and second youngest fully expanded leaf blades were wrapped in aluminium foil and frozen in liquid N\(_2\) and then lyophilized, after which dry masses were recorded. The roots, dead leaves, and ‘other’ tissues were placed in paper bags and dried at 60 °C and the dry masses recorded.

**Growth analysis**

The whole plant relative growth rate (RGR) was calculated from the dry mass data taken at initial and final harvests, using the formula given by Venus and Causton (1979). The percentage of dead leaf material of the whole shoot dry mass was assessed.

**Analyses of Na\(^+\), Cl\(^-\), and K\(^+\) in variously aged leaf blades**

The lyophilized tissues of the expanding, youngest fully expanded, and second youngest fully expanded leaf blades were ground to a fine powder in a ball mill and subsamples of approximately 0.02 g dry mass were used. Ions were extracted in 10 ml hot water (70 °C for 3 h) and samples were then shaken at room temperature for 2 d. The concentrations of Na\(^+\) and K\(^+\) in dilutions were determined by flame photometry (Flame Photometer 410; Corning, Halstead, UK). Cl\(^-\) concentrations were determined using a Buchler-Cotlove Chloridometer (Model 4-2000: Buchler Instruments, New Jersey USA). Recoveries of Na\(^+\), Cl\(^-\), and K\(^+\) from plant standards (State Chemistry Laboratory, Victoria, Australia) were, respectively, 85, 83, and 87%. The data presented have not been adjusted.
Determination of organic solute concentrations in leaf blades of *H. vulgare* and *H. marinum*

Lyophilized and ground subsamples of expanding, youngest fully expanded and, second youngest fully expanded leaf blades were used for the organic solute analyses. Tissues were extracted using the procedure described by Fan et al. (1993). In short, 3 ml of ice-cold 5% (v/v) perchloric acid was added to ~0.1 g of ground tissue, mixed, and centrifuged at 12 096 g for 30 min. Supernatant was collected and stored in a glass vial on ice. The pellet was again extracted in 3 ml of ice-cold 5% (v/v) perchloric acid, and the second supernatant was combined with that collected previously. pH of the supernatant was adjusted to 3.5±0.05 using K₂CO₃ to precipitate the perchlorate, after which the sample was again centrifuged, the supernatant collected, and the volume determined. The extract was passed through a 0.2 µm filter prior to injection into a HPLC system. The HPLC system (Waters Corporation, Milford, MA, USA) consisted of a 600E pump, 717 auto-sampler, 996 photodiode array detector, and Millennium Chromatography Manager software. The system was equipped with a Sugar-Pak column (300 mm length×6.5 mm diameter) (Waters Corporation) in a column heater maintained at 90 °C. The methods were as described by Naidu (1998), except that Ca-EDTA in the mobile phase was increased from 5 to 7.5 mg l⁻¹. Recoveries of Asn, glycinebetaine, and Pro from spiked samples of *Hordeum* shoot tissue were, respectively, 82, 96, and 94%. The data presented have not been adjusted.

Expressed leaf sap osmotic potential (Ψₑ)

To determine Ψₑ for expanding and youngest fully expanded leaf blades, frozen leaf samples were allowed to thaw to room temperature while in their sealed vials, before being pressed. The expressed sap was analysed using a dew-point depression osmometer (WESCOR Inc., Logan, UT, USA; C-52 sample chamber and HR-33T dew-point microvoltmeter).

Estimated contributions of ions and organic solutes to leaf sap Ψₑ in *H. vulgare* and *H. marinum*

The Ψₑ of a solution is given by –nRT/V, where n=number of solute molecules, R=the universal gas constant, T=temperature in °K, and V=volume in litres. In saline conditions, both solute accumulation and a lower water content can cause Ψₑ to become more negative. The contribution of reduced tissue water content was estimated by comparing leaf water content (ml g⁻¹ dry mass) of plants grown in the non-saline and 300 mol m⁻³ NaCl solutions. The contributions of ions (Na⁺, Cl⁻, and K⁺) and organic solutes (Asn, glycinebetaine, and Pro) to Ψₑ were calculated for *H. vulgare* and *H. marinum* from the data on ion and organic solute concentrations in variously aged leaf blades (µmol g⁻¹ dry mass) and leaf water contents (ml g⁻¹ dry mass) of plants grown in the non-saline and 300 mol m⁻³ NaCl solutions.

Statistical analyses

An ANOVA was performed on the growth parameters (shoot and root dry masses, RGR, and proportion of dead leaf material) to determine whether species influenced responses of these parameters to the various NaCl treatments. The leaf Ψₑ, tissue Na⁺, Cl⁻, and K⁺ concentrations, K⁺:Na⁺ ratios, and organic solute concentrations were evaluated using ANOVA to determine the influence of species and NaCl treatment. The following regression analyses were performed to determine whether inherent growth rate (i.e. vigour) was related to salt tolerance: whole plant dry mass in 150, 300, or 450 mol m⁻³ NaCl (as % of control), against whole plant dry mass (control); RGR in 150, 300, or 450 mol m⁻³ NaCl (as % of control), against RGR (control). Means were compared using the LSD (P=0.05), and all means presented in the tables and figures are accompanied by standard errors (GenStat, 6th edn; VSN International; www.vsni-intl.com).

Results

Growth analyses

**Shoot and root dry mass:** In the non-saline solution, *H. vulgare* had shoot and root dry masses approximately 5-fold higher than those of the other *Hordeum* species (Fig. 1; P<0.001). *Hordeum marinum* and *H. marinum* also had relatively high shoot (2.3 and 1.4 g, respectively) and root (0.8 and 0.5 g, respectively) dry masses, compared with the remaining species which ranged from 0.7 to 0.3 g for shoots, and 0.4 to 0.1 g for roots. The NaCl treatments caused shoot and root dry masses to decline in all species; however, *H. marinum*, *H. marinum* and, in particular, *H. vulgare* maintained the largest shoot and root dry masses throughout all NaCl concentrations. *Hordeum bogdani*, *H. lechleri*, and *H. jubatum* had the lowest shoot and root dry masses in all the NaCl treatments.

In terms of percentage reductions in dry mass, compared with controls, the NaCl treatments affected *H. marinum* and *H. intercedens* least, with whole plant dry masses in the 150 mol m⁻³ NaCl reduced by 33% and 34%, respectively. In all the other species, the 150 mol m⁻³ NaCl solution caused a 50% or greater reduction in whole plant dry mass. In the 300 mol m⁻³ NaCl solution, *H. marinum* had the smallest reduction in dry mass, which was 58%, compared with the remaining species in which dry masses were reduced by 70–80%. In the 450 mol m⁻³ NaCl, the reduction in dry mass for *H. marinum* was 75%, while the reduction in dry mass ranged from 81–91% in the remaining species.

Regression analysis of whole plant dry mass (as % of control) in 150, 300, or 450 mol m⁻³ NaCl against whole plant dry mass (control) was not significant (not shown; P>0.05, r²=0.05); showing that the variation in salt tolerance (as % of control) was not merely due to inherent differences in size of the various species.

**Relative growth rate:** Whole plant RGRs did not vary greatly amongst the species when in the non-saline solution, with the majority of the species having rates between 184 and 200 mg g⁻¹ d⁻¹ (Table 2). *Hordeum marinum* and *H. intercedens* were exceptions, having rates of 240 and 211 mg g⁻¹ d⁻¹, respectively, in the non-saline solution. NaCl caused a decline in RGR in all species (P<0.001).

In terms of the percentage reductions in RGRs when in the saline solution, compared with non-saline controls, *H. marinum* and *H. intercedens* had the smallest reductions (34% and 38% in the 450 mol m⁻³ NaCl solution, respectively) (Table 2; P<0.001). These two species also had the smallest reductions in RGR when in the 150 and 300 mol m⁻³ NaCl solutions; RGR in *H. marinum*, for example, was reduced by 10% and 21%, respectively. By contrast, *H. vulgare*, *H. jubatum*, and *H. lechleri* experienced the largest reductions in RGR in the NaCl treatments; for example, RGR decreased by ≥50% in the 450 mol m⁻³ NaCl solution. These species also experienced the largest...
reductions in RGR in the other NaCl treatments, with RGR in *H. vulgare*, for example, reduced by 17% and 39% in the 150 and 300 mol m\(^{-3}\) NaCl solutions, respectively.

Regression analysis of RGR (as % of control) in 150, 300, or 450 mol m\(^{-3}\) NaCl against RGR (control) was not significant (not shown; \(P >0.05\), \(r^2=0.33\)); showing that the differences amongst the genotypes in salt tolerance (RGR as % of control) were not merely related to inherent differences in RGR.

Table 2. *Whole-plant relative growth rates (mg \(g^{-1} d^{-1}\)) of Hordeum species in nutrient solution containing 0.2, 150, 300, or 450 mol m\(^{-3}\) NaCl.*

Species are listed in order according to tolerance (% of control) at 450 mol m\(^{-3}\) NaCl. Plants were raised to the 2.0–2.5-leaf stage in non-saline nutrient solution before NaCl was added at 50 mol m\(^{-3}\) \(d^{-1}\) until the final concentrations were reached. Plants were at these final concentrations for 16–21 d. Values are means ± standard error for three pots (pot means on a per plant basis, and each pot held three plants). Values for plants grown in the 450 mol m\(^{-3}\) NaCl solution are also presented as a percentage of the control plants grown in 0.2 mol m\(^{-3}\) NaCl. (n.a. = not available due to poor germination of this accession). ***, \(P <0.001\).

<table>
<thead>
<tr>
<th>Species</th>
<th>NaCl concentration (mol m(^{-3}))</th>
<th>450 mol m(^{-3}) NaCl-treated plants as % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2</td>
<td>150</td>
</tr>
<tr>
<td><em>H. marinum</em></td>
<td>196±4.1</td>
<td>177±0.1</td>
</tr>
<tr>
<td><em>H. intercedens</em></td>
<td>211±5.9</td>
<td>191±3.7</td>
</tr>
<tr>
<td><em>H. secalinum</em></td>
<td>200±5.1</td>
<td>170±4.5</td>
</tr>
<tr>
<td><em>H. patagonicum</em></td>
<td>187±7.0</td>
<td>155±4.8</td>
</tr>
<tr>
<td><em>H. bogdianii</em></td>
<td>184±0.5</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>H. muriatum</em></td>
<td>240±3.8</td>
<td>208±4.4</td>
</tr>
<tr>
<td><em>H. jubatum</em></td>
<td>184±6.6</td>
<td>137±1.2</td>
</tr>
<tr>
<td><em>H. lechleri</em></td>
<td>186±2.1</td>
<td>137±1.7</td>
</tr>
<tr>
<td><em>H. vulgare</em></td>
<td>199±3.9</td>
<td>166±4.4</td>
</tr>
</tbody>
</table>

LSD (species×NaCl) = 9***

Proportion of dead leaf material: In the non-saline and 150 mol m\(^{-3}\) NaCl solutions, the amount of dead leaf material (as a percentage of shoot dry mass) on any of the genotypes was at most 4% (Fig. 2). In the 300 mol m\(^{-3}\) NaCl solution, the percentages of dead leaf material in *H. vulgare* and *H. patagonicum* (11% and 7%), were higher than for the majority of the other species (\(P <0.001\)). In the 450 mol m\(^{-3}\) NaCl, differences amongst the species were even larger, with the percentages of dead leaf material in *H. vulgare*,...
H. patagonicum, and H. murinum being approximately double those in any other species. In particular, the percentage of dead leaf material in H. vulgare in the 450 mol m\(^{-3}\) NaCl was 17%, almost six times that in H. marinum, the species with the lowest amount of dead leaf material, at 3% \((P < 0.001)\).

Concentrations of Na\(^+\), Cl\(^-\), and K\(^+\) in variously aged leaf blades

\(\text{Na}^+\): For plants in the non-saline solution, there was no difference amongst species in leaf blade Na\(^+\) concentrations (Fig. 3). NaCl treatments increased the concentration of Na\(^+\) in leaf blades of all species, well above those in non-saline controls \((P < 0.001)\). With the exceptions of H. vulgare, H. murinum, and H. patagonicum, the other species showed relatively little variation in leaf blade Na\(^+\) concentrations in the 150, 300, or 450 mol m\(^{-3}\) NaCl solutions. For example, in the youngest fully expanded leaf blades of H. secalinum, Na\(^+\) concentrations in plants in the 150, 300, or 450 mol m\(^{-3}\) NaCl solutions were 387, 416, and 440 \(\mu\text{mol g}^{-1}\) dry mass, respectively. However, in H. vulgare and H. murinum (and to a lesser extent, H. patagonicum) increases in external NaCl to 300 mol m\(^{-3}\) or above, caused marked increases in Na\(^+\) concentrations in leaf tissues. For example in the youngest fully expanded leaf blades of H. secalinum, Na\(^+\) concentrations in the plants in 150, 300, or 450 mol m\(^{-3}\) NaCl were 127, 438, and 1835 \(\mu\text{mol g}^{-1}\) dry mass, respectively.

In general, H. vulgare, H. murinum, and H. patagonicum had higher Na\(^+\) concentrations in all leaf tissues and at all NaCl treatments, compared with the other species. For plants in the 450 mol m\(^{-3}\) NaCl solution, for example, the expanding leaf blades of H. vulgare, H. murinum, and H. patagonicum contained 2132, 1286, and 497 \(\mu\text{mol Na}^+\ g^{-1}\) dry mass, respectively, whereas in the other...
species it ranged from 285 (H. bogdanii) to as low as 132 (H. marinum) μmol g⁻¹ dry mass.

Na⁺ concentrations appeared to increase with leaf age in some species; however, the species×NaCl×leaf age interaction was not significant in any NaCl treatment.

Cl⁻: Cl⁻ concentrations in the various leaf blades of all species under the three NaCl treatments showed trends very similar to those of Na⁺ (Fig. 4). The Na⁺:Cl⁻ ratio (averaged for the species and variously aged leaf tissues) of plants grown in 150, 300, or 450 mol m⁻³ NaCl were 1.1, 0.9, and 0.8, respectively. As examples of genotypic differences, at 300 mol m⁻³ NaCl, Cl⁻ concentrations in the youngest fully expanded leaf blades of H. vulgare and H. murinum were 2107 and 1050 μmol g⁻¹ dry mass, respectively; whereas in this leaf in all other species, Cl⁻ ranged from 452 μmol g⁻¹ dry mass in H. patagonicum to as low as 181 μmol g⁻¹ dry mass in H. secalinum.

In the plants grown in non-saline solution, however, leaf Cl⁻ concentrations were higher than those of Na⁺ (the non-saline nutrient solution contained 0.2 mol m⁻³ Na⁺ and 0.05 mol m⁻³ Cl⁻). In H. vulgare and H. murinum grown in non-saline solution, Cl⁻ concentrations in the expanding leaf blades were, on average, 5-fold higher than those of Na⁺. In all other species, Cl⁻ concentrations were between 9- and 27-fold higher than those of Na⁺. This was paradoxical, since when the plants were exposed to 150 mol m⁻³ NaCl and above, H. vulgare and H. murinum had considerably higher concentrations of Cl⁻ in the leaf tissues than all the other species (P < 0.001).

As with the Na⁺ concentrations in leaves of plants in increasing NaCl, there was relatively little difference in Cl⁻ concentrations in most species when external NaCl was increased to 150 mol m⁻³ and above; in the expanding leaf blades of H. marinum, for example, Cl⁻ concentrations in the 150, 300, and 450 mol m⁻³ NaCl solutions were 205, 275, and 233 μmol g⁻¹ dry mass, respectively. Hordeum vulgare, H. murinum, and H. patagonicum were again the exceptions, showing marked increases in Cl⁻ concentrations with increasing external NaCl. In H. vulgare, in particular, Cl⁻ concentrations in the expanding leaf blades rose from 26 μmol g⁻¹ dry mass in the non-saline solution, to 491, 1039, and 1936 μmol g⁻¹ dry mass in the 150, 300, and 450 mol m⁻³ NaCl treatments, respectively.

Cl⁻ concentrations generally increased with leaf blade age in all NaCl treatments; for example at 450 mol m⁻³ NaCl, Cl⁻ concentrations were 1.9-fold higher in the second youngest fully expanded leaf blades compared with those in the expanding leaf blades (P < 0.001).

K⁺: In the non-saline solution, H. marinum, H. patagonicum, and H. secalinum had higher leaf K⁺ concentrations than the other species, with 1336, 1297, and 1287 μmol g⁻¹ dry mass, respectively, compared with an average for the others of 1027 μmol g⁻¹ dry mass (P < 0.001). When exposed to NaCl, leaf blade K⁺ concentrations (averaged for all species at all three NaCl treatments) declined by between 19% and 27% (P < 0.001; Fig. 5). In the NaCl treatments, K⁺ concentrations were lower in H. vulgare than in the wild Hordeum species (with the exception of

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![Fig. 4. Chloride concentrations in variously aged leaf blades of Hordeum species grown in nutrient solution containing 0.2, 150, 300, or 450 mol m⁻³ NaCl for 21 d. See Fig. 1 legend for treatment details. Error bars not visible when smaller than the size of the symbols. *, Values not available due to poor germination of this accession. P(species×NaCl) < 0.001; LSD=244.](https://academic.oup.com/jxb/article-abstract/56/419/2365/531979)
For example, K⁺ concentrations in the expanding leaf blades of the wild *Hordeum* species were 31% higher, on average, than in *H. vulgare* (*P* < 0.001). More specifically, in *H. marinum* and *H. vulgare*, species with the lowest and highest Na⁺ and Cl⁻ concentrations, respectively, K⁺ concentrations in the expanding leaf blades were, respectively, 1395 and 846 μmol g⁻¹ dry mass in the non-saline solution, and these declined to 926 and 783 μmol g⁻¹ dry mass in the 300 mol m⁻³ NaCl solution.

For plants in the non-saline or in the 150 mol m⁻³ NaCl treatments there was a general trend for leaf tissue K⁺ concentrations to decrease with increasing leaf age (Fig. 5); however, this trend was not observed in plants at the higher NaCl treatments and the species × NaCl × leaf age interaction was not significant.

**K⁺:Na⁺ ratio:** The pattern of K⁺:Na⁺ in leaf tissues versus increasing external NaCl was relatively similar for most of the *Hordeum* species, the exceptions being *H. marinum* and *H. vulgare* (calculated from data (not shown) in Figs 3 and 5; *P* < 0.001). For example, in the 150 mol m⁻³ NaCl solution, the K⁺:Na⁺ ratios in the expanding leaf blades of *H. intercedens*, *H. jubatum*, *H. lechleri*, *H. marinum*, *H. patagonicum*, and *H. secalinum* ranged from 9 to 13 and fell more or less linearly to between 2 and 7 in plants at 450 mol m⁻³ NaCl. *H. marinum* and *H. vulgare* were the exceptions to this trend (*P* < 0.001); in *H. marinum*, the K⁺:Na⁺ ratio remained relatively high in the expanding leaf blade of plants at 150 mol m⁻³ NaCl solution, but then fell to 3 and 0.5 in the 300 and 450 mol m⁻³ NaCl solutions, respectively; well below the values in the other species. By contrast, the K⁺:Na⁺ ratio in *H. vulgare* started well below the values in the other species; in the 150 mol m⁻³ NaCl solution, the value was 1.5 for expanding leaf blades, and it became even lower in plants at 300 and 450 mol m⁻³ NaCl, being 0.9 and 0.3, respectively. These genotypic differences in the K⁺:Na⁺ ratios were also evident for the next two youngest leaf blades, although the magnitude progressively declined.

**Organic solute concentrations in leaf blades of *H. vulgare* and *H. marinum***

The tissue samples of many of the wild *Hordeum* species were too small to enable a complete analysis of organic solutes. Therefore, the two most contrasting species in the parameters discussed above (*H. vulgare* and *H. marinum*) were selected for this part of the study (Fig. 6).

**Glycinebetaine:** There was no difference between *H. vulgare* and *H. marinum* in glycinebetaine concentrations in leaves of plants in the non-saline solution (Fig. 6). In both species, glycinebetaine concentrations increased in response to increases in external NaCl. The pattern of increase in glycinebetaine concentrations in response to NaCl treatment.
Salt tolerance in wild Hordeum species

Fig. 6. Concentrations of glycinebetaine, asparagine and proline in variously aged leaf blades of *H. marinum* and *H. vulgare* grown in nutrient solution containing 0.2, 150, 300, or 450 mol m\(^{-3}\) NaCl. See Fig. 1 legend for treatment details. Error bars not visible when smaller than the size of the symbols. Glycinebetaine: P(species×NaCl×leaf blade)<0.01; LSD=46. Proline: P(species×NaCl×leaf blade) ≤0.05; LSD=52. Asparagine: P(species×NaCl×leaf blade) <0.01; LSD=15.

was similar in the expanding, and recently expanded leaf blades, although, generally glycinebetaine decreased with leaf age (P <0.01). In the 450 mol m\(^{-3}\) NaCl solution, for example, glycinebetaine concentrations in the expanding leaf blades of *H. marinum* and *H. vulgare* were 1.3–2.2-fold higher, respectively, than in the second youngest fully expanded leaf blades.

Proline: Proline concentrations did not differ between leaves of *H. vulgare* and *H. marinum* in the non-saline solution (Fig. 6). Although increasing external NaCl concentrations caused an increase in Pro in both species, the increase was much larger in *H. vulgare* (being 17-fold in the expanding leaf blades) than in *H. marinum* (8-fold in the expanding leaf blades) at 450 mol m\(^{-3}\) NaCl. In *H. vulgare*, Pro concentrations were highest in the expanding leaf blade (P <0.05).

In *H. marinum*, Pro concentrations were lower than those of glycinebetaine in all NaCl treatments; being 13–79% of the glycinebetaine concentrations. In *H. vulgare* in the non-saline, 150 and 300 mol m\(^{-3}\) NaCl treatments, Pro concentrations were also lower than those of glycinebetaine. However, in *H. vulgare* in the 450 mol m\(^{-3}\) NaCl treatment, Pro concentrations were 1.6–2.8-fold higher than those of glycinebetaine.

Asparagine: In plants in the non-saline solution, Asn concentrations in leaves of *H. vulgare* and *H. marinum* were below 8 μmol g\(^{-1}\) dry mass in both species (Fig. 6). With increasing NaCl in the external solution, Asn concentrations in the variously aged leaf blades of *H. marinum* did not significantly increase. In *H. vulgare*, Asn concentrations increased in all leaf blades with increased external NaCl concentration; for example, in the expanding leaf blades, Asn concentrations increased from 2 μmol g\(^{-1}\) dry mass in the non-saline solution to 15, 35, and 80 μmol g\(^{-1}\) dry mass in the plants in 150, 300, and 450 mol m\(^{-3}\) NaCl solutions, respectively.

In both species, and in all NaCl concentrations, Asn decreased with leaf age (P <0.001); for example, concentrations of Asn in the plants in 450 mol m\(^{-3}\) NaCl solution were 1.8- and 3.3-fold higher in the expanding leaf blades of *H. marinum* and *H. vulgare*, respectively, compared with those in the second youngest fully expanded leaf blades.

Asn concentrations were low compared with those of glycinebetaine and Pro in both species at all NaCl concentrations; being 2–36% of glycinebetaine, and 4–80% of Pro concentrations.

Expressed leaf sap osmotic potential (\(\Psi_n\))

For plants in non-saline solution, there was no difference in leaf \(\Psi_n\) amongst the species (Fig. 7). The 150, 300, and 450 mol m\(^{-3}\) NaCl treatments decreased the \(\Psi_n\) of the nutrient solution (\(\Psi_{\text{external}}\)) from −0.05 MPa to −0.69, −1.37, and −2.05 MPa, respectively. This resulted in a decline in leaf \(\Psi_n\) in all species (P <0.001). In general, this decline was approximately linear; except in *H. vulgare* and *H. marinum*, where the decline in leaf \(\Psi_n\) was larger than in the other species and increased sharply (i.e. almost doubling) from the 300 to the 450 mol m\(^{-3}\) NaCl treatment. At 450 mol m\(^{-3}\) NaCl (Δ\(\Psi_{\text{external}}\)=−2.0 MPa), the Δ\(\psi_{\text{leaf}}\) (salt treated minus control) was −3.52 and −3.02 MPa for *H. vulgare* and *H. marinum*, respectively, compared with −0.96 to −1.62 MPa for the other species. However, all of the species maintained leaf \(\Psi_n\) below \(\Psi_{\text{external}}\), suggesting that the much larger decline in leaf \(\Psi_n\) in *H. vulgare* and *H. marinum* exceeded that necessary for ‘osmotic adjustment’. Nevertheless, *H. vulgare* had the smallest reduction in tissue water content with the addition of NaCl. For example, in the expanding leaf blades of *H. vulgare*, the water content was reduced by 21% in the 300 mol m\(^{-3}\) NaCl (data not shown). Expanding leaf blades of *H. marinum* had a tissue water content in the non-saline solution of 5.6 ±0.1 ml g\(^{-1}\) dry mass, similar to that of *H. vulgare* (5.0 ±0.1 ml g\(^{-1}\) dry mass), but *H. marinum*...
showed a relatively large reduction in leaf tissue water content with increasing NaCl. For example, for *H. marinum* plants in the 300 mol m$^{-3}$ NaCl treatment, leaf tissue water content was reduced by 46% in the expanding leaf blades (data not shown). The average reduction in tissue water content for expanding leaf blades of the other wild species, when treated with the 300 mol m$^{-3}$ NaCl, was 43%, similar to that in *H. marinum*. This indicates that when exposed to NaCl, reduced tissue water content made a larger contribution to lowering leaf $\Psi_\pi$ in *H. marinum*, and the other wild species, compared with *H. vulgare*.

**Estimated contributions of ions and organic solutes to leaf sap $\Psi_\pi$ in *H. vulgare* and *H. marinum***

The contributions of selected ions (K$^+$, Na$^+$, and Cl$^-$) and organic solutes (Asn, glycinebetaine, and Pro) to $\Psi_\pi$ in the expanding and youngest fully expanded leaf blades of *H. vulgare* and *H. marinum* treated with non-saline and 300 mol m$^{-3}$ NaCl solution were calculated as described in the Materials and methods. K$^+$ plus Na$^+$ and Cl$^-$, accounted for 65% of $\Psi_\pi$ in the expanding leaf blades of *H. vulgare* grown in 300 mol m$^{-3}$ NaCl solution, of which K$^+$ accounted for 19%, Na$^+$ for 21%, and Cl$^-$ for 25% (data not shown). By comparison, these three ions accounted for 51% of $\Psi_\pi$ in the expanding leaf blades of *H. marinum* grown in 300 mol m$^{-3}$ NaCl solution; with K$^+$ contributing 35%, whereas Na$^+$ and Cl$^-$ each accounted for only 6% and 10%, respectively. The contributions of these three ions to $\Psi_\pi$ in the youngest fully expanded leaf blades were similar to those in the expanding leaves for both species. Although leaf blade organic solute concentrations (dry mass basis) were quite similar in *H. vulgare* and *H. marinum* in the 300 mol m$^{-3}$ NaCl solution, organic solutes made a significantly larger contribution to $\Psi_\pi$ in *H. marinum* (15% and 9% in the expanding and youngest fully expanded leaf blades, respectively), compared with *H. vulgare* (8% and 3%, respectively), due to the lower water content in *H. marinum*. In both *H. marinum* and *H. vulgare*, glycinebetaine made the largest contribution to $\Psi_\pi$ in the 300 mol m$^{-3}$ NaCl, at 8% and 6%, respectively, in the expanding leaves, followed by Pro (6% and 2%, respectively). These trends were similar for plants in the other NaCl concentrations; however, the contrasts between the two species became most prominent in the 450 mol m$^{-3}$ NaCl solution. Asn contributed $\approx$1% to $\Psi_\pi$ in expanding leaf blades of both species at all NaCl concentrations.

**Discussion**

This study evaluated the tolerance of eight wild *Hordeum* species, and cultivated barley, to growth in salinized nutrient solution, and compared leaf tissue concentrations of Na$^+$, Cl$^-$, and K$^+$, and leaf $\Psi_\pi$. The wild *Hordeum*
species had superior ‘exclusion’ of Na\(^+\) and Cl\(^-\) from the leaves and better maintenance of tissue K\(^+\) concentrations, compared with *H. vulgare*, when exposed to NaCl salinity. A number of these wild *Hordeum* species also had lower reductions in growth when in saline conditions, compared with *H. vulgare*, indicating substantial variation in salt tolerance, and associated traits, within the genus *Hordeum*.

The Na\(^+\) and Cl\(^-\) concentrations in the leaf tissues of the wild *Hordeum* species were markedly lower than those for *H. vulgare*. For plants grown at 150 mol m\(^{-3}\) NaCl, Na\(^+\) and Cl\(^-\) concentrations in the youngest fully expanded leaf blades of the wild *Hordeum* species were, on average, similar to those found for *H. jubatum* (Suhaayda et al., 1992; Huang and Redmann, 1995), and were significantly lower than those previously recorded for several wild Triticeae species when grown at 150 mol m\(^{-3}\) NaCl; being 76% and 72% lower, respectively, than those found in the progenitor of barley, *Hordeum vulgare* ssp. spontaneum (Gorham et al., 1990a; Forster et al., 1994), 45% and 34% lower, respectively, than in *Thinopyrum elongatum* (Greenway and Rogers, 1963), and 27% and 39% lower, respectively, than in *Thinopyrum bessarabicum* (Gorham et al., 1986). Na\(^+\) and Cl\(^-\) concentrations in the wild *Hordeum* species were on average 62% and 57% lower, respectively, than in durum wheat (*Triticum turgidum*) (Munns et al., 2000; Rivelli et al., 2002; Husain et al., 2004), but Na\(^+\) was similar to that in bread wheat (*Triticum aestivum*) (Munns et al., 2000; Rivelli et al., 2002; Husain et al., 2004) for plants at 150 mol m\(^{-3}\) NaCl. Moreover, Na\(^+\) (and Cl\(^-\)) ‘exclusion’ by several of the wild *Hordeum* species was still achieved even at high salinity (i.e. 300 and 450 mol m\(^{-3}\)), which was in stark contrast to *H. vulgare* (Fig. 3).

Cl\(^-\) concentrations in leaves of *H. vulgare* increased almost linearly with increasing salinity (Fig. 4). Greenway (1965) demonstrated that Cl\(^-\) entered roots of *H. vulgare* via an ‘energy-independent’ flow at high external NaCl, whereas the ‘energy-dependent’ component of Cl\(^-\) uptake, typical of all plants when exposed to low Cl\(^-\) (White and Broadley, 2001), was decreased. The ‘energy-independent’ uptake of Cl\(^-\) increased linearly with external concentration (Greenway, 1965). In contrast to the high concentrations of Cl\(^-\) in *H. vulgare*, several of the wild *Hordeum* species still maintained relatively low concentrations of Cl\(^-\) in leaves, even at high salinity (i.e. 300 and 450 mol m\(^{-3}\) NaCl). Leaf Cl\(^-\) concentrations in the wild *Hordeum* species were also markedly lower than those reported for bread wheat. For example, the Cl\(^-\):Na\(^+\) ratio in the leaf tissues of bread wheat cv. ‘Janz’ was 5 (Husain et al., 2004) compared with 1.2 in the wild *Hordeum* species. This suggests that the genus *Hordeum* contains significant variation for ‘exclusion’ of both Na\(^+\) and Cl\(^-\) under saline conditions, whereas there appears to be little variation in Cl\(^-\) ‘exclusion’ between other species in the Triticeae (Shah et al., 1987; Husain et al., 2004).

As a result of superior Na\(^+\) ‘exclusion’ and better maintenance of leaf K\(^+\) concentrations in saline conditions, the wild *Hordeum* species exhibited higher leaf K\(^+\):Na\(^+\) ratios compared with *H. vulgare*. In the wild *Hordeum* species, the average K\(^+\):Na\(^+\) ratio in the youngest, fully expanded leaf blades of plants at 150 mol m\(^{-3}\) NaCl was 5.2, compared with 0.8 in *H. vulgare*. Many *Triticum* species have good Na\(^+\) ‘exclusion’ and better K\(^+\)/Na\(^+\) selectivity compared with *H. vulgare*, as evidenced by high leaf K\(^+\):Na\(^+\) ratios (Gorham et al., 1990a; Gorham, 1993). For example, Gorham et al. (1990a) measured a K\(^+\):Na\(^+\) ratio of 5.9 in the youngest, fully expanded leaf blades of *Triticum aestivum* and one of its progenitors, *Triticum tauschii*, grown in 160 mol m\(^{-3}\) NaCl for 7 d. The high K\(^+\):Na\(^+\) ratio in leaves of *T. aestivum* and *T. tauschii* is partially attributed to the ‘enhanced K\(^+\)/Na\(^+\) discrimination’ character (Gorham et al., 1990b; Gorham, 1993; Dvora´k et al., 1994). Similarly high K\(^+\):Na\(^+\) ratios in leaves of the wild *Hordeum* species indicates mechanisms for Na\(^+\) ‘exclusion’ and K\(^+\)/Na\(^+\) selectivity in these species. However, the wild *Hordeum* species (with the exception of *H. murinum*) appear to maintain K\(^+\)/Na\(^+\) selectivity even at high salinity (i.e. 450 mol m\(^{-3}\) NaCl). By contrast, the K\(^+\)/Na\(^+\) discrimination trait in *T. aestivum* and *T. tauschii* is most apparent at relatively low salinities (i.e. ~50 mol m\(^{-3}\)) (Gorham, 1993; Gorham et al., 1997).

The measurements of leaf Ψ\(_n\) for plants grown in the NaCl treatments indicated that all of the wild *Hordeum* species maintained Ψ\(_n\) below Ψ\(_{\text{external}}\), despite having relatively low Na\(^+\) and Cl\(^-\) accumulation compared with *H. vulgare* (although *H. murinum*, with relatively high leaf Na\(^+\) and Cl\(^-\) concentrations, was an exception) (Fig. 7). The response of the wild *Hordeum* species (except *H. murinum*), in which entry of Na\(^+\) and Cl\(^-\) appears to be well-regulated so that leaf Ψ\(_n\) only declined by that required to maintain it below Ψ\(_{\text{external}}\), is similar to that of *Thinopyrum bessarabicum* (Gorham et al., 1985). The decline in leaf Ψ\(_n\) in *H. maritimissimum* in the saline solution resulted from reduced tissue water content, and some entry of Na\(^+\) and Cl\(^-\) with maintenance of K\(^+\) concentrations (dry mass basis) and increased production of organic solutes. Glenn (1987) found a similar response to NaCl stress amongst halophytic grasses compared with glycophytic grasses; several halophytes showed reduced tissue water contents and higher K\(^+\):Na\(^+\) ratios, while still maintaining growth in the saline conditions (Glenn, 1987). The consequences of this strategy for the plant are poorly understood. Some authors propose that reduced tissue water content may be regarded as an ameliorating factor that lowers osmotic potential whilst limiting Na\(^+\) and Cl\(^-\) uptake (Glenn and O’Leary, 1984; Glenn, 1987). However, a reduction in leaf water content may affect turgor, depending on wall elasticity, although moderate reductions in turgor might not always impact on growth (Munns, 1993). Moreover, reduced tissue water content would itself

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increase the concentration of ions in the cellular fluids and this is what presumably determines toxicity. Data on concurrent changes in tissue elasticity (i.e. cell walls contracting as water is lost), and possible changes in wall extensibility under saline conditions as speculated by Greenway and Munns (1980), are limited for monocots [e.g. Spartina alterniflora (Drake and Gallagher, 1984), Ammophila arenaria and Elymus mollis (Pavlik, 1984)] and elasticity either did not change or decreased. Nevertheless, the common occurrence of reduced leaf water content and restricted ion uptake in halophytic monocots in response to NaCl stress makes the phenomenon worthy of further study.

Calculations of contributions of ions and organic solutes to leaf $\Psi_{w}$ indicated that $H. \text{marinum}$ has a higher proportion of organic solutes to osmotically ‘balance’ Na$^+$ and Cl$^-$ (which are presumably sequestered in the vacuole) compared with $H. \text{vulgare}$. For example, when at 150 mol m$^{-3}$ NaCl, the ratio of glycinebetaine to Na$^+$ in expanding leaf blades of $H. \text{marinum}$ was 1.1 compared with 0.2 in $H. \text{vulgare}$. Moreover, in expanding leaf blades of plants grown in 300 mol m$^{-3}$ NaCl, organic solutes contributed ~15% of $\Psi_{w}$ in $H. \text{marinum}$, compared with ~8% in $H. \text{vulgare}$. Interestingly, when exposed to salinity, Pro was higher in $H. \text{vulgare}$ (dry mass basis) and also in several other species in the Triticeae [e.g. T. aestivum cv. ‘Chinese Spring’ (Gorham et al., 1986; Colmer et al., 1995), $H. \text{jubatum}$ (Huang and Redmann, 1995), and Thinopyrum elongatum (Gorham et al., 1986)], than in $H. \text{marinum}$.

$H. \text{marinum}$, $H. \text{intercedens}$, and to a lesser extent $H. \text{secalinum}$, were the most salt-tolerant species in this study, having the smallest reductions (% of control values) in shoot and root dry masses and RGR in the NaCl treatments. In comparison to non-saline controls, whole plant dry mass in $H. \text{marinum}$ (the most tolerant species) was 67% in the 150 mol m$^{-3}$ NaCl treatment, compared with 46% in $H. \text{vulgare}$. The value measured here for $H. \text{vulgare}$ ssp. vulgaris was similar to that previously found for a number of other cultivars (Greenway, 1962; Storey and Wyn Jones, 1978b), and its progenitor, $H. \text{vulgare}$ ssp. spontaneum (dry mass in 150 mol m$^{-3}$ NaCl was 40% of control) (Forster et al., 1994). However, the distinction between the wild $Hordeum$ species and $H. \text{vulgare}$ was less for growth parameters, compared with those for tissue ion concentrations, when in the NaCl treatments. Differences amongst the species for growth in saline conditions might become even larger in the longer term (NaCl exposure in the present experiment was up to 21 d). Na$^+$ and Cl$^-$ accumulate as leaves age since ions are constantly delivered in the transpiration stream, hence salt concentrations will be highest in the older leaves (Greenway and Munns, 1980), which eventually die due to ion toxicity (Munns, 2002). Growth differences between closely related genotypes in saline conditions might take time to develop, as different rates of salt accumulation in the leaves impact on leaf area (Munns et al., 1995; Munns, 2002). The large difference in the proportion of dead leaf material between the wild $Hordeum$ species and $H. \text{vulgare}$, when exposed to NaCl (Fig. 2), supports the suggestion that differences in growth might become even larger when exposed to 450 mol m$^{-3}$ NaCl solution, the dead leaf material (as a % of shoot dry mass) in $H. \text{vulgare}$ was 17%, compared with 3–11% in the other species. Munns et al. (1995) found for wheat that, when the percentage of dead leaves reached about 20% of the total, the rate of leaf production slowed down dramatically and some plants died. It is likely that the present experiments underestimate the actual variation in salt tolerance between the species studied.

In conclusion, the growth parameters and tissue Na$^+$ and Cl$^-$ concentrations measured under NaCl treatments in this experiment indicate large differences in salt tolerance of several wild $Hordeum$ species as compared with $H. \text{vulgare}$. The genus $Hordeum$ is comprised of four genomes: $H. \text{vulgare}$ has the I genome, $H. \text{marinum}$ the Y genome, $H. \text{intercedens}$ and $H. \text{secalinum}$, the X genome as compared with $H. \text{vulgare}$. The genus $Hordeum$ was found that tolerance to waterlogging and associated root aeration traits were related to genome type. Species with the I or Y genome were generally much less tolerant to waterlogging than those with the X or H genome (Garthwaite et al., 2003). The present findings on salinity tolerance in wild $Hordeum$ species indicate that the X and H genomes are also of interest for traits associated with salt tolerance. The results provide evidence for the reputed salt tolerance of several wild species in the genus $Hordeum$, and demonstrate the significant diversity in salt tolerance amongst $Hordeum$ species.

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