The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator

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Background and Aims Seed germination is negatively affected by salinity, which is thought to be due to both osmotic and ion-toxicity effects. We hypothesize that salt is absorbed by seeds, allowing them to generate additional osmotic potential, and to germinate in conditions under which they would otherwise not be able to germinate.

Methods Seeds of barley, Hordeum vulgare, were germinated in the presence of either pure water or one of five iso-osmotic solutions of polyethylene-glycol (PEG) or NaCl at 5, 12, 20 or 27 °C. Germination time courses were recorded and germination indices were calculated. Dry mass, water content and sodium concentration of germinating and non-germinating seeds in the NaCl treatments at 12 °C were measured. Fifty supplemental seeds were used to evaluate the changes in seed properties with time.

Key Results Seeds incubated in saline conditions were able to germinate at lower osmotic potentials than those incubated in iso-osmotic PEG solutions and generally germinated faster. A positive correlation existed between external salinity and seed salt content in the saline-incubated seeds. Water content and sodium concentration increased with time for seeds incubated in NaCl. At higher temperatures, germination percentage and dry mass decreased whereas germination index and sodium concentration increased.

Conclusions The results suggest that barley seeds can take up sodium, allowing them to generate additional osmotic potential, absorb more water and germinate more rapidly in environments of lower water potential. This may have ecological implications, allowing halophytic species and varieties to out-compete glycophytes in saline soils.

Key words: Barley, Hordeum vulgare, germination, osmotic potential, PEG, salt, seed, sodium, seed water content.

INTRODUCTION

More than 900 million hectares of land world-wide, approx. 20 % of the total agricultural land (FAO, 2007), are affected by salt, accounting for more than 6 % of the world’s total land area. NaCl is the predominant salt causing salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation (Munns and Tester, 2008). Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress (Chapman, 1974; Unger, 1995), the mechanism(s) of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in vegetative plants (Hester et al., 2001; Garthwaite et al., 2005; Hu et al., 2005; Ren et al., 2005; Kanai et al., 2007).

Salinity affects seed germination through osmotic effects (Bliss et al., 1986), ion toxicity (Hampson and Simpson, 1990) or a combination of the two (Huang and Redmann, 1995). In vegetative plants, salt stress causes reduced cell turgor and depressed rates of root and leaf elongation (Werner and Finkelstein, 1995; Fricke et al., 2006), suggesting that environmental salinity acts primarily on water uptake. Furthermore, high intracellular concentrations of both Na+ and Cl− can inhibit the metabolism of dividing and expanding cells (Neumann, 1997), retarding germination and even leading to seed death. Ionic effects may be distinguished from osmotic effects by comparing the effect of salt solutions and iso-osmotic solutions of an inert osmoticum such as polyethylene-glycol (PEG; technically primarily a matricum) that cannot penetrate the cell wall. Inhibition of germination in PEG-treated seeds is attributed solely to osmotic effects, and any difference in germination of salt-treated relative to PEG-treated seeds is generally attributed to ionic effects (Dodd and Donovan, 1999).

Plants can be classified into two main groups based on their response to saline stress, salt-tolerant halophytes and salt-intolerant glycophytes. However, this classification is somewhat artificial as the implied discreteness of response does not exist in reality, with responses occurring along a gradient...
Salinity-induced reduction in the germination of halophytes is mainly due to osmotic effects only, whereas glycophytes are more likely to exhibit additional ion toxicity (Romo and Haferkamp, 1987; Dodd and Donovan, 1999). Furthermore, the seeds of salt-tolerant species tend to have lower osmotic potentials, allowing them to absorb water from the environment. This decrease in osmotic potential can be achieved in two ways: exclusion of salt from the cells while maintaining osmotic potential using organic solutes, or by allowing Na\(^+\) and Cl\(^-\) to enter the cells and using them as osmolytes while having mechanisms for mitigating the toxic effects of salt within the cell.

Cellular NaCl concentration is a function of the fluxes into, and out of, the cell. Glycophytic plants tend to have limited regulation of NaCl entry, whereas halophytes typically have good regulation with ion-gated channels regulating the entry of NaCl into cells (Glenn et al., 1999). Export of Na\(^+\) from the cell requires specialized plasma-membrane Na\(^+\)/H\(^+\) antiporters (Horie and Schroeder, 2004). Some halophytic species accumulate Na\(^+\) and Cl\(^-\) in the vacuole, mediated by tonoplast Na\(^+\)/H\(^+\) anti-porters, allowing the cytoplasm to be maintained at substantially lower ion concentrations while avoiding metabolic inhibition (Rea et al., 1992; Serrano and Gaxiola, 1994). Over-expression of tonoplast Na\(^+\)/H\(^+\) anti-porter genes (NHX genes), or the proton pumps (ATPase) supplying them with energy, has been shown to increase the salt tolerance of the very glycophytic Arabidopsis (Apse et al., 1999; Gaxiola et al., 2001). However, all these mechanisms of salt tolerance – excretion, osmotic adjustment using organic solutes and compartmentalization of Na\(^+\) – are metabolically energetic and compete with plant growth for resources (i.e. sugars). Thus, if these mechanisms, elucidated in vegetative plant cells, also operate within the cells of seeds germinating in saline environments, it suggests conflicting demands for carbon reserves, both osmotic balance and growth, the balance between which must determine the likelihood of successful germination.

Likewise, temperature is crucial in determining germination rate and timing of non-dormant seeds in most species. Although high environmental salinity inhibits germination, the detrimental effects of salinity are generally reduced at optimum temperatures (De Villiers et al., 1994; Aiazzi et al., 2002; Khan et al., 2002), with decreased germination noted in various species at either supra- or sub-optimal temperatures (Gulzar et al., 2001; Khan and Gulzar, 2003). Decreases in germination have been attributed to increased evaporation of moisture at high temperatures, increasing salt concentration by capillary movement (Khan and Ungar, 1996). Although the specific reasons for reduced germination at low temperatures under salt stress are unclear, it may be that the physiological mechanisms involved in the ability to tolerate salt are less effective. In some species, salt tolerance, or the lack thereof, is not temperature dependent (Khan and Gul, 1998). In wheat and rice, salinity has been shown to negatively affect the rate of starch remobilization by causing a decrease in \(\alpha\)-amylase activity, although PEG had similar effects (Lin and Kao, 1995; Almansouri et al., 2001). These negative effects of salt on \(\alpha\)-amylase activity were genotype specific, which may explain genotypic variation in germination responses to salt. Salinity–temperature interactions may have significant eco-physiological implications in terms of germination rates and date under field conditions (Ungar, 1995).

This paper details an experiment conducted for two barley varieties, in which differential salinity responses were noted in a pre-trial (on the effects of salinity and temperature) and seeks to provide detailed information on the germination responses. Barley, a commercially important species, can tolerate about 5 g L\(^{-1}\) NaCl and can be considered a marginal halophyte (Glenn et al., 1999). We aim to investigate the hypothesis that sodium is sequestered by the cells of halophytic seeds and used as a cellular osmoticum. We hope to differentiate clearly between osmotic and ionic effects, and to clearly illustrate increased germination rates and percentages as a result of sodium uptake by the seeds.

**MATERIALS AND METHODS**

**Plant material**

Two varieties of barley (Hordeum vulgare) were used: ‘Cask’ and ‘County’ (Cropmark Seeds Ltd, Christchurch, New Zealand). Seeds were stored in a cool, dark room prior to the start of the experiment. The seeds in both samples were approx. 18 months old when the experiments were carried out (April 2006). Approximately 85% of ‘Cask’ seeds and 80% of ‘County’ seeds were germinable.

**Germination experiments**

Germination studies were conducted in incubators with a 12-h photoperiod (Sylvania cool white fluorescent lamps, 25 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) photosynthetically active radiation). Two osmotica [NaCl and PEG, in reverse osmosis (RO) purified water], six water potentials (0, −0.45, −0.88, −1.32, −1.76 and −2.2 MPa) and four temperature regimes (5, 12, 20 and 27°C) were used in the experiment, for each variety. The osmotic potentials of these solutions were corrected for the effects of temperature using the relationships determined by Michel (1983). A randomized complete block design was applied at each temperature, with four replicates of 25 seeds each. The equivalent water potentials of NaCl and PEG solutions were determined using a dew point micro-voltmeter (HR33T; Wescor Inc., Logan, UT, USA). Seeds were arranged on a blotter to which 5 mL of the test solution was added. Pairs of blotters were sealed in lidded plastic containers to minimize evaporative losses. Seeds were considered to have germinated upon emergence of the radicle. Initially, the seeds were checked every 4 h for 3 d; however, as the germination rates decreased this was increased to every 8 h for 7 d, then once a day. When no seeds germinated in a treatment for 5 d, we considered that germination had completed for that treatment. Treatments in which none of the seeds germinated were incubated under their respective conditions until no further germination was noted for any treatment in the entire experiment for 5 d.

The germination index was estimated using the following equation (Maguire, 1962):

\[
\sum_{i=1}^{k} \frac{n_i}{t_i}
\]
were calculated before being digested using HCl and H2O2. These treatments were also sampled. The seeds were dried at the end of the experiment, seeds that had not germinated in temperatures, and those germinating in –0.88 MPa PEG at 20 and 27°C were also removed, weighed and labelled (very few seeds germinated in –0.88 MPa NaCl at 5 or 12°C). At the end of the experiment, seeds that had not germinated in these treatments were also sampled. The seeds were dried at 70°C, their dry masses were determined and water contents were calculated before being digested using HCl and H2O2 at 200°C, and their sodium content was measured by atomic absorbance spectrometry.

Fifty supplemental seeds of ‘Cask’ and ‘County’ were incubated in –0.88 MPa NaCl at 12°C, allowing water content, seed dry mass and sodium concentration to be determined throughout the first few hours of the experiments. Three seeds were sampled randomly every 4 h. Germination incidence was not taken into consideration.

Table 1. Final germination percentages for ‘Cask’ and ‘County’ barley incubated under a range of osmotic potentials generated by either NaCl or PEG 6000

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>NaCl</th>
<th>PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>-0.44</td>
</tr>
<tr>
<td>‘Cask’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>83 (2)</td>
<td>79 (2)</td>
</tr>
<tr>
<td>12</td>
<td>86 (3)</td>
<td>85 (6)</td>
</tr>
<tr>
<td>20</td>
<td>72 (2)</td>
<td>67 (2)</td>
</tr>
<tr>
<td>27</td>
<td>37 (1)</td>
<td>39 (4)</td>
</tr>
<tr>
<td>‘County’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>73 (3)</td>
<td>68 (7)</td>
</tr>
<tr>
<td>12</td>
<td>79 (2)</td>
<td>79 (2)</td>
</tr>
<tr>
<td>20</td>
<td>71 (5)</td>
<td>70 (3)</td>
</tr>
<tr>
<td>27</td>
<td>67 (5)</td>
<td>57 (8)</td>
</tr>
</tbody>
</table>

Values in parentheses are standard errors of the mean.

where ni is the percentage of seeds germinating on the ith day, ti is the number of days counted from the start of the experiment (i) to k, the last day on which seeds germinated. Higher values represent a more rapid rate of germination. The relationship between germination index and water potential was determined by linear regression.

Seed property measurements

To look more closely at the relationships between salinity and germination, germinating seeds of all NaCl treatments including control at 12°C were removed, rinsed in RO water, dried with blotting paper, weighed and labelled. Furthermore, seeds germinating in –0.88 MPa NaCl at all temperatures, and those germinating in –0.88 MPa PEG at 20 and 27°C were also removed, weighed and labelled (very few seeds germinated in –0.88 MPa PEG at 5 or 12°C). At the end of the experiment, seeds that had not germinated in these treatments were also sampled. The seeds were dried at 70°C, their dry masses were determined and water contents were calculated before being digested using HCl and H2O2 at 200°C, and their sodium content was measured by atomic absorbance spectrometry.

Data analysis

Seeds incubated at 12°C were selected to measure seed properties as it was considered to be the most ecologically relevant of the four germination temperatures used. Differences between directly comparable treatments were estimated by either a one-way or a two-way ANOVA as appropriate with a post-hoc Holm–Sidak test, while regression analyses between germination index and water potential or between seed properties and time were carried out in SigmaPlot version 11.2 (Systat Inc., San Jose, CA, USA).

RESULTS

At 5°C, both ‘Cask’ and ‘County’ control seeds (0 MPa) had high germination percentages: 83 ± 1.9% and 73 ± 3%, respectively (Table 1). Seeds exposed to higher salt concentrations started later and took longer to germinate, although, apart from the –1.76 and –2.2 MPa treatments in ‘Cask’ and the –2.2 MPa treatment in ‘County’, they eventually reached similar final germination percentages. The –0.88 MPa NaCl treatment had a slightly higher final value than the control in both varieties. Final germination percentages decreased significantly with increasing salinity in both ‘Cask’ (two-way ANOVA, F(3,72) = 99.81, P < 0.001) and ‘County’ (two-way ANOVA, F(3,72) = 47.60, P = 0.001). Similarly, as the incubation temperature was increased, germination rates increased but final germination percentage further decreased in both varieties, particularly in NaCl solutions below –1.32 MPa for ‘County’ and –1.76 MPa for ‘Cask’. Significant inter- and intra-specific differences were much greater in ‘Cask’ than in ‘County’. At high salinities, this trend was reversed, with ‘County’ being more affected than ‘Cask’.

In the PEG treatments, only seeds in treatments with osmotic potentials above –0.88 MPa germinated at 5 and 12°C for both varieties. At 20 and 27°C, germination percentages increased and seeds at –1.32 MPa could germinate, but the percentages were very low except in ‘Cask’ at 20°C. Significant differences between treatments were noted, with germination percentages decreasing with decreasing water potential (two-way ANOVA, ‘Cask’, F(3,72) = 154.73, P < 0.001; ‘County’, F(3,72) = 704.83, P < 0.001), while the optimum temperature for germination in PEG was 20°C, with other treatments significantly lower than the maxima (two-way ANOVA, ‘Cask’, F(3,72) = 178.42, P < 0.001; ‘County’, F(3,72) = 63.63, P < 0.001). In the water controls the temperature-related decrease in germination percentages was much greater in ‘Cask’ than in ‘County’. At high salinities, this trend was reversed, with ‘County’ being more affected than ‘Cask’.
where it was much lower. However, the amplitude increased with temperature, with an optimum at 20 °C. In ‘County’, however, the gradient of the lines was significantly affected by increased temperature, with seeds at low water potentials failing to germinate. No significant difference in the relationship between water potential and germination index was noted between the NaCl and PEG treatments at 27 °C.

At 12 °C, there were no significant differences in the dry mass between germinating and non-germinating ‘Cask’ seeds (Fig. 2A). Conversely, in ‘County’, seeds germinating under control, −0.45 and −1.76 MPa NaCl treatments were significantly heavier than non-germinating seeds (Fig. 2B), but not at other water potentials. Significant differences were noted in seed water content between germinating and non-germinating seeds incubated in NaCl for the two varieties (Fig. 2C, D). Germinating seeds of all the NaCl treatments reached a water content of approx. 0.45 mg H₂O g⁻¹ dry weight (d. wt) for both varieties. A positive relationship between water potential and seed water content of non-germinating seeds was noted in ‘Cask’. In ‘County’ the seed water content was significantly higher in non-germinating seeds than in those which germinated, except at −2.2 MPa where it was significantly lower in the non-germinating seeds. No significant differences were found in the sodium concentration (μg mg⁻¹ d. wt) of germinating and non-germinating seeds in either ‘Cask’ or ‘County’ water control seeds (i.e. 0 MPa, Fig. 2E, F). Seed sodium

\[ \text{Germination index plotted against external water potential for ‘Cask’ (A, C, E, G) and ‘County’ (B, D, F, H) barley seeds at 5 (A, B), 12 (C, D), 20 (E, F) and 27 °C (G, H). Symbols represent seeds germinated in NaCl or in PEG, as indicated.} \]
concentration increased with increasing external NaCl concentration for both varieties, both in germinating and in non-germinating seeds. However, in non-germinated seeds it increased only until –1.32 MPa in 'Cask' and –0.88 MPa in 'County', then held steady at approx. 7.5–8 mg gm⁻¹ d. wt.

Non-germinating seeds had significantly higher sodium concentrations than germinating seeds at every water potential except –2.2 MPa treatments for ‘County’. The concentration of salt in the seed expressed as a fraction of the water content also increased with increasing external salt concentration (Fig. 2G, H). As the seed salt content was relatively constant at high salinities, this increase can only be attributed to the decreases in seed water content as a result of osmotic considerations.

In both varieties incubated in –0.88 MPa NaCl solution, significant negative correlations were noted between seed dry mass and time, although r² values were low (F₁,₄₇ = 6.63, P = 0.047, r² = 0.08 and F₁,₄₇ = 6.63, P = 0.013, r² = 0.12 for ‘Cask’ and ‘County’ respectively; Fig. 3A, B). Seed water content was initially low, approx. 18% (‘Cask’) and 29% (‘County’) of fresh mass 8 h after the start of the experiment, initially increasing rapidly, although progressively less so, to water contents of 54 and 48% for the two varieties.
after 72 h (Fig. 3C, D). Three parameter exponential models increasing to a maximum were fitted to the data, revealing significant relationships between seed water content and time ($F_{2,41} = 12.94$, $P < 0.001$, $r^2 = 0.378$ and $F_{2,41} = 55.92$, $P < 0.001$, $r^2 = 0.635$ for ‘Cask’ and ‘County’, respectively). Significant positive relationships were also noted for both varieties between seed sodium concentration and time ($F_{1,46} = 26.20$, $P < 0.001$, $r^2 = 0.368$ and $F_{1,47} = 40.00$, $P < 0.001$, $r^2 = 0.461$ for ‘Cask’ and ‘County’, respectively), although ‘County’ accumulated sodium faster than ‘Cask’ (Fig. 3E, F). A significant positive relationship between seed solution sodium concentration and time was noted in ‘County’ (Fig. 3H; $F_{1,47} = 11.58$, $P = 0.001$, $r^2 = 0.201$), but not in ‘Cask’ (Fig. 3G). The lack of increase in ‘Cask’ appears to be related to the relatively rapid increase in water content, coupled with the relatively modest increase in salt content.

**DISCUSSION**

At each temperature seeds germinated more rapidly and to lower water potentials in the NaCl treatments than the isotonic PEG treatments. This suggests that seeds incubated in NaCl were less negatively affected by osmotic potential than PEG-incubated seeds, or were more able to adjust to the decreased water potentials (Table 1, Fig. 1). For both ‘Cask’ and ‘County’, seed sodium concentration increased as the external NaCl concentration increased (water potential decreased, Fig. 2E, F). In the –0.88 MPa NaCl treatment,
both the seed sodium concentration and the seed water content increased quickly with time (Fig. 3C–F). The observations that the NaCl seeds were able to germinate faster and to higher percentages than the PEG seeds, and that they take both salt and water up rapidly and to levels determined by the external salt concentration, are consistent with our hypothesis that sodium was absorbed by seeds, facilitating water uptake and allowing germination under osmotic conditions which would otherwise prevent germination.

Using salt as an osmoticum in saline environments appears to allow seeds to germinate more rapidly, and at lower osmotic potentials than they might otherwise be able to. This may have functional ecological effects, increasing these plants’ ability to compete temporally with other species, germinating faster and shading seedlings which do not germinate as rapidly. Salt has been shown to be an order of magnitude metabolically ‘cheaper’ than sugars for generating osmotic potential in vegetative plants (Raven, 1985), and may be similarly used in seeds, provided toxicity issues can be resolved. In vegetative plant cells, toxicity is resolved by vacuolar compartmentalization (Qiu et al., 2007), and recent reports suggest that Na$^+$ can be bound in starch granules (Kanai et al., 2007). In seeds, however, this compartmentalization would be energy intensive, while binding sodium to starch would prevent the starch from being used to provide energy for germination. Thus, it would seem metabolic tolerance to salt would be more important in seeds than at other life stages, due to their limited carbohydrate reserves.

Seed mass would seem to be a good indicator of C-reserve size. Seed dry mass decreased with time (Fig. 3A, B) and the dry mass of germinating seeds incubated in –0.88 MPa NaCl treatment was lower than the dry mass of seeds incubated in an isotonic PEG solution at either 20 and 27 °C (data not shown). This may suggest that the mechanism of sodium absorption as an osmoticum by seeds consumes C reserves. Alternatively, large seeds may be more able to generate osmotic potential within the developing embryo than smaller seeds, mitigating the need for sodium uptake, or facilitating the exclusion or compartmentalization of sodium from the cells. Previous research found that germination rates were positively correlated with seed size under saline conditions in Frankenia (Easton and Kleindorfer, 2009), while small sunflower seeds germinated to higher rates than their larger counterparts under saline conditions (Kaya and Day, 2008). In our study, the larger ‘Cask’ seeds germinated at similar rates to the smaller ‘County’ seeds, and it is unclear whether size-dependent changes in germination rate only operate within varieties, or between varieties. However, the larger ‘Cask’ seeds were able to germinate in higher salt concentrations (Table 1).

Fairly consistently, germinating seeds had a water content of approx. 0.45 mg H$_2$O g$^{-1}$ dry matter, irrespective of the external salt concentration (Fig. 2C, D) or temperature (data not shown) for both varieties. The specific cause for the lack of germination in non-germinating seeds is unclear, although the water content and sodium concentration of those treated with NaCl was generally higher than the germinating seeds (Fig. 2C–H), suggesting that osmotic limitation of water influx is unlikely to be the causal agent. It seems plausible that membrane integrity may be compromised, or that salt accumulation may have caused a loss of tonoplast Na$^+$/H$^+$ anti-porter function, thus preventing germination. Previous studies that report recovery of germination upon transfer of seeds from NaCl solution to pure water generally attribute the initial repression of germination and its subsequent recovery to osmotic factors, without exploring alternative hypotheses, such as the export of sodium from the seeds upon transfer to pure water (Debez et al., 2004). Likewise, it is unclear whether the high sodium concentrations seen here in non-germinating seeds are physiologically relevant (i.e. a causal factor), or simply a result of the longer duration that non-germinating seeds spent in their respective solutions. However, it should be noted that in seeds cultured in –0.88 MPa NaCl solution at 12 °C, the sodium concentration increased by 2 – 3 μg mg$^{-1}$ d. wt for ‘Cask’ and 4 – 5 μg mg$^{-1}$ d. wt for ‘County’ over the first 72 h of incubation. This suggests that the seeds were able to reach their final sodium concentration relatively quickly under those conditions, and potentially faster at higher salinities or temperatures. Thus, the high salt levels in non-germinating seeds may accumulate rapidly. It seems likely that salt accumulation is the causal factor preventing germination under saline conditions, with subsequent recovery of the seeds upon transfer to water facilitated by salt efflux from the cells.

As in previous studies, germination proceeded more rapidly as temperature increased (Gummersson, 1986). However, final germination percentages tended to decrease with increases in temperature, suggesting that optimal germination temperature is a trade-off between these two processes in barley. Although the maximum germination percentage was realized at low temperatures, it seems clear that there must be a minimum germination temperature, or more likely a distribution of minimum germination temperatures. At 20 and 27 °C, the negative effects of osmolarity were partially mitigated in the –0.88 and –1.32 MPa PEG treatments, and although this may be partially due to slight decreases in the water potential of the solutions at these temperatures, it seems likely that there were some changes in the physiological status of the seeds at higher temperatures. Germination indices revealed a similar picture, with germination indices in water generally increasing with increasing temperature (between 20 and 27 °C in ‘Cask’ is an obvious exception). In ‘Cask’, increasing temperature did not affect the relationship between the external water potential and germination index, with the regression gradients remaining largely unchanged. However, in ‘County’, a water potential by temperature interaction was notable, with the minimum water potential for germination increasing at higher temperatures, as in other studies (Alvarado and Bradford, 2002). Specifically, increased temperature led to the loss of any positive ‘ion effects’ on germination rates in the NaCl seeds, perhaps as plasma membranes become more fluid, and therefore leak more, at high temperatures. Seed sodium concentration at 5 °C was higher than that at 12 °C, although this may have been due to the longer exposure in the saline conditions. In PEG treatments, increases in seed sodium concentrations were noted in –0.88 MPa at 20 and 27 °C (data not shown) compared with the control at 12 °C for both varieties. Although the seeds were washed before being weighed and
dried, some adhesion of salt to the seed coat/cell wall must be anticipated. Dose-dependent uptake of salt by sorghum carp- ytopses has been noted previously (Wahid et al., 1998).

In conclusion, barley seeds appear to acquire Na\(^+\) from the saline environment prior to and during germination, which has the net effect of allowing them to absorb more water and germinate faster than seeds in an isotonic PEG solution, and to be able to germinate under osmotic conditions in which they would otherwise not be able to. Seed sodium concentration increased with time and with increases in environmental salt concentra-
tion. Temperature had a significant effect, with a general increase in germination rate and seed sodium concentration with increasing temperature, and a decrease in germination pro-
centage and seed dry mass. It is unclear whether the germinating
seeds germinate because they are more able to regulate sodium levels in their cytoplasm, perhaps by compartmentalization into the vacuole, whilst also being able to generate significant turgor, or whether their sodium concentrations are merely lower as a result of the decreased duration in which they are in the
sodium solution.

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