## Title

Tolerance of combined salinity and $O_2$ deficiency in *Hordeum marinum* accessions from the grain-belt of Western Australia



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**Publication Date**
2009-04-15

Peer reviewed

## Introduction

Waterlogging of soil is a wide-spread problem in wheat production areas around the world (Setter and Waters, 2003) and large areas of saline agricultural land are also prone to waterlogging. Crops with tolerance of both these stresses are required (Barrett-Lennard, 2003). Soil waterlogging reduces plant growth by reducing the availability of $O_2$ in the root zone and by increases in toxic products from anaerobic metabolism by microorganisms (Armstrong, 1979). Salinity reduces plant growth by osmotic stress and ion toxicity (Greenway and Munns, 1980). Combined salinity and waterlogging increased greatly the concentrations of $Na^+$ and $Cl^-$ in shoots of wheat, as compared with salinity alone (Barrett-Lennard, 1986; Malik *et al*., 2009), and large increases were also documented for several other species (Barrett-Lennard, 2003). Furthermore, $K^+$ uptake by plants can be reduced by waterlogging (Wiengweera and Greenway, 2004) or salinity (Greenway and Munns, 1980) alone; so, when root hypoxia and salinity occur together, $K^+$ uptake is reduced even further (Drew *et al*., 1988).

Amongst the wild relatives of wheat, *Hordeum marinum* (sea barleygrass) has been reported to be tolerant of salinity and waterlogging (Garthwaite *et al*., 2003; 2005). Wide hybridization of *H. marinum*, with wheat, produced an amphiploid with improved tolerance of salinity (Islam *et al*., 2007). The potential to use *H. marinum* as a donor for salt- and waterlogging-tolerance into wheat (Colmer *et al*., 2006), prompted us to collect and screen *H. marinum* accessions from the grain-belt of Western Australia for tolerance to salinity, waterlogging and these stress combined.

In a recent paper we reported on the leaf $Na^+$ and $Cl^-$ concentrations in these accessions from Western Australian collection, along with additional information on responses of 17 accessions from several countries and obtained from the Nordic Gene Bank (Malik *et al*., 2009). Here we present for the first time data on growth responses, aerenchyma formation and the formation of a barrier to radial $O_2$ loss in the accessions from Western Australia, as well as $K^+$ concentrations in the shoots in response to salinity, waterlogging and the two stresses combined.

## Materials and methods

Seventeen accessions of *H. marinum* were collected from the grain-belt of Western Australia. Single heads were collected, seeds were raised, and all were diploids (root tip squashes and chromosome counts by AKMR Islam). Wheat (cv. Chinese Spring) was also included in the experiment as a salt- and waterlogging-sensitive check species.

The experiment was carried out in a controlled environment room with photoperiod of 12 h, irradiance 400-500 µmol quanta $m^{-2}$ $s^{-1}$ PAR at plant height, temperature 20/15°C, and relative humidity 60/80 % (light/dark). Four root-zone treatments were imposed: aerated non-saline (control), aerated saline (200 mol $m^{-3}$ NaCl), stagnant non-saline, and stagnant plus saline (200 mol $m^{-3}$ NaCl). The experimental design was: 4 treatments x 17 accessions (plus wheat as a sensitive check) x 3 replicates, in a completely randomized block design. Due to space limitations in the controlled environment room, the experiment was carried out as a series of 3 complete blocks staggered with time. Pots were completely randomized within each block and to minimize the effects of possible environmental gradients within the controlled environment room, the pots were re-randomized weekly.

Details of plant culture treatment period, harvest procedures, measurements of root porosity and radial $O_2$ loss, and chemical analyses, were as described in Malik *et al*. (2009).

## Results

Whole plant RGR of the 17 *H. marinum* accessions in aerated non-saline nutrient solution (range 0.14-0.17 g $g^{-1}d^{-1}$) was similar to RGR of wheat (0.14 g $g^{-1}d^{-1}$) (Fig 1A). The stagnant non-saline treatment reduced RGR of the *H. marinum* accessions to 67-98% of the aerated controls, and wheat to 65% (Fig 1B). The aerated saline treatment reduced RGR of the *H. marinum* accessions to 73-96% of the controls, and wheat to 66%. The combined stagnant plus saline treatment reduced RGR (Fig 1C) of the *H. marinum* accessions to 43-58% of the control, and for wheat to only 22% of the control (Fig 1D).

Adventitious root porosity of the *H. marinum* accessions was 17-25% when in aerated non-saline solution, whereas in wheat it was 10% (Table 1). In stagnant non-saline solution, root porosity increased by up to 1.8-fold for *H. marinum* accessions, but in wheat it increased 2.3-fold. Salinity in aerated solution did not change the mean root porosity value across all accessions, however, in some accessions it increased and in other it decreased. In stagnant medium, salinity decreased the root porosity mean values; however, the accession varied in response, with some showing increased porosity and others decreased porosity.

Aerenchyma was also quantified in cross sections taken at 50 mm behind the root tip. *H. marinum* accessions had constitutive aerenchyma up to 12%, and wheat had 5% when grown in aerated non-saline solution. Increases and/or decreases in the porosity of adventitious roots in different treatments (Table 1) were reflected by the amounts of aerenchyma in the adventitious roots (data not shown).

Adventitious roots of *H. marinum* accessions WA6, WA23 and WA11 formed a 'tight' barrier to radial $O_2$ loss, whereas all other accessions formed a partial barrier to ROL when in stagnant non-saline solution (Fig 2). ROL at 10 mm behind the root tip was up to 6-fold higher than at the basal root zones (70-90 mm behind root tip). Roots of plants in the stagnant plus saline solution, compared with those in stagnant non-saline treatment, had 48-98% of the ROL at 10 mm behind root tip (Fig 2). By contrast with *H. marinum* roots, wheat did not form barrier to ROL and had higher ROL at the basal zones compared to the tip (i.e. 10 mm behind the tip).

$K^+$ concentration (i.e. [$K^+$]) in the youngest fully-expanded leaf of *H. marinum* accessions when grown in aerated non-saline solution were similar, or slightly below, that in wheat (Fig 3). Aerated saline treatment decreased leaf [$K^+$] to 77-92% in the *H. marinum* accessions and for wheat it declined to 41% of the control. Stagnant plus saline treatment decreased leaf [$K^+$] further, but it remained higher in *H. marinum* accessions than in wheat (Fig 3).

## Conclusion

*H. marinum* accessions showed variation in tolerance to combined saline plus stagnant treatment; however, compared with wheat, *H. marinum* was more tolerant. *H. marinum*-wheat amphiploids have been produced by AKMR Islam (The University of Adelaide) and those analysed to date have demonstrated improved tolerance of salinity (Islam *et al*., 2007) and waterlogging (Malik *et al*., unpublished). The most tolerant *H. marinum* accessions have been used to produce *H. marinum*-wheat amphiploids; physiological characterisation of these new amphiploids for tolerance to salinity, waterlogging and these two stress combined, is currently in progress.

## Acknowledgements

We thank the Australian Grains Research and Development Corporation (GRDC) for funding this research. AIM thanks JSPS for financial support to attend the 2009 IPNC in California.

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**Table 1.** Adventitious root porosity of *Hodeum marinum* accessions collected from Western Australia, and *Triticum aestivum* cv. Chinese Spring, in aerated or stagnant nutrient solution containing 0.2 or 200 mM NaCl. Adventitious root porosity was measured on roots of 100-150 mm length. Values are the means of three replicates plants grown in different pots. The LSDs refers to the influence of accession type on adventitious root porosity within different treatments at the 5% level. *, $p<0.05$; ***, $p<0.001$; ns, not significant.; n.a., not available as tissue sample was insufficient.

| | Adventitious root porosity (% gas volume per unit root volume) | | | |
|---|---|---|---|---|
| | Aerated | | Stagnant | |
| Species/ Accession code | 0.2 mM NaCl (control) | 200 mM NaCl | 0.2 mM NaCl | 200 mM NaCl |
| WA2 | 24 ± 5 | 18 ± 1 | 22 ± 1 | n.a |
| WA3 | 17 ± 1 | 22 ± 2 | 24 ± 2 | 22 ± 2 |
| WA5 | 19 ± 1 | 22 ± 2 | 23 ± 2 | n.a |
| WA6 | 22 ± 2 | 17 ± 1 | 30 ± 1 | 30 ± 5 |
| WA9 | 18 ± 1 | 18 ± 2 | 26 ± 1 | 29 ± 2 |
| WA11 | 18 ± 1 | 19 ± 1 | 26 ± 1 | 24 ± 3 |
| WA12 | 17 ± 1 | 19 ± 1 | 26 ± 3 | 25 ± 3 |
| WA16 | 17 ± 1 | 18 ± 1 | 24 ± 4 | 23 ± 3 |
| WA17 | 18 ± 1 | 19 ± 3 | 25 ± 3 | 18 ± 2 |
| WA21 | 15 ± 2 | 17 ± 1 | 24 ± 2 | 22 ± 6 |
| WA23 | 24 ± 0 | 23 ± 1 | 28 ± 1 | 29 ± 1 |
| WA25 | 24 ± 3 | 19 ± 3 | 28 ± 1 | 32 ± 2 |
| WA26 | 22 ± 1 | 21 ± 1 | 28 ± 2 | 23 ± 8 |
| WA28 | 19 ± 2 | 22 ± 1 | 26 ± 2 | 21 ± 4 |
| WA29 | 18 ± 0 | 24 ± 2 | 25 ± 0.3 | 25 ± 4 |
| WA31 | 18 ± 1 | 20 ± 3 | 29 ± 1 | 30 ± 1 |
| WA34 | 16 ± 1 | 18 ± 2 | 28 ± 0.5 | 28 ± 3 |
| *T. aestivum* | 10 ± 0 | 7 ± 1 | 23 ± 2 | 13 ± 3 |
| Mean | **19** | **19** | **26** | **24** |
| LSD | 5.3*** | 5.0*** | ns | 8.3** |

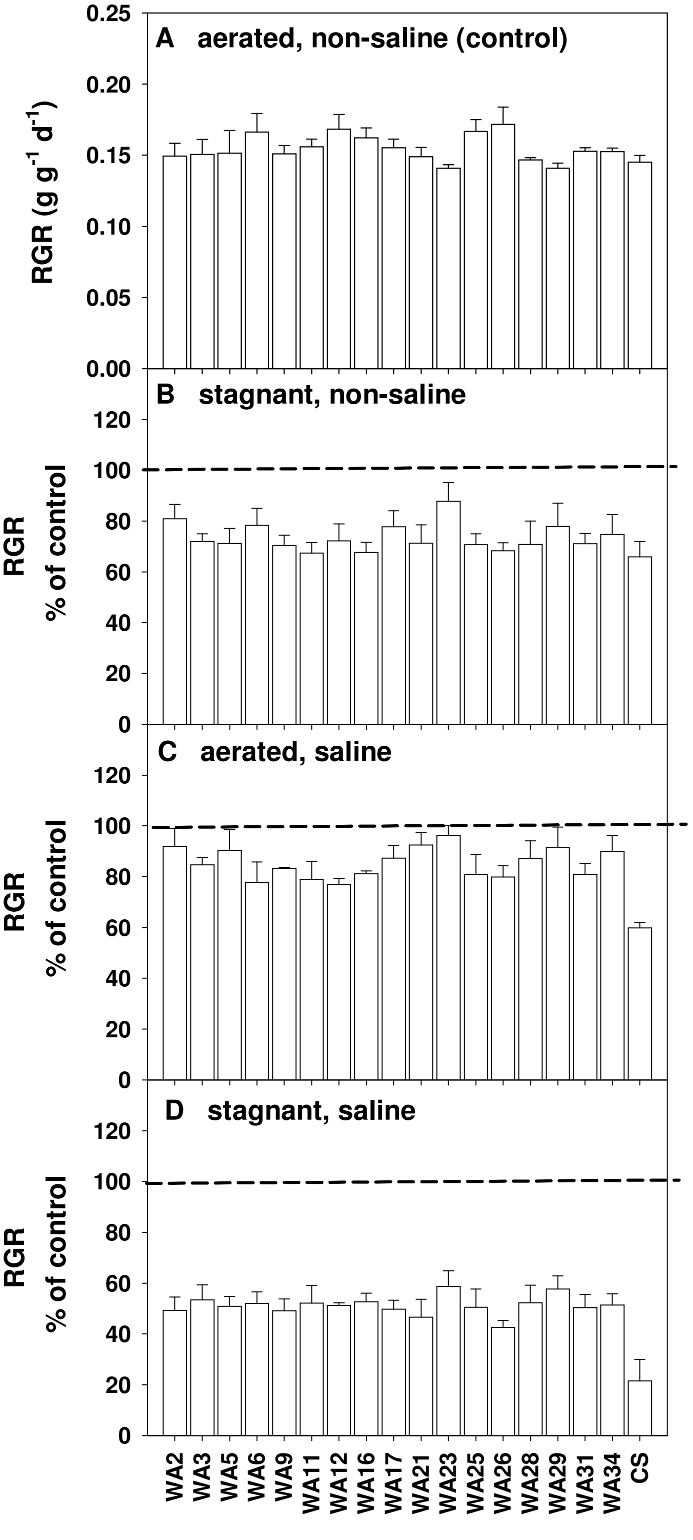


Fig 1. Relative growth rate (RGR) of whole plants for 17 accessions of *Hordeum marinum* and one wheat genotype (cv. Chinese Spring, CS) in (A) aerated non-saline nutrient solution (control). RGR as percentage of controls for plants in nutrient solutions with treatments: (B) deoxygenated stagnant agar, non-saline, (C) aerated 200 mol $m^{-3}$ NaCl, and (D) deoxygenated stagnant agar plus 200 mol $m^{-3}$ NaCl. Values are means of three replicates ± standard errors.

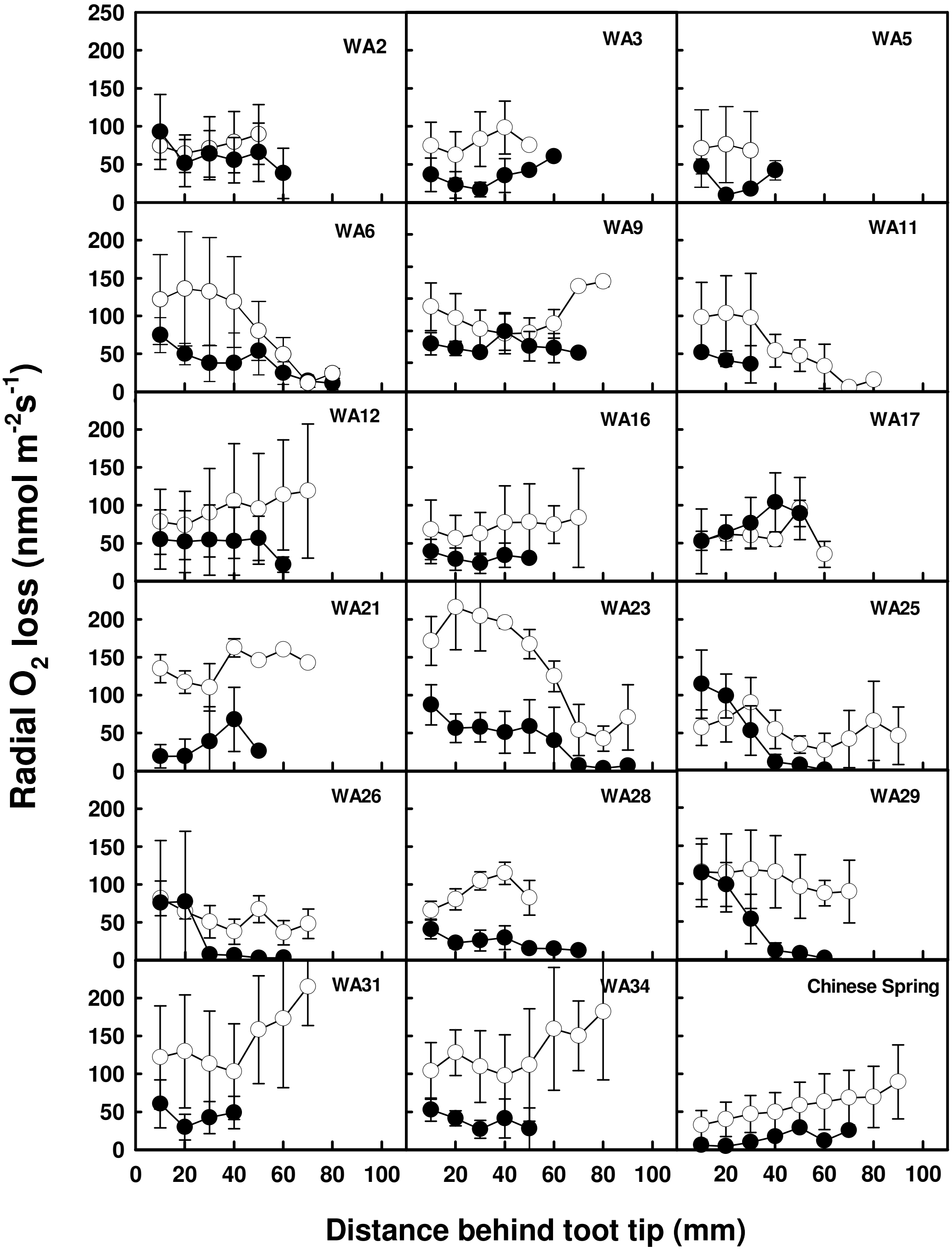


Fig 2. Rates of radial $O_2$ loss (ROL) along adventitious roots for 17 accessions of *Hordeum marinum* and one wheat genotype (cv. Chinese Spring, CS), when in an $O_2$-free medium, with shoots in air. Plants were grown in stagnant deoxygenated nutrient solution that was either non-saline (open circles) or contained 200 mol $m^{-3}$ NaCl (closed circle) for the final 25-28 d. Measurements were taken in freshly prepared solutions of the same compositions as the growth medium, at 20°C. Lengths of roots measured were 108 ± 2 mm (non-saline) and 99 ± 2 mm (200 mol $m^{-3}$ NaCl). Values are means ± standard errors of three replicates.

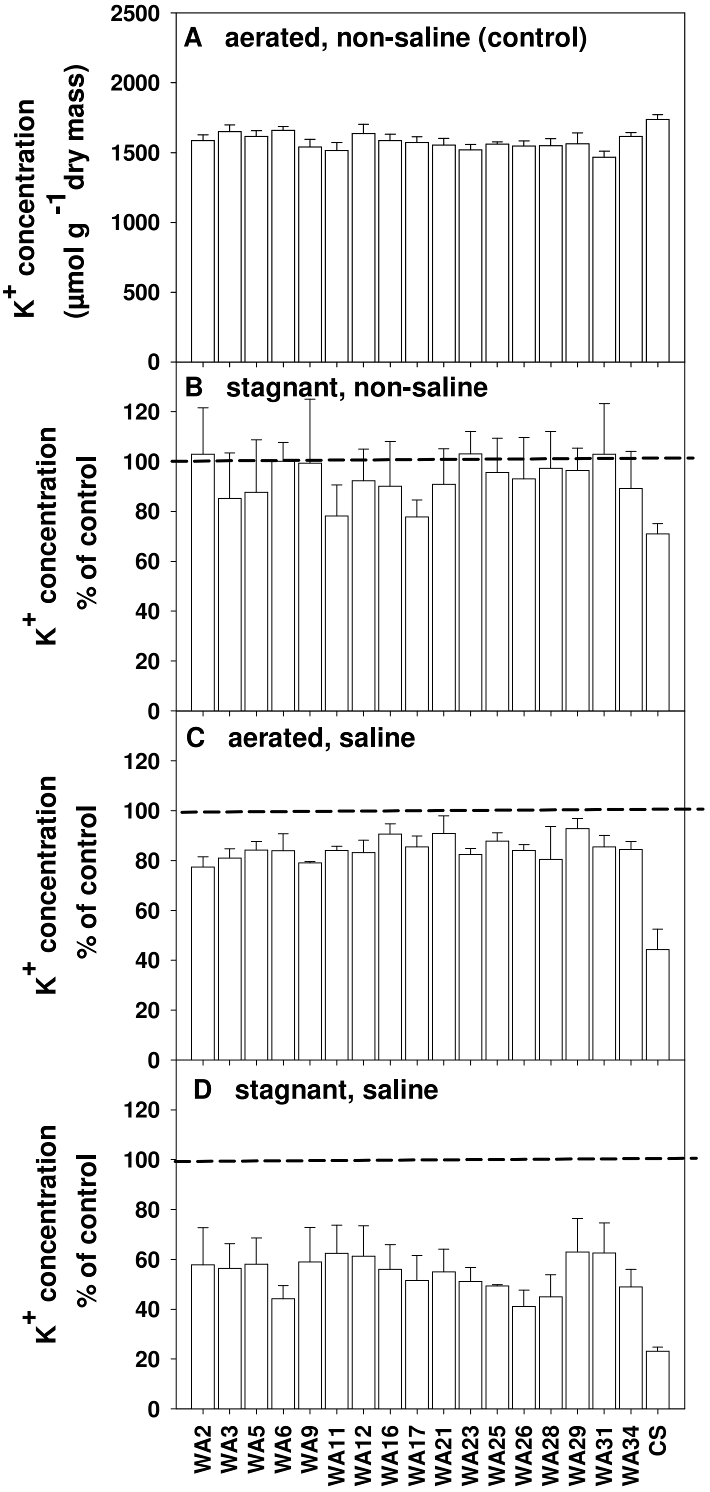


Fig 3. $K^+$ concentration in the youngest fully-expanded leaf for 17 accessions of *Hordeum marinum* and one wheat genotype when grown in (A) aerated non-saline nutrient solution (control). Responses to treatments are given as percentages of controls for: (B) deoxygenated stagnant agar, non-saline, (C) aerated 200 mol $m^{-3}$ NaCl, and (D) deoxygenated stagnant agar plus 200 mol $m^{-3}$ NaCl. Values are the means of three replicates $\pm$ standard errors.