DEVELOPMENT OF A CEREAL TOLERANT TO SALINITY AND WATERLOGGING

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ABSTRACT

Large areas of agricultural land in Australia have, and will, become saline. Land affected by dryland salinity is often prone also to waterlogging, so productive use of these areas requires plants with a high tolerance to salinity and waterlogging. Substantial improvements in tolerance, above the levels in current cultivars of barley or wheat, are required. 'Wild' species, related to wheat and barley, that inhabit salt marshes may provide sources of tolerance. Wide-crossing of *Hordeum marinum* (Sea Barleygrass) with bread wheat is proposed as a strategy to develop a cereal with substantial tolerance to salinity and waterlogging. *Hordeum marinum* has a high degree of salt tolerance and it also possesses mechanisms for root aeration that contribute to waterlogging tolerance. Aerenchyma and a barrier to radial O_2 loss in roots act synergistically to enhance O_2 diffusion towards the root tip, promoting root growth into anaerobic substrates. A *Hordeum marinum*-wheat amphiploid has been produced, demonstrating the feasibility of at least the first stages of our programme to use *Hordeum marinum* in the development of cytogenetical stocks to transfer traits associated with salt- and waterlogging-tolerance into bread wheat.

INTRODUCTION

Salinisation is a threat for up to one-third of agricultural land in Australia (National Land & Water Resources Audit). In WA alone, 1,800,000 ha of the wheat belt is already affected by dryland salinity, with estimates of up to 6,000,000 ha at risk. The productive use of saline land is currently limited to fodder production for livestock. Additional options for farming on salt-affected lands are needed, particularly in times of fluctuating prices for livestock products. Moreover, as the area of saline land increases, flexibility in enterprise diversification at the whole farm level will decrease, unless more options are developed for farming on saltland. Development of a salt-tolerant crop would fill this need.

Substantial improvements in salt tolerance, above the levels in current cultivars of barley or wheat, are required. In addition, since areas affected by dryland salinity are also prone to waterlogging (*e.g.* WA wheatbelt, Malcolm, 1980), waterlogging tolerance is also needed. Undomesticated or 'wild' species within the Triticeae (same tribe as wheat and barley) are a potential source of genes for salt- and waterlogging-tolerance that could be introduced into wheat (Omelien *et al*., 1991; McDonald *et al*., 2001b).

Amongst the 'wild' relatives in the Triticeae, a number of *Hordeum* species that inhabit salt marshes (von Bothmer *et al*., 1991) are of particular interest to us as potential sources of tolerance. Some of these species are highly salt-tolerant, being capable of growth at salinity levels approaching sea water (Mano & Takeda, 1998). Furthermore, since several *Hordeum* x wheat hybrids have been reported (Islam & Shepherd, 1990), it may be possible to use some species of *Hordeum* in cereal improvement. In fact, a new cereal, tritordeum, has been developed from *Hordeum chilense* x wheat hybrids (Martin *et al*., 1999). Unfortunately, the goal of Martin and co-workers has been to develop a cereal with improved resistance to diseases, and the *Hordeum* parent used was not one of the particularly salt- or waterlogging-tolerant species.

Despite the potential to make use of *Hordeum* species in cereal breeding, knowledge about these plants is relatively poor. If *Hordeum* species are to be used in the development of salt- and waterlogging-tolerant cereals, information is required on: (i) the levels of salt- and waterlogging-tolerance in the various species, (ii) the traits confering tolerance, (iii) the ability to hybridise these species with wheat, and (iv) once introduced into wheat, expression of key traits in this genetic background. *Hordeum marinum* (Sea Barleygrass) is a species of particular promise, due to its high tolerance of waterlogging (McDonald *et al*., 2001b) and salinity (Mano & Takeda, 1998). Moreover, *Hordeum marinum* can be hybridised with bread wheat (Jiang & Dajun, 1987). In this paper we report on progress towards using *Hordeum marinum* as a donor of key traits to improve salt- and waterlogging-tolerance in bread wheat.

A CYTOGENETICAL APPROACH TO IMPROVE SALT- AND WATERLOGGING-TOLERANCE IN WHEAT

The tribe Triticeae contains wheat, barley and rye, as well as a number of non-cultivated (*ie.* 'wild') species. Wide-crossing between wheat and a number of these species has been successful, including several species of *Hordeum* (Islam & Shepherd, 1990). Our strategy for the initial phases of the cytogenetical work required to transfer salt- and waterlogging-tolerance from *Hordeum marinum* into a wheat background, is shown in Figure 1.

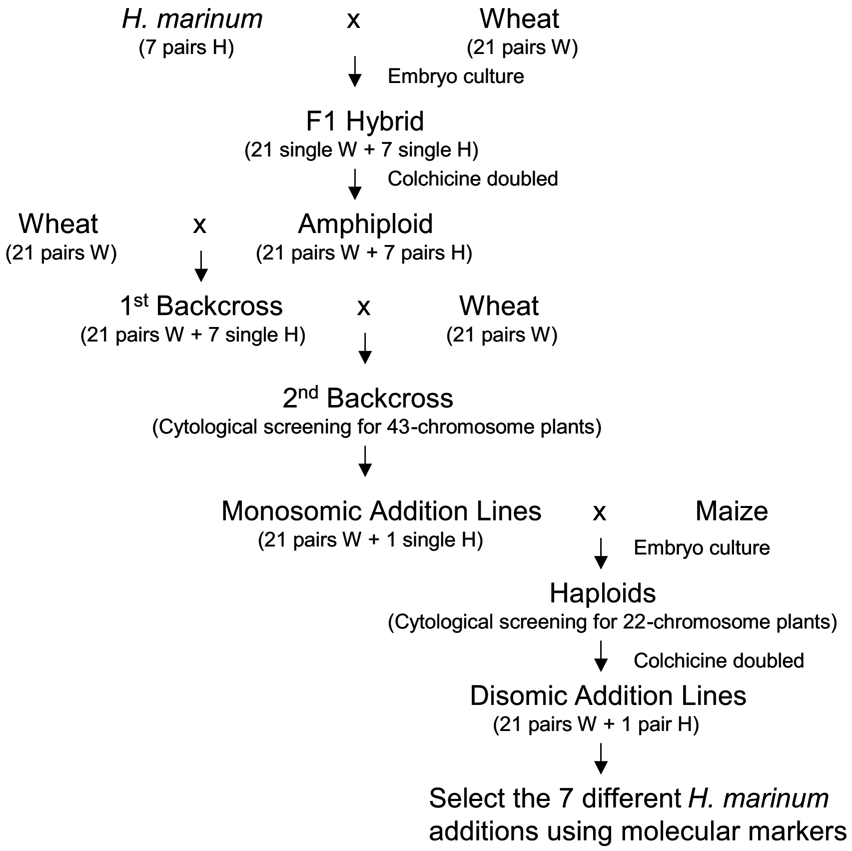

Figure 1. Diagram showing the steps involved in production of *Hordeum marinum*-wheat disomic addition lines. H = *Hordeum marinum* chromosomes (diploid sub-species; genome XX); W = wheat chromosomes (hexaploid wheat; genome AABBDD).

The initial cytogenetical work focused on producing a hybrid between the *Hordeum marinum* line already selected for key traits associated with waterlogging tolerance, and bread wheat (*Triticum aestivum*). It has been possible to hybridise these two species using *Hordeum marinum* as the female parent, and F1 plants were obtained from hybrid embryos cultured on nutrient agar medium. Amphiploid seeds were subsequently produced from colchicine doubled sectors in F1 plants. The amphiploid was used as the pollen parent to backcross to wheat, and the first backcross seeds are about to mature.

The backcross plants will be pollinated with wheat to produce a second backcross generation. The progeny plants will be cytologically screened for 43-chromosome monosomic addition lines of individual *Hordeum marinum* chromosomes to wheat. These monosomic addition plants will be pollinated with maize pollen and embryos will be cultured to obtain haploids. Cytological screening of the potted haploid plants will search for occasional 22-chromosome plants having an extra *Hordeum marinum* chromosome as well as the full complement of wheat. These haploids will be treated with colchicine to double the chromosome number and thus produce disomic addition lines. These addition lines will be characterised using molecular markers for all seven Triticeae chromosomes.

Physiological analyses of these addition lines should identify the *Hordeum marinum* chromosome(s) on which traits for salt- and waterlogging-tolerance are located. The addition of a whole *Hordeum marinum* chromosome to wheat may also carry some undesirable genes along with the desired ones. This problem can be overcome by minimising the size of the *Hordeum marinum* chromosome added to wheat, by producing recombinant lines to incorporate only small segments of the alien chromosome(s) carrying the desired traits. The recombinant lines would then need to be crossed with locally adapted cultivars prior to release of new salt- and waterlogging-tolerant cultivars to farmers (these steps are not shown in Figure 1).

SALT TOLERANCE

Wheat, like all other crop species except sugar beet, is a non-halophyte. Although considerable variation in salt tolerance exists among non-halophytes (Greenway & Munns, 1980), the range is from extreme sensitivity to moderate tolerance. Barley is regarded as one of the most salt tolerant crops, but even barley cropping becomes unviable at relatively low levels of soil salinity. Halophytes are plants that naturally inhabit saline environments such as salt marshes or salt lakes, and these species are much more tolerant of salinity than those used as crops (Greenway & Munns, 1980). Fortunately, some members of the Triticeae (same tribe as wheat and barley) are halophytes; for examples *Agropyron elongatum* (syn. *Lophopyrum elongatum*; Tall Wheatgrass) and *Hordeum marinum* (Sea Barleygrass). Wide-crossing of these halophytic ‘wild’ species (*e.g.* Wheatgrasses, Omielan *et al*., 1991; Gorham, 1994) with wheat, has been proposed as a strategy to enable substantial gains in salt tolerance.

Salt tolerance in plants involves a suite of traits, expressed at several levels of organisation (Greenway & Munns, 1980; Munns, 2002). The ability to restrict the rate of entry of the potentially toxic Na^+ (and Cl^-) into the shoots, often termed ‘ion exclusion’, is regarded as a key trait. Maintenance of K^+ uptake, even in the face of very high Na^+:K^+ in the soil solution, is also vital since K^+ is a macronutrient essential for enzyme functioning and is also a major osmoticum. Osmotic adjustment, the accumulation of solutes per unit of tissue water, enables tolerant species to live where high concentrations of salts in the soil make the external water potential very low. Na^+ and Cl^- that does enter the leaves is compartmentalised into vacuoles to prevent ion toxicity inhibiting enzymes in the cytoplasmic compartments. Synthesis of organic solutes, ‘compatible’ with enzyme functioning, prevents dehydration of the cytoplasmic compartments when large concentrations of ions are stored in the vacuoles (Wyn Jones *et al*., 1977).

The traits contributing to salt tolerance in *Hordeum marinum*, and the possibility to enhance expression of these in a wheat background by producing *Hordeum marinum*-wheat lines, should be determined. Ability to exclude Na^+ is superior in *Hordeum marinum* and *Agropyron elongatum*, in comparison to wheat (Table 1). When grown at 250 mM NaCl for 21 days, Na^+ concentrations in leaf tissues of both ‘wild’ species were lower than in wheat, with those in *Hordeum marinum* being the lowest (Table 1).

Table 1. Na^+ concentrations in leaves of *Hordeum marinum*, *Agropyron elongatum*, and wheat (*Triticum aestivum* cv. Chinese Spring) grown for 21 days at 250 mM NaCl. Plants were grown at 20°C/15°C day/night in aerated solution culture (composition given in McDonald *et al.*, 2001a; except Ca^{2+} was raised to 5 mM). All leaves on each plant, not including sheaths, were pooled for the analyses. Data given are means of 4 replicates ± SE. (Kinnane & Colmer, Unpublished)

Genotype	Leaf Na^+ concentration* (μmol g^{-1} dry weight)	Leaf K^+:Na^+
Hordeum marinum (tetraploid sub-species)	380 ± 20	3.5
Agropyron elongatum	850 ± 420	3.3
Triticum aestivum	2050 ± 560	1.0

*Na^+ concentrations in leaves of controls grown at 1 mM NaCl were at most 35 μmol g^{-1} dry weight.

Our cytogenetic work to incorporate chromosomes from *Hordeum marinum* into bread wheat is still in the early stages (see the section above on ‘cytogenetics’). Seeds of our *Hordeum marinum*-wheat amphiploid have only recently become available, and we have not yet tested the amphiplolid for Na^+ ‘exclusion’ or salt tolerance. By contrast, a complete set of addition and substitution lines of *Agropyron elongatum* chromosomes in bread wheat was produced during the early 1970’s (Dvorak & Knott, 1974). When grown in field plots irrigated with saline water, the *Agropyron elongatum*-wheat amphiploid and the substitution lines containing chromosome 3 from *Agropyron elongatum*, showed superior Na^+ ‘exclusion’ and grain yield, when compared to the wheat parent (Table 2).

Table 2. Na^+ concentration in the flag leaf of wheat (*Triticum aestivum* cv. Chinese Spring), an *Agropyron elongatum*-wheat amphiploid and derived chromosome substitution lines, when grown in field plots irrigated with saline water. Plots were irrigated with water of ‘low’ or ‘high’ salinity, so that soil EC in a saturated paste extract was 1.1-1.2 dS m^{-1} in control plots and 13.9-15.6 dS m^{-1} in saline plots. Data given are the ranges of the means of 3 replicates for each of the substitution lines for each *Agropyron elongatum* chromosome (from Omielan *et al.*, 1991).

Genotype	Flag leaf Na^+ concentration (μmol g^{-1} dry weight)		Grain yield	
	Control	Saline	Control (t ha^{-1})	Saline (% of control)
Triticum aestivum	9	522	2.3	8
Agropyron-wheat amphiploid	8	96	2.6	46
Substitution lines 1E(1A), 1E(1B), 1E(1D)	5-11	354-533	1.7-2.3	15-21
Substitution lines 2E(2A), 2E(2B), 2E(2D)	5-8	381-532	2.6-3.2	10-15
Substitution lines 3E(3A), 3E(3B), 3E(3D)	5-8	107-195	2.7-3.1	25-37
Substitution lines 4E(4A), 4E(4B), 4E(4D)	9-25	303-645	2.5-2.7	6-10
Substitution line 5E(5D)	7	420	1.9	10
Substitution lines 6E(6A), 6E(6B), 6E(6D)	5-7	372-492	2.2-3.2	8-13
Substitution lines 7E(7A), 7E(7B), 7E(7D)	6-12	373-471	2.2-2.8	14-18

The relatively high degree of salt tolerance in the substitution lines containing chromosome 3 from *Agropyron elongatum* is impressive (Table 2), but unfortunately the lines were as sensitive to waterlogging as the wheat parent (McDonald *et al.*, 2001a). By contrast, *Hordeum marinum* is salt tolerant and is also waterlogging tolerant (see below), so our objective is to use *Hordeum marinum* as a donor of tolerance to salinity and waterlogging to bread wheat. The importance of waterlogging tolerance for the development of a viable cropping option for land affected by dryland salinity is discussed below.

WATERLOGGING TOLERANCE

Waterlogged soils are usually anaerobic (*ie.* devoid of O_2) and can also be chemically reduced. The lack of O_2 results from the low diffusivity of gases in water preventing replacement of O_2 consumed by microorganisms in the soil. Roots of plants require O_2 for respiration to provide sufficient energy for growth, maintenance, and nutrient uptake processes. Roots of most plant species can only survive for at most a few days in the absence of O_2. Species tolerant of waterlogging develop extensive adventitious root systems that contain aerenchyma (Jackson & Drew, 1984). The aerenchyma provide a low resistance internal pathway for movement of O_2 (and other gases) within the roots. In addition to having extensive aerenchyma, the roots of many wetland species also contain a barrier to radial O_2 loss (ROL) in the basal zones (Armstrong, 1979; Jackson & Armstrong, 1999; Colmer 2002). These traits act synergistically to enhance O_2 diffusion to the root tip, and thus root growth into anaerobic substrates (Armstrong, 1979). Thus, features that enhance root aeration are considered to be key traits contributing to waterlogging tolerance in plants (Armstrong, 1979; Jackson & Drew, 1984).

The relatively poor waterlogging tolerance in the *Agropyron elongatum*-wheat lines (McDonald *et al*., 2001a) prompted us to screen a wider range of 'wild' relatives in the Triticeae (McDonald *et al*. 2001b). We found that *Hordeum marinum* contains a key trait for root aeration, found also in numerous wetland plants but never before in dryland crops or their close relatives. Roots of *Hordeum marinum* formed high constitutive porosity when grown in aerated solution (Table 3), and more importantly contained a barrier to ROL in basal zones of the roots when plants were grown in stagnant solution (Figure 2). Growth in stagnant solution resulted in root porosity being increased in all genotypes, but the levels in roots of *Hordeum marinum* were slightly higher than the values in wheat, and considerably higher than in barley or in *Agropyron elongatum* (Table 3). Root porosity in the *Agropyron elongatum*-wheat amphiploid did not differ from that in wheat.

Table 3. Porosity (% gas spaces per unit volume) for adventitious roots of selected species in the Triticeae, when grown in aerated or stagnant deoxygenated nutrient solution for the final 21 days. The maximum distances roots penetrated into an anaerobic medium are also given. Stagnant de-oxygenated nutrient solution contained 0.1% agar to mimick the changes in gas composition in waterlogged soils (Wiengweera *et al*., 1997). Data given are means of 3 replicates ± SE. (From McDonald *et al*. 2001a & 2001b)

Genotype	Porosity (% gas spaces/volume)		Maximum length of roots in an anaerobic medium (mm)
	Aerated	Stagnant	
Hordeum vulgare (Stirling)	6.7	15.8	160
Triticum aestivum(Chinese Spring)	5.7	21.6	192
Agropyron elongatum	7.6	13.5	215
Agropyron-wheat amphiploid	5.5	21.0	237
Hordeum marinum (tetraploid sub-species)	13.8	25.4	325

Losses of O_2 from the roots, to the soil (*ie*. ROL), can be substantial for some species; and ROL reduces the longitudinal diffusion of O_2 in the aerenchyma towards the root tip. For roots of wheat, ROL was highest from the most basal zones and decreased towards the root tip (Figure 2); this pattern is typical for a dryland species (Jackson & Drew, 1984; Armstrong, 1979). By contrast, the roots of many wetland species contain a barrier to ROL (Jackson and Armstrong, 1999; Colmer, 2002), restricting O_2 loss from the aerenchyma and thus enhancing O_2 diffusion towards the root tip. Such a 'wetland pattern' of ROL was present for roots of *Hordeum marinum* (Figure 2). Thus, the relatively high porosity (constitutive and inducible) together with an inducible barrier to ROL, result in greater capacity for internal aeration in roots of *Hordeum marinum*, as compared with wheat. This superior capacity for internal aeration presumably contributes to the greater rooting depths (Table 3) and waterlogging tolerance in *Hordeum marinum* when compared to wheat (McDonald *et al*., 2001b). Finding superior root aeration traits in a 'wild' relative of wheat (namely, *Hordeum marinum*) is a major step forward, since it is now feasible to attempt to transfer these traits into bread wheat, using the cytogenetical strategy outlined in a previous section of this paper.

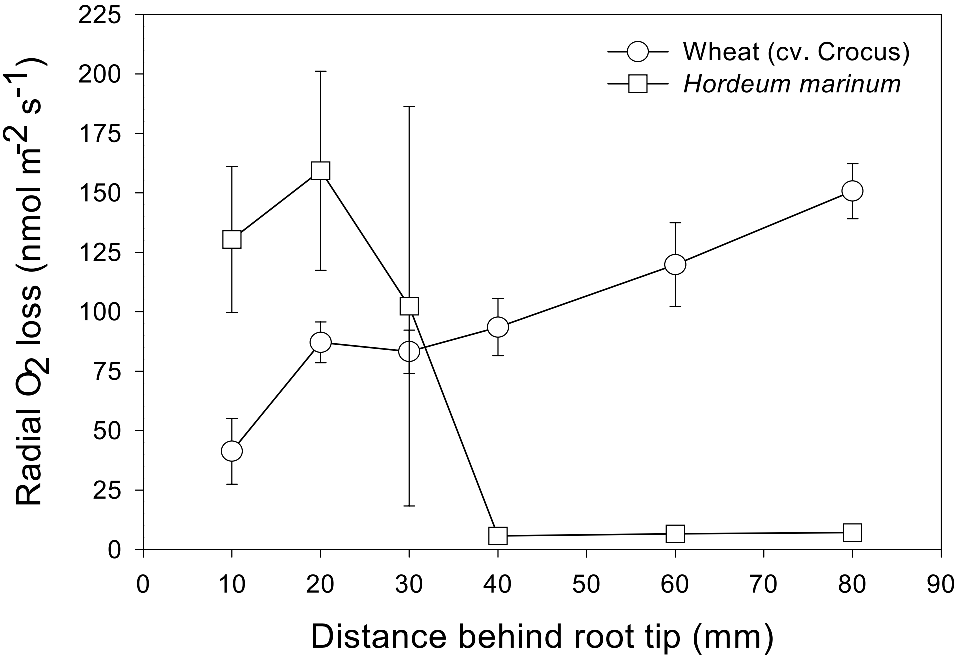

Figure 2. Profiles of radial O_2 loss (ROL) along adventitious roots of wheat (*Triticum aestivum* cv. Crocus) and *Hordeum marinum* (diploid sub-species) when in an O_2-free rooting medium with shoots in air. Plants were grown in stagnant deoxygenated nutrient solution for 21 d prior to the measurements taken at 20 °C. Composition of the nutrient solution was as given in McDonald *et al.* (2001a). Roots were 100 to 112 mm in length. Data given are the means of 3 replicates ± SE. (Colmer & Islam, Unpublished).

SALINITY X WATERLOGGING INTERACTIONS

The co-occurrence of salinity and waterlogging can result in severe damage, or even death, of poorly adapted plant species such as wheat (Barrett-Lennard *et al.*, 1999). A lack of O_2 in the root zone means that respiration in root tissues is inhibited, unless the roots can access sufficient levels of O_2 via the aerenchyma. Energy shortage in the root cells inhibits energy-dependent ion transport across membranes, resulting in 'breakdown' of Na^+ 'exclusion' in O_2-deficient roots (*e.g.* wheat, Barrett-Lennard *et al.*, 1999; corn, Drew & Lauchli, 1985). Increased delivery of Na^+ (and Cl^-) to the shoots, together with a decrease in K^+ uptake, means ion toxicity will occur more quickly in plants faced with a combination of salinity and root-zone O_2 deficiency, especially if shoot growth and therefore 'dilution' of the incoming ions is also decreased.

We hypothesize that root aeration traits that confer tolerance to waterlogging should be a prerequisite for tolerance to combined salinity and waterlogging. Internal aeration, via the aerenchyma, would enable cellular respiration to continue so that energy is available for maintenance of membrane transport processes essential for Na^+ exclusion and K^+:Na^+ selectivity. Our physiological screening of the *Hordeum marinum*-wheat lines will evaluate salt tolerance, waterlogging tolerance, and tolerance of these stresses when combined.

CONCLUSIONS

A *Hordeum marinum*-wheat amphiploid has been produced, demonstrating the feasibility of at least the first stages of our programme to use *Hordeum marinum* in the development of cytogenetical stocks to transfer traits associated with salt- and waterlogging-tolerance into bread wheat. However, our programme is only in its second year and much work remains.

Further cytogenetical manipulations are required to produce chromosome addition lines and eventually recombinant lines. Screening progeny to track salt- and waterlogging-tolerance to particular chromosomes and chromosome segments is required, to enable production of wheats containing minimal amounts of alien chromosome segments. It is also possible that since salt- and waterlogging-tolerance both involve several traits, further crossing to recombine traits into a single recombinant *Hordeum marinum*-wheat line may be needed, before materials are ready for entry into wheat breeding programmes. The recombinant lines would then need to be crossed with locally adapted cultivars, to produce new salt- and waterlogging-tolerant cultivars capable of extending cropping onto soils with salinity levels too high for existing cereals. Even so, cropping is unlikely to be viable on the most severely salt-affected lands, where fodders would remain the most appropriate option.

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