Attempts to transfer salt- and waterlogging-tolerances from Sea barleygrass (*Hordeum marinum* Huds.) to wheat

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ABSTRACT

Wheat crops are adversely affected by salinity and water logging in areas of southern Australia. Sea barleygrass (*Hordeum marinum*), a wild distant relative of wheat inhabiting salt marshes, has a high degree of tolerance to both salinity and waterlogging. The initial aim of our work was to cross *H. marinum* and wheat and to produce the amphiploid from the hybrid. The amphiploid showed improved salt- and waterlogging-tolerances than the wheat parent. Since the current amphiploids are all in *H. marinum* cytoplasm, they suffer a loss in fertility due to cytoplasmic male sterility induced by the *H. marinum* cytoplasm. The cytoplasm of selected amphiploids, showing better tolerances, is currently being transferred back to the wheat cytoplasm to restore normal fertility. The aim initially is to develop salt- and waterlogging-tolerant feed grain which will allow cropping to be extended onto mildly affected salt land. Furthermore, in a parallel program, 6 out of the 7 possible disomic additions lines (1*Hm*, 2*Hm*, 4*Hm*, 5*Hm*, 6*Hm* and 7*Hm*) of individual *H. marinum* chromosome to CS wheat have been produced. These addition lines will be useful in determining the chromosomal controls of salt-and waterlogging-tolerance in *H. marinum.*

INTRODUCTION

Salinity is an increasing problem for crop production in many regions of the world[11]. In addition, many salt-affected areas are also prone to waterlogging thus making these soils particularly unproductive[1]. Improvement of salt tolerance in wheat can be achieved by introducing genes from salt-tolerant wild relatives[3,10]. Among the halophytic wild relatives of wheat, tall wheat grass (*Thinopyrum* species) has received the earlier attention as donors of salt tolerance. The amphiploid produced between these two showed higher salt tolerance[6,9] but still lacked in waterlogging tolerance[8]. Sea barleygrass (*Hordeum marinum*), another halophytic wild relative that grows in salt marshes[2] shows tolerance to waterlogging[4] and salinity[5]. Therefore, attempts were made to produce an amphiploid between wheat and *H. marinum* and then a set of *H. marinum* addition lines to wheat.

MATERIALS AND METHODS

Two accessions of *Hordeum marinum* Hudson subs. *marinum* namely H21 and H90 were imported from Sweden (courtesy of Dr Roland von Bothmer, Swedish University of Agricultural Sciences) and after being released from quarantine were grown in the greenhouse at the Waite Campus, Urrbrae, South Australia. H21 was crossed with Chinese Spring (CS) wheat and H90 with commercial wheats. Because of the small anther size, *H. marinum* had to be used as the female parent in the cross.

Beginning one day after pollination, florets were treated with gibberellic acid solution to promote embryonic growth. Since the embryos do not mature on plants, they had to be dissected out under aseptic conditions and cultured on nutrient agar medium to produce F1 hybrid plants. Potted seedlings at 3-4 tillering stage were uprooted from soil and after washing in water the root and crown region was treated in an aerated colchicine solution for chromosome doubling to produce amphiploids.

Chromosome counts of amphiploid, BC1 and BC2 plants were done following Feulgen staining procedure. Alien chromosome addition lines were selected among BC2 progeny using representative RFLP probes previously mapped by others to each of the seven Triticeae homoeologous chromosome groups.

RESULTS AND DISCUSSION

F1 hybrids

A total of 1340 florets of *H. marinum* H21(Fig 1a,b) were pollinated with CS wheat to give 49 seeds (3.6%). 10 small embryos were cultured on nutrient agar medium and 6 F1 hybrid plants were obtained. These plants were planted in pots and the hybrid status of some of these plants was determined from observing 28 somatic chromosomes in root tip cells which exhibited mostly 28 univalents (28’) at meiosis. A small number of seed was produced in colchicine-doubled sectors of all F1 hybrid plants.

Similarly 1150 florets of *H. marinum* pollinated with commercial wheats (Westonia, Wyalkatchem, Camm) produced 28 (2.4%) seeds and 9 F1 hybrid plants were obtained. These were all treated with colchicine as before and again seeds were obtained from them in colchicine-doubled sectors.

Amphiploids

56-chromosome amphiploid plants (Figs 1c,d) were selected from seeds obtained in doubled sectors and planted in large pots for seed increase. The fertility of the amphiploid plants were low due to cytoplasmic male-sterility induced by the *Hordeum* cytoplasm. This was particularly more noticeable in amphiploids with commercial wheats like Wyalkatchem. This low fertility in amphiploids can be attributed to cytoplasmic male-sterility induced from interaction of *Hordeum* cytoplasm with the wheat genome.

First backcross (BC1) plants

BC1 plants were obtained by pollinating wheat as the female parent with pollen from the amphiploid plants. Several 49-chromosome BC1 plants were planted in large pots and their BC1 status was confirmed from observing 21" + 7' at meiosis. Since the BC1 plants are in wheat cytoplasm, these were, as expected, much more self fertile.

Second backcross (BC2) plants

BC2 progeny was only produced from amphiploid involving CS wheat thus far. The somatic chromosome constitution of 147 BC2 plants was determined from root tip chromosome counts in a search for putative 43-chromosome monosomic addition of individual *H. marinum* chromosomes to wheat. Altogether 32 plants with 43 chromosomes were obtained. These were planted in small pots and DNA from these plants was probed with RFLP markers mapping all seven *H. marinum* chromosomes. 1*Hm*, 2*Hm*, 4*Hm*, 5*Hm*, 6*Hm* and 7*Hm* monosomic additions were detected among these plants.

All of these monosomic additions were pollinated with maize pollen to produce haploids. These were planted in small pots and at 3-4 tillering stage were uprooted and root tip chromosome counts taken to select 22-chromosome aneuhaploids. These aneuhaploids were treated with colchicine for chromosome doubling and disomic additions (1*Hm*, 2*Hm*, 4*Hm*, 5*Hm*, 6*Hm* and 7*Hm*) of the individual *H. marinum* chromosome to CS wheat were obtained.

Salinity and waterlogging tolerance

Salt tolerance in the *H. marinum*-wheat (CS) amphiploid was intermediate to that of its parents[7]. The improved salt tolerance in the amphiploid, as compared with wheat, was related to 'exclusion' of Na^+ and Cl^-. Waterlogging tolerance of the amphiploid will be reported in a future publication.

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Figure 1. (a) *H. marinum* plant, (b) *H. marinum* spike, (c) CS wheat plant (left) and H21xCS Amphiploid plant (right), (d) CS wheat spike (left) and amphiploid spike (right).