Mapping quantitative trait loci associated with salinity tolerance in synthetic derived backcrossed bread lines

Oghonnaya FC¹,², Huang S⁴,⁵, Steadman E¹, Livinus Emehiri¹, Dreccer F³, Lagudah ES⁴, Munns R⁴

¹Biosciences Research Division, Department of Primary Industries, Grains Innovation Park, Natimuk, Horsham Vic. 3401, Australia
²International Centre for Agricultural Research in the Dry Areas, PO Box 5466, Aleppo, Syria
³CSIRO Plant Industry, Cooper Laboratory, PO Box 863, University of Queensland, Warrego Highway, Gatton, Queensland, 4343
⁴CSIRO Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory, 2601, Australia
⁵ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, 35 Stirling Highway, WA 6009, Australia

INTRODUCTION

Soil salinity is one of the major environmental problems affecting agricultural production in arid and semi-arid regions of the world, both in irrigated and dryland agriculture. Improving salinity tolerance of wheat is a key target for many wheat breeding programs worldwide. Amongst the synthetic hexaploid wheats (SHWs) (2n=6x=42, AABBDD) derived from crosses between Triticum turgidum L. ssp. durum (2n=4x=28, genome AABB) and Aegilops tauschii (syn Ae. squarrose, T. tauschii; 2n=2x=14, genome DD), significant variation was observed for salinity tolerance. To investigate natural allelic variants contributing to quantitative variation for salinity tolerance in hexaploid wheat, a quantitative trait mapping approach was used to analyse a population of BC₁F₅ synthetic-derived backcrossed lines (SBLs) derived from crossing SHWs to bread wheat. The SBLs were screened for salinity tolerance based on the sodium exclusion mechanism. QTLs were detected on chromosomes 2B, 2D, 3D, 4B, 4D, 6D, 7A and 7D that were significantly associated with sodium exclusion. Of these, only the chromosome 4D region has previously been reported as associated with sodium exclusion in bread wheat and thereby contributing to salt tolerance (Dubcovsky et al. 1996). The SBLs proved to be a promising tool to identify, characterize and introgress different salinity tolerance genes into adapted wheat genetic backgrounds.

MATERIALS AND METHODS

Plant materials: A mapping population of 121 BC₁F₅ derived from a cross between ‘Aus29639’ and ‘Yitpi’ was used in this study. Aus29639 is synthetic hexaploid wheat (SHW) with significantly lower Na⁺ exclusion than current bread wheat standards (Dreccer et al. 2004). Aus29639 was used as the female parent while the recurrent parent Yitpi, an Australian wheat, was used as the pollen donor. F₁ plants were backcrossed to Yitpi (as male) to produce 121 lines which were selfed for five generations by single-seed descent (SSD) without selection to produce the BC₁F₅ population.

Assessment of salinity tolerance: BC₁F₅ derived lines were grown in the glasshouse with a day/night temperatures of 20°C/12°C in a hydroponic system following the protocol established by Munns and James (2003). Before planting, seeds were vernalised for three weeks and pre-germinated in Petri dishes lined with wetted filter paper. The experiments were laid out as row-column design with three replicates. Pre-germinated seeds were planted in pots filled with rinsed 5-10mm coarse quartz gravel and placed in stainless steel trays which were automatically sub irrigated every 30 minutes. Seedlings were grown under half-strength modified Hoagland solution for three days after which they were grown on full strength. 25 mM NaCl was added twice daily until the target concentration of 150 mM NaCl was reached. Supplemental Ca²⁺ was added as CaCl₂ to give a final Na⁺: Ca²⁺ ratio of 15:1. The timing of the appearance of the third leaf was recorded. The hydroponic solution was changed between 7 and 10 days while maintaining and/or adjusting the pH at 5.5 daily. The third leaves were harvested ten days later, dried, weighed and used for determining Na⁺ and K⁺ concentrations by flame photometry.

DNA extraction and microsatellites (SSRs) markers screening and Bulk Segregant Analysis:

D DNA was extracted from 3- to-4 week old seedlings of the two parents and BC₁F₅ SBLs using a standard phenol/chloroform method as described by Oghonnaya et al. (2001). SSR markers available in the public domain (http://wheat.pw.usda.gov/GG2/index.shtml) were tested on the parents to determine polymorphism. PCR amplification for SSRs were performed in a final volume of 10μl containing 5 μl premix D (FailSafe™ PCR PreMix-Epicentre), 0.5μm forward primer, 0.5μm reverse primer, 0.1 μl 2.5 units of FailSafe™ DNA polymerase, 25-50ng of template DNA and distilled water. PCR amplifications were performed on an Eppendorf Mastercycler® Gradient, using a touchdown
cycle consisting of 1 cycle of 94°C for 1min, followed by 18 cycles of 94°C for 30sec; 64°C for 30sec, decreasing 0.5°C each cycle; 72°C for 30sec. An additional 28 cycles followed, consisting of 94°C for 30sec; 55°C for 30sec; 72°C for 30sec. A final extension of 72°C for 5min was performed before samples were placed at 4°C. Amplification products (DNA fragments) were resolved by polyacrylamide gel electrophoresis using vertical unit manufactured by C.B.S Scientific Co. USA.

Based on the results from salinity screening of BC1F5 SBLs, ten lines each were selected from putative salt tolerant and intolerant extremes of the populations and designated salt resistant or susceptible (S) lines. Resistant and susceptible ‘DNA bulks’ were formed by pooling 1 µg of DNA from each of the 10 most resistant and 10 most susceptible lines, respectively and used for bulk segregant analysis (BSA). Both bulks including the parents Aus29639 and Yitpi were screened with 900 SSRs. For RFLP analysis, DNA from Chinese Spring nullitetrasomic aneuploids lines of chromosome 7 (Sears et al. 1954) as well as controls of Chinese Spring, Cranbrook, Halberd, Sunstate and Westonia were used. These were digested with five restriction enzymes – EcoRI, EcoRV, HindIII, NcoI and XbaI. To ascertain the co-segregation of HKT2;1 with salinity tolerance, DNA of selected synthetic backcross derived BC1F5 lines that displayed low exclusion and high accumulation, including Aegilop tauschii, Triticum durum and bread wheat and synthetic hexaploid were digested with EcoRI and probed with HKT2;1. Southern blotting and DNA hybridization was carried out according to Ogbonnaya et al (2001).

RESULTS

Salinity tolerance amongst parents and the SBLs: The summary of parental genotypes and BC1F5 population for salinity tolerance expressed as geometric mean of the third leaf ten days after growing in 150 mM NaCl are presented in Figure 1. There were significant (P <0.01) genotypic differences amongst the SBLs. The third leaf shoot Na+ concentrations of the BC1F5 population ranged from 138 to 1212 Na+ µmol g DW-1 compared to ‘Aus29639’ the salinity tolerant parent with 312 Na+ µmol g DW-1, and Yitpi, the moderately saline tolerant parent with 415 Na+ µmol g DW-1. Transgressive segregation was evident in both directions indicating the presence of BC1F5 lines with phenotypic values outside the range of the parental genotypes (Figure 1). Seven lines with enhanced sodium tolerance were selected and re-evaluated in BC1F6 generation against the recurrent parent, Yitpi. In all cases the 7 BC1F5 SBLs displayed elevated levels of salinity tolerance when compared to Aus29639 and Yitpi. The most sodium tolerant SBL had leaf 3 Na+ content of 182 (µmol gDW-1) compared to the recurrent parent with 358 Na+ µmol gDW-1. This result provides independent confirmation of the development of SBLs with enhanced low sodium exclusion than the recurrent parent. The magnitude of salinity tolerance from the SBLs with the lowest Na+ exclusion is twice that of its recurrent parent and serves as a useful germplasm in breeding for salinity tolerance.

From 900 SSR markers tested on ‘Aus29639’and ‘Yitpi’, 403 (representing 40% of the SSR tested to date) were polymorphic between the two parents across the three genomes. Of these, 157 were found to be polymorphic between the resistant and susceptible bulks and are currently being used to genotype the BC1F6 mapping population including an additional validation population. Preliminary simple interval mapping revealed that several quantitative trait loci (QTLs) located on chromosomes 2B, 2D, 3D, 4B, 4D, 6D, 7A and 7D were significant determinants of salinity tolerance with phenotypic variation ranging from 5 to 25%.

Lines of the BC1F6 family of the Aus29639 and Yitpi cross were analysed with an EST-RFLP (restriction fragment length polymorphism) probe for HKT2;1 which occurs on the group 7 chromosomes (Huang et al. 2008). This was found to be linked to the QTL on chromosome 7A in bread wheat. The allele that was linked to the Na− came from Yitpi.

DISCUSSION

Results from this study indicate that some of the SBLs were associated with significantly superior Na+ exclusion relative to the recurrent ‘Yitpi’ parent. This is due to transgressive variation associated with complementary alleles from the SHW parent that enhanced the performance of the elite recurrent wheat parent, ‘Yitpi’. These SBLs provides us with the opportunity to explore what happens when ‘adapted gene complexes’ are disrupted, giving rise to positive transgressive genetic variation. We also found that HKT2;1 was linked to the QTL on chromosome 7A in bread wheat that mediate Na+ exclusion.
There are several reports showing that HKT transporters (High-affinity K\(^+\) Transporter) mediate Na\(^+\)-specific transport or Na\(^+\)-K\(^+\) co-transport, and play a key role in regulation of Na\(^+\) and K\(^+\) homeostasis in plants (Munns and Tester 2008). These are essential transporters for controlling the movement of Na\(^+\) from roots to shoots and are important in determining the salt tolerance of plants (Munns and Tester 2008). In a recent study by Huang et al. (2008), HKT gene sequences were mapped on chromosomal arms of wheat and barley using wheat chromosome substitution lines and barley-wheat chromosome addition lines. In addition, HKT gene members in the wild diploid wheat ancestors, *T. monococcum* (A\(^b\) genome), *T. urartu* (A\(^u\) genome) and *Ae. tauschii* (D\(^k\) genome) were investigated. Variation in copy number for individual HKT gene members was observed between the barley, wheat and rice genomes, and between the different wheat genomes. HKT2;1/2-like, HKT2;3/4-like, HKT1;1/2-like, HKT1;3-like, HKT1;4-like and HKT1;5-like genes were mapped to the wheat-barley chromosome groups 7, 6, 2 and 4, respectively (Huang et al. 2008).

A QTL for Na\(^+\) exclusion was previously reported on chromosome 4D of bread wheat in the same region as the QTL from the Aus29639/ Yitpi cross. This was named Kna1 for its ability to enhance the accumulation of K\(^+\) over Na\(^+\) in the shoot (Dubreva et al. 1996). The candidate gene for Kna1 is an HKT transporter of the HKT1:5 gene family (Byrt et al. 2007).

The QTL for Na\(^+\) exclusion on chromosome 7A was linked to the HKT transporter *HKT2:1*, there being insufficient sequence difference between *HKT2:1* and *HKT2:2* to distinguish them with the probe used (Huang et al. 2008). This is of the same gene family as the original HKT transporter identified, TaHKT1 (Schachtman and Schroeder 1994). This has been renamed *TaHKT2:1* because it belongs to the group 2 HKT family which are Na\(^+\)-K\(^+\) co-transporters, in contrast to the group 1 family which are specific Na\(^+\) transporters (Huang et al. 2008). The original *TaHKT2:1* is probably located on chromosome 7B (Huang et al. 2008), so the chromosome 7A gene member represents a homoeologue.

We conclude that a marker associated with the Na\(^+\) exclusion trait on chromosome 7A may provide a tool that allows the screening of bread wheat varieties for Na\(^+\) exclusion and hence salinity tolerance.

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**REFERENCES**


