Identification of a QTL on chromosome 7AS for sodium exclusion in bread wheat

Edwards J[1], Shavrukov Y[1], Ramsey C[1], Tester M[1], Langridge P[1], Schnurbusch T[1,2].
[1]Australian Centre for Plant Functional Genomics, University of Adelaide, Urrbrae, SA 5064, Australia.
[2]current address: Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany

ABSTRACT

The purpose of this study was to define the chromosomal location of QTLs associated with sodium (Na^+) exclusion in bread wheat. Two F_1-derived doubled haploid (DH) mapping populations made from crosses between Cranbrook/Halberd, (160 lines) and Excalibur/Kukri, (233 lines), were used in this study. Shoot Na^+ accumulation was measured in both supported hydroponics and in field trials at Roseworthy, SA. The hydroponics experiments were carried out twice and the field trial once for both populations. A QTL located on the short arm of chromosome 7A was present in both environments (hydroponics and field trials) and both populations. This QTL was suggestive to significant (LOD = 1.65 to 7.36) and accounted for 3% to 41% of the total phenotypic variation in both populations, with the favourable (Na^+ exclusion) allele coming from Cranbrook and Excalibur.

INTRODUCTION

Salt affected soils occur in more than 100 countries of the world in every climatic zone, exceeding 6% of the world's total land area [3,9]. Different types of salinization, with a prevalence of Na^+ salts, affect 30% of the land area in Australia. Ground water associated salinity and irrigation salinity affect approximately 16% of the agricultural area and recent investigations suggest that 67% has the potential to develop transient salinity. This occurs when salts derived from rainfall and soil weathering reactions accumulate in soils where evapotranspiration exceeds precipitation, causing salts to rise up the profile through capillary action. Salinity imposes severe limitations to Australian agricultural productivity, particularly dry land agriculture, and is one of the major contributing factors that results in yields lower than the climate related potential [8,9]. Salinity stresses plants in two ways. High concentrations in the soil creates an osmotic stress, making it harder for plants to extract water, and salts within the plant affect many aspects of plant metabolism and high concentrations can be toxic [5,8]. As salinity is a soil condition characterised by a high concentration of Na^+ salts, it is not surprising that plants have evolved mechanisms to regulate its accumulation and to select against it in favour of other nutrients commonly present in low concentrations, such as potassium. Bread wheat is a very efficient Na^+ excluder. When grown in 50 mM NaCl, bread wheat cultivars excluded 97% to 99% of the Na^+ in the soil solution [4]. In this study we screened two bread wheat populations with the aim of discovering QTLs responsible for increased Na^+ exclusion.

MATERIALS AND METHODS

Plant materials: Two F_1-derived DH mapping populations were used in this study. The Excalibur/Kukri ACPFG drought mapping population and the Cranbrook/Halberd population consist of 233 and 160 DH lines respectively. The entire Excalibur/Kukri population was evaluated in both environments. The Cranbrook/Halberd population segregates for the *Rht-B1b* semi-dwarfing locus. Although no QTLs for Na^+ exclusion were located on chromosome 4B, where *Rht-B1b* is located, it was found to have an epistatic effect on Na^+ exclusion. The population was divided in to two sub-populations (tall and semi-dwarf) using the *Rht-B1b* marker from Ellis *et al.*[2]., data from the "semi-dwarf sub-population" are presented here. In the Cranbrook/Halberd field experiment, a subset of lines was selected at random from the entire population.

Field experiments: The field experiments were both grown at Roseworthy, South Australia (34.57°S, 138.74°E, 87m altitude), during the 2006 growing season and the management regime followed local practice. The Excalibur/Kukri field experiment was randomised using a nearest neighbour design with two replicates of each DH line, with additional plots of the parental lines and control varieties. The plots were 1.3 m wide and 3.2 m long with six rows. Twenty youngest fully emerged leaves were collected from each plot during tillering (Zadoks growth stages 20 to 29). Two leaves of similar weight were selected for analysis from each plot. The Cranbrook/Halberd field experiment was a randomised complete block design with two replicates. The plots were 1 m wide and 4 m long with four rows. Two bulks of twelve leaves were collected from each plot at the three leaf stage (Zadoks growth stage 13).

Hydroponics assays: Seeds were germinated for 4 days on moist filter paper before being transferred to a supported hydroponic system. Seedlings were transplanted directly into 20 L containers with 3 mm diameter polycarbonate fragments, used as a soil substitute. Plastic netting with 10 mm squares separated the individual plants. Plants were supplied with a growth solution containing; 5 mol m^{-3} KNO_3, 2 mol m^{-3} $Ca(NO_3)_2$, 2 mol m^{-3} $MgSO_4$, 0.5 mol m^{-3} Na_2SiO_3, 0.2 mol m^{-3} NH_4NO_3, 0.1 mol m^{-3} KH_2PO_4, 0.05 mol m^{-3} NaFe(III)EDTA and micronutrients (50 mmol m^{-3} H_3BO_3, 10 mmol m^{-3} $ZnSO_4$, 5 mmol m^{-3} $MnCl_2$, 0.5 mmol m^{-3} $CuSO_4$ and 0.1 mmol m^{-3} Na_2MoO_4), pH =

6.5-7.0, in a 20 min pump/20 min drain cycle, for 10 days, until the third leaf emerged. At third leaf emergence, NaCl was added twice daily in 25 mol m^{-3} steps until a final concentration of 100 mol m^{-3} NaCl were achieved. Additional $CaCl_2$ was added to maintain the constant Ca^{2+} activity. After 10 days of Na^+ stress the third leaf from each individual plant was harvested.

Sodium analysis: Leaf fresh and dry weights were recorded and all leaves were dried over night at 85°C, before digestion in 1% HNO_3 at 85°C for 4 hrs. Concentrations of Na^+ in the leaf were determined by flame photometry and expressed as mmol Na^+ $litre^{-1}$ (on a tissue water basis) and μmol Na^+ g dry $weight^{-1}$.

Statistical analysis: Data were analysed using linear mixed models which were fitted using the residual maximum likelihood (REML) procedure in Genstat 10.0 [7]. Initially the DH lines and checks were considered to be random to calculate estimates of the variance components, which were used to estimate the broad sense heritability on a line mean basis. The analyses were then repeated with the DH lines and checks as fixed effects to estimate best linear unbiased estimators (BLUEs). These spatially-adjusted line means were used for the QTL mapping presented in this study.

QTL mapping: The Excalibur/Kukri genetic map and its construction are currently un-published. The Cranbrook/Halberd genetic map has been described by Chalmers *at al.* [1]. QTL analysis was carried out using mixed linear composite interval mapping in QTLNetwork 2.0 [10] and the QTL chromatograms were plotted using Qgene 4.0 [6]. The critical LOD significance thresholds for QTL detection were calculated using 1000 permutations and genome wide error rates of 0.1 (putative) and 0.05 (significant).

RESULTS AND DISCUSSION

While numerous QTLs for Na^+ exclusion were detected depending on the population and environment only one QTL on the short arm of chromosome 7A was consistently detected in all six experiments. The QTL was not significant in three experiments (Table 1), but within each population the QTL chromatograms and map location were consistent across experiments (Figure 1). The microsatellite marker locus *Xwmc083-7A*, common to both populations, was the closest marker to the QTL in all of the experiments. The percentage of the phenotypic variation explained in each of the experiments ranged from 3% to 41% depending on the sampling environment, population and population size (R^2 in Table 1). The R^2 is clearly influenced by the selection of DH lines in the Cranbrook/Halberd population, with the 65 semi-dwarf DH lines selected for the presence of the *Rht-B1b* locus. Hence the epistatic effect on the 7A Na^+ exclusion QTL, associated with the "tall phenotype," is removed. This epistatic effect can be attributed to the *Rht-B1b* locus reducing biomass production. The effect of biomass (dry weight of the leaf samples analysed) on the Na^+ content of the 38 Cranbrook/Halberd DH lines that were selected at random from the whole population was highly significant ($P < 0.001$ data not shown). This QTL is of potential interest as it has been detected here in two unrelated populations and in both the controlled environment of a supported hydroponics system and under field conditions. Therefore, results from both environments are an excellent validation for the hydroponics system, which is a repeatable, high through put and cost-effective method of screening cereal germplasm for differences in their Na^+ excluding ability. This QTL is also very relevant in the current environment, where the land area affected by salinity is rapidly increasing due to land use practices and climate change. However, further field evaluation is required to validate whether the effect of this QTL on Na^+ exclusion has a significant positive effect on grain yield in high salinity environments and that it does not have a deleterious effect on yield in the broader mega environment targeted by a particular breeding program.

REFERENCES

1 Chalmers, K. J., Campbell, A. W., Kretschmer, J., Karakousis, A., Henschke, P. H., Pierens, S., Harker, N., Pallotta, M., Cornish, G. B., Shariflou, M. R., Rampling, L. R., McLauchlan, A., Daggard, G., Sharp, P. J., Holton, T. A., Sutherland, M. W., Appels, R. and Langridge, P. (2001) Construction of three linkage maps in bread wheat, (*Triticum aestivum*). *Australian Journal of Agricultural Research* 52, 1089-1119.

2 Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzke, G. J. and Richards, R. A. (2002) "Perfect" Markers for the *rht-b1b* and *rht-d1b* dwarfing genes in wheat. *Theoretical and Applied Genetics* 105, 1038-1042.

3 FAO (2007) FAO land and plant nutrition management service. Available at http://www.fao.org/ag/agl/agll/spush/intro.htm.

4 Munns, R. (2005) Genes and salt tolerance: Bringing them together. *New Phytologist* 167, 645-663.

5 Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59, 651-681.

6 Nelson, J. C. (1997) Qgene: Software for marker-based genomic analysis and breeding *Molecular Breeding* 3, 239-245.

7 Payne, R.W., *et al.* (2007) Genstat® release 10 reference manual (Published by VSN International). http://www.vsni.co.uk.

8 Rengasamy, P. (2002) Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: An overview. *Australian Journal of Experimental Agriculture* 42, 351-361.

9 Rengasamy, P. (2006) World salinization with emphasis on Australia. *Journal of Experimental Botany* 57, 1017-1023.

10 Yang, J., Zhu, J. and Williams, R. (2007) QTL mapping method and software: Mapping the genetic architecture of complex traits in experimental populations. . *Bioinformatics* 23, 1527-1536.

Table 1 Summary of the chromosome 7AS Na^+ exclusion QTL results**.** For each environment the marker interval, number of DH lines, narrow sense heritability (h^2), LOD, phenotypic variance accounted for (R^2) and the additive effect are included. The favourable parental allele is given in parenthesis.

Population	Environment	Marker interval	DH lines	h^2	LOD	R^2	Add
Excalibur/Kukri							
QNax.aww-7AS	Hydroponics 2006	*Xwmc083-Xstm511ctg*	233	0.60	1.65#	3.0	-10.58
(Excalibur)	Hydroponics 2007	*Xwmc083-Xstm511ctg*	233	0.40	4.18*	7.4	-7.66
	Field trial 2006	*Xwmc083-Xstm511ctg*	233	0.26	4.13*	7.1	-0.56
Cranbrook/Halberd							
QNax.aww-7AS	Hydroponics 2005	*Xwmc083-Xcdo595*	65	0.32	7.36*	41.0	-7.09
(Cranbrook)	Hydroponics 2006	*Xwmc083-Xcdo595*	65	0.26	2.84#	18.0	-3.27
	Field trial 2006	*Xwmc083-Xcdo595*	38	0.60	1.95#	21.0	-1.03

*QTL significant at the P = 0.05 level.
#QTL significant at the P = 0.10 level.

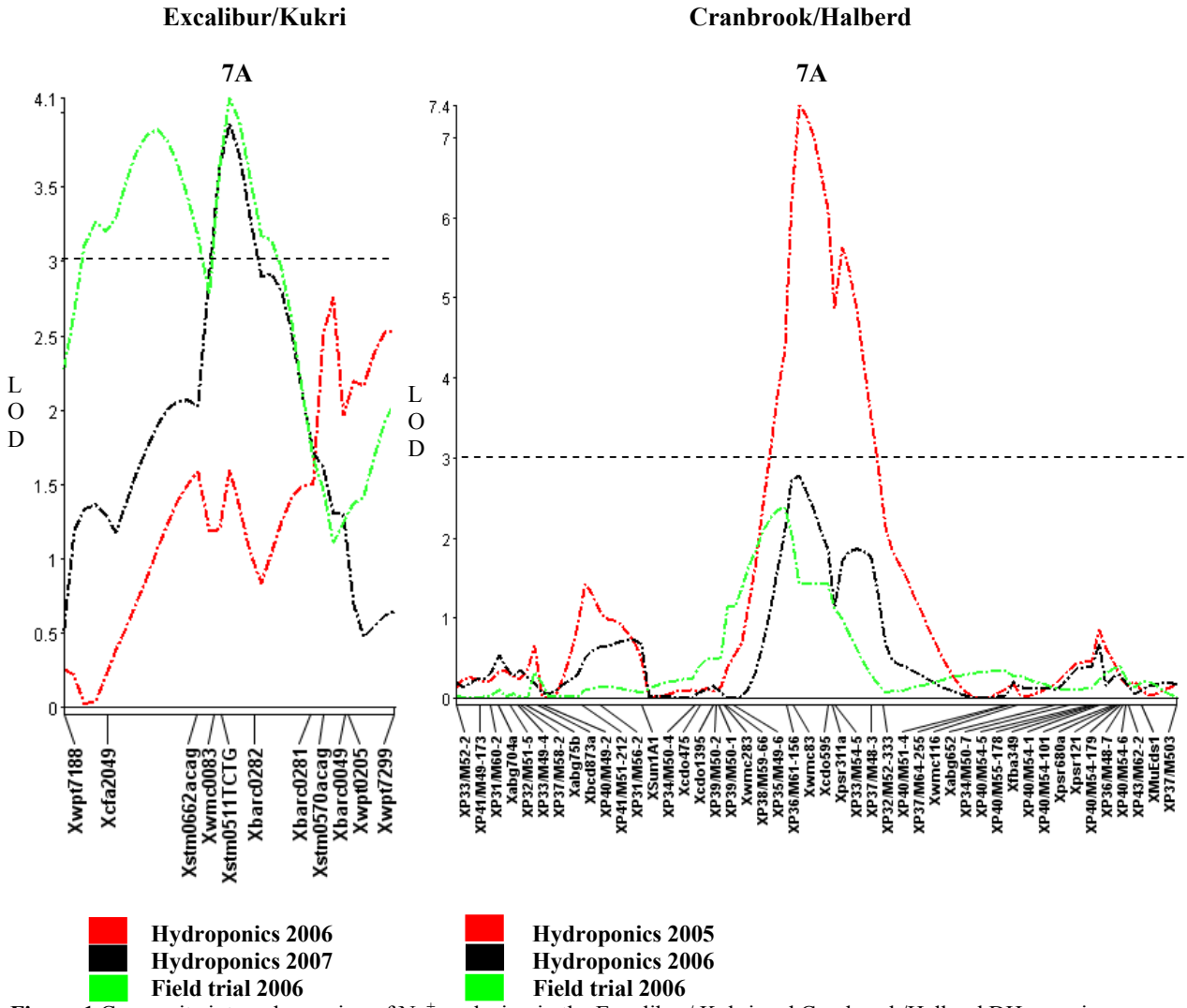

Figure 1 Composite interval mapping of Na^+ exclusion in the Excalibur/ Kukri and Cranbrook/Halberd DH mapping populations. The approximate 5% significance level is represented by a dashed line.