Genes for sodium exclusion in wheat

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A dissertation submitted to the University of Adelaide
in accordance with the requirements of
the degree of PhD
in the Faculty of Science,
School of Agriculture, Food and Wine

October 2008

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Acknowledgements

I would like to thank my supervisors, Rana Munns (CSIRO) and Mark Tester (University of Adelaide), for all their help, advice and support throughout my PhD, and for being fantastic supervisors. I would like to thank Richard James, Wolfgang Spielmeyer, Shaobai Huang, Evans Lagudah, Matthew Gilliham and Steve Tyerman for their help and guidance.

I am very grateful to have had the opportunity to work at Plant Industry in the Crop Adaptation building with so many brilliant people and good role models. I am thankful for the support of friends both at CSIRO and ACPFG.

I would like to thank Paul and my family for their constant support throughout my PhD.

List of publications from this thesis

Journal publications

Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R (2007) HKT1;5-like cation transporters linked to Na^+ exclusion loci in wheat, *Nax2* and *Kna1*. Plant Physiology. 143:1918-1928

Byrt CS, Munns R (2008) Living with salinity. New Phytologist 179:903-905.

Patents

Byrt CS, James RA, Lagudah ES, Munns R, Platten JD, Spielmeyer W (2008) Polynucleotides and methods for enhancing salinity tolerance in plants. Wipo Patent WO/2008/006169

Byrt CS, Huang S, James RA, Lagudah ES, Munns R, Spielmeyer W (2006) Salt tolerant plants. Australian provisional application No. 20069047 filed 31/9/06

Conference posters

Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R (2006) Is *Nax2* an HKT transporter? Gordon Research Conference, Salt and water stress in plants. Sept 3-7th, Oxford, UK.

Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R (2007) Is *Nax2* an HKT transporter? 4th Annual ACPFG Research Symposium: The Genomics of Drought. 22-24 October, Adelaide, South Australia.

Abbasov M, Byrt C, Spielmeyer W, Street K, Shavrukov, Aliyev R, Tester M, Munns R (2008) Screening diploid wheats with various eco-graphic distributions for two salt tolerance genes. 5th International Crop Science Congress. April 13-18, ICC Jeju, Korea.

Oral presentations

Hare RA, James RA, Huang S, Byrt CS, Lagudah ES, Lindsay EP, Spielmeyer W, Rathjen AJ, Munns R (2008) Breeding for salt tolerance in durum wheat. International durum wheat symposium: From Seed to Pasta, The Durum Wheat Chain. June 30 – July 3, Bologna, Italy.

Byrt CS, James RA, Spielmeyer W, Munns R, Gilliham M, Tester M (2008) Investigation of the function of a sodium transporter from wheat, HKT1;5. ComBio 2008, 22-25 September, Canberra, Australia.

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List of abbreviations

ABARE	Australian Bureau of Agriculture and Resource Economics
ACPFG	Australian Centre for Plant Functional Genomics
bp	base pair
cDNA	complementary DNA
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv.	cultivar
2,4-D	2,4-dichlorophenoxyacetic acid
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organisation
GUS	β-glucuronidase
HKT	High-affinity potassium (K^+) transporter
kb	kilobase
kDa	kilodalton
LB	Luria-Bertani medium
mRNA	messenger RNA
NIL	near isogenic line
NLWRA	National Land and Water Resources Audit
PCR	polymerase chain reaction
PPM	Plant preservative mixture
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
RT-PCR	reverse-transcriptase polymerase chain reaction
SEM	standard error of the mean
spp.	species (plural)
ssp.	subspecies
TE	Tris-EDTA buffer
TRIS	tris(hydroxymethyl)methylamine
U	enzyme unit
USDA	United States Department of Agriculture

Nomenclature

The current nomenclature for HKT transporters as described by Platten et al. (2006) and Huang et al. (2008)

New	Old	QTL
AtHKT1;1	*AtHKT1*	
OsHKT1;5	*OsHKT8*	*SKC1* (Ren et al., 2005)
OsHKT2;1	*OsHKT1*	
TaHKT2;1	*TaHKT1*	
TmHKT1;4	*TmHKT7*	*Nax1* (Huang et al., 2006)
TmHKT1;5	*TmHKT8*	*Nax2* (Byrt et al., 2007)
TaHKT1;5	*TaHKT8*	*Kna1* (Byrt et al., 2007)

Abstract

Salinity stress limits the growth and productivity of agricultural crops in many regions of the world. Whole plant tolerance to soil salinity involves numerous processes in many different tissues and cell types. For many cereals, sensitivity to salinity is due to the accumulation of sodium (Na^+) to toxic concentrations in the leaves. This thesis investigates a mechanism of control of Na^+ accumulation in leaves of wheat.

Bread wheat excludes sodium from the leaves better than durum wheat. Bread wheat is hexaploid (AABBDD) whereas durum wheat is tetraploid (AABB). The D-genome in bread wheat carries a major locus for sodium exclusion, *Kna1*, which may contribute to the differences in sodium exclusion between bread wheat and durum wheat.

An unusual durum wheat, Line 149, excludes sodium to a similar degree as bread wheat. Line 149 was derived from a cross between a *Triticum monococcum* (accession C68-101; AA) and a durum wheat (*T. turgidum* ssp. *durum* cv. Marrocos; AABB). Line 149 had been found to contain two major genes for sodium exclusion, named *Nax1* and *Nax2*, which appeared to retrieve sodium from the xylem sap in the roots and so prevent it reaching the leaves. Line 149 had been crossed with the durum wheat cv. Tamaroi, which accumulates high concentrations of Na^+ in the leaves, and near-isogenic single-gene mapping populations had been developed for *Nax1* and *Nax2*. *Nax1* had been located on chromosome 2A. The objective of this thesis was to map *Nax2* and identify a candidate gene.

Nax2 mapped to chromosome 5AL based on linkage to microsatellite markers. A high-affinity potassium (K^+) transporter (HKT)-like gene, *HKT1;5* was considered as a candidate gene for *Nax2*, based on similarity of the phenotype to a rice orthologue. Sequence information from a wheat *HKT1;5*-like expressed sequence tag in the public database was used to develop a probe for use in Southern hybridsation. A *HKT1;5*-like fragment was identified in Line 149 and *T. monococcum* C68-101, but was absent in Tamaroi. The *HKT1;5*-like gene, named *TmHKT1;5-A*, co-segregated with *Nax2* in the *Nax2* single-gene mapping population. The *HKT1;5* probe identified three putative *HKT1;5*-like genes on the long arm of chromosome 4B, and one *HKT1;5*-like gene on the long arm of chromosome 4D, in Langdon (*T. turgidum* ssp. *durum*) substitution lines, and in Chinese Spring (*T. aestivum*) ditelomeric lines. No A-genome *HKT1;5* like gene was identified in Langdon or Chinese Spring.

The D-genome *HKT1;5* gene, named *TaHKT1;5-D*, was found to co-locate with *Kna1*, the gene for sodium exclusion in bread wheat, in Chinese Spring chromosome 4D deletion lines. *Nax2* (*TmHKT1;5-A*) was found to be homoeologous with the gene for sodium exclusion in bread wheat, *Kna1* (*TaHKT1;5-D*). *TmHKT1;5-A* and *TaHKT1;5-D*, and their

promoters, were 94% identical, and both were expressed in the roots of wheat plants. This is consistent with the genes being located in the stele of the roots and retrieving Na^+ from the xylem sap as it flows towards the shoot, and so excluding Na^+ from the leaves.

A marker for *TmHKT1;5-A* was developed to track this gene in durum wheat breeding programs. A study of the *HKT1;5* gene in diploid ancestors of wheat indicated that this gene is present in most *Triticum monococcum* accessions, some *T. boeoticum* accessions, but not present in any *T. urartu* accessions. *T. urartu* is the likely A genome ancestor of modern wheat. This may explain the absence of *HKT1;5* in the A genome of modern wheat.

The protein encoded by *TaHKT1;5-D* transported sodium when expressed in *Xenopus laevis* oocytes. The inward currents were specific to Na^+, but at particular mole fractions of Na^+ and K^+ outward currents were observed that were consistent with outward K^+ transport. These data were consistent with the putative physiological function, of retrieving Na^+ from the xylem sap as it flows to the leaves, and resulting in a net exchange with K^+.

A construct designed to silence the expression of *TaHKT1;5-D* was introduced to bread wheat cv. Bob White. Nineteen putative transgenic plants were developed. The leaf Na^+ concentrations and genotype of the T_1 individuals were assayed. The data from two of the transgenic plants indicated that *TaHKT1;5-D* may have been silenced and that this may have lead to the increase in Na^+ accumulation in the leaves. However, this data is not conclusive at this time.

The information gained from this study will assist the introduction of the Na^+ exclusion trait into current durum cultivars, which are poor at excluding Na^+ and are salt sensitive. This information will also contribute to the body of knowledge of ion transport in plants and salinity tolerance in wheat.