Toward Map-based Cloning of a Na^+ Exclusion Gene From Barley (*Hordeum vulgare* L.)

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ABSTRACT

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ABSTRACT

Salinity is a major abiotic stress reducing crop productivity around the world. Mechanisms of salt tolerance are still largely unknown, and more work is needed to unravel the systems plants use to tolerate toxic levels of salt in their growing environment. The diploid species barley is relatively salt tolerant and therefore represents a useful model for studying salinity tolerance in the cereals. The ability to exclude Na^+ from the shoot is one component of salinity tolerance, and can be studied by measuring Na^+ accumulation in the shoot using ICPOES (inductively coupled plasma optical emission spectrometer) or flame photometry.

A population of 150 barley doubled-haploid (DH) lines generated from a cross between Australian cultivar Clipper and the Algerian landrace Sahara 3771 was developed at the Waite Campus of the University of Adelaide. Four separate soil-based experiments by three researchers found that this population segregated for Na^+ accumulation in the shoot, and that this trait was controlled by a major QTL (quantitative trait locus) on the long arm of chromosome 1H, which was named Hv*Nax3* (Chapter 3). The locus accounted for 80, 84, 77 and 40% of the total variation in shoot Na^+ concentration in these experiments. Interestingly, another experiment revealed no Na^+ accumulation effect of this QTL at all (Quinn, 2003), indicating that expression of the QTL was strongly dependent on the environmental conditions. Considering the phenotype distribution of non-recombinants for the Hv*Nax3* interval and the phenotype distribution of recombinants, it was possible to map Hv*Nax3* as a Mendelian locus.

Sequences of markers in the Hv*Nax3* region were used to identify a 6.6 Mb corresponding interval on rice chromosome 5 (Chapter 4). Co-linearity between the rice and barely genomes was used to identify barley ESTs and rice genes to generate new CAPS markers in the Hv*Nax3* region. Mapping and polymorphisms surveys with the marker fragments suggested that the Hv*Nax3* region is a region of high recombination frequency but low polymorphism. Mapping newly generated markers reduced the interval on rice chromosome 5 to about 2 Mb. Comparative mapping revealed that co-linearity was interrupted by duplication, inversions and transpositions. The existence of these inversions and the low polymorphism frequency in this region hampered the generation of markers.

Experiments in hydroponics, sand and soils were carried out to characterize the effect of the Hv*Nax3* locus on Na^+, K^+ accumulation in organs or whole shoot (Chapter 5). The experiments were conducted in growth chambers, glasshouse and in the field. Individual organs or the whole shoot were analysed using flame photometry or ICPOES. Conditions under which the Hv*Nax3* was expressed were identified, and one such setup was used in a subsequent exercise to fine-map the locus.

Hv*Nax3* was fine-mapped using an F_2 population of 125 plants developed by crossing two Clipper × Sahara (CS) doubled haploid lines (Chapter 6). The Hv*Nax3* genotypes of the eleven F_2 recombinants were determined by scoring F_3 progeny families for shoot Na^+ accumulation and markers. The combined information was used to further delimit Hv*Nax3* to a 2.8 cM marker interval in barley and a corresponding interval in rice of 222 kb on rice chromosome 5. Marker Hv*CLP*, a close homologue of At*SOS3* which contributes to salinity tolerance in *Arabidopsis*, co-segregated perfectly with the Hv*Nax3* locus, and was therefore considered as a plausible candidate for the Hv*Nax3* gene.

Hv*CLP* RNA expression was studied using Clipper (Na^+ excluder) and Sahara (Na^+ non-excluder) parents; CS DH line 134, Golden promise and BC_1F_2 derived sib lines carrying contrasting Hv*Nax3* alleles (Chapter 7). These studies revealed that Hv*CLP* expression was higher in Clipper than Sahara and higher in root than shoot. However, further experiments on BC_1F_2-derived lines suggested that the Hv*CLP* alleles do not differ inherently in their expression levels.

BAC (bacterial artificial chromosomes) clones containing candidate genes were identified and full length cDNA and genomic sequences of the Hv*SOS3* homologue (Hv*CLP*) from Clipper and Sahara were obtained using 5′ RACE PCR and polymorphisms were identified (Chapter 7). One amino acid difference (Alanine to Threonine) was identified between the Clipper and Sahara sequences, which could potentially account for phenotypic differences (Na^+ exclusion) between the Sahara (non-excluding) and Clipper (excluding) Hv*Nax3* alleles, consistent with Hv*CLP* being the Hv*Nax3* gene. 3D modelling of the Clipper and Sahara HvSOS3 proteins using the known *Arabidopsis* SOS3 structure as a template indicated that the overall shape and the distribution of the secondary structure elements were highly conserved in these proteins (Appendix C).

Twenty-four BC_1F_2 -derived lines containing mostly a Clipper genetic background, but homozygous for either the Clipper or Sahara allele of Hv*Nax3* (12 of each type), were generated. In two field trial sites in South Australia, the lines carrying the Hv*Nax3* Clipper allele averaged 48.5 and 38% less leaf Na^+ accumulation and 30 and 18.5% more grain yield, than the lines carrying the Sahara allele, respectively. Therefore, the Na^+ exclusion Hv*Nax3* allele appears to have considerable value for improving the salinity tolerance of barley in commercial South Austrian field conditions. This gene could be better utilized in breeding programs by using the linked PCR markers to select for the gene, or could be used to engineer tolerant varieties by transformation.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Alireza Rivandi and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Alireza Rivandi: ………………………………

Date: ……………………

DEDICATION

This thesis is deidicated to my family and all my teachers.

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Appendix: A

Salinity (primary and secondary) and sodicity in Australia.

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B.1 Analysis of variance table - First calcareous soil and Newbark mix experiment

B.2 Analysis of Variance table – First Glenthorne soil experiment

B.3 Analysis of Variance table – Second Glenthorne soil experiment

B.4 Analysis of Variance table – Third Glenthorne soil experiment

B.5 Analysis of Variance table – Minnipa soil experiment

Appendix: C

Phylogenetic analysis and construction of SOS3 models from barley and rice proteins.

Figure 1: An un-rooted radial phylogenetic tree of selected SOS3 proteins.
Amino acid sequences were aligned with ClustalX (Thompson et al., 1997) and branch lengths are drawn to scale. Circle colors indicate selected branches of cereal (yellow and pink) and plant dicotyledonous SOS3 proteins (green). Two letter prefixes for sequence identifiers indicate species of origin and a full list of all SOS3 proteins is specified in Materials and Methods. The tree was bootstrapped using N-J algorithm (Thompson et al., 1997). The barley Clipper and Sahara, rice and *Arabidopsis* sequences (in bold) were used for construction of molecular models and are highlighted in dark pink (Fig. 1).

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(B), Stereoview of ribbon representations of the Clipper (steel blue) and Sahara (pink) SOS3 models shows the disposition of secondary structure elements. The two models are superposed with rmsd value of 0.29 Å in Cα positions over 185 residues.

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Appendix: D

ANOVA was conducted using GenStat program (edition 6.1).

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D.2: Analysis of variance table - Bird proof cage experiment

D.3: Analysis of variance table – experiments in the field

Georgetown field trial
Whitwarta field trial

LIST OF ABBREVIATIONS

Acronyms/symbols	**Definition**
ABI:	Applied Biosystems Incorporated
ACPFG:	Australian Centre for Plant Functional Genomics
AFLP:	Amplified Fragment Length Polymorphism
AGRF:	Australian Genome Research Facility
ANOVA:	Analysis of Variance
ATP:	Adenosine Triphosphate
BAC:	Bacterial Artificial Chromosome
BC_1F_2:	Backcross one F_2 Derived Lines
bp:	Base Pairs
BSA:	Bovine Serum Albumin
CAPS:	Cleaved Amplified Polymorphism Sequence
cDNA:	Copy Deoxyribonucleic Acid
cM:	Centimorgan
cm:	Centimetre
CS:	Clipper × Sahara
CS DH:	Clipper × Sahara Double Haploid Lines
DW:	Dry Weight
DH:	Double Haploid
DMF	Dimethylformamide
DNA:	Deoxyribonucleic Acid
dNTP:	Deoxyribonucleic Triphosphate
EDTA:	Ethylene-Diamine-Tetra-Acetic-Acid
EST:	Expressed Sequence Tags
EDTA:	Ethylene-Diamine-Tetra-Acetic-Acid
EST:	Expressed Sequence Tags
g:	Gram
GP:	Golden Promise
HC:	Homozygous Clipper
HS:	Homozygous Sahara

LIST OF ABBREVIATIONS

ICPOES:	Inductively Coupled Plasma Optical Emission Spectrophotometry
IPTG:	Isopropyl β-D-1-thiogalactopyranoside
InDel:	Insertion-Deletion
IRGSP:	International Rice Genome Sequencing Program
kb:	Kilobase
kg:	Kilogram
KOAc:	Potassium Acetate
Kv:	Kilovolt
L:	Litre
LOD:	Logarithmic Odds Ratio
LSD:	Least Significant Difference
m:	Mili
M:	Molar
MAS:	Marker Assisted Selection
mg:	Milligram
min:	Minute
mins:	Minutes
mm:	Milimiter
mRNA:	Messenger Ribonucleic Acid
NCBI:	National Centre of Biotechnology Information
NILs:	Near Isogenic Lines
ng:	Nanogram
ºC:	Degree Celsius
ORF:	Open Reading Frame
PCR:	Polymerase Chain Reaction
pH:	Negative Logarithm of Hydrogen Ion Concentration
PVPP:	Polyvilylpolypyrrolidone
QTL:	Quantitative Trait Loci
R^2:	Measure of Association
RACE:	Rapid Amplification of cDNA Ends

LIST OF ABBREVIATIONS

RAPD:	Randomly Amplified Fragment Length Polymorphism
RFLP:	Restriction Fragment Length Polymorphism
RNA:	Ribonucleic Acid
RO:	Reverse Osmosis
rpm:	Revolutions Per Minute
SARDI:	South Australian Research and Development Institute
SE:	Standard Error
s:	Second
SOS:	Salt Overly Sensitive
SNP:	Single Nucleotide Polymorphism
SSC:	Sodium Chloride Citrate
SSR:	Simple Sequence Repeats
SMART (5'-):	Switching Mechanism at 5' End of RNA Transcript
TAE:	Tris-Acetate-EDTA
TAIR:	The *Arabidopsis* Information Resources
TE:	Tris-EDTA
TILLING:	Targeting Induced Local Lesions In Genomes
UV:	Ultraviolet
X-Gal:	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
YAC:	Yeast Artificial Chromosome
µL:	Microliter
µM:	Micromolar
µg:	Microgram
mm:	Millilitre
mM:	Millimolar
Ω:	Ohm