# Genetic Control of Sodium Exclusion in Tetraploid Wheat

A thesis submitted in fulfilment of the requirements for the degree of
Master of Agricultural Science at the University of Adelaide

By

**Muhammad Shefatur Rahman**
M.S. in Biotechnology and B.Sc.Ag (Hon's)
Bangladesh Agricultural University, Bangladesh

Australian Centre for Plant Functional Genomics
School of Agriculture, Food and Wine
Faculty of Sciences
The University of Adelaide

August 2011

# List of Abbreviations

| | |
|---|---|
| ACPFG | Australian Centre for Plant Functional Genomics |
| AFLP | Amplified fragment length polymorphism |
| AGRF | Australian Genome Research Facility |
| BLUE | best linear unbiased estimator |
| BLUP | best linear unbiased predictor |
| bp | base pair |
| CAPS | cleaved amplified polymorphic sequence |
| CHX | cation hydrogen exchanger |
| CIMMYT | International Maize and Wheat Improvement Centre |
| cM | centi Morgan |
| CSIRO | Commonwealth Scientific and Industrial Research Organisaion |
| DArT | Diversity Array Technology ™ |
| DAT | days after transplantation |
| DH | doubled haploid |
| DLW | dry leaf weight |
| DNA | deoxyribonucleic acid |
| EC | electrical conductivity |
| EST | expressed sequence tag |
| FLW | fresh leaf weight |
| HKT | high affinity potassium Transporter |
| LKC | leaf $K^+$ concentration |
| LNC | leaf $Na^+$ concentration |

**List of Abbreviations (Continued)**

| | |
|---|---|
| LRS | likelihood ratio statistic |
| MRT | Multiplex Ready Technology ™ |
| NIL | near- isogenic line |
| PCR | polymerase chain reaction |
| QTL | quantitative trait locus |
| RFLP | restriction fragment length polymorphism |
| rfu | relative fluorescence units |
| RIL | recombinant inbred lines |
| SNP | single nucleotide polymorphism |
| SOS | salt overly sensitive |
| SSR | simple sequence repeat |

# Table of Contents

# List of Tables

**List of Tables (Continued)**

## List of Figures

**List of Figures (Continued)**

**List of Figures (Continued)**

# Abstract

Worldwide, soil salinity is one of the major abiotic stress factors limiting crop production. Durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.]) is a relatively salt sensitive species. Its sensitivity to salt is thought to be due to its poor ability in limiting accumulation of toxic $Na^+$ in leaf tissues. The present research was undertaken to investigate the genetic control of sodium exclusion in a tetraploid wheat population of recombinant inbred lines developed from a durum wheat cultivar Atil and a cultivated emmer wheat (*Triticum turgidum* L. ssp. *dicoccum* [Schrank].Thell.) accession PI94628. In order to estimate the nature and extent of variation for sodium exclusion within the progeny, two experiments were carried out using a supported hydroponic system. In both cases, $Na^+$ and $K^+$ concentration were determined from the fourth leaf of plants which had imposed a 100 mM NaCl stress for 10 days. In the first experiment, screening of replicated parental lines and a subset of inbred lines (24 RILs) indicated the existence of significant genetic variation for sodium exclusion within the population. It was also found that the spatial variation within the experimental equipment contributed only 8-9% of the total observed phenotypic variation. In the second experiment, screening of the entire population indicated transgressive segregation for both of the $Na^+$ and $K^+$ accumulation traits, with the durum wheat line Atil found to be the better sodium excluder. A significant negative correlation ($r$ = -0.7) was found between leaf $Na^+$ and $K^+$ concentration, however, neither of these traits was found to be correlated with the shoot biomass of 30-day-old seedlings (21 days under salt stress). To construct a genetic linkage map, 1057 markers (916 DArT and 141 SSRs) were used. Of these, 467 markers were eliminated from the linkage analysis due the segregation distortion. The remaining 495 DArTs and 95 SSR markers were used in map construction. They provided reasonable genome coverage (2136.5 cM),

with a marker density of 3.6 cM/marker. The markers were distributed on 34 linkage groups representing parts of 14 chromosomes, with gaps of greater than 15 cM still remaining in 12 of the chromosomes. The majority of these markers showed conserved locations and orientation when compared to those described in previous genetic linkage maps of tetraploid and hexaploid wheat. Marker polymorphisms in two regions on chromosome 5A and 5B were significantly associated phenotypic variation for both $Na^+$ and $K^+$ accumulation in the leaf tissues. In both of these QTL regions, the durum wheat parent was contributed the alleles for lower $Na^+$ concentration and higher $K^+$ concentration. The two QTLs explained 28% and 19% of total phenotypic variation for $Na^+$ and $K^+$ accumulation, respectively. Both QTLs were mapped in the centromeric regions of the chromosomes. No QTLs for these traits have previously been reported in these regions. A highly significant QTL for shoot biomass was also mapped on chromosome 5A; explaining 16% of the phenotypic variation but this was over 32.4 cM from the leaf $Na^+$ or $K^+$ concentration QTLs. Furthermore, the QTLs were not found to be associated with traits related to vigour and/or vernalisation requirement. The molecular markers in the QTL regions detected here could serve as starting points for further characterisation of these genomic regions to elucidate the physiological and molecular bases of these QTLs.

# 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 Muhammad Shefatur Rahman 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.

I give consent to this copy of the thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of the thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Thesis program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

**Muhammad Shefatur Rahman**
August, 2011

**Acknowledgements**

First and foremost, I would like to express my sincere gratitude to my supervisors, Associate Professor Ken Chalmers and Professor Diane Mather, who have guided, supported and encouraged me throughout the course of my study. I would also like to profoundly acknowledge them for their support in setting up my research goals and giving me the opportunity to conduct the research in 'Genetic Analysis and Molecular Breeding (GAMB)' laboratory. I would specially like to acknowledge and thank to my primary supervisor Associate Professor Ken Chalmers for his continuous support, constructive criticisms and suggestions during the preparation of this thesis. I would also like to acknowledge his immense effort in editing the thesis. Thanks also due to my advisor Dr. Stuart Roy, for his valuable suggestions and advice.

I would like to express my sincere thanks to Julien Bonneau, for all of his help and support regarding genetic analysis experiments. I would also like to acknowledge him for providing the initial data of the linkage map. I would like to acknowledge all the members of the GAMB laboratory, particularly, Dr. Julia Brueggemann, Dr. Genet Mekuria, Elise Tucker, Elysia Vassos and Rebecca Fox for their assistance with various aspects of laboratory work throughout the study. I am also grateful to Professor Mark Tester for the access to his laboratory while conducting the sodium exclusion analysis. Thanks are also due to Dr. Yuri Shavrukov for his technical advice for the hydroponic experiment.

I am grateful to the AusAid for providing me a scholarship and Australian Centre for Plant Functional Genomics (ACPFG) for providing research funding. I am also thankful to ACPFG for providing excellent scientific environment to accomplish my study. I

would also like to acknowledge Bangladesh Agricultural Research Institute (BARI) and the Ministry of Agriculture, Bangladesh for study permission.

In addition, I would like to express my gratitude to the following people:

- Greg Lott for his technical advice with computing the genetic linkage map.
- Paul Eckermann (University of Adelaide) for his support in statistical analysis.
- Dr. Manahil Baho, Shashiprabha Goonetilleke and Nawar Shamayar for providing support in hydroponic experiment.
- Dr. Yusuf Genc for technical advice for sodium exclusion analysis.
- Dr. Mahmood Hassan for his advice regarding manuscript writing.
- Post-graduate coordinator Professor Otto Schmidt, Dr. Christopher Ford for their advice and support.

I would like to acknowledge my mentor; Dr. Andrew Jacob for his support during my difficult times. My gratitude extends to my colleagues Rezwan Molla and Mirza Naser at the Bangladesh Agricultural Research Institute of Bangladesh for their support.

I would like to acknowledge all of my friends living in Adelaide and the members from my cricket club, Adelaide Bangladesh Tigers (ABT) for their immense support in my overseas life. I would like to acknowledge my wife and daughter for their patience and support throughout my study. Last but not the least; I would like to express my deepest gratitude to my parents for their support and encouragement in all aspect of my career and life.