Allelic variation analysis and development of gene-specific molecular markers conferring acid soil tolerance in barley (*Hordeum vulgare* L.)

by

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Submitted in fulfilment of the requirement for the Degree of Doctor of Philosophy

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Dec 2013
Declaration

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Acknowledgments

First of all, I would like to thank my motherland - People`s Republic of China; and I also thank The Commonwealth of Australia for offering me this great opportunity to study abroad. During the three years, I did enjoy the scientific atmosphere, the multiple western cultures and the hospitality of its people. I hope the friendships between the two countries could be tightened by the scientific cooperation and student exchange. Thanks to the GRDC (the Australian Grains Research and Development Corporation) for providing founding to my research project and the University of Tasmania for offering the scholarship while I undertook this degree.

Next, a deep thanks to my supervisory team—A/Prof Meixue Zhou (UTAS), Prof Chengdao Li (DFWA), Prof Sergey Shabala (UTAS), Prof Dongfa Sun (HZAU)—for countless hours spent and endless patience directing, guiding, encouraging and helping me during the past three years. Thank you all for your enthusiasm and inspiration for science making me brave and assured when I was weak and puzzled.

I owe my great thanks to Ms Sue Broughton (DFWA) and Ms Irene Waters (DFWA) for assisting me with collecting the phenotypic data. Without your strong assistance, I am not able to finish my Ph D within 3 years. I would like to thank Ms Sharon Westcott (DFWA), Miss Vera Limadinata (Murdoch Univ.) and Dr Xiaoqi Zhang (Murdoch Univ.) for supporting me with the techniques of DNA extraction, SSR, SSCP, sequencing and countless samples analysing for me. Thanks to Dr Tefera Angessa (UWA) for helping me with collecting the seeds. I really appreciate...
the suggestions from Prof Rudi Appeals (Murdoch Univ.), Dr Dean Deprevean (Murdoch Univ.), Dr Mike Francki (DFWA), Dr Qisen Zhang (DFWA), Dr Xue Gong (UWA), Dr Reetinder Gill (Murdoch), Dr Fengqi Li (CAAS, China), Ms Yiming Guo (UWA) for helping with the data analysis and paper writing during my Ph.D.

I would like to thank all the members in barley and wheat groups including Steve Brown, Dora Li, Esther Walker, Fiona Brockman, Junhong Ma, Julie Uhlmann in State Agriculture Biotechnology Centre, Murdoch. Thank you for your help and accompany during the three years. I also would like to express my deep thanks to Prof Mike Jones (Murdoch Univ.), Ms Beelay Adis (Murdoch Univ.), Ms Frances Brigg (Murdoch Univ.) and Dr Jingjuan Zhang (Murdoch Univ.) for your kindness help while I am at the Murdoch University. And I also appreciated the kind suggestions for my scientific career from Prof Wujun Ma (Murdoch Univ.), Dr Sheng Chen (UWA).

To my fellow Ph D candidates, who shared this journey with me—Daniel Kolleh (Murdoch Univ.), Hollie Webster (Murdoch Univ.), Xue Gong (UWA), Chandima H. Appuhamilage (UWA), Shahidul Islam (UWA) and Shunli Wang (NCU, China), Ke Wang (NCU, China), Rui Qiu (NWSUAF, China)—a massive thanks for your friendship, advice, encouragement, accompany and many other enjoyable games which make my Ph D colourful.

Thanks to my parents for having me. I really appreciate the life which you gave to me. Thank you for all your support, patience, love and kindness to me while I was doing my Ph D.
Finally, huge thanks to all the friends and relatives who gave support and help during the three years. Especially, I would like to thank Mr Fei Li. I owe you so much. Thank you for your love, patience, and good temper which makes me brave on my way.
Publications


Abstract

Acid soil is a prevalent problem over the world. The high concentration of Al in the acid soil is one of the major production limiting factors to many plants. Decades of studies have resulted in a significant progress in revealing the mechanism of Al tolerance in plants. Several key genes have been identified and the Al tolerance was validated to be related with gene sequence variations in some plants. Barley (Hordeum vulgare L.) is one of most sensitive cereals to aluminum toxicity. This thesis was aimed at revealing the mechanisms of Al tolerance in barley using the marker development, QTL, association mapping and sequencing techniques. The major findings of the thesis are listed below:

1. The genetics of barley Al tolerance was studied in two double haploid populations, Hamelin/Svanhals and Br2/Hamelin, through QTL mapping. The phenotypic vitiation was investigated in both hydroponic and acid soil experiments. The phenotypic result suggested that a single gene is responsible for Al tolerance in barley. Al tolerance is controlled by single QTL on chromosome 4H and flanked by commonly used SSR makers.

2. Gene-specific markers were developed covering the whole sequence of HvAACT1 gene (also named as HvMATE). The polymorphic gene-specific markers were incorporated with the other commonly used SSR markers to conduct the QTL mapping. The result showed that the QTL interval for acid soil tolerance was narrowed and the phenotypic variations explained by the QTLs were increased. The gene-specific markers could also explain more phenotype variation than these
commonly used SSR markers. These new gene-specific markers provide effective and simple molecular tools for marker assisted selection in acid soil tolerant barley breeding.

3. The genetic diversity analysis and candidate gene association mapping based on HvAACT1 gene were conducted using accessions with different Al tolerance. Twenty eight gene-specific markers were polymorphic among different accessions. The sequencing analysis showed that these polymorphisms among accessions varied from over 1Kb to one SNP. These markers clearly identified the genetic relationship of different accessions using cluster analysis. Several gene specific markers were found to be associated with the Al tolerance in accessions. These significant polymorphisms detected by these markers could be considered candidate variation sites related to Al tolerance.

4. The allele variations of HvALMT were also studied in different accessions. Ten pairs of primers (6 in the coding region, 4 in the upstream region) showed polymorphisms. Sequencing analysis showed that these polymorphisms varied from one SNP to over 400bp insertion/deletion. Stepwise regression analysis revealed one gene-specific marker, UA21, was significantly correlated with the phenotypic variation of root length in acid soil and the relative root length in acid soil, as well as acid soil treated with lime.

In conclusion, the findings from this thesis suggest that: 1) While citrate exudation was validated to be responsible for Al tolerance in barley, it cannot fully explain the genetic variability in Al tolerance in barley; 2) Malate exudation may play an important role in detoxifying Al in some barley accessions; 3) The gene specific markers developed from HvAACT1 and HvALMT can be used in molecular
marker assisted selection in breeding; and 4) The significant polymorphisms detected by the association mapping can be used for further gene expression study.
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