Assessment of stress-induced and developmentally-induced DNA methylation changes in barley (Hordeum vulgare L.)

Moumouni Konate

A thesis submitted to the University of Adelaide for the degree of

Doctor of Philosophy

School of Agriculture, Food and Wine

Faculty of Sciences, The University of Adelaide

April 2017

Declaration

I, undersigned Moumouni Konate, certify that:

- This work is original and has not been accepted for the award of any other degree or diploma in any university or other tertiary institution to Moumouni Konate and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due acknowledgement is made in the text
- No part of this work will be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of the degree except where due reference has been made in the text;
- I give consent for the thesis to be made available for loan and photocopying after it has been examined and placed in the library, subject to the provisions of the Copyright Act 1968. For manuscripts included in this thesis (Chapters 3, 4 and 5), the authors acknowledge that the copyright resides with the copyright holder of those works.
- I give consent for the digital version of my thesis to be made available on the web, via the University's digital research repository, the library catalogue, The Australian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Date: ……3 April 2017…….

Signature: …… ……

Moumouni Konaté

Table of Contents

Acknowledgments

The thesis is the culmination of four years of research apprenticeship, with excitement and challenges along the way. This would not have been possible without the guidance of my supervisors Dr Bettina Berger, Dr Carlos Marcelino Rodriguez Lopez, Professor Eileen Scott and Professor Michael Wilkinson. I would like here to express my gratitude and admiration for being a recipient of their time, support and scientific direction. I am particularly indebted to Dr Carlos Marcelino Rodriguez Lopez for discussions, and for his dedication to this project.

My sincere thanks to Professor Diane Mather, for being my Independent Advisor, and for her wise advice during my studies. I am thankful to Associate Professor Kenneth Chalmers, for being my Postgraduate Coordinator.

I would like to acknowledge Dr Olena Olena Kravchuk and Dr Julian Taylor, both at the Biometry Hub (School of Agriculture, Food and Wine), for assistance with experimental design and statistical analyses. I also acknowledge Steve Pederson, Head of the Bioinformatics Hub (The University of Adelaide), his colleague Dr James Breen and their PhD student Benjamin Mayne, for their assistance in sequencing data analyses. Also, special thanks to the staff of the Plant Accelerator for their enthusiastic help during my experiments.

I would like to thank Dr Jacob Sanou, former Regional Director of INERA, and Mrs Julienne Gue, Sociologist at INERA (Bobo-Dioulasso, Burkina Faso) for their encouragement and constant support for my PhD project in Australia. I am grateful to Dr Bruce Pengelly (CSIRO EcoSciences Precinct Brisbane) and Dr Peter Carberry (Deputy Head ICRISAT, India), for their interest in and support for my PhD project.

I would like to thank past and present lab. members, from all of whom I have benefited in one way or another during my time here: Dr Adam Croxford, Dr Caroline Ford, Ms Amanda Camp, Mr Pastor Fabre, Mr Kiflu Gebremicael Tesfamicael, and many other students.

I acknowledge the Australian Agency for International Development (AusAID) for providing me with an Australian Awards Scholarship to undertake this PhD project. Many thanks to Niranjala Seimon, AusAID Student Support, for her continuous assistance and support throughout my stay in Australia.

Finally, I would like to thank my parents for their support and prayers; my wife Sali for her support, both intellectually and emotionally; my sons Hamza and Moubarack, and my new-born daughter Amira, for the special times they provided for me in Adelaide.

ᘐ *This thesis is dedicated with love to my mother, Tene Mema Cisse and the memory of my dad Zakaria Konate who passed away while this thesis was under review* ᘓ

Thesis abstract

DNA methylation is involved in both plant development and adaptation to environmental stress. Changes in DNA methylation can affect the expression of genes that are important for both plant tissue differentiation and stress response. Characterisation of tissue and stress specific methylation markers generates an invaluable tool for epiallele discovery that can be used for future functional and crop improvement studies.

We used barley as a plant model, and salinity as a stress model, to study methylation markers that discriminate the plant tissues and that are specific to salinity stress. This choice presented the advantage of using a crop plant with a reference genome sequence, which allows for genomic analyses; and an abiotic stress factor that is relatively easy to control.

Nine barley varieties subjected to mild salt stress (75 mM NaCl) were studied for their response to the stress by measuring phenotypic traits, such as biomass, yield and ion accumulation in the leaves. Then, Methylation Sensitive Amplified Polymorphisms (MSAP) were used to analyse changes induced by salt stress in their DNA methylation profiles, which were tested for correlation with the phenotypic data from the same plants. This study revealed that, although the MSAP approach can detect differentially methylated markers induced by a mild salt stress in barley, it presented a limitation in the number of differentially methylated markers (DMMs) detected. This study also revealed that the detection of DMMs by MSAPs was significantly influenced by genotypic differences among varieties. Finally, analysis of the epigenetic variability detected by MSAP indicated that microclimatic differences experienced by different plants in the study contributed to what was previously considered to be stochastic variability.

The results from the MSAP suggested an alternative approach was required to identify DMMs that are conserved across barley varieties. Using the high throughput DNA sequencing approach methylation-sensitive genotyping by sequencing (ms-GBS), we detected thousands of salt-induced DMMs and similar numbers of tissue-specific DMMs. Ms-GBS-generated DMMs were potentially universal, since they were conserved in five barley varieties used in the study. Sequence analysis of the ms-GBS generated DMMs indicate that both tissue-specific and salt-

induced changes in DNA methylation happen preferentially in repeat regions, but also target other gene types, such as protein-coding and Transfer RNA genes. Ontology analysis of differentially methylated protein-coding genes revealed that many are likely to play a role in stress response and organ-specific functions. However, further studies, including expression analyses, are needed to link gene methylation to gene expression.

List of Figures

List of Tables

Abbreviations

µl	microlitre(s)
µM	micromolar
ACPFG	Australian Centre for Plant Functional Genomics
AFLP	Amplified Fragment Length Polymorphism
AGRF	Australian Genome Research Facility
ANOVA	Analysis of variance
AP2/DREB	Activating Protein 2 / dehydration-responsive element-binding
bp	Base pair(s)
b-ZIP	Basic Leucine Zipper Domain
BSA	Bovine Serum Albumin
cm	Centimetre(s)
DAS	Day(s) after sowing
DE	Differentially expressed
DF1, DF2,	Discriminant Factor 1, 2
DM	Differentially Methylated
DMM	Differentially Methylated Marker
DNA	deoxyribonucleic acid
dNTP	Dinucleotide tri-phosphate
FDR	False Discovery Rate
GO	Gene Ontology
HCA	Hierarchical Cluster Analysis
HKT	High affinity potassium transport
HNO3	Nitric acid
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
INERA	Environment and Agriculture Research Institute (Burkina Faso)
K^+	Potassium ion
Kb	Kilo base pair(s)
L	Litre(s)
logFC	Logarithm 2 of fold-change
LSD	Fisher's Least significant difference
m	Metre(s)

mg	Milligrams(s)
ml	Millilitre(s)
mM	Millimolar(s)
MSAP	Methylation-Sensitive Amplification Polymorphism
ms-GBS	Methylation-Sensitive Genotyping By Sequencing
Na^+	Sodium ion
NaCl	Sodium Chloride
NEB	New England Biolabs
ng	Nanogram(s)
NHX	Na+/H+ exchanger
Pa	Pascal(s)
PAR	Photosynthetic active radiance
PC-LDA	Principal Components – Linear Discriminant Analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
Phi-ST	Phi Statistics
pmol	Pico Mole(s)
REVIGO	Result Visualisation of Gene Ontology
RGB	Red Green Blue
RH	Relative humidity
SEM	Standard Error of the Mean
SVP	Saturated Vapour Pressure
TE	Transposable elements
TES	Transcription End Site
TSS	Transcription Start Site
UC Davis	University of California at Davis
UTR	Untranslated Region
v/v	volume/volume
VPD	Vapour pressure deficit
w/w	weight/weight
WRKY	a protein starting with amino-acids Tryptophan (W)- Arginine (R)- Lysine (K)- Tyrosine (Y).