

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

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Doctor of Philosophy

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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.

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Abbreviations

μl	microlitre(s)
μM	micromolar
ACPPFG	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
HNO ₃	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)
log ₂ FC	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).