

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

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## Table of Contents

Declaration.....	i
Acknowledgments .....	viii
Thesis abstract.....	ix
List of Figures.....	xi
List of Tables .....	xiii
Abbreviations.....	xiv
Chapter 1: Literature review and research aims .....	1
1.1. Introduction.....	1
1.2. Concepts and mechanisms of epigenetics.....	2
1.2.1. Histone modifications .....	3
1.2.2. Small interfering RNA.....	5
1.2.3. DNA methylation.....	6
1.3. Biological functions of DNA epigenetic variations.....	7
1.3.1. DNA methylation as a developmental script .....	7
1.3.2. DNA methylation as a defence mechanism.....	8
1.3.3. DNA methylation as a regulator of transposons and plant plasticity .....	9
1.3.4. DNA methylation as a driver of evolution.....	11
1.4. Epigenetic profiling methods.....	12
1.5. Plant responses to stress.....	13
1.6. Salinity induced alteration of plant methylation patterns .....	16
1.6.1. Salinity induces DNA hypomethylation in roots .....	16
1.6.2. Salinity induces hypermethylation in shoots .....	17
1.6.3. Factors affecting salinity-induced alteration of DNA methylation .....	17
1.6.4. Epigenetic regulation of gene expression during salinity stress .....	19
1.7. Project objectives.....	22

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1.8. Linking statement .....	24
Chapter 2: Assessment of the effect of mild salt stress on barley phenotypes and epigenomes	25
2.1. Introduction.....	25
2.2. Material and methods.....	27
2.2.1. Plant material and greenhouse conditions .....	27
2.2.2. Measurement of phenotypic parameters .....	28
2.2.3. MSAP analysis.....	29
2.3. Results.....	34
2.3.1. Effect of mild salinity on barley varieties.....	34
2.3.2. Salt-induced DMMs.....	40
2.3.3. Estimation of epigenetic differentiation between salt treatments .....	42
2.3.4. Correlation between salinity symptoms and DNA methylation .....	43
2.4. Discussion.....	45
2.4.1. The effect of mild salt stress is genotype dependent .....	45
2.4.2. Salt stress induces both qualitative and quantitative DMMs in barley .....	46
2.4.3. No universal salt-induced DMMs in barley under mild salinity .....	48
2.4.4. Correlation between salinity symptoms and DNA methylation .....	48
2.5. Conclusion .....	49
Chapter 3: Patterns of salt-induced differentially methylated markers in barley ( <i>Hordeum vulgare</i> ) genome as revealed by Methylation-sensitive Genotyping-By-Sequencing.....	50
Abstract.....	54
3.1. Introduction.....	55
3.2. Results.....	57
3.2.1. Methylation-sensitive Genotyping-By-Sequencing (ms-GBS) .....	57
3.2.2. Salt-induced DNA methylation changes is tissue and concentration specific ...	58
3.2.3. Stability of salt-induced DMMs across treatments.....	60

---

3.2.4.	Distribution of salt-induced DMMs around repeat regions and genes .....	63
3.2.5.	Gene ontology analysis of salt-induced DMMs .....	67
3.2.6.	Differentially expressed genes in barley roots.....	71
3.3.	Discussion.....	75
3.3.1.	Salt-induced DMMs are not that stochastic .....	75
3.3.2.	Salt-induced DMMs are more abundant in leaves but more intense in roots ....	76
3.3.3.	Salt-induced DNA methylation may be involved in gene regulation.....	77
3.3.4.	Salt-induced DMMs correlate with stress related genes.....	78
3.3.5.	Conclusion .....	79
3.4.	Material and methods.....	80
3.4.1.	Plant material and stress treatment .....	80
3.4.2.	DNA extraction.....	81
3.4.3.	Methylation Sensitive genotyping by sequencing (ms-GBS).....	81
3.4.4.	Data analysis .....	82
3.4.5.	Salinity induced differentially methylated markers in barley.....	83
3.4.6.	Distribution of salt-induced DMMs around genomic futures.....	83
3.4.7.	Gene ontology of differentially methylated genes.....	84
3.4.8.	Gene expression and ontology analysis of root transcriptome .....	84
	References.....	86
	Chapter 4: Atlas of tissue and age specific patterns of DNA methylation during early development of barley ( <i>Hordeum vulgare</i> ).....	95
	Abstract.....	99
4.1.	Introduction.....	100
4.2.	Results.....	101
4.2.1.	Methylation-sensitive genotyping by sequencing (ms-GBS).....	101
4.2.2.	Estimation of “tissue and age”-dependent epigenetic differentiation.....	102
4.2.3.	Analysis of DNA methylation differences between roots and leaves .....	105

---

4.2.4.	Analysis of DNA methylation differences between leaf blades and sheaths ..	108
4.2.5.	Distribution of tissue-specific DMMs around genes .....	109
4.2.6.	Distribution of tissue-specific DMMs near repeat regions .....	110
4.2.7.	Distribution of genes around differentially methylated (DM) repeats) .....	111
4.2.8.	Gene ontology of differentially methylated genes.....	112
4.2.9.	Gene ontology of genes near differentially methylated repeats .....	115
4.3.	Discussion .....	117
4.3.1.	Extensive DMMs between roots and leaves .....	117
4.3.2.	Minor association of DNA methylation with organ ageing in barley seedlings 119	
4.3.3.	Tissue-specific DNA methylation preferentially targets repeat regions in the barley genome.....	120
4.3.4.	DMMs between roots and leaves, target genes that are relevant to plant tissue identity	121
4.3.5.	Conclusion .....	122
4.4.	Material and methods.....	123
4.4.1.	Plant material and growth conditions .....	123
4.4.2.	Methylation sensitive genotyping by sequencing (ms-GBS) .....	123
4.4.3.	Principal component – linear discriminant analysis (PC-LDA) .....	124
4.4.4.	DMMs detection in barley .....	124
4.4.5.	Distribution of DMMs around genomic features and gene ontology .....	124
References.....		126
Chapter 5:	Greenhouse spatial effects detected in the barley ( <i>Hordeum vulgare</i> L.) epigenome may underlie the stochasticity of DNA methylation .....	133
ABSTRACT.....		138
INTRODUCTION .....		139
MATERIAL AND METHODS .....		141

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Plant material and experimental design .....	141
Greenhouse environmental conditions.....	144
DNA extraction.....	145
MSAP.....	145
MSAP data analysis .....	145
Assessment of correlations between epigenetic profiles and plant phenotypes .....	146
RESULTS .....	147
Microclimatic variability in the greenhouse .....	147
Correlation between DNA methylation profile and plant position in the greenhouse.....	149
Correlations between barley phenotype, epigenome and position .....	155
DISCUSSION .....	158
Stochastic DNA methylation is explained by microclimatic differences .....	158
Positional effect affects salt stress-induced DNA methylation changes in barley .....	159
Phenotypic differences associated to greenhouse microclimates correlate with epigenetic differences.....	159
CONCLUSION.....	160
ACKNOWLEDGMENTS .....	161
AUTHOR CONTRIBUTIONS: .....	161
CONFLICTS OF INTEREST:.....	161
SUPPORTING INFORMATION.....	161
REFERENCES .....	162
Chapter 6:    General discussion .....	167
6.1.    Summary of the thesis project .....	167
6.1.1.    Salt-induced and tissue specific- DMMs in barley.....	168
6.1.2.    Stochasticity of DNA methylation.....	170
6.1.3.    Generating genotype-independent DMMs.....	170
6.1.4.    DMMs induced simultaneously by salt stress and tissue identity .....	171

6.1.5. DMMs target repeat regions of the barley genome .....	172
6.1.6. DNA methylation profiling for gene discovery .....	172
6.2. Outlook work .....	173
Appendices.....	175
References (Chapters 1, 2 and 6) .....	220



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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.

## List of Figures

Figure 1.1: Different types of epigenetic mechanism.....	4
Figure 1.2: Involvement of epigenetic mechanisms in plant plasticity. ....	10
Figure 1.3: Sensitivity of isoschizomers MspI and HpaII to DNA cytosine methylation in their recognition site 5'-CCGG-3'. ....	13
Figure 1.4: Schematic representation of mechanisms controlling plant responses to stresses ..	15
Figure 2.1 Leaf [Na <sup>+</sup> ] and [K <sup>+</sup> ] of eight barley varieties. ....	35
Figure 2.2: Projected shoot area of eight barley varieties under control (0 mM NaCl, white bars) and stress (75 mM NaCl, grey bars) conditions.....	38
Figure 2.3: Salt tolerance of eight barley varieties. ....	39
Figure 3.1: Number of salt-induced differentially methylated markers (DMMs) in barley leaves and roots.....	58
Figure 3.2: Tissue-specific response intensity and directionality of salt-induced DNA methylation changes. ....	59
Figure 3.3: Venn diagram showing the number of differentially methylated markers (DMMs) induced by different salt concentrations in barley leaves and roots. ....	61
Figure 3.4: Hierarchical clustering of the fold changes in read counts of DMMs stable across salt concentrations.....	62
Figure 3.5: Distribution of salt-induced differentially methylated markers (DMMs) around repeat regions and genes. ....	65
Figure 3.6: Distribution of salt-induced differentially methylated markers (DMMs) around UTRs, exons and tRNA genes. ....	66
Figure 3.7: Summary treemaps of GO (gene ontology) term representatives for the category “biological process” obtained from salt-induced differentially methylated genes in barley leaves. ....	69
Figure 3.8: Summary treemaps of GO (gene ontology) term representatives for the category “biological process” obtained from salt-induced differentially methylated genes in barley roots: ....	70
Figure 3.9: Summary treemaps of GO (gene ontology) term representatives for the category “biological process” obtained from salt-induced differentially expressed genes in barley roots. ....	73

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Figure 4.1: Analysis of the differentiation of DNA methylation profiles of barley roots, leaf sheaths and leaf blades.....	104
Figure 4.2: Analysis of the number of DMMs among three barley tissues. ....	106
Figure 4.3: Directionality of the methylation in tissue-specific DNA methylation markers...	107
Figure 4.4: Hierarchical clustering analysis of the DMMs. ....	108
Figure 4.5: Distribution of tissue-specific differentially methylated markers (DMMs) around genes. ....	110
Figure 4.6: Distribution of tissue-specific differentially methylated markers (DMMs) around repeats. ....	111
Figure 4.7: Distribution of genes around differentially methylated repeat regions. ....	112
Figure 4.8: Summary treemaps of GO (gene ontology) term representatives for the category “biological process” obtained from differentially methylated genes between roots and leaves. ....	115
Figure 4.9: Representative GO enrichment summary treemaps obtained from genes near DM repeats between roots and leaves. ....	117
Figure 5.1: Experimental layout and plan of the greenhouse (24 m <sup>2</sup> ). ....	143
Figure 5.2: Average daily environmental conditions in the greenhouse. ....	148
Figure 5.3: Principal coordinates analysis (PCoA) of MSAP (methylation sensitive amplified polymorphism) markers in barley variety Commander. ....	151
Figure 5.4: Correlation between pairwise epigenetic distance (Epi GD) and plant position in the greenhouse. ....	153
Figure 5.5: Exemplars of MSAP (methylation sensitive amplified polymorphism) alleles that show significant differences in peak height between positions in the greenhouse.....	154
Figure 5.6: Box plots showing biomass and grain yield range per position (P1-5) in the greenhouse (n = 9). ....	155
Figure 5.7: Correlation between pairwise epigenetic distance (EpiGD) and pairwise difference in grain yield between plants of the variety Schooner. ....	156

## List of Tables

Table 2.1: Adapter and primer sequences used for the MSAP (Rodríguez López et al., 2012). .....	30
Table 2.2: Composition of the master mixture for restriction of genomic DNA and ligation of adapters. ....	30
Table 2.3: Composition of the solution for the pre-amplification PCR.....	31
Table 2.4: Composition of the solution for the selective amplification PCR.....	32
Table 2.5: Number of qualitative salt-induced DMMs in barley.....	41
Table 2.6: Number of quantitative salt-induced DMMs in barley.....	41
Table 2.7: Pairwise Phi-ST (Phi statistics) and P-value (in brackets) between control and salt stress samples (respectively 0 mM and 75 mM NaCl). ....	42
Table 2.8: Pairwise Phi-ST (Phi statistics) and P-value (in brackets) between control and salt stress samples (respectively 0 mM and 75 mM NaCl). ....	43
Table 2.9: Coefficient of determination ( $R^2$ ) between epigenetic distance and salt-induced variation in leaf $[Na^+]$ , $[K^+]$ , biomass (Biom) and grain yield (Yield). ....	44
Table 3.1: Data yields of the ms-GBS, generated using the Illumina HiSeq 2500 platform.....	57
Table 3.2: Number of genes differentially methylated and associated GO terms in barley leaves and roots.....	68
Table 3.3: Number of genes differentially expressed (DE genes) and associated GO terms in barley roots. ....	72
Table 3.4: List of differentially methylated DE genes in barley roots. ....	74
Table 4.1: Data yields from ms-GBS, generated using the Illumina HiSeq 2500 platform. ...	102
Table 4.2: Number of Differentially Methylated Markers in barley tissues of different ages.	105
Table 4.3: Number of differentially methylated DM genes and associated gene ontology (GO) terms.....	113
Table 5.1: List and description of barley genotypes used in this study .....	142
Table 5.2: Summary descriptives of the Vapour Pressure Deficit (VPD) and light integral by sensor node (Node A-D). ....	149
Table 5.3: Correlation between pairwise epigenetic distance and physical distance. ....	152
Table 5.4: Correlation between epigenetic distance and grain yield of nine barley varieties.	157

## 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 <sub>3</sub>	Nitric acid
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
INERA	Environment and Agriculture Research Institute (Burkina Faso)
K <sup>+</sup>	Potassium ion
Kb	Kilo base pair(s)
L	Litre(s)
log <sub>2</sub> FC	Logarithm 2 of fold-change
LSD	Fisher's Least significant difference
m	Metre(s)

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mg	Milligrams(s)
ml	Millilitre(s)
mM	Millimolar(s)
MSAP	Methylation-Sensitive Amplification Polymorphism
ms-GBS	Methylation-Sensitive Genotyping By Sequencing
Na <sup>+</sup>	Sodium ion
NaCl	Sodium Chloride
NEB	New England Biolabs
ng	Nanogram(s)
NHX	Na <sup>+</sup> /H <sup>+</sup> 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).