METABOLOMICS AUSTRALIA

Plant Metabolomics

Prof Ute Roessner

Head of School, School of BioSciences
Node Leader, Metabolomics Australia
Past Immediate President, International Metabolomics Society

BIOPLATFORMS AUSTRALIA

NCRIS National Research Infrastructure for Australia
An Australian Government Initiative

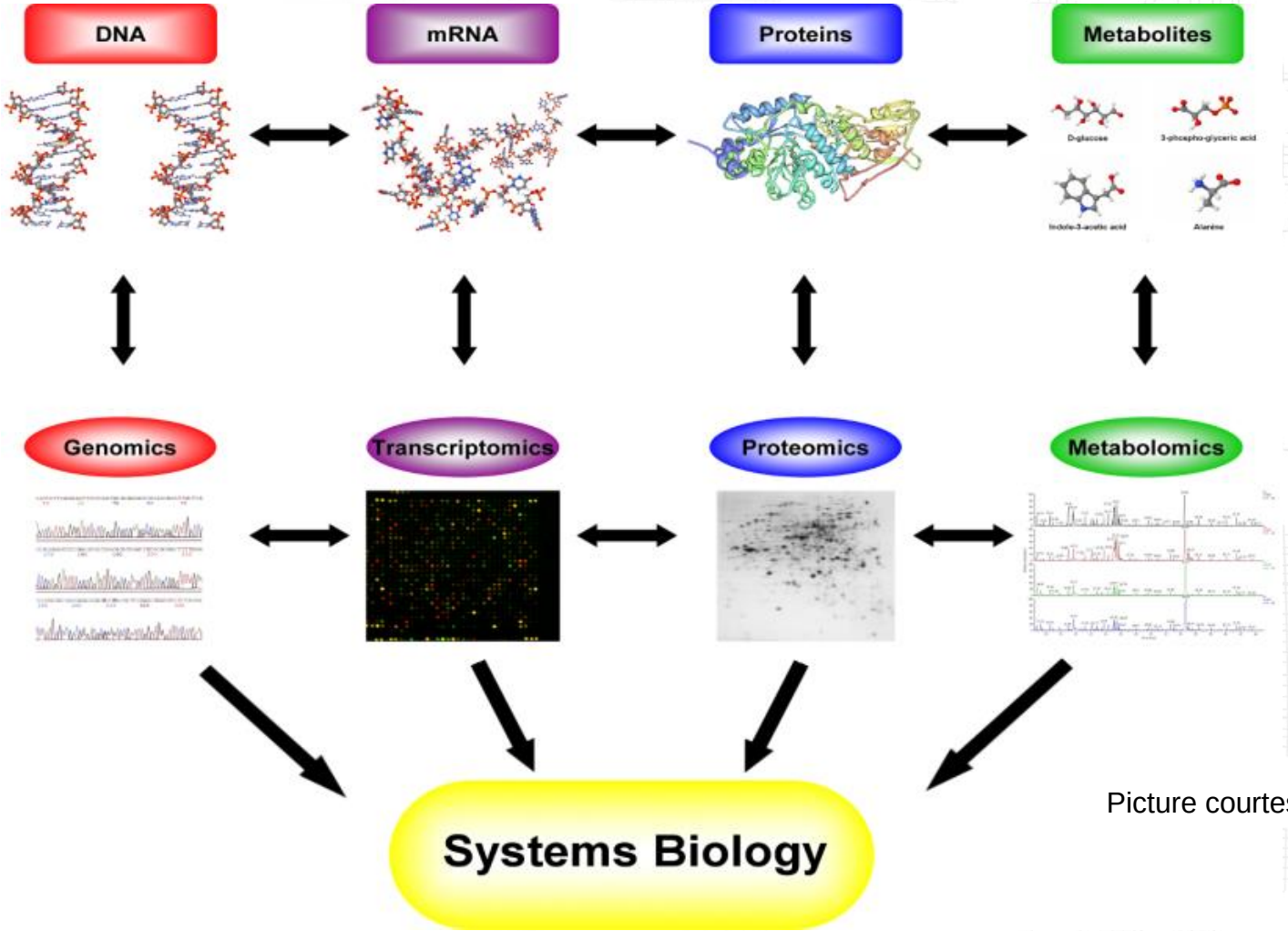
THE UNIVERSITY OF MELBOURNE

What can happen

What appears to be happening

What makes it happen

What is happening and has happened



Picture courtesy J. Bowne

What is Metabolomics about?

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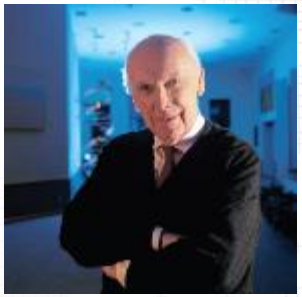
This is the analytical approach of a large-scale, non-targeted and high-throughput determination of metabolites in a biological system

The real challenge of metabolomics is to comprehensively cover the analysis of many compounds having many different chemical structures

"Think metabolism... If I were doing a PhD [in cancer research], I'd be doing it in metabolomics".



Prof. James Watson, Nobel Laureate
Co-discoverer of the structure of DNA

The “Other” Metabolomes

(courtesy of Prof. David Wishart)

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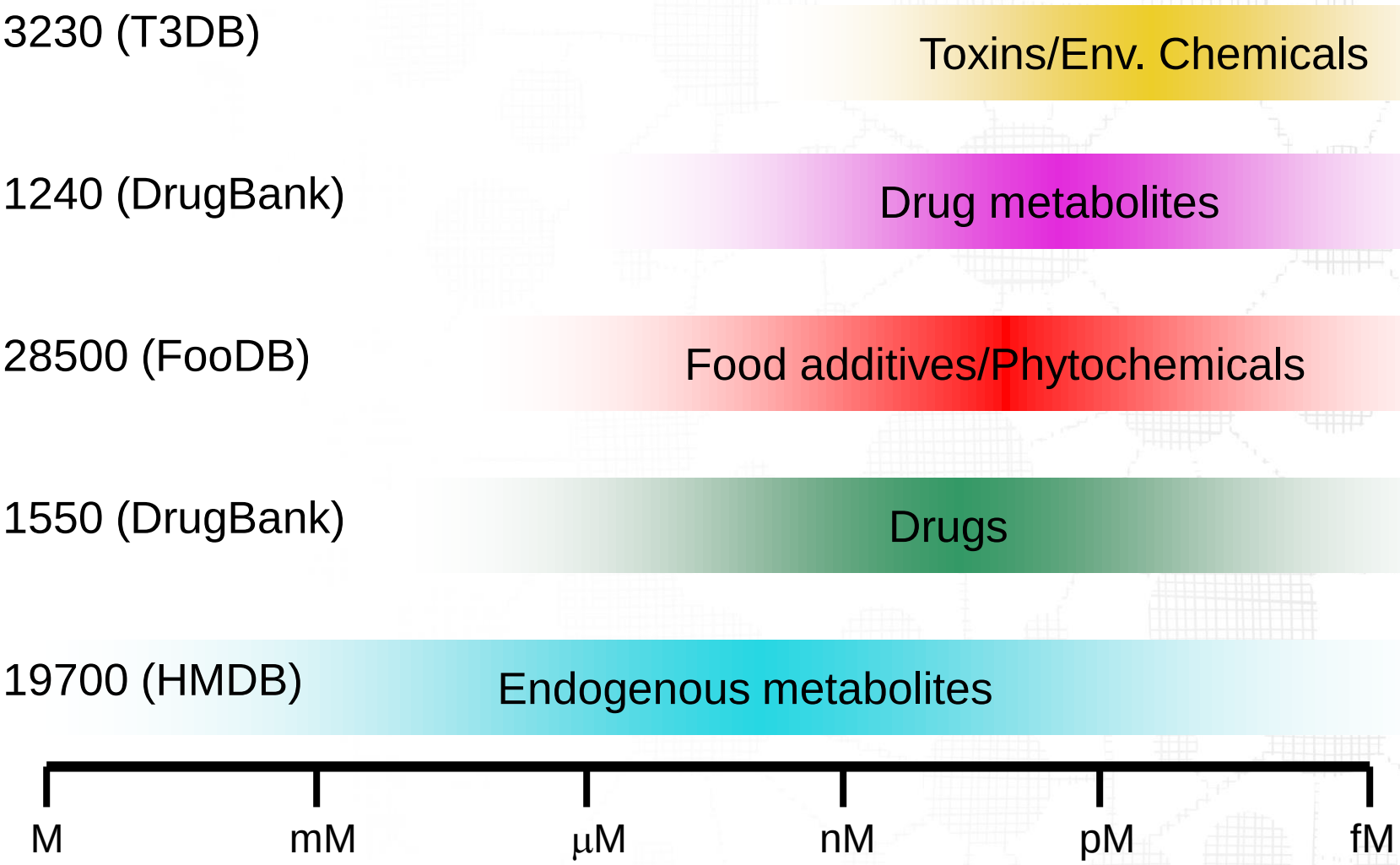

Courtesy Prof David Wishart

Metabolomics Workflow

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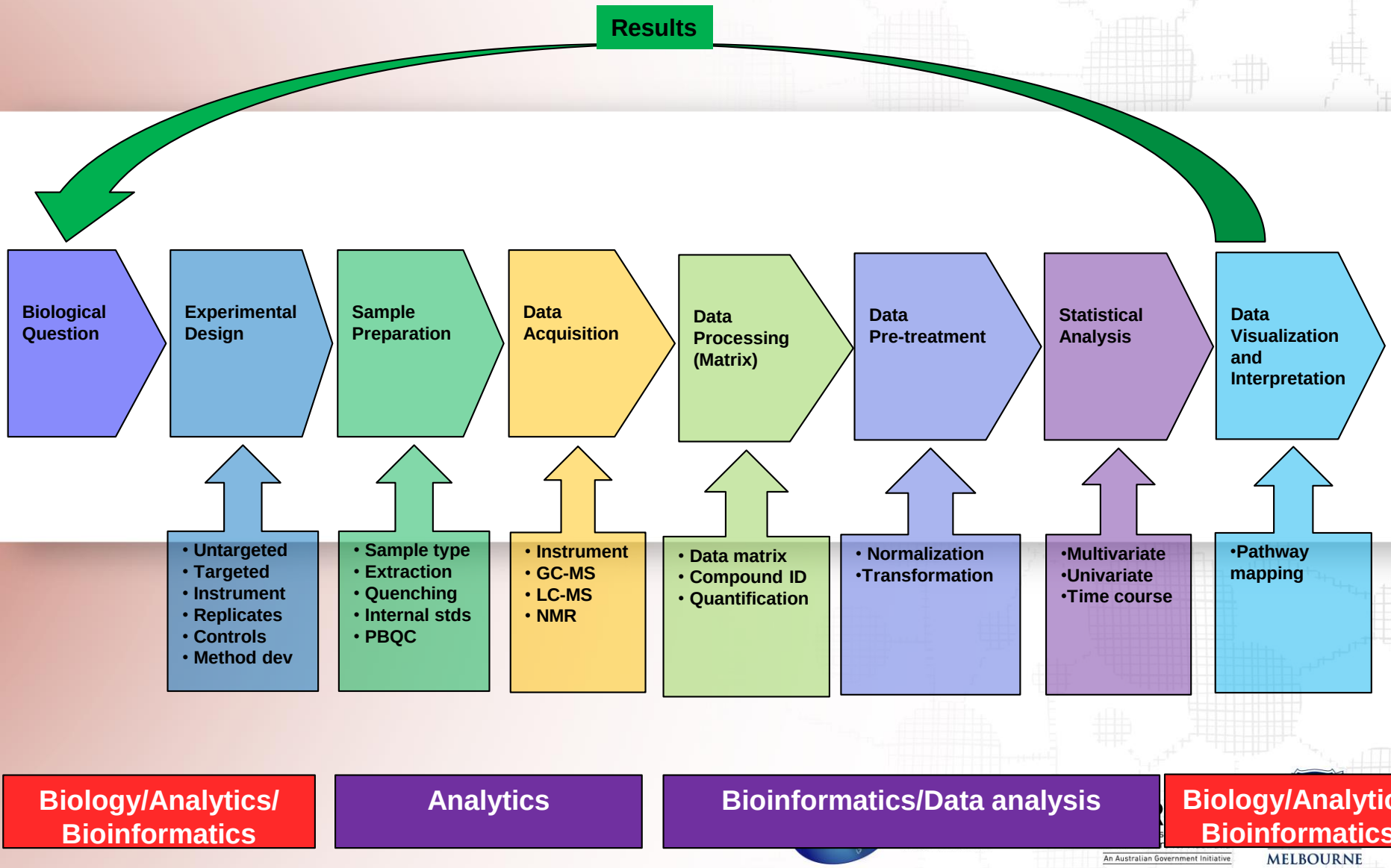

What types of metabolites can be analysed?

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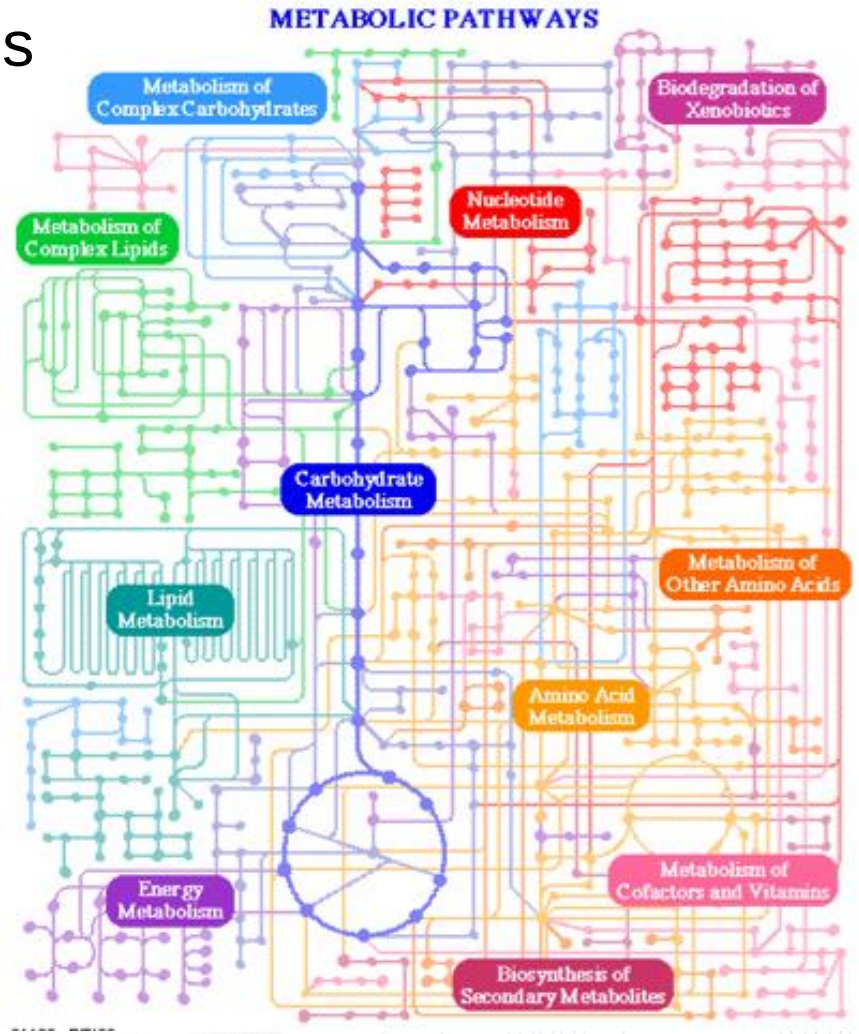
All low molecular weight metabolites (<1500 Daltons)

Includes, but not limited to:

Sugars, sugar phosphates
Organic acids
Amino acids,
Fatty acids
Nucleosides
Lipids (i.e. sterols, phospholipids)
Small peptides
Other secondary metabolites
Drugs, insecticides, other xenobiotics



Total number of metabolites > 500,000

metabolites per cell ~ 1,000 to 6,000

Comprehensive workflow

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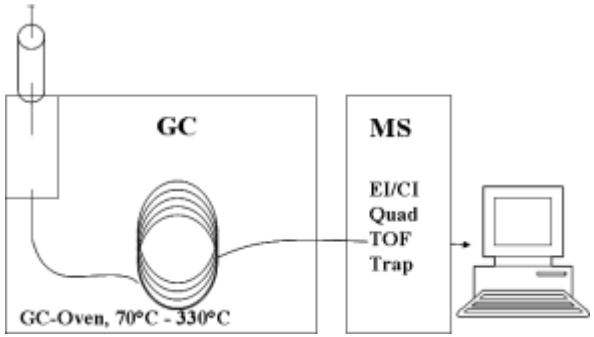
tissue harvest

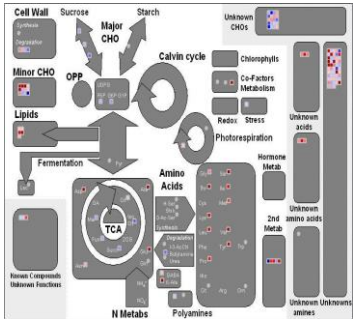
shock freeze

homogenization

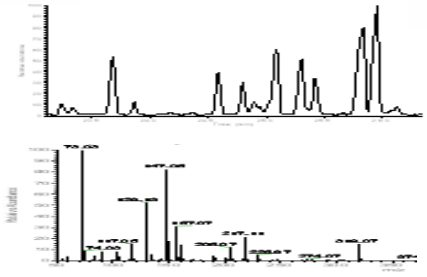
extraction procedure



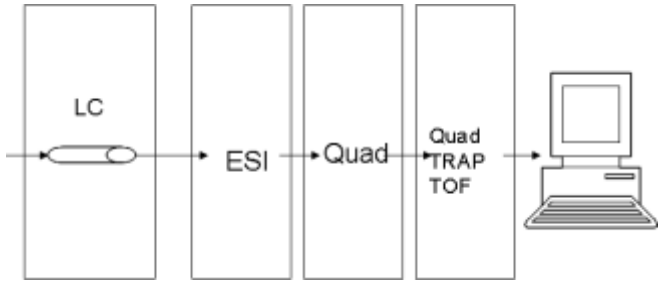
derivatisation



data interpretation



data analysis



Integrated Biology workflow

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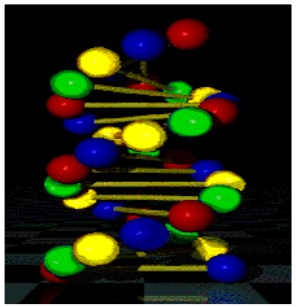
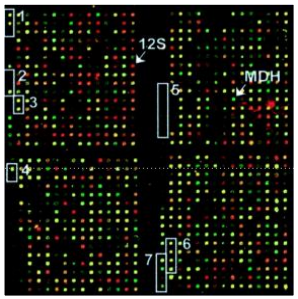
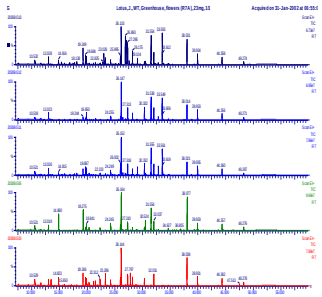
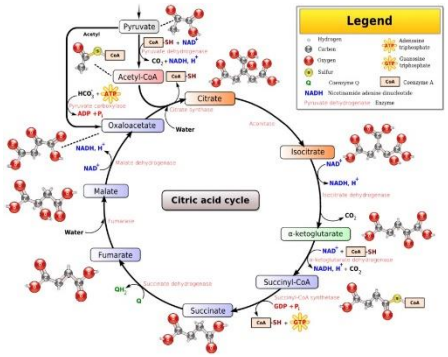
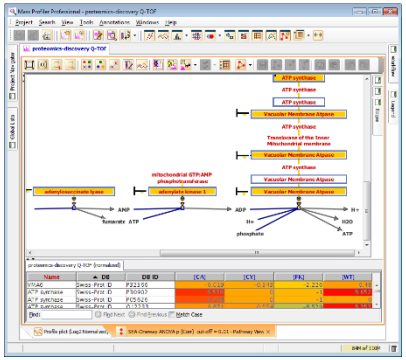
tissue harvest shock freeze homogenization extraction procedure

Genomics Transcriptomics Proteomics Metabolomics



Example: LC-MS approach

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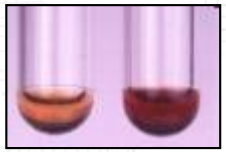
Sample



Polar extract (e.g. MeOH)

Lipid extract (e.g. chloroform)

Apolar compounds

Polar compounds

Lipid profile

Lipid species quantifictaion

C18RP

+/- ESI QTOF

HILIC (ANP)

+/- ESI QTOF

LC-QTOF

MRM using QQQ

Tandem LC-QqTOF-MS

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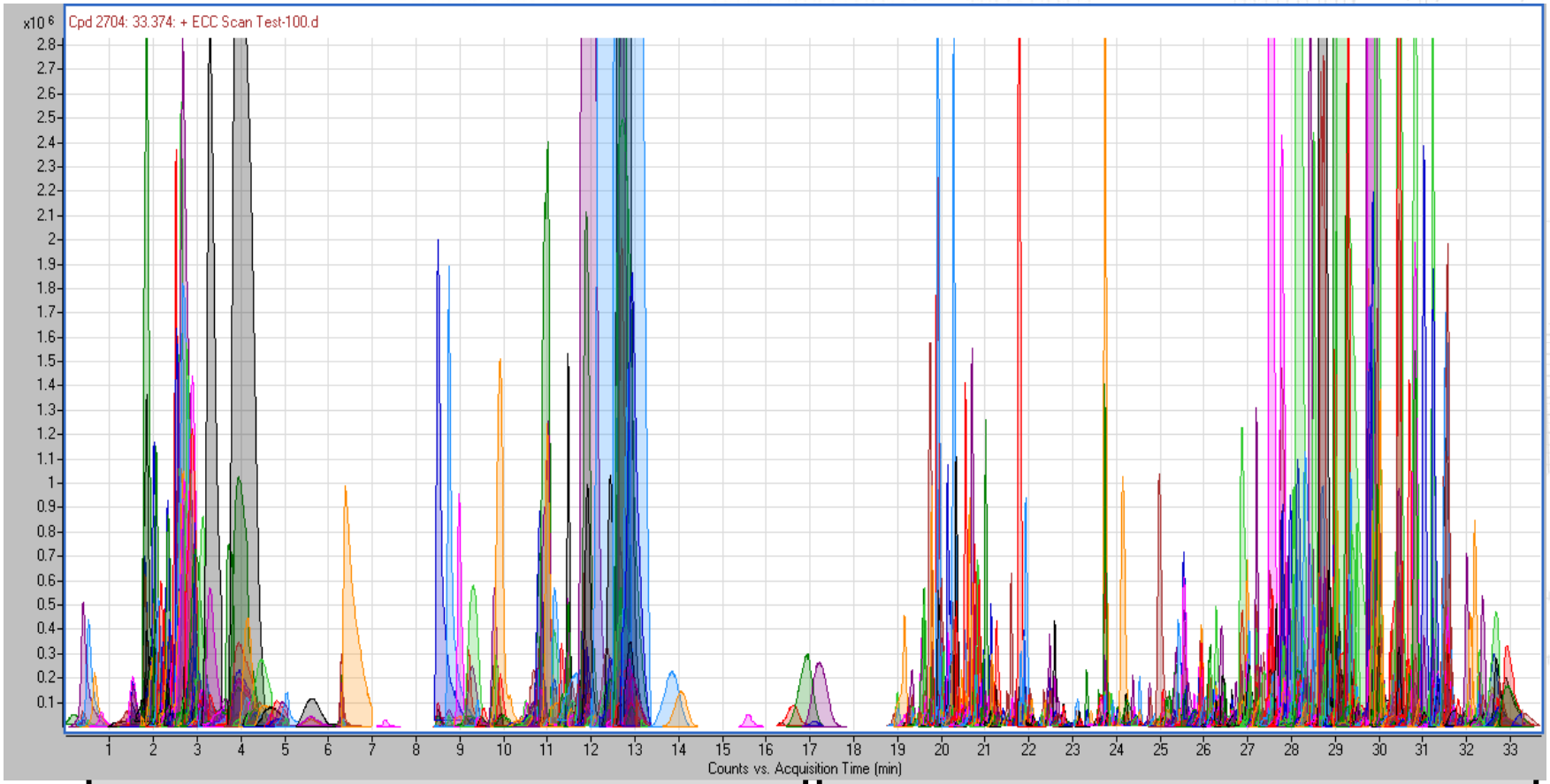
~ 2700 compounds



Targeted *vs* Untargeted

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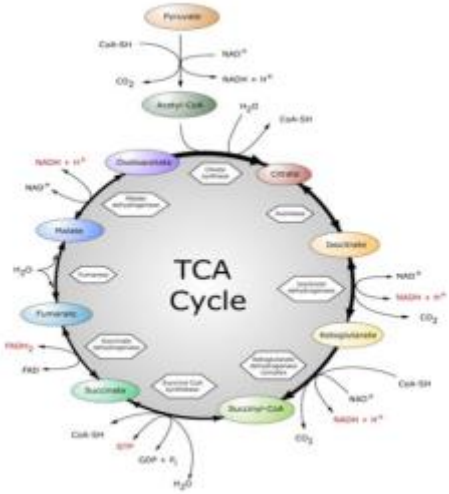
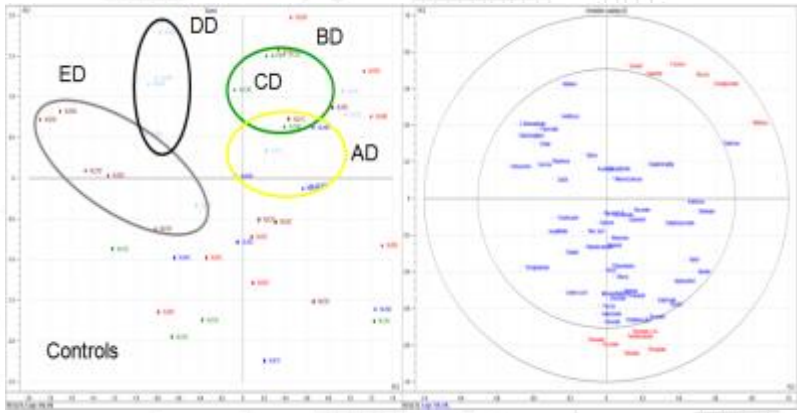
“detect the expected”

Absolute quantification
Pool sizes
You know what you measure
Not comprehensive
More accurately
Better sensitivity
Easier to interpret
Pathway mapping



“detect the unexpected”

Semi-quantitative
X-fold comparison
Biomarker discovery
More comprehensive
Loads of unknowns
Multivariant analyses



Quantitative metabolomics

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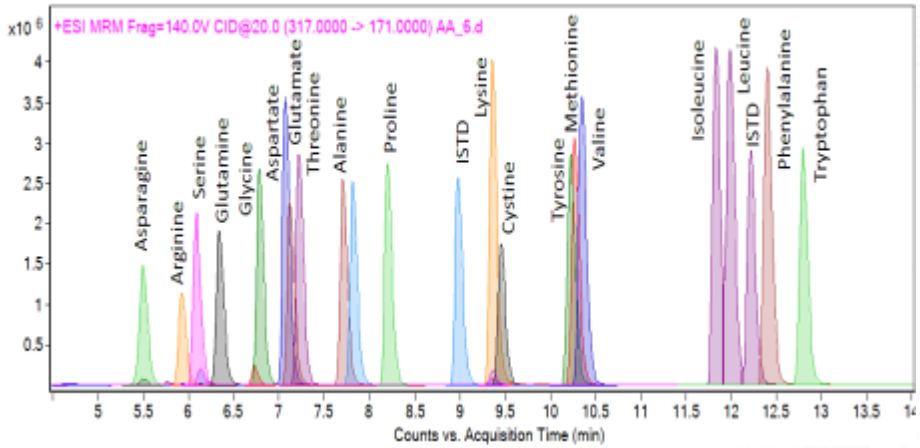
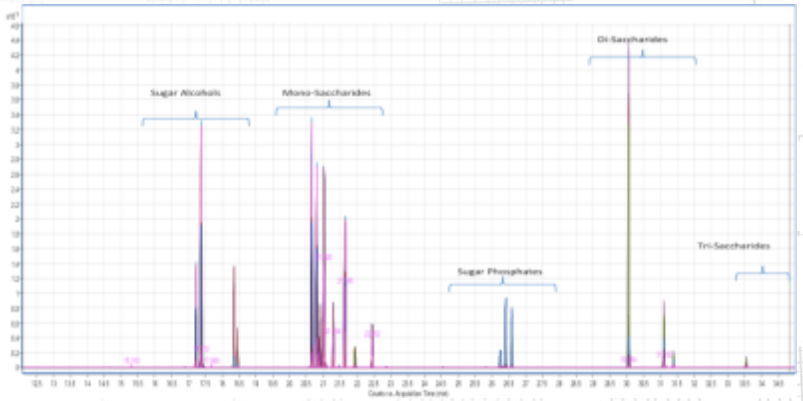
Boughton et al. 2011

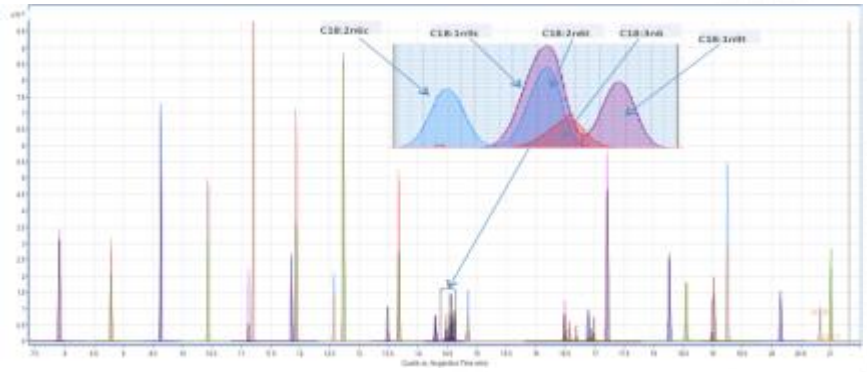


36 amino acids and 30 amines

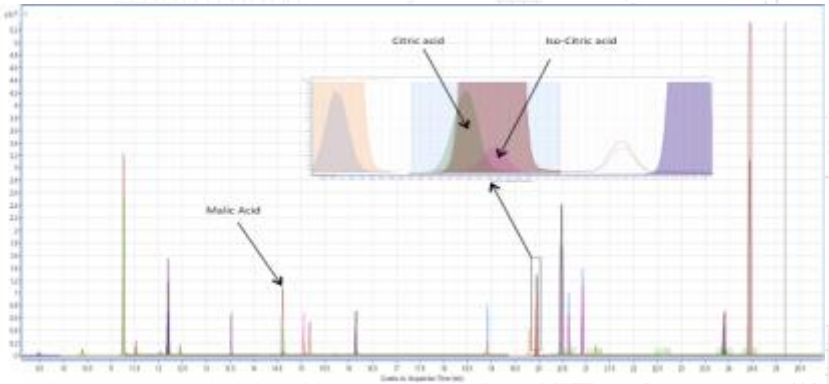
Dias et al. 2015



47 sugars and sugar alcohols



36 fatty acids



22 organic acids

Identifying novel salinity tolerance mechanisms by spatial analysis of lipids in barley roots

THE UNIVERSITY OF MELBOURNE

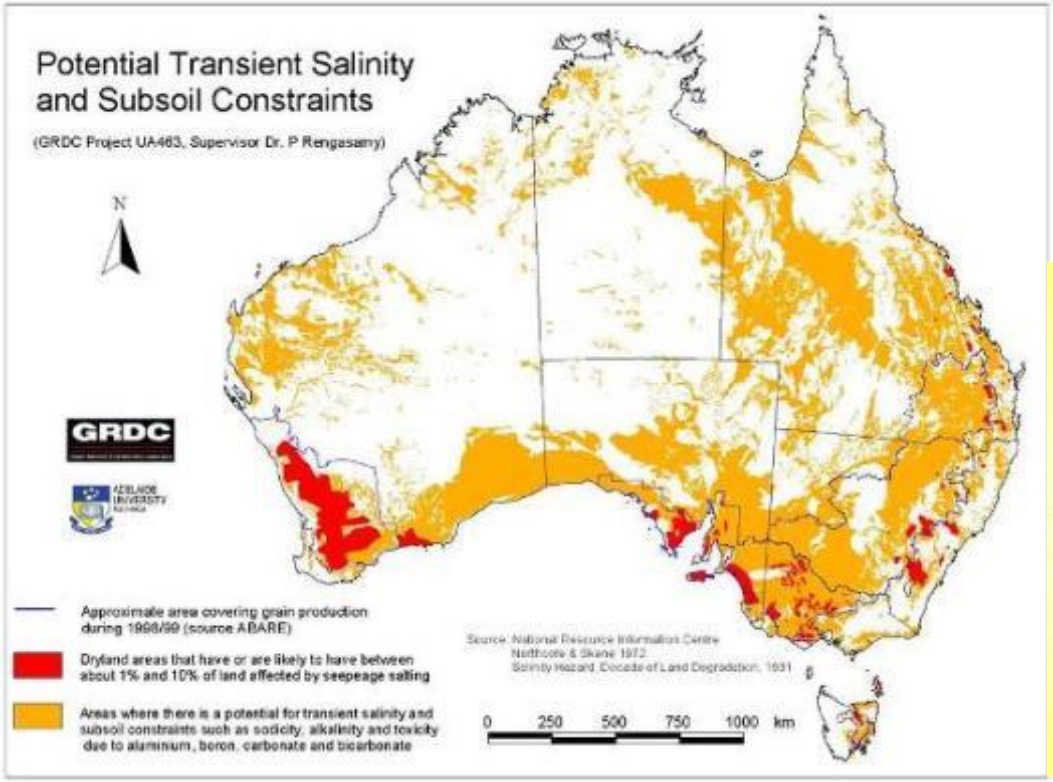
SCHOOL OF BOTANY

Australian Government

Australian Research Council

Metabolomics Australia

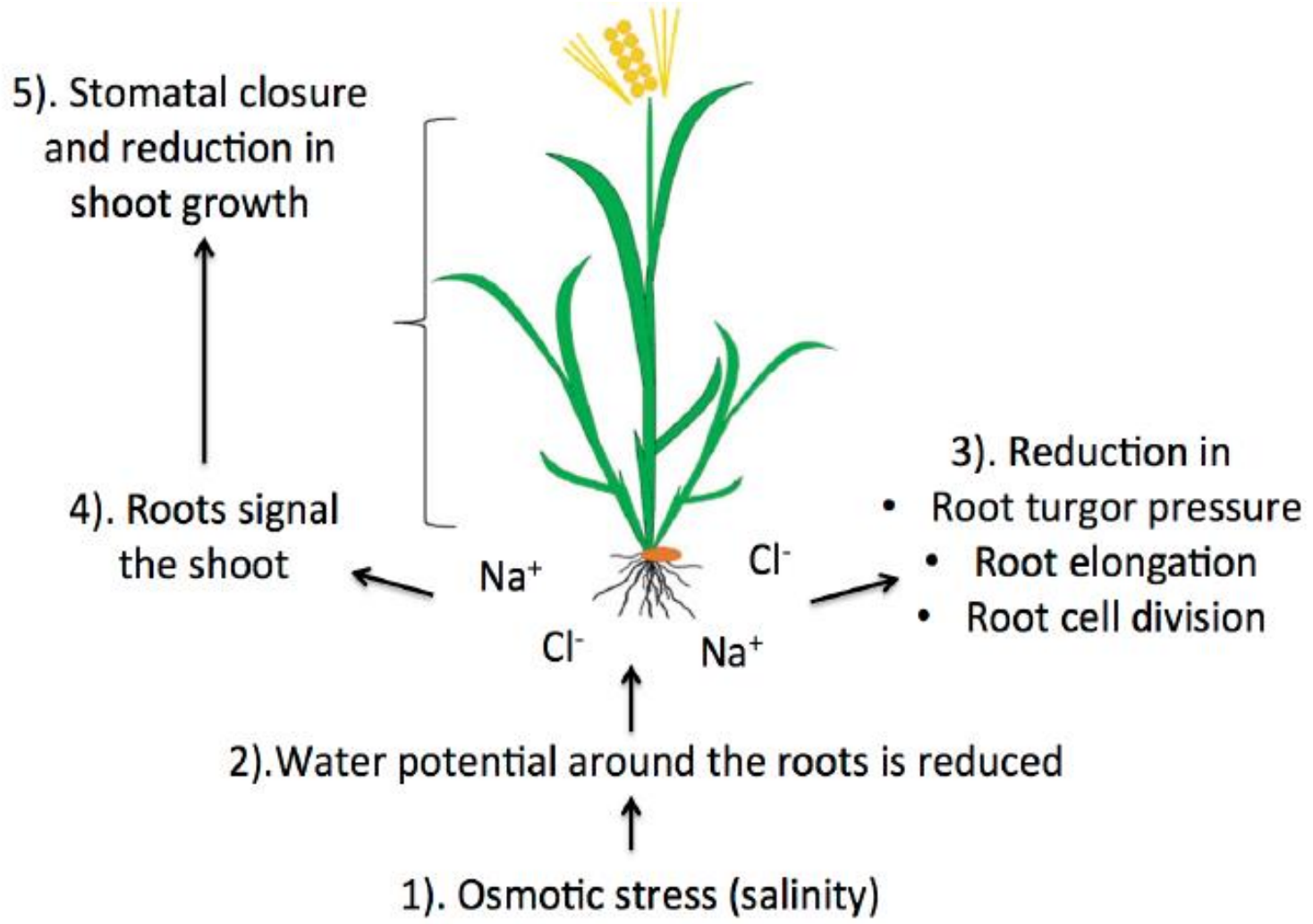
Abiotic stress affects agricultural productivity in Australia



Australian wheat belt significantly affected by salinity
In danger: Australian wheat exports worth ~ $4 billion p.a.

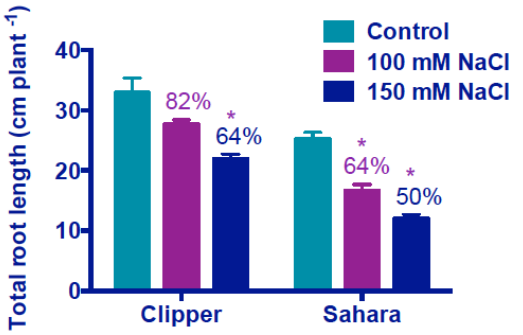
Rengasamy (2002) *Aust. J. Exp. Agric.* **42:** 351-361

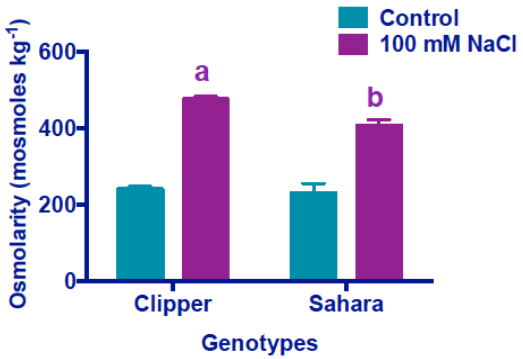
Effect of salt stress on plants

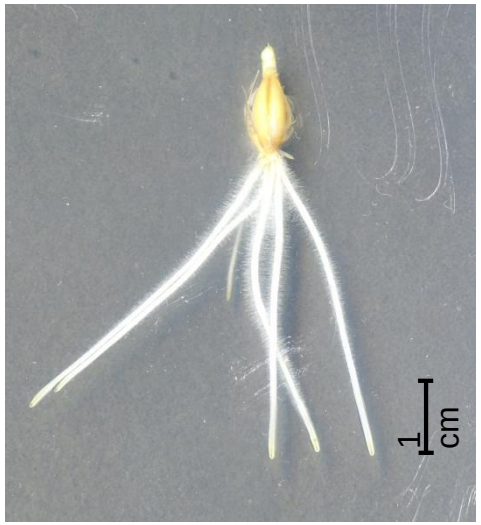


Roy et al, 2012

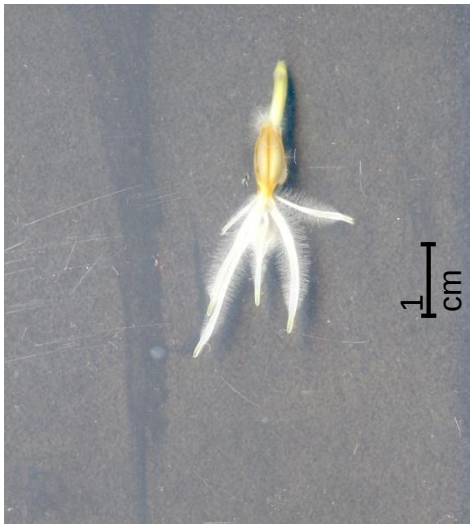
Effect of salt stress on roots







Control



Salt

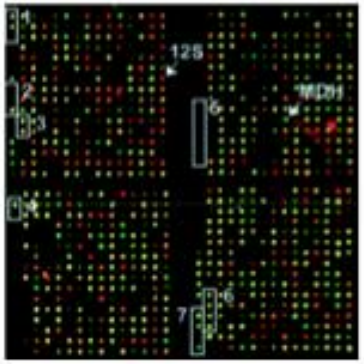
CSIRO PUBLISHING
Functional Plant Biology
http://dx.doi.org/10.1071/FP12290

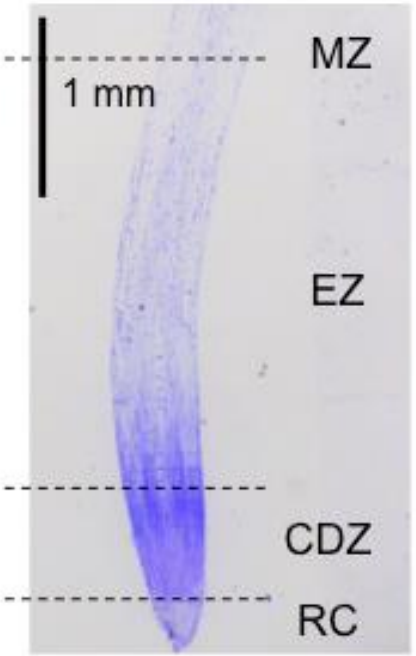
Genetic variation in the root growth response of barley genotypes to salinity stress

Megan C. Shelden[A,E], *Ute Roessner*[A], *Robert E. Sharp*[D], *Mark Tester*[B] *and Antony Bacic*[C]

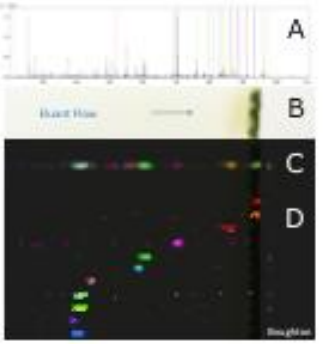
Project AIM: Understand early tissue-specific salt responses

mRNA

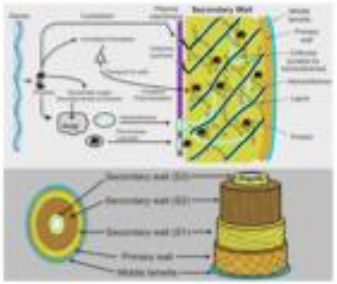




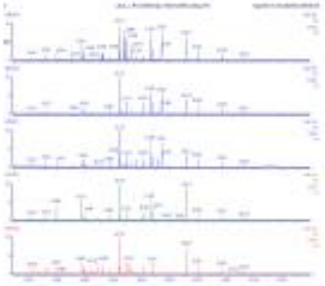
Lipids



Cell Wall



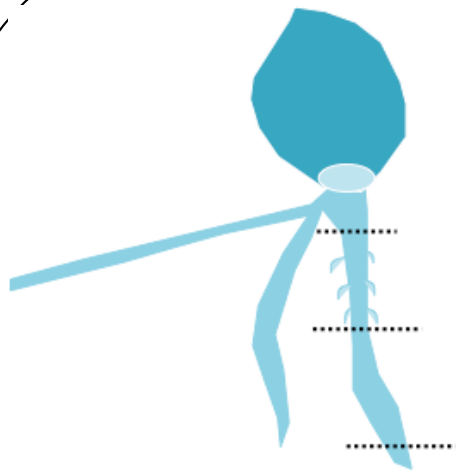
Metabolites

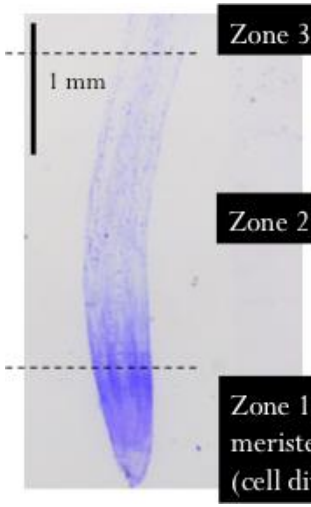


In collaboration with Plant Cell Wall Centre
T. Bacic, M. Doblin, A. Cassin

In collaboration with MA

Study Design





Barley Cultivars: Clipper and Sahara

Growth and Treatment on Agar Plates

Root Tissue Harvest
(3 Zones: Root Cap, Elongation and Maturation Zone)

1. Cell Wall Polysaccharide Analysis

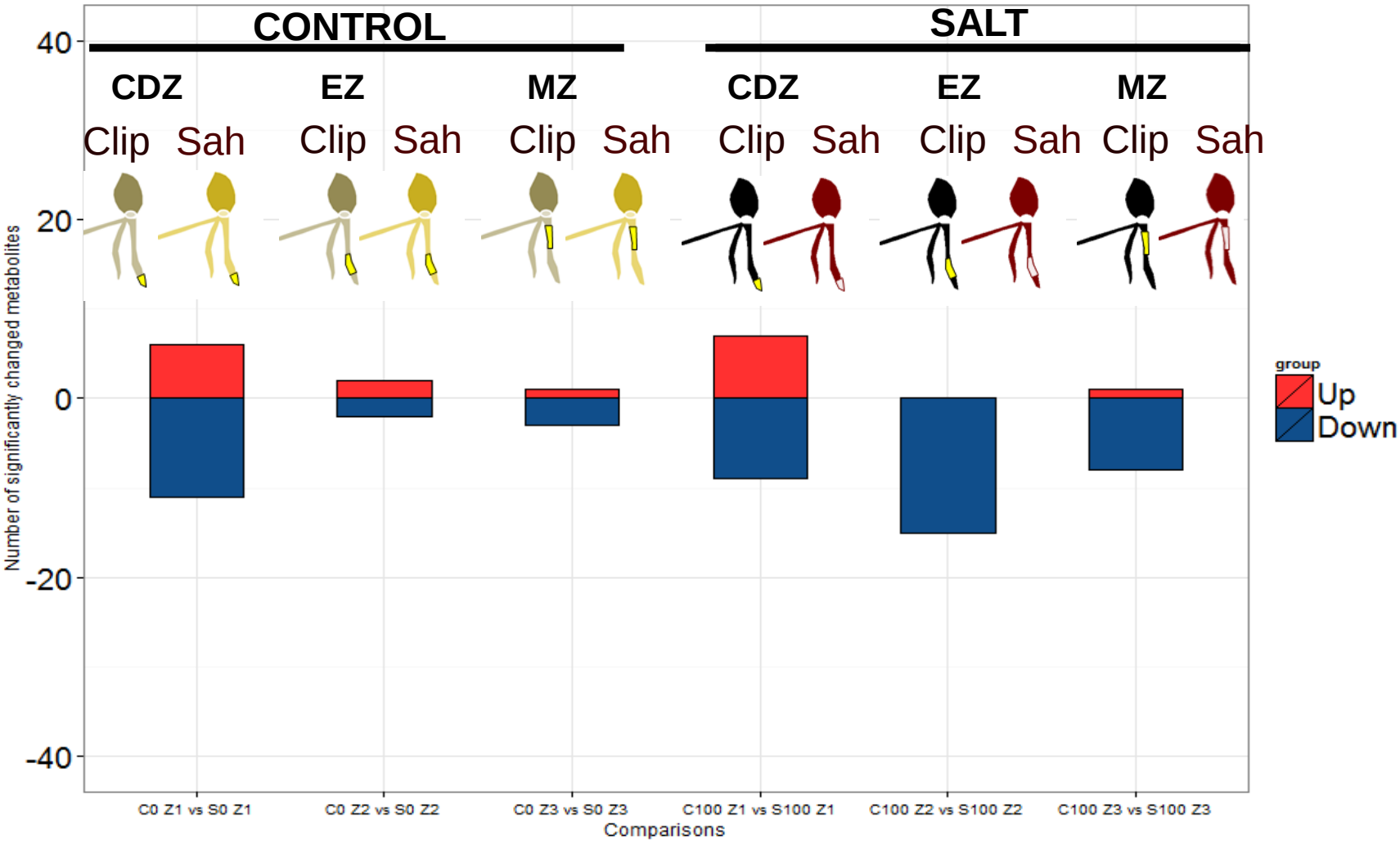
2. Targeted Metabolite Profiling

3. RNA-Seq Analysis

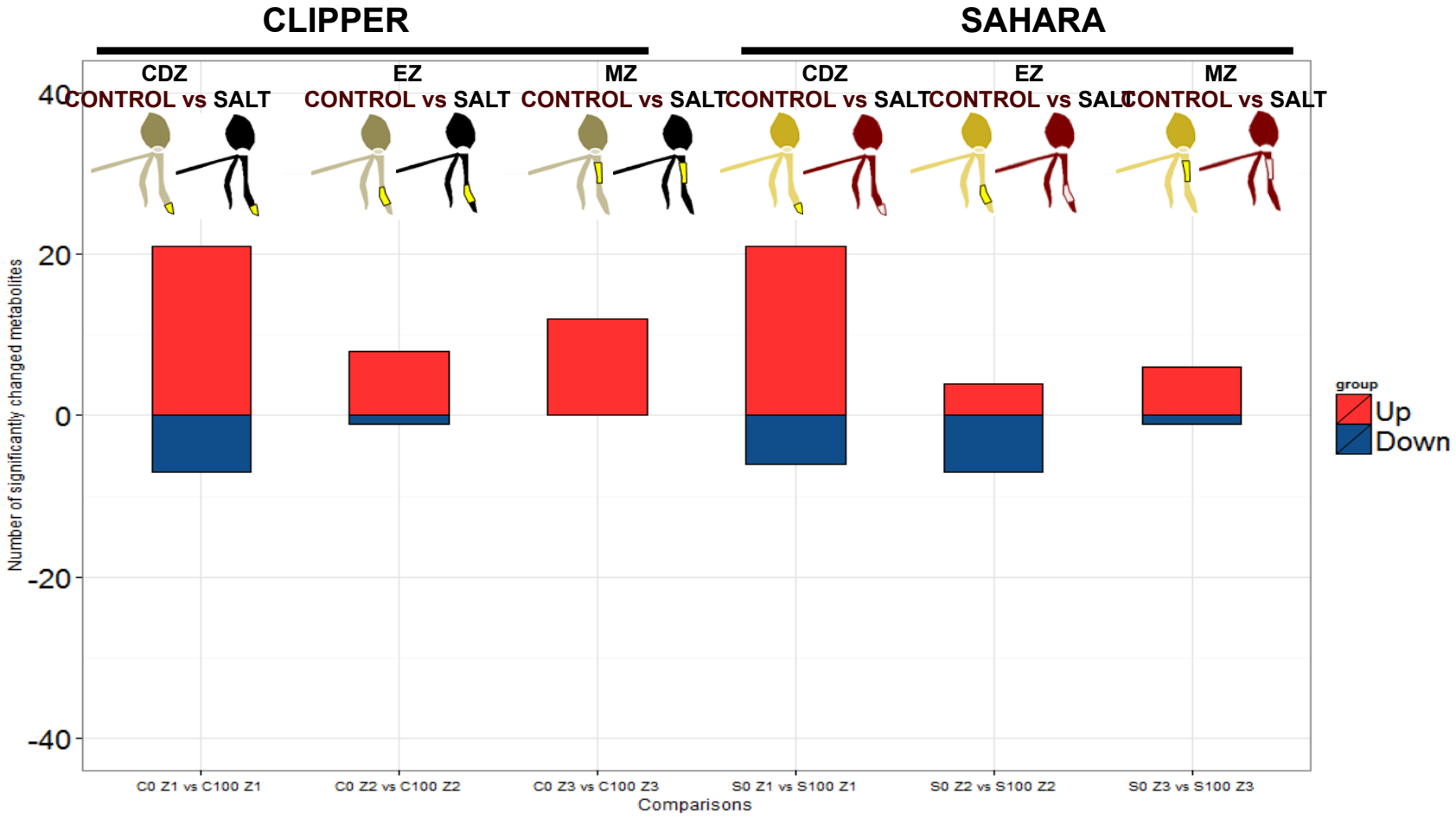
Data Analysis and Statistics

Data Integration and Biological Interpretation

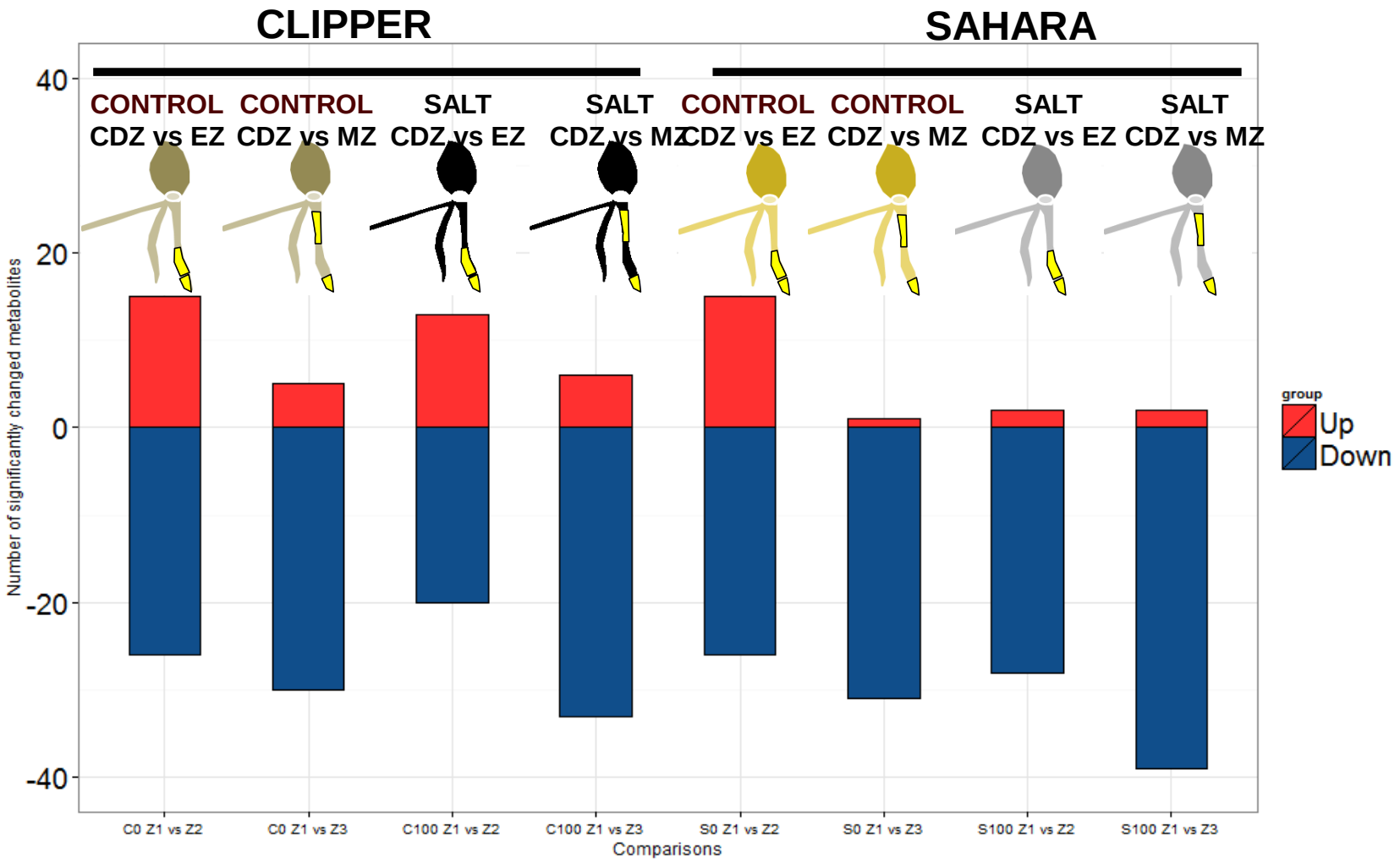
Significant differences (Sug, OA, AA) between cultivars



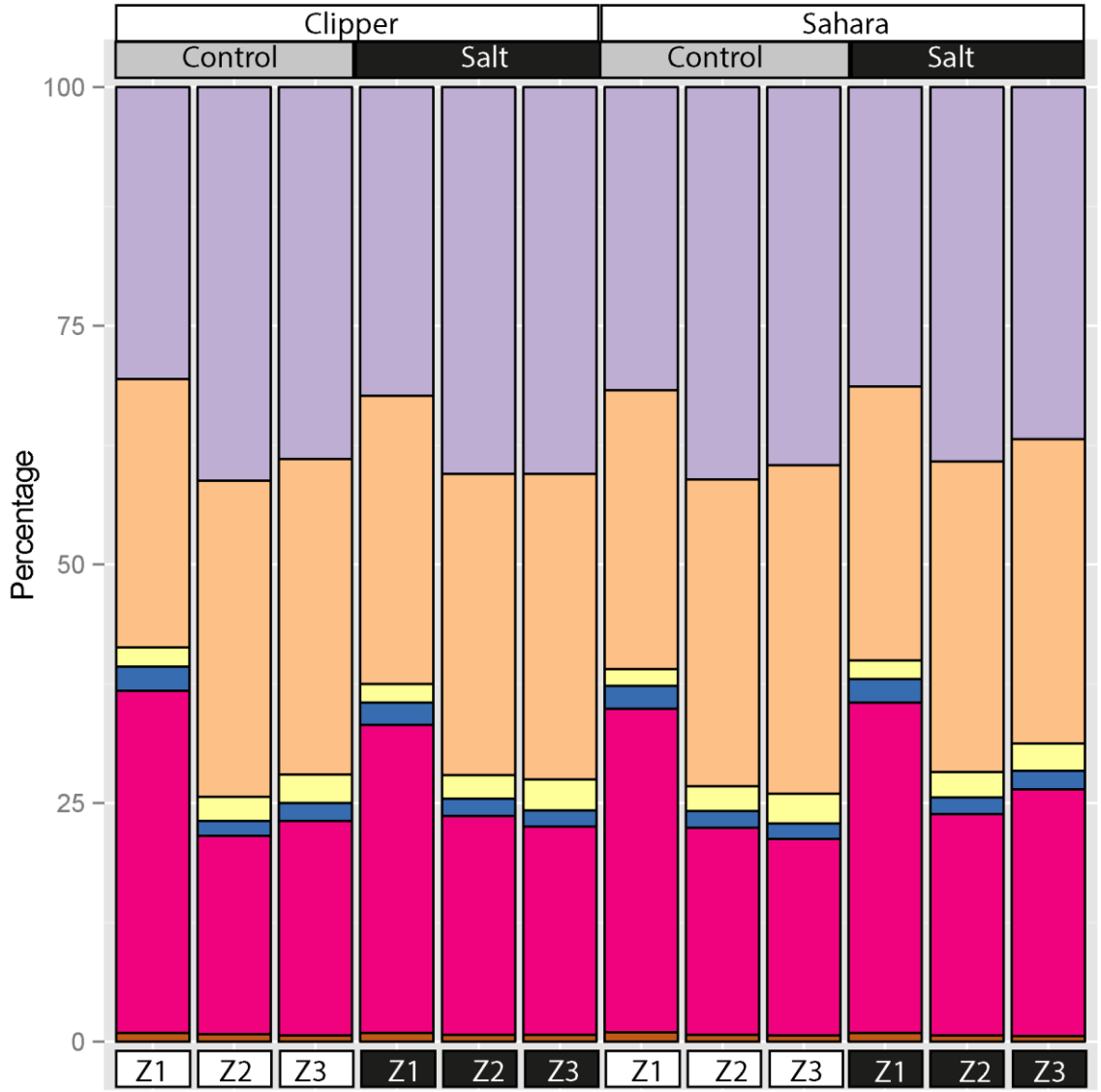
Significant changes (Sug, OA, AA) following treatment

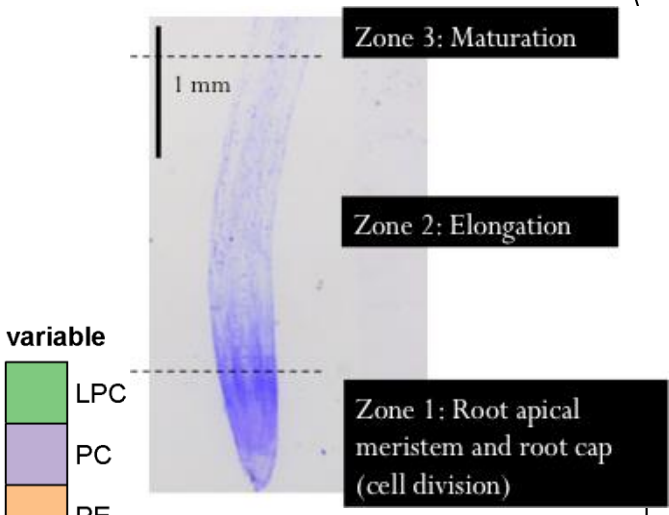


Significant differences (Sug, OA, AA) between zones

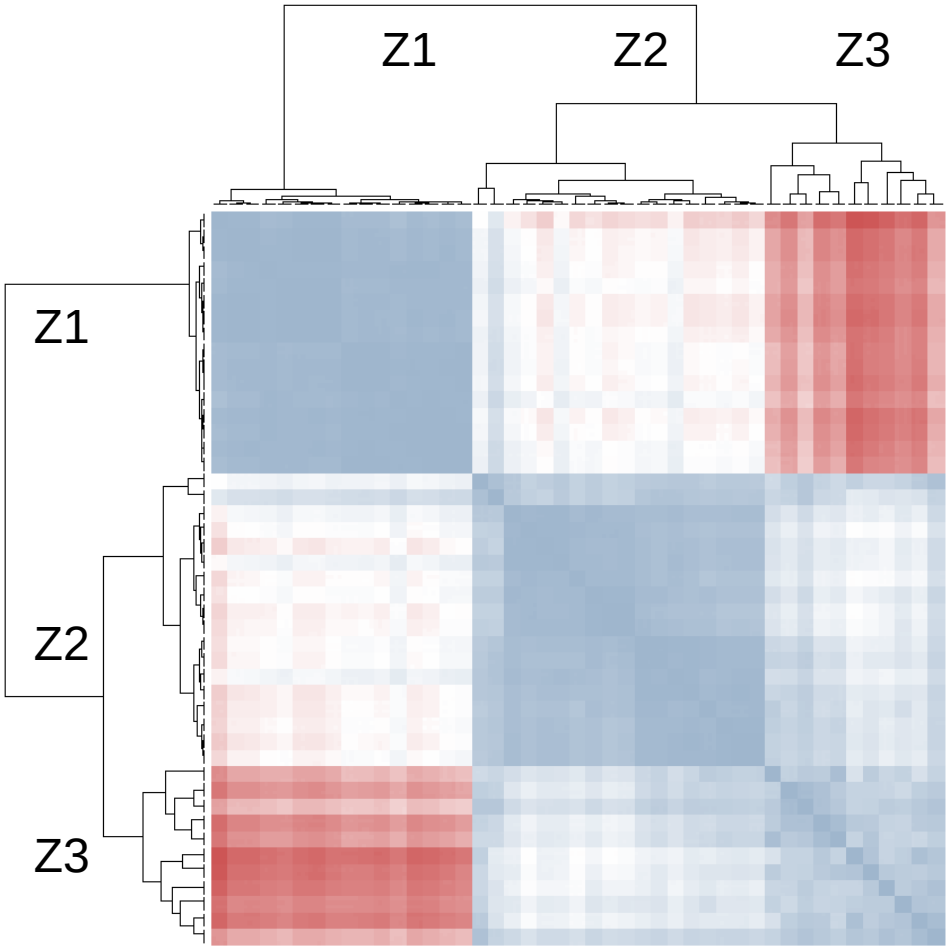


Tissue-specific lipid analysis





Next-Generation RNA Sequencing



A clear per-zone (horizontal axis) response is observed in this Pearson's correlation plot illustrating cell differentiation along the root zone.

Hill, C. B., et al. (2016). Sci Rep **6**: 31558.

Transcriptomics analysis (short-term salt stress)

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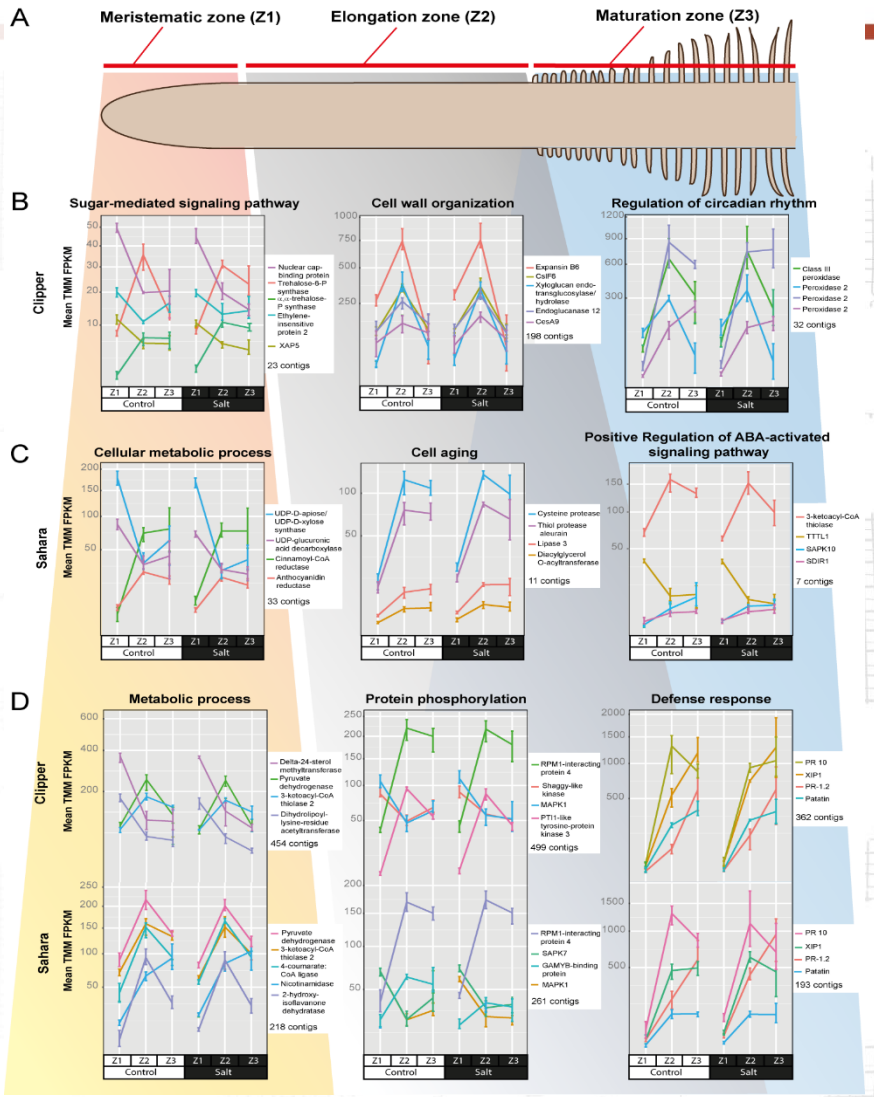
- Root zones are highly specialized in their biological function
- Transcriptional differences are much larger between zones than between treatments.
- Biochemical processes are controlled in a root-zone specific manner



Hill, C. B., et al. (2016). Sci Rep **6**: 31558.

Significant metabolites - Clipper

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Z1 only

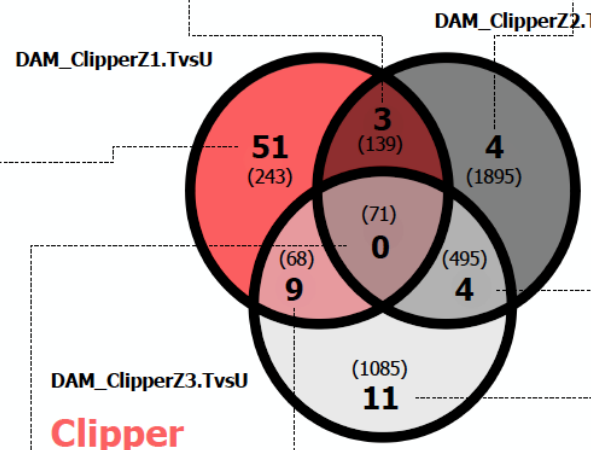
	DAM	FC
Sugars (16)	Glucose 6-P	-3.57
	Maltose	+2.53
	Arabinose	-2.27
	Trehalose	-2.22
	Raffinose	-1.89
	Melibiose	-1.85
	Galactitol	-1.82
	Xylitol	-1.79
	Glucose	+1.78
	Inositol	-1.75
	Arabitol	-1.75
	Fucose	-1.72
	Fructose 6-P	-1.67
	Galactose	-1.64
	Turanose	-1.61
	Xylose	-1.56
Organic acids (14)	Fumarate	-4.00
	Oxoglutarate	-3.23
	Succinate	-3.13
	Malonate	-2.22
	Salicylate	-2.08
	2-keto gluconic acid	-2.00
	Malate	-2.00
	Aconitate	-1.96
	Pipecolate	+1.92
	Citrate	-1.89
Organic acids	Glucuronate	-1.85
	Nicotinic acid	-1.72
	Quinate	-1.61
	Caffein acid	-1.59
Amines (12)	Phenethylamine	-3.13
	Serine	-2.86
	Citruline	-2.63
	Homoserine	-2.50
	Proline	+2.43
	Glutaminic acid	-2.22
	Glycine	-2.13
	Octopamine	-2.08
	Histidine	-2.04
	Aspartic acid	-2.00
	Ornithine	-1.89
	Agmatine	+1.65
Lipids (9)	PC (34:3)	+2.78
	LPC (18:3)	+2.34
	PG (32:0)	+2.48
	PC (34:1)	+2.18
	PE (36:3)	+2.85
	PC (36:0)	+3.2
	PC (38:5)	+2.18
	PG (36:3)	-3.45
	LPC (18:1)	+1.87

Z1 or Z2

	DAM	(Z1) FC	(Z2) FC
Amines (2)	GABA	+2.24	+2.17
	Arginine	+1.71	+2.16
Lipids	PI (34:1)	-1.72	-2.44

Z2 only

	DAM	FC
Amines	Asparagine	+2.67
Fatty acids (3)	Elaidic acid (C18:1n9t)	+1.62
	Linoleic acid (C18:2n6c)	+1.60
	Palmitic acid (C16:0)	+1.45



Z2 or Z3

	DAM	(Z2) FC	(Z3) FC
Amines	Tyrosine	-2.22	-3.03
Fatty acids (3)	Nervonic acid (C24:1)	+1.29	-1.54
	Lauric acid (C12:0)	-1.25	-1.92
	Myristic acid (C14:0)	-1.19	-1.92

Z3 only

	DAM	FC
Sugars	Sucrose	+2.71
Amines (4)	Valine	-2.04
	Tryptophan	-1.92
	Isoleucine	-1.89
	beta-alanine	+1.84
Fatty acids (6)	Erucic acid (C22:1n9)	-2.22
	Heptadecanoic acid (C17:0)	-2.13
	cis-10-pentadecanoic acid (C15:1)	-1.79
	Stearic acid (C18:0)	-1.61
	Arachidic acid (C20:0)	-1.35
	Pentadecanoic acid (C15:0)	-1.32

Z1 or Z2 or Z3

	DAM	FC
N/A	N/A	N/A

Z1 or Z3

	DAM	(Z1) FC	(Z3) FC
Amines (7)	Tyramine	-3.23	-2.50
	Glutamine	-2.94	-2.00
	Threonine	-2.70	-2.56
	Cysteine	-2.22	-1.82
	Methionine	-2.00	-3.57
Amines	Putrescine	-1.96	-4.55
	Leucine	-1.82	-3.12
Fatty acids (2)	Behenic acid (C22)	+2.65	+1.27
	Lignoveric acid (C24:0)	+1.77	+1.43

Significant metabolites - Sahara

METABOLOMICS AUSTRALIA

Z1 only

	DAM	FC
Sugars (2)	Arabinose	+3.63
	Raffinose	+2.58
Organic acids	Pipecolate	+4.89
Amines (10)	Proline	+4.03
	Ornithine	-2.70
	Putrescine	-2.13
	Citruline	-2.08
	4-hydroxyproline	+2.02

	DAM	FC
Amines	Glutamine	-1.79
	Homoserine	-1.75
	Glycine	-1.75
	Phenylalanine	-1.59
	Threonine	-1.54
Lipids (3)	PA (39:0)	-1.41
	PC (33:3)	-2.38
	PC (36:6)	+2.09

Z1 or Z2

	DAM	(Z1) FC	(Z2) FC
Amines (8)	Tyramine	-2.44	+2.33
	Serine	-1.79	+5.07
	Glutaminic acid	-1.67	+4.11
	Arginine	+1.63	+1.93
	Taurine	-1.61	+4.64
	Octopamine	-1.59	+2.77
	Aspartic acid	-1.52	+2.34
	Cysteine	+1.44	-3.57
Lipids	PI (34:1)	-2.04	-3.57

Z2 only

	DAM	FC
Sugars	Sucrose	+4.76
Amines (3)	Agmatine	+3.72
	Phenethylamine	+3.62
	Lysine	+1.92
Fatty acids	Stearic acid (C18:0)	-4.00

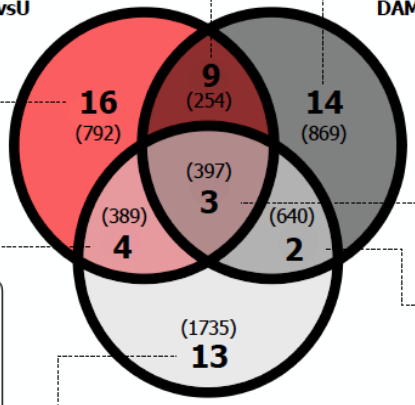
	DAM	FC
Lipids (9)	PI (34:3)	+6.31
	PA (46:6)	+6.08
	PC (38:5)	+4.88
	PE (34:3)	+4.33
	SM (41:1)	+3.94
	PE (32:2)	+3.73
	PC (34:1)	+3.63
	PG (32:0)	+3.08
	LPC (18:3)	+3.07

Z1 or Z3

	DAM	(Z1) FC	(Z3) FC
Amines (4)	Alanine	+1.69	+2.49
	Valine	+1.55	-2.63
	Tryptophan	-1.56	-2.94
	Histidine	-1.52	-2.78

DAM_SaharaZ1.TvsU

DAM_SaharaZ2.TvsU



DAM_SaharaZ3.TvsU

Z1 or Z2 or Z3

	DAM	(Z1) FC	(Z2) FC	(Z3) FC
AmineAA (3)	Tyrosine	-2.33	-3.57	-2.08
	Methionine	-1.45	-4.76	-4.76
	beta-alanine	+1.40	+6.56	+2.48

Z2 or Z3

	DAM	(Z2) FC	(Z3) FC
FattyAcids	Nervonic acid (C24:1)	-2.50	-1.69
Lipids	PI (36:4)	-3.45	-2.38

Z3 only

	DAM	FC
Organic acids	Malate	-3.57
Amines	Leucine	-2.13
Fatty acids (10)	Heptadecanoic acid (C17:0)	-1.75
	Erucic acid (C22:1n9)	-1.64
	Myristic acid (C14:0)	-1.49
	cis-10-pentadecanoic acid (C15:1)	-1.47
	Lauric acid (C12:0)	-1.49

	DAM	FC
Fatty acids	Lignoveric acid (C24:0)	+1.39
	Elaidic acid (C18:1n9t)	+1.40
	Behenic acid (C22)	+1.59
	Palmitic acid (C16:0)	+1.26
	Pentadecanoic acid (C15:0)	-1.25
Lipids	PI (36:5)	-3.03

Sahara
(treatment-specific)

Salt signalling

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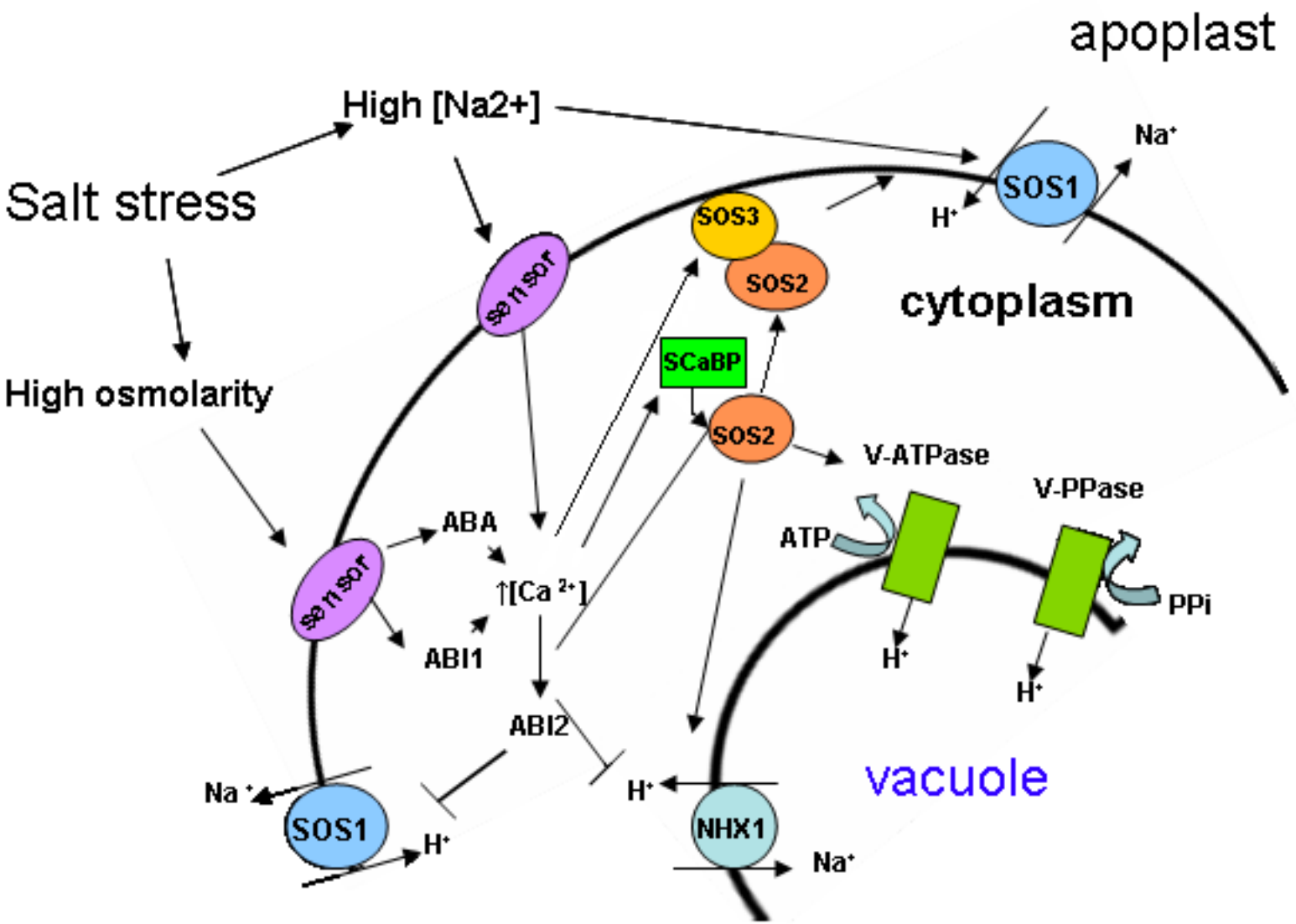
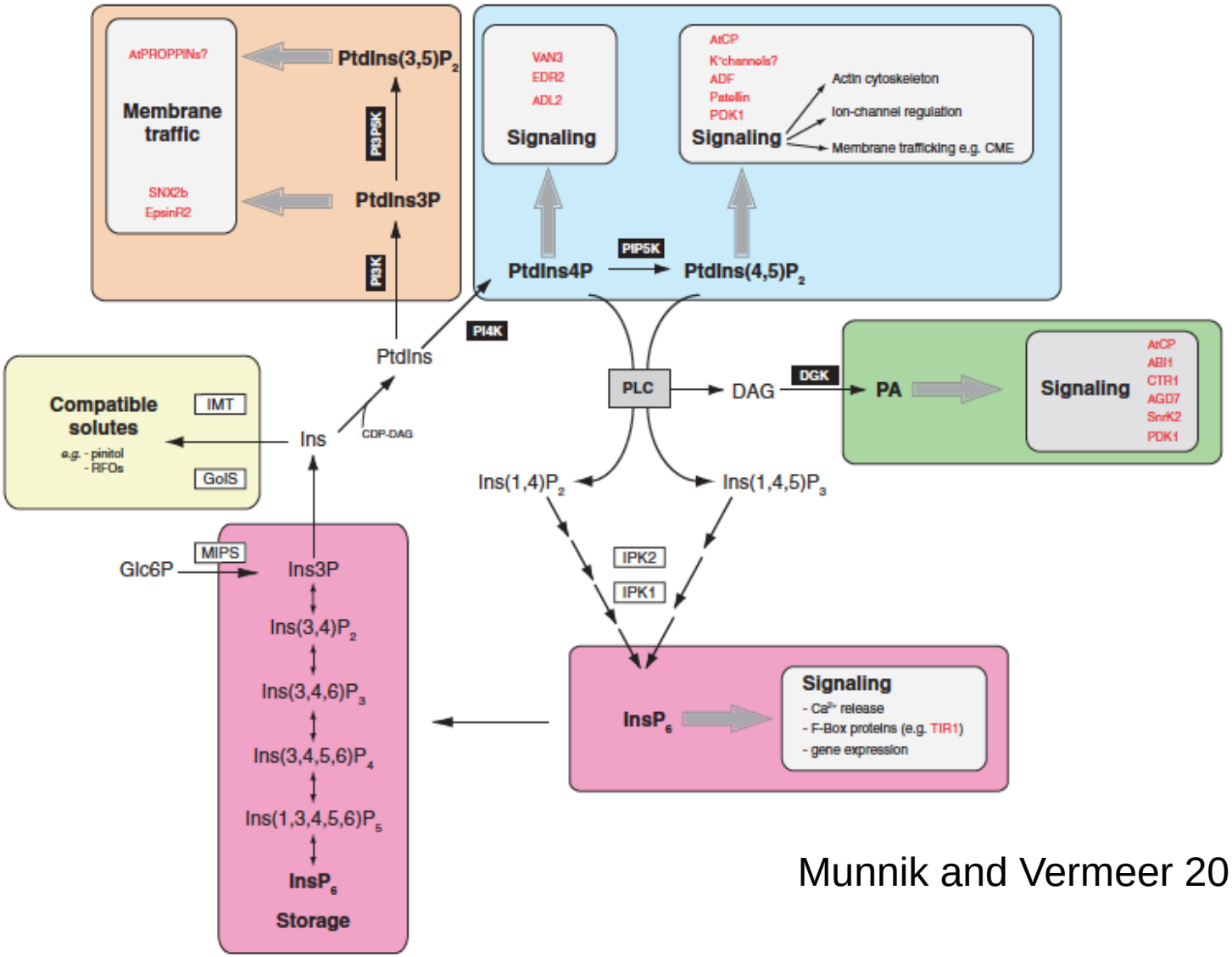


Proposed lipid based signalling

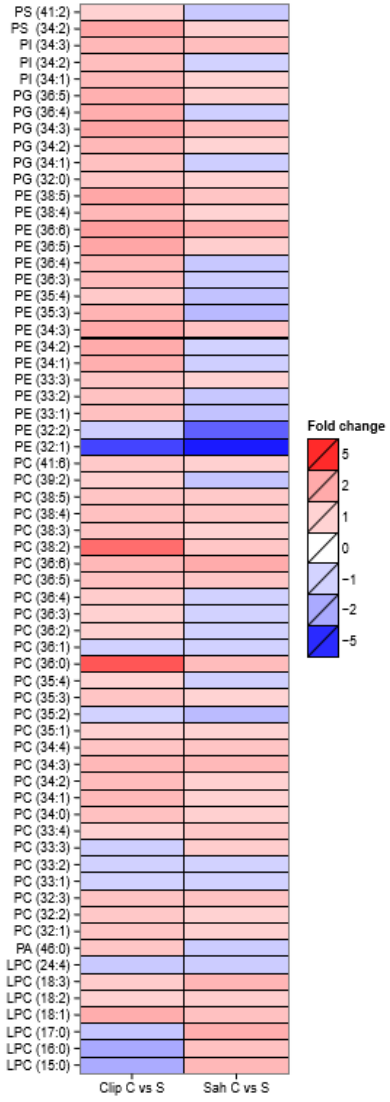
METABOLOMICS AUSTRALIA

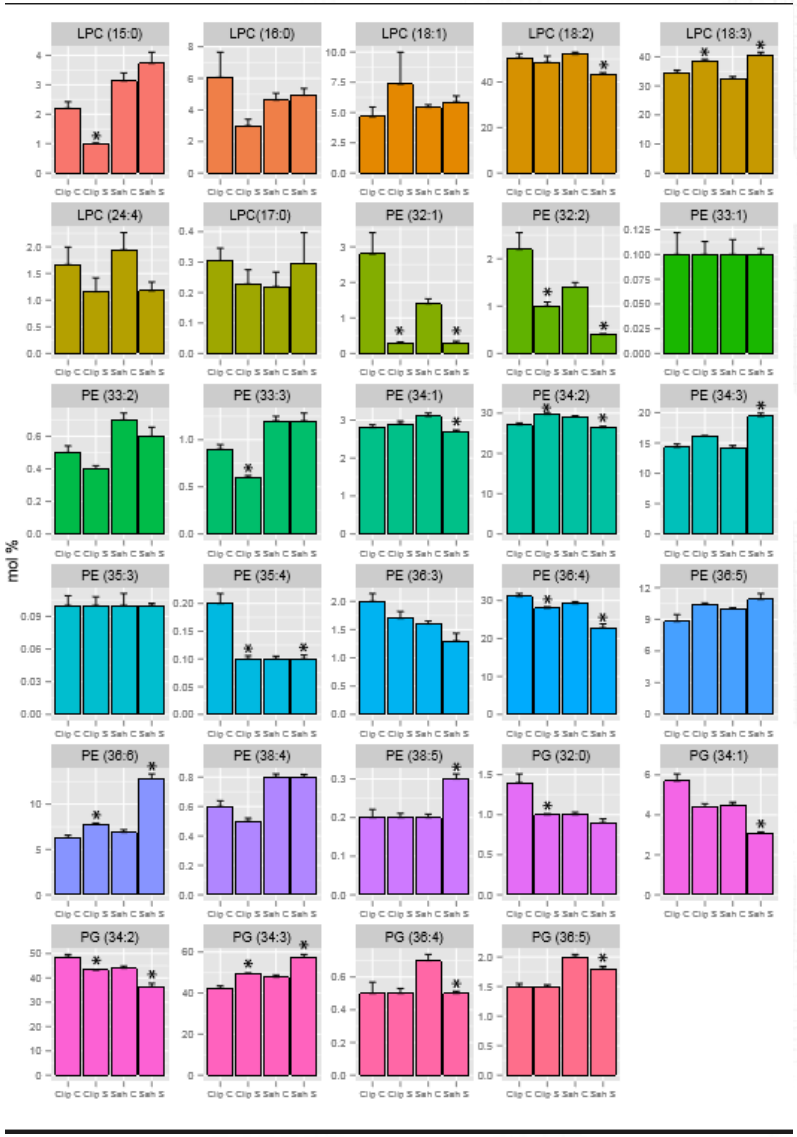


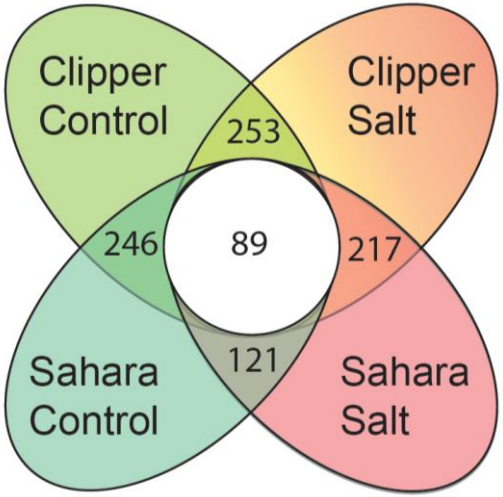
Munnik and Vermeer 2010

Total lipid analysis in salt treated roots

METABOLOMICS AUSTRALIA





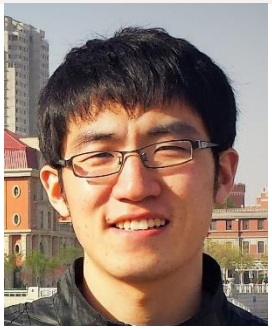


Natera et al, 2016, Funct Plant Biol

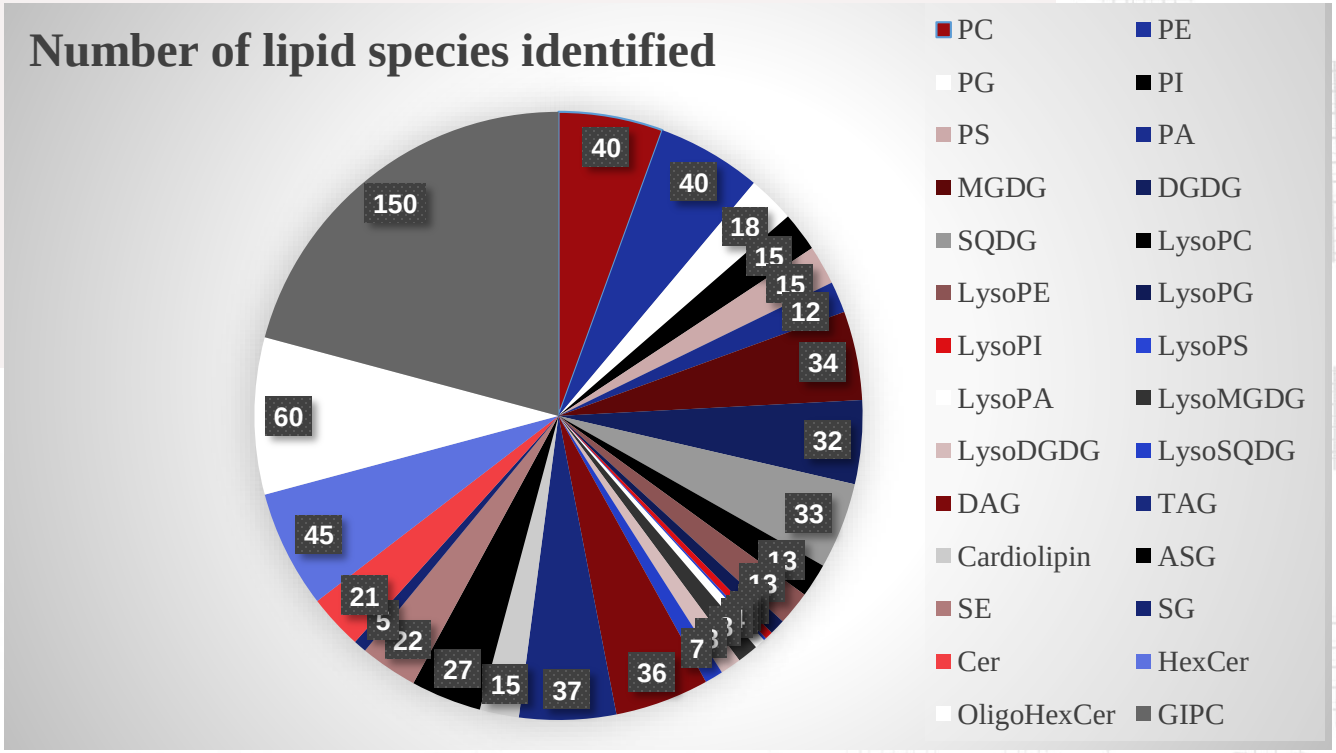
The barley root lipidome

METABOLOMICS AUSTRALIA

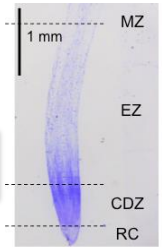
Gradient Category and Source Polarity			
Gradient 1/Neg	**Gradient 2/Pos**	**Gradient 3/Pos**	**Gradient 4/Pos**
PC	Ceramide	GIPC	TAG
PE	HexCer	OHC	SE
PG	DAG		
PS	ASG		
PI	SG		
SQDG			
DGDG			
MGDG			
Cardiolipin			
LysoGlycerolipid			
PA			



Yu et al 2018

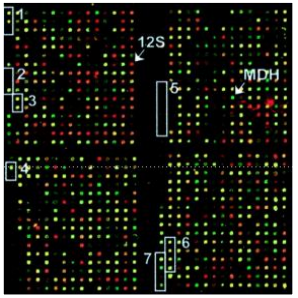


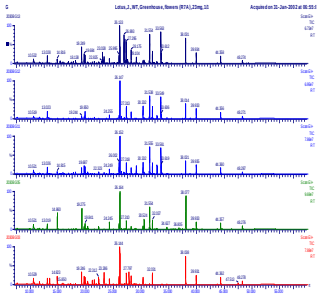
Where to next?

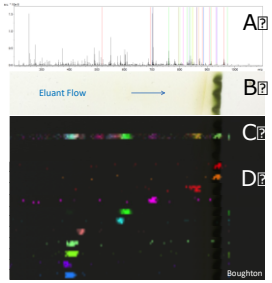


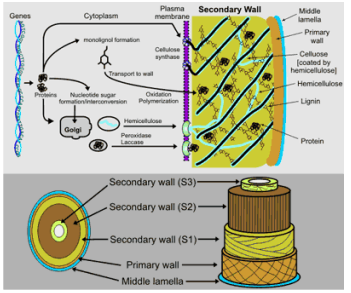
tissue harvest shock freeze homogenization extraction

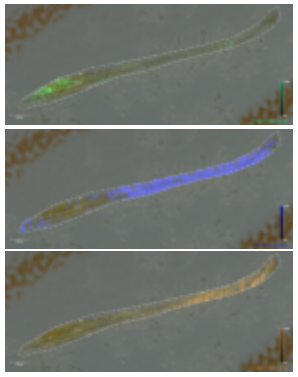
Transcriptomics Metabolomics Lipidomics Cell wall

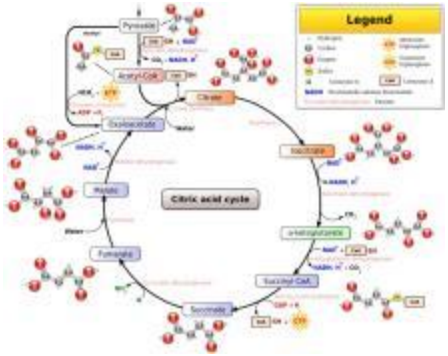




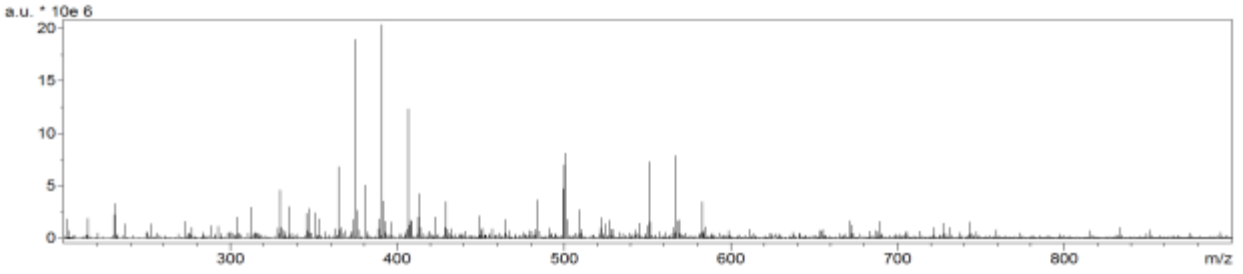


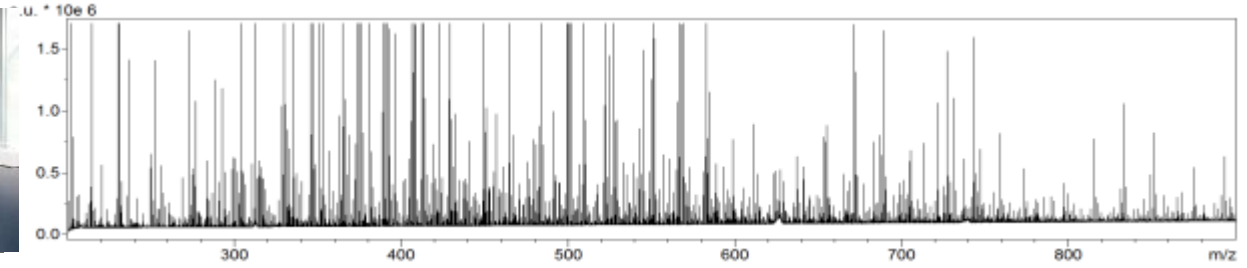






MALDI-IMS of barley roots





Workflow

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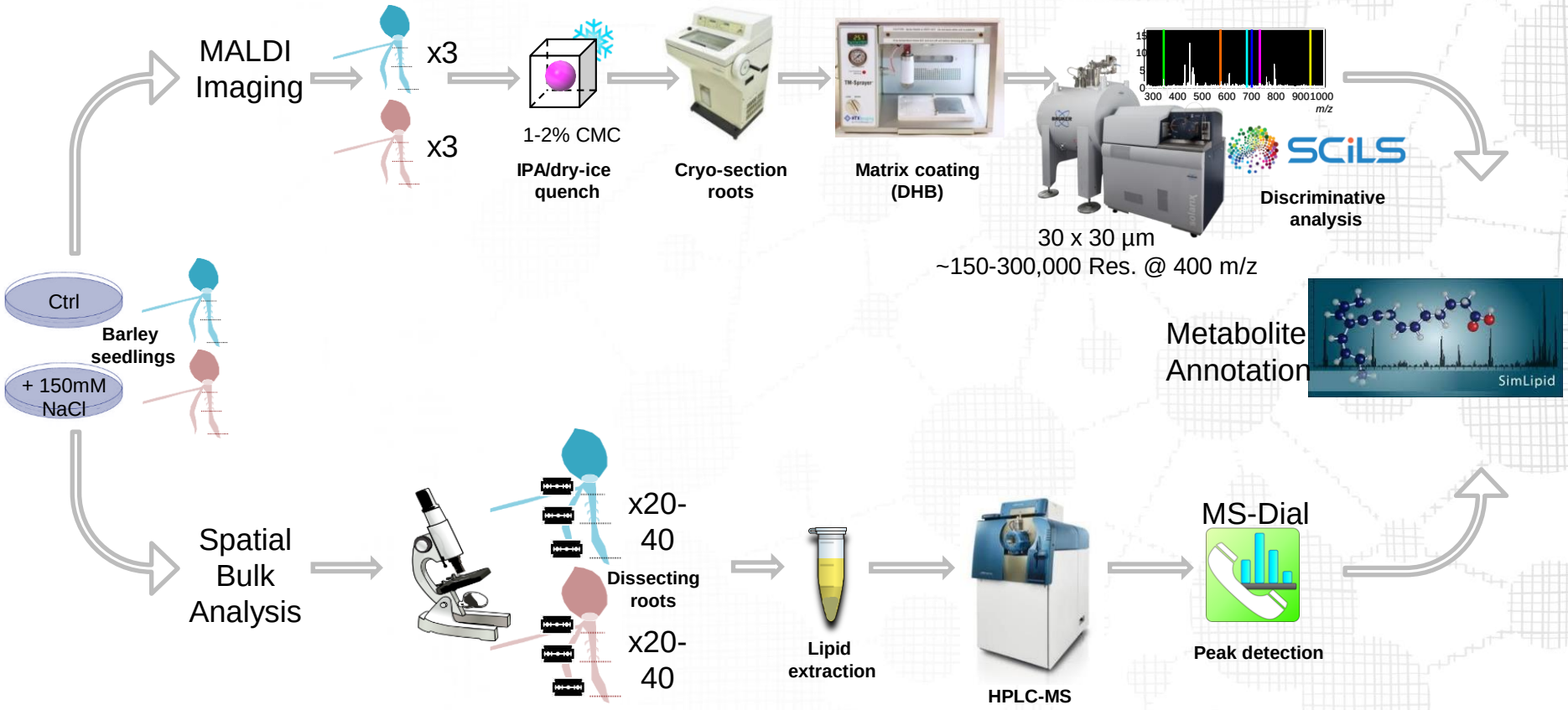
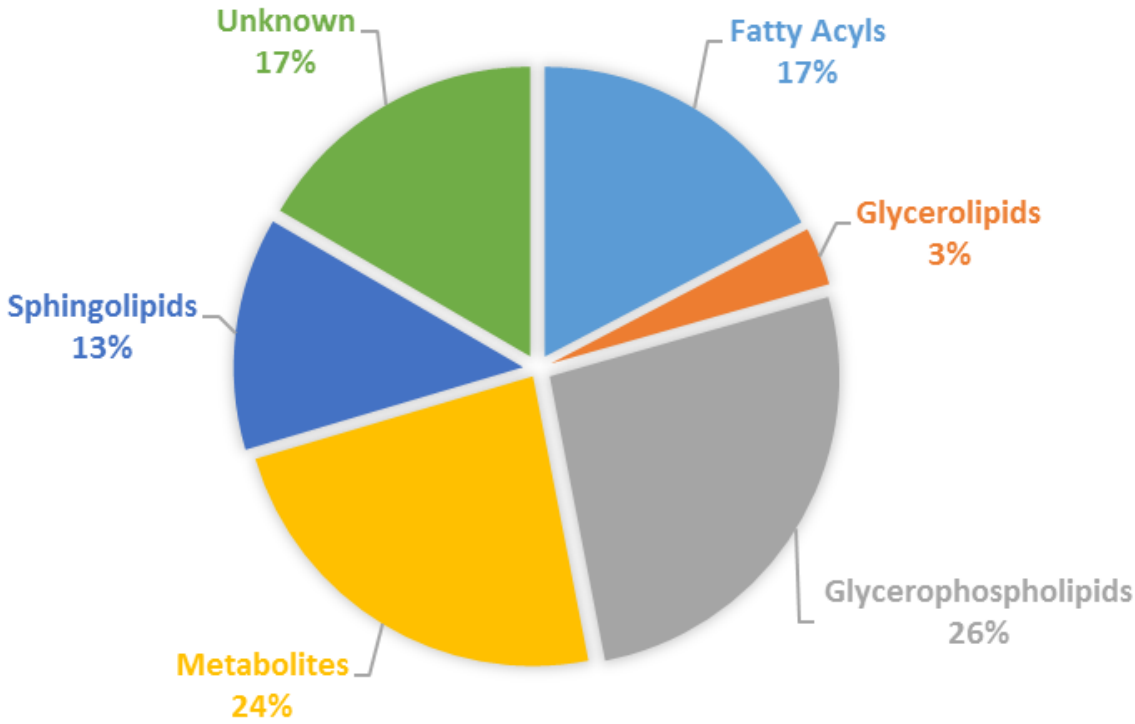


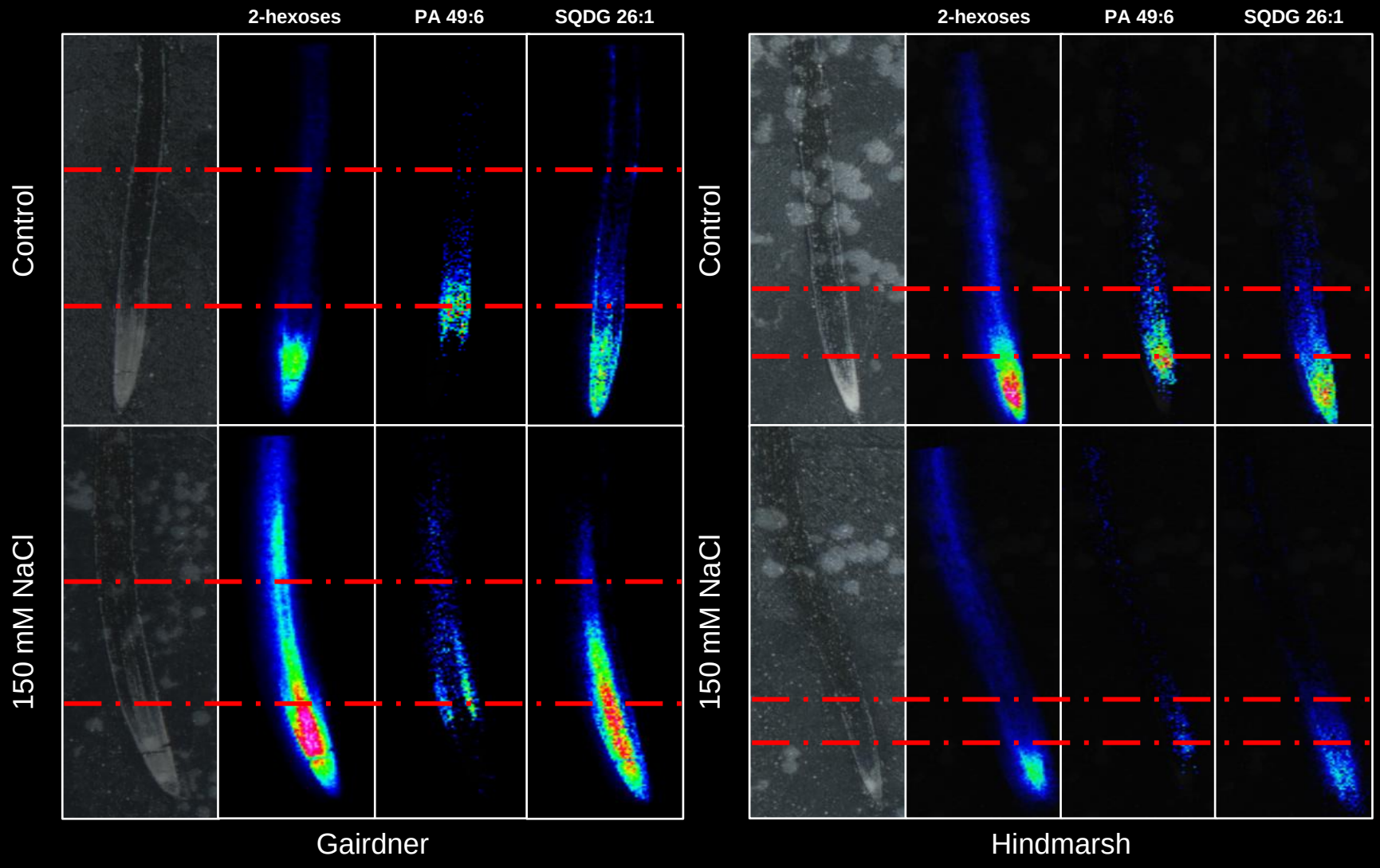
Tentative Annotations via MSI

METABOLOMICS AUSTRALIA

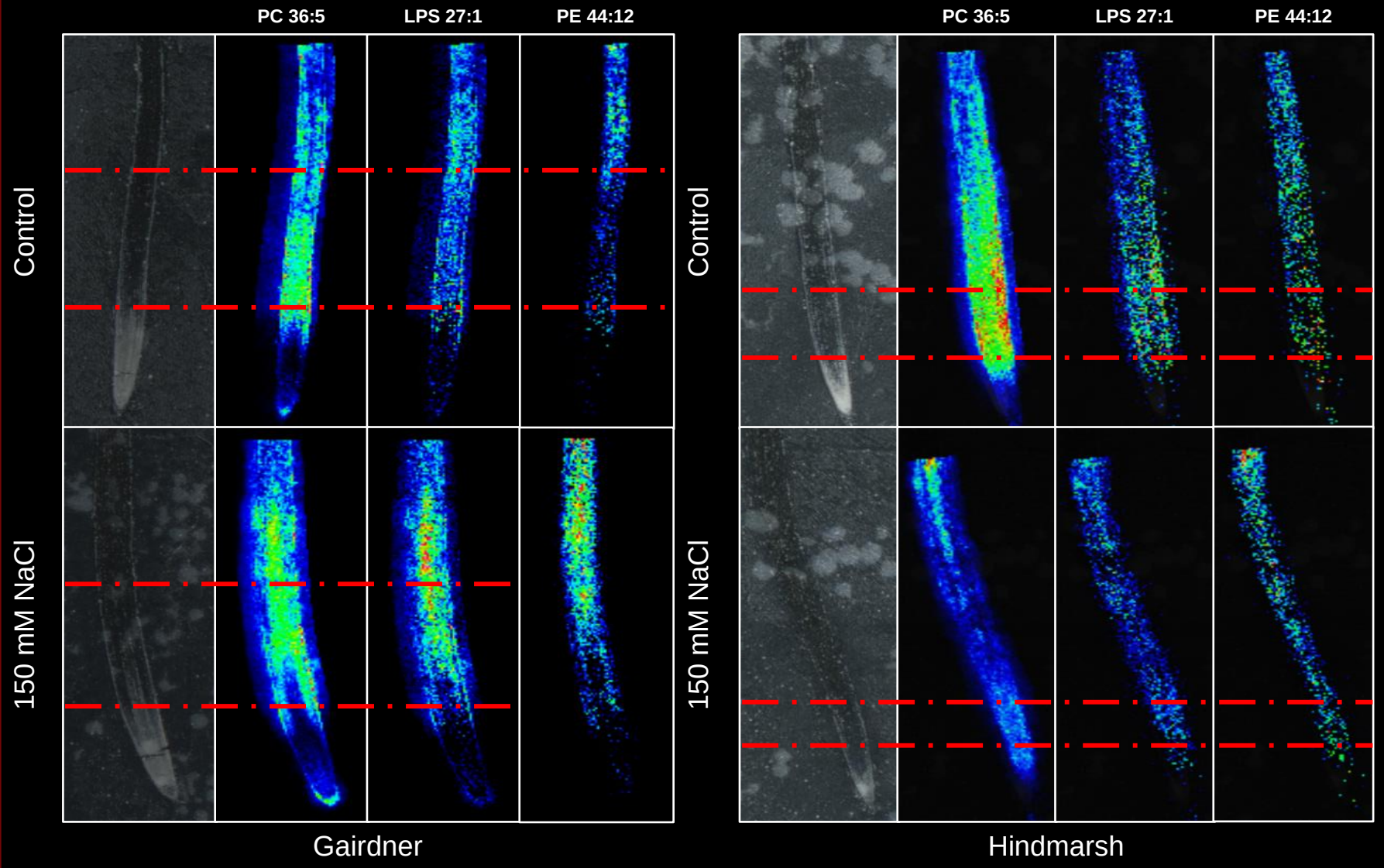
- **230** spatially distributed ions across different barley cultivars (Gairdner and Hindmarsh)
- Annotated using SimLipid and Metlin (5 ppm), and curated for multiple adducts & isobars



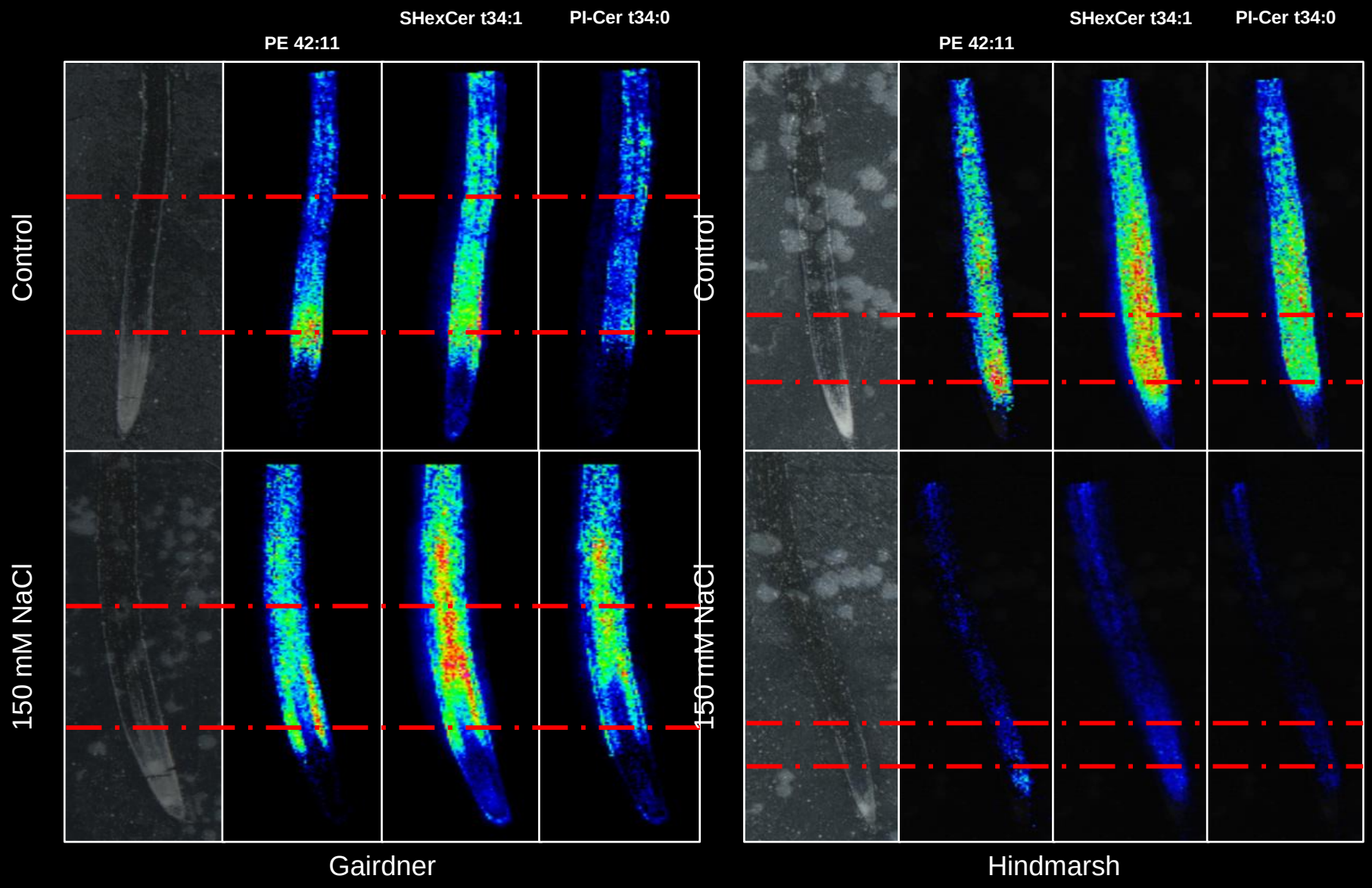
Results


2-hexoses
PA 49:6
SQDG 26:1
Control
150 mM NaCl
Gairdner
2-hexoses
PA 49:6
SQDG 26:1
Control
150 mM NaCl
Hindmarsh

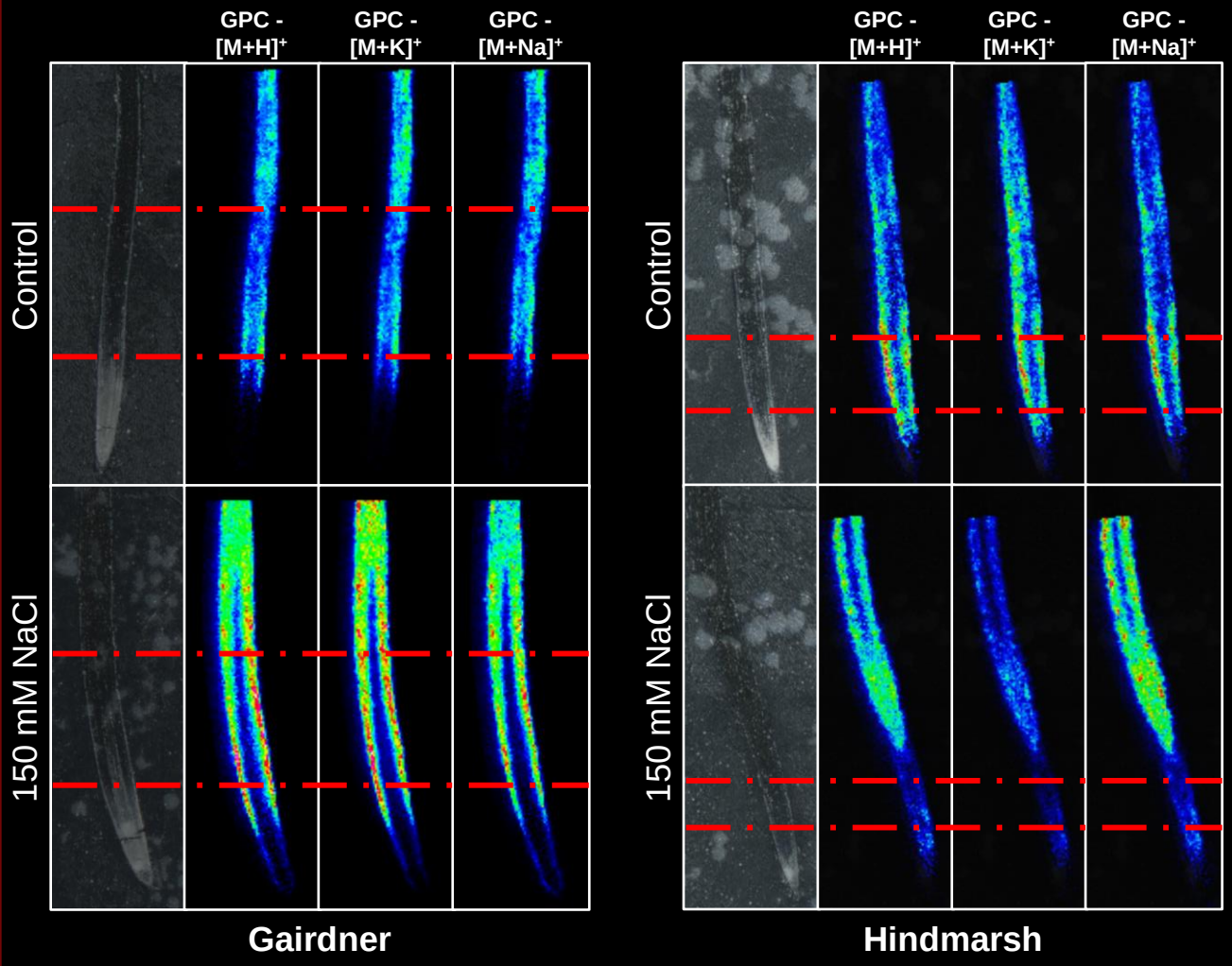
Results


PC 36:5
LPS 27:1
PE 44:12
Control
150 mM NaCl
Gairdner
PC 36:5
LPS 27:1
PE 44:12
Control
150 mM NaCl
Hindmarsh

Results


PE 42:11
SHexCer t34:1
PI-Cer t34:0
PE 42:11
SHexCer t34:1
PI-Cer t34:0
Control
Control
150 mM NaCl
150 mM NaCl
Gairdner
Hindmarsh

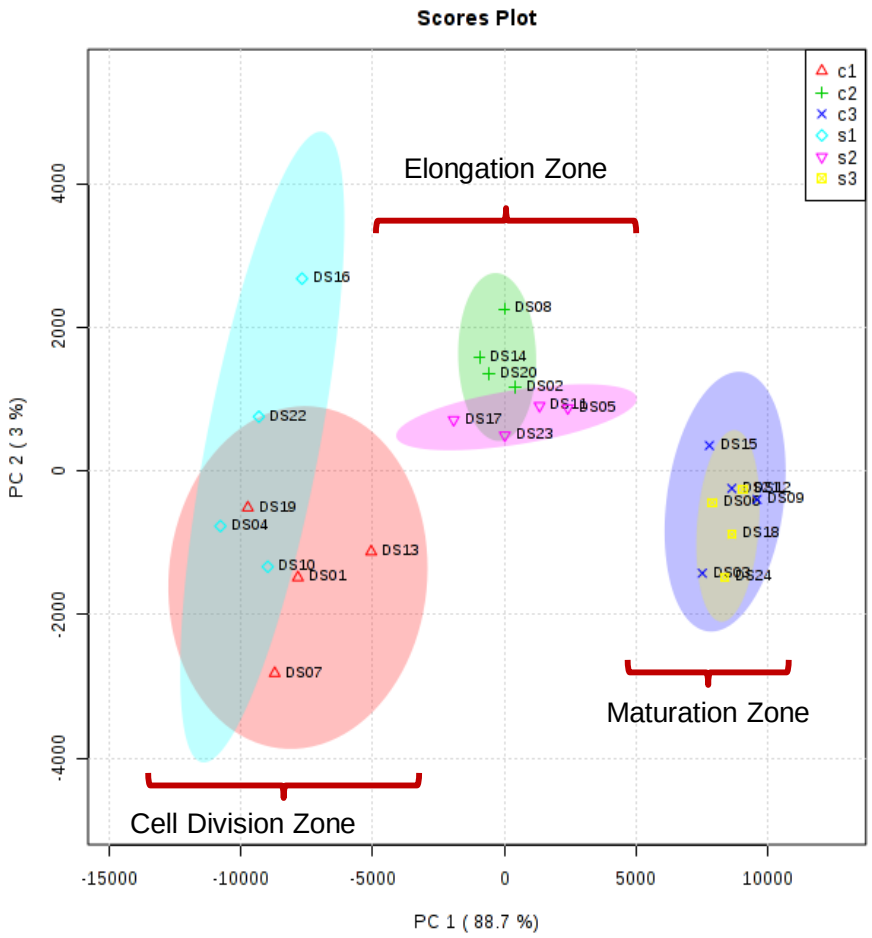
Results

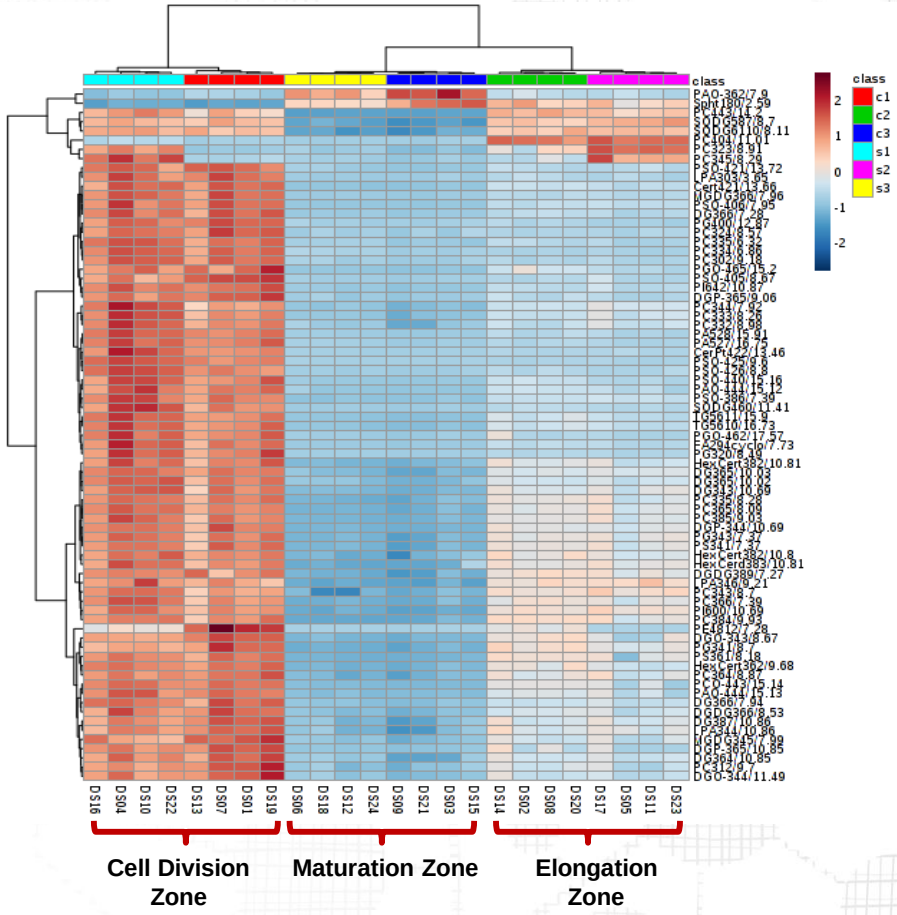


- GPC is a degradation product of phosphatidylcholine, resulting from the removal of fatty acids by the activity of phospholipase A1, phospholipase A2 and lysophospholipase (Sahsah et al., 1998).
- The presence of GPC is generally interpreted as a stress-induced membrane turnover or degradation (Aubert et al., 1996).

Spatial Bulk Analysis – LC-MS

METABOLOMICS AUSTRALIA

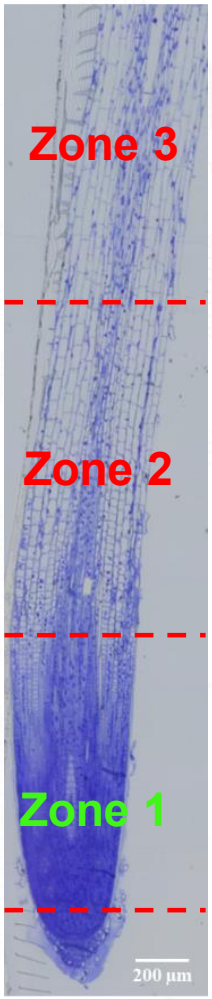




Summary

METABOLOMICS AUSTRALIA

Gairdner		Hindmarsh
230 Curated Features	Zone 3	277 Curated Features
145 Tentative ID's SimLipid 48 Tentative ID's Metlin	Zone 2	166 Tentative ID's SimLipid 65 Tentative ID's Metlin
71 Discriminatory Salt vs Ctrl		51 Discriminatory Salt vs Ctrl
Metabolites and FA's are discriminatory		Metabolites and FA's are discriminatory
3 Discriminatory Ctrl vs Salt	Zone 1	22 Discriminatory Salt vs Ctrl
FA's and SL are discriminatory		GP's and SL's are discriminatory



Discriminatory Features Associated with Zones 2 and 3

Summary

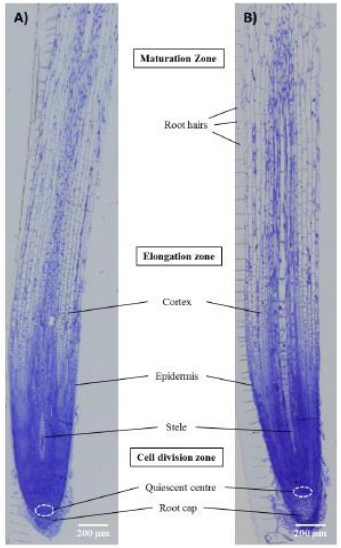
METABOLOMICS AUSTRALIA

- Tissue type-resolved analyses demonstrate tissue-specificity of metabolism
- Large differences between genotypes of the same species
- Lipids are important players in salinity response
- Imaging MS provides new means of tissue-specific metabolite analysis

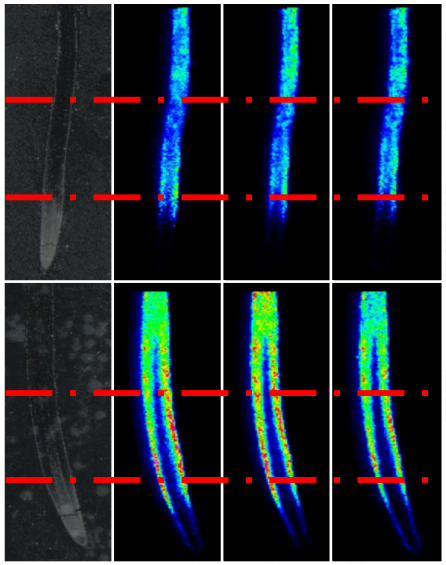
Future for spatial analysis

METABOLOMICS AUSTRALIA

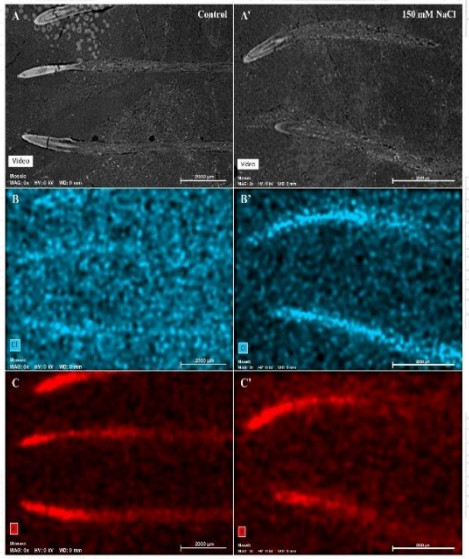
Microscopy



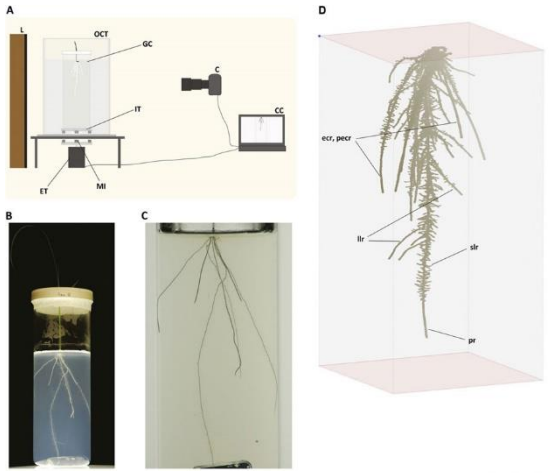
Imaging MS



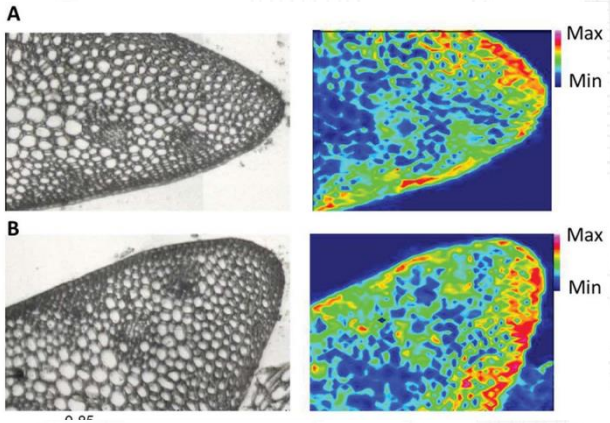
Micro-XRF



Root phenotyping



FT-IR



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