SC 813 761 Biochemical Seminar I

Topic : Metabolic contribution to salinity stress response in grains of two barley cultivars with contrasting salt tolerance

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Today's discussion

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Metabolic contribution to salinity stress response in grains of two barley cultivars with contrasting salt tolerance

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Background

- The big picture – what is the societal or socioeconomical problem(s) to be solved?
- Barley
- Salinity stress
  - What is it?
  - Why is this important?

Let's go through the Introduction!

Background : Barley and soil salinity

- Barley is one of cereal species. It ranks fourth in quantity produced in the world.
- Barley has many uses in the food industry, such as malting, making beer and animal food. Therefore, barley is in great demand.
- One of the problems affecting barley yields is soil salinity.

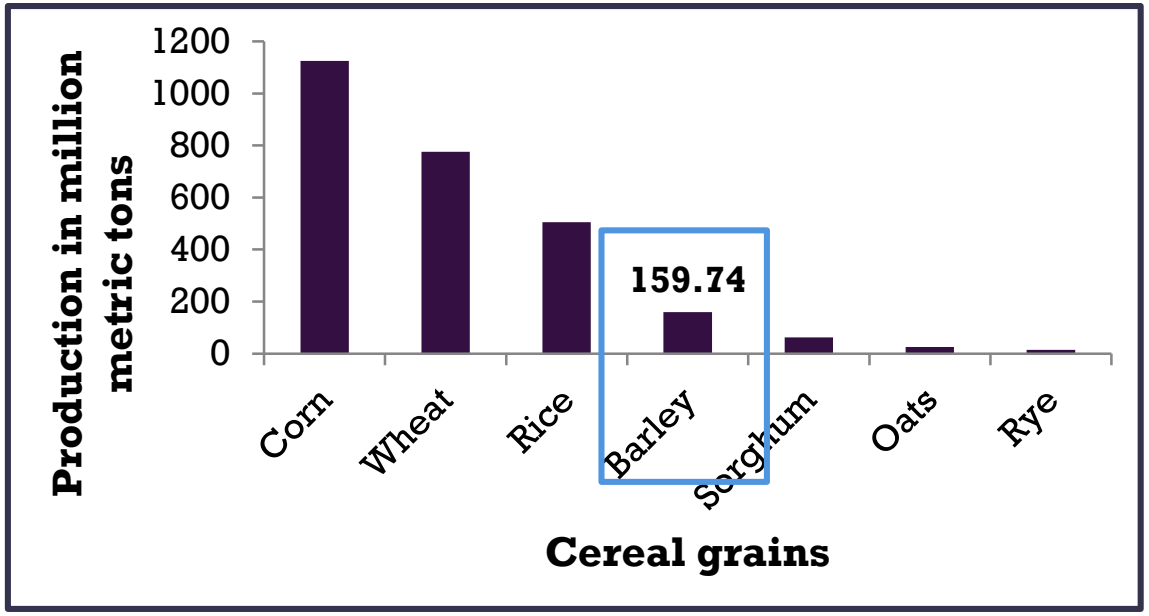


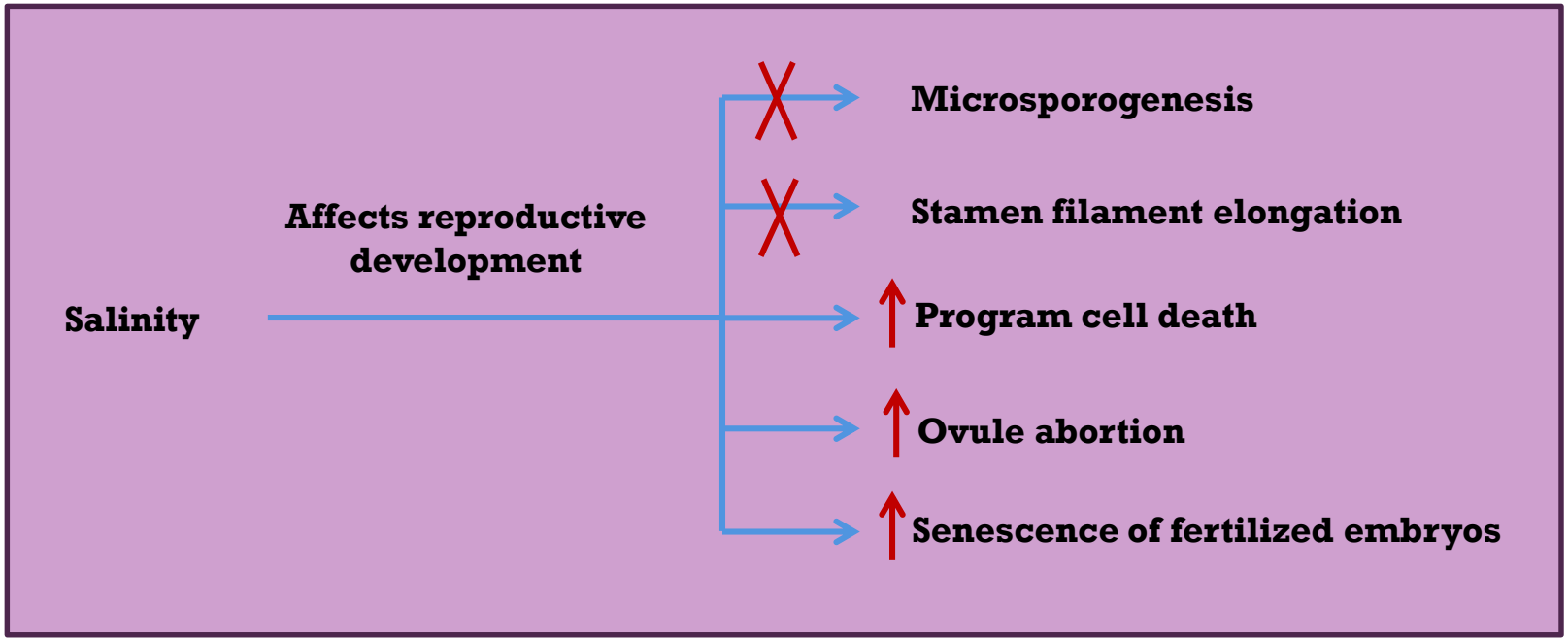
Figure 1: Worldwide production of grain in 2020/21

https://www.statista.com/statistics/263977/world-grain-production-by-type/

- Saline soils have electrical conductivity (EC) of about 4 dS/m (40 mmol/L NaCl).
- Over 800 million hectares of land are affected by salinity around the world and tend to increase.

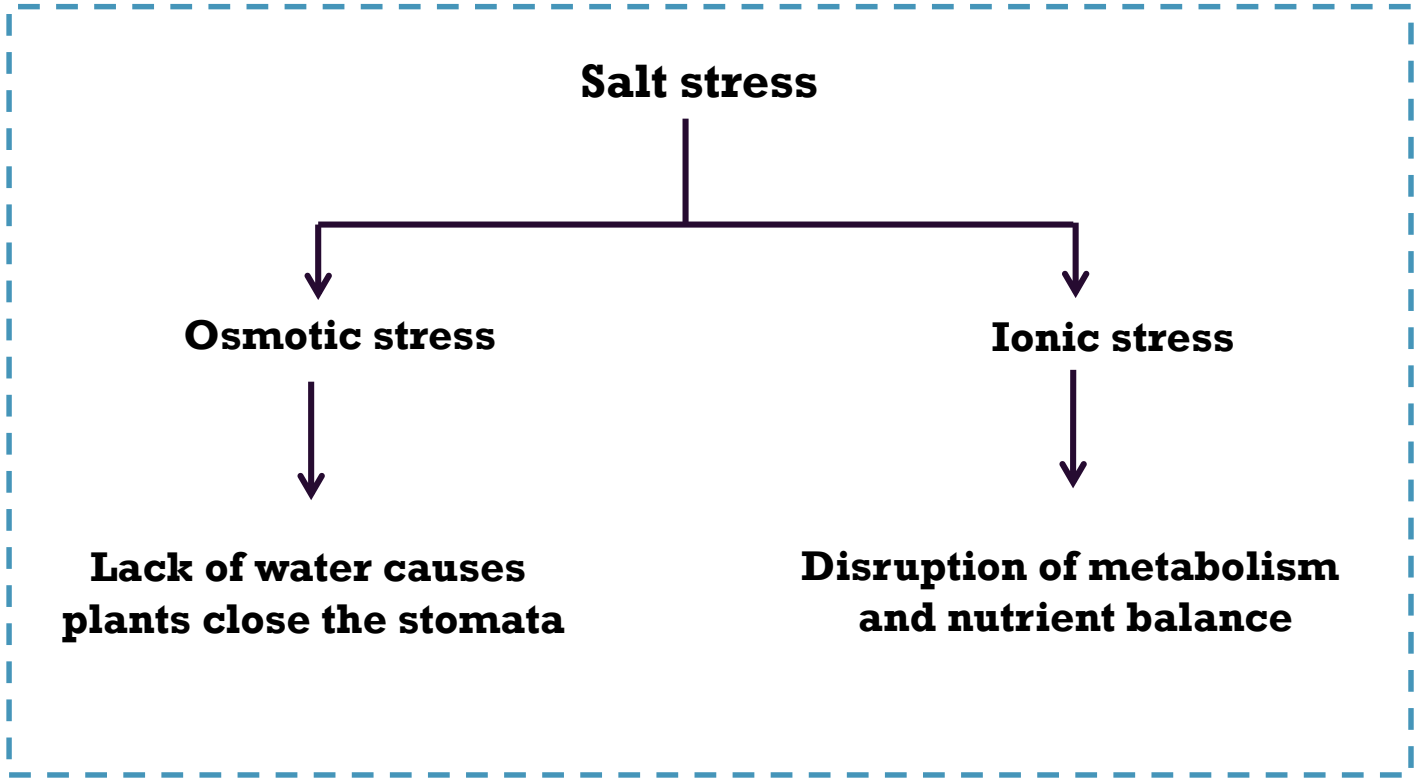
Salinity stress

- Salinity impacts soil deterioration leading to decrease plant growth and yield of grain crops worldwide.
- Salinity is a major problem that affects reproductive development of plant.
- The impact of salt stress on plant can divide into two phase including osmotic and ionic stress.



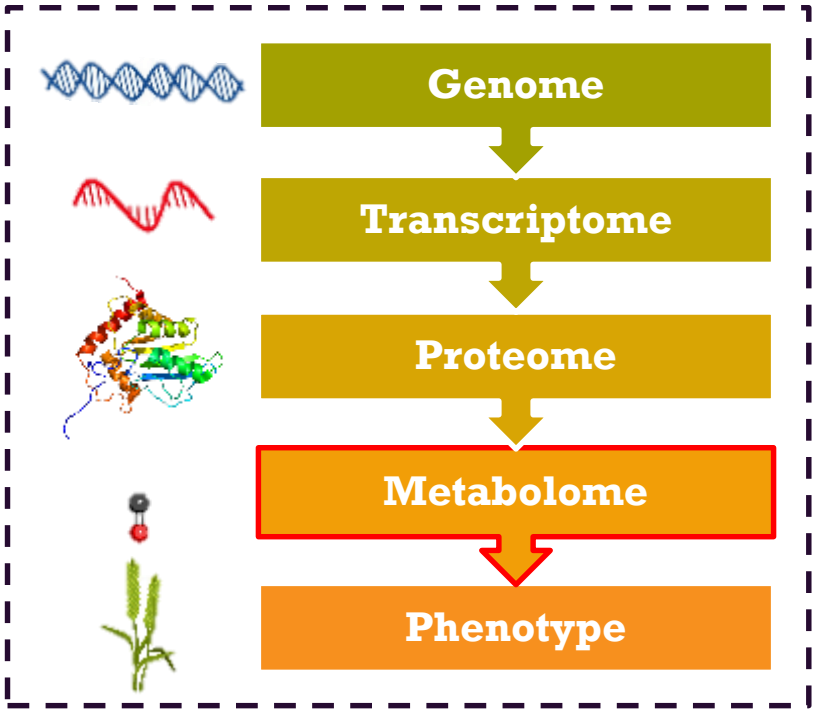
Salinity stress

- The first phase is physiological anomaly caused by decrease of water potential and depends on salt concentration around the cell.
- The second phase occurs from accumulation of ions due to long-term salt stress, affecting metabolism and nutrient availability of plant.



The metabolome

- Since salinity stress affects the physiology of leaves, roots, and plant growth parameters, molecular or biochemical studies to provide a comprehensive understanding of biological processes for the manipulation of the phenotypes are required for crop improvement under stress and to differentiate tolerant and sensitive lines.
- Molecular studies are conducted at several levels, including the genome, transcriptome, proteome, and metabolome.



Xu et al. (2014)

- Changes in genes and proteins under stress often do not give accurate phenotype compared to changes in cellular metabolites.
- The metabolome is closely-related to the phenotype. Thus, metabolite values may be used for diagnosis and prediction of plant symptoms under salt stress.

The Novelty : let's get back to the introduction!

"A few researchers have investigated the effects of drought, heat and salinity stresses on seed yield and seed metabolites of the global major crops, soybean (Iqbal et al., 2018), canola (Elferjani and Soolanayakanahally, 2018) and wheat (Datir et al., 2020). However, there are only a handful of reports on barley grain developmental pathways and/or modulations in these under abiotic stress conditions."

" The objective of the present study was to apply metabolite profiling to identify metabolites that might serve as biomarkers for barley cultivars differently adapted to salinity stress. Therefore, a study was undertaken on a salt-tolerant and a salt-sensitive cultivar under long-term salinity stress (100 mM NaCl) up to seed maturation stage and led to observations of characteristic patterns of metabolites.

What is the biological question(s) being asked?

Let's brainstorm and write on the board!

What approach was chosen to address the question?

- Scope of the study
  - What was the plant used?
    - What genotypes were used?
  - What was the study approach?
  - What traits were characterized?
    - Were they molecular, cellular, biochemical, physiological or morphological?

What is the hypothesis?

Let's brainstorm and write on the board!

What are the techniques used and what type(s) of data generated?

Let's go through the M&M!

Scope of Work

Short term salinity stress

Rice culture

Seeds of 11 Australian accredited malting cultivated varieties of barley such as Bass, Commander, Fathom, Flinders, GrangeR, Hindmarsh, LaTrobe, Litmus, Oxford, Scope and Westminister

- Growth parameters
- Analysis of Na+ and K+

Long term salinity stress

Rice culture

Seeds of 2 barley varieties cv. GrangeR and Scope selected from Short term salinity stress.

Metabolite Profiling

- Untargeted metabolomics analysis
- Chemometric analysis of GC-MS data

What are the techniques used and what type(s) of data generated?

- Method 1: Measuring growth and development

Samples
- **Leaves**
- **Root**

Growth parameter

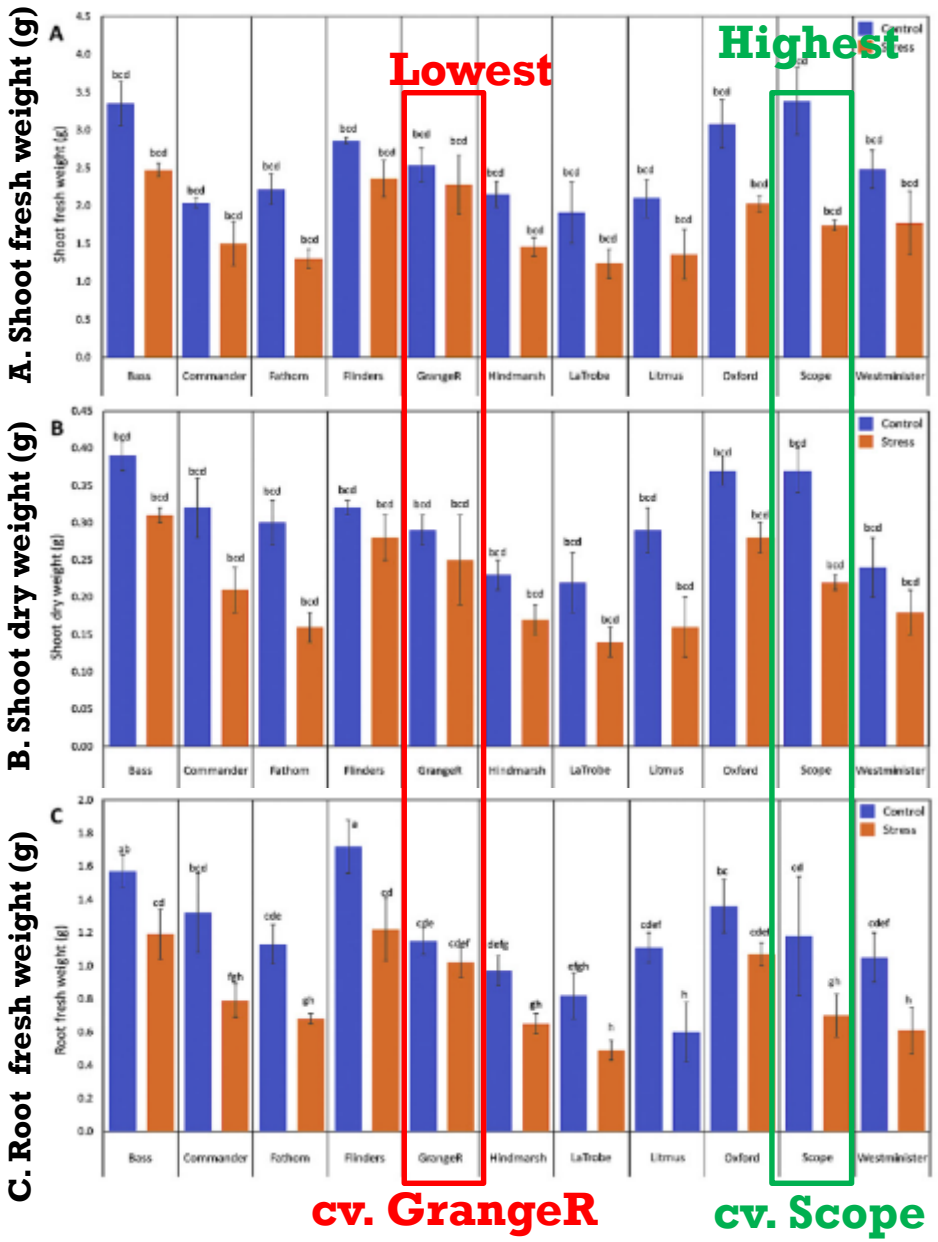
- **Shoot fresh weight**
- **Root fresh weight**
- **Shoot length**
- **Root length**
- **Shoot dry weight**
- **Root dry weight**

dry at 80 °C for 48 h.

What is the expected outcome?

Let's brainstorm!

Method 1: Measuring growth and development



- Under the EC15 condition, the plants showed lower shoot and root FW and shoot DW than the control.
- In salt stress, shoot and root weight of GrangeR display the lowest reduction while Scope display the highest.
- Plant growth is reduced under salinity. It is mainly affected by ion toxicity, osmotic stress and nutrient disorder.

Figure 2: Effect of 150 mM NaCl stress on growth parameters of eleven barley cultivars. A. Shoot fresh weight; B. Shoot dry weight; C. Root fresh weight.

Method 1: Measuring growth and development

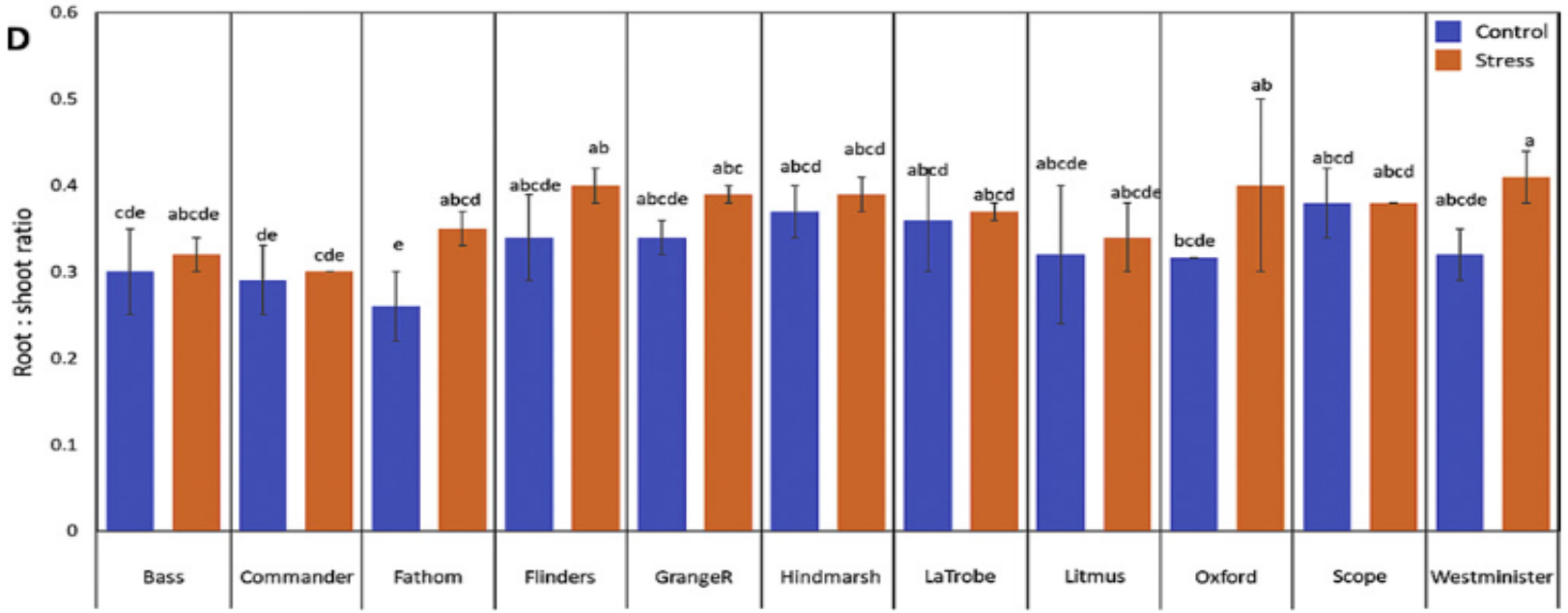


Figure 3: Effect of 150 mM NaCl stress on growth parameters of eleven barley cultivars. D. Root to shoot ratio.

- The root and shoot ratio of all barley lines under salt stress are increased except for Scope.
- An increase in root and shoot ratio is an adaptation mechanism to salt stress and enhances water absorption.

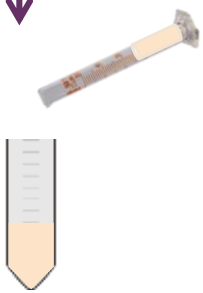
What are the techniques used and what type(s) of data generated?

- Method 2: Measuring Na^+ and K^+ concentrations

0.7 g of dry samples
- **leaves**
- **root**

Dry to ash at 550 °C for 12 hrs.

2.5 ml mixture of 10 M HCl and 16 M HNO_3 (1:4)



Adjust to 25 ml with deionized water

Sonicate for 10 min

Centrifuge at 4000 rpm, 5 min

Analyze with Atomic absorption spectrometer

What is the expected outcome?

Let's brainstorm!

Method 2: Measuring Na^+ and K^+ concentrations

A. Na^+:K^+ ratio in shoots

B. Na^+:K^+ ratio in roots

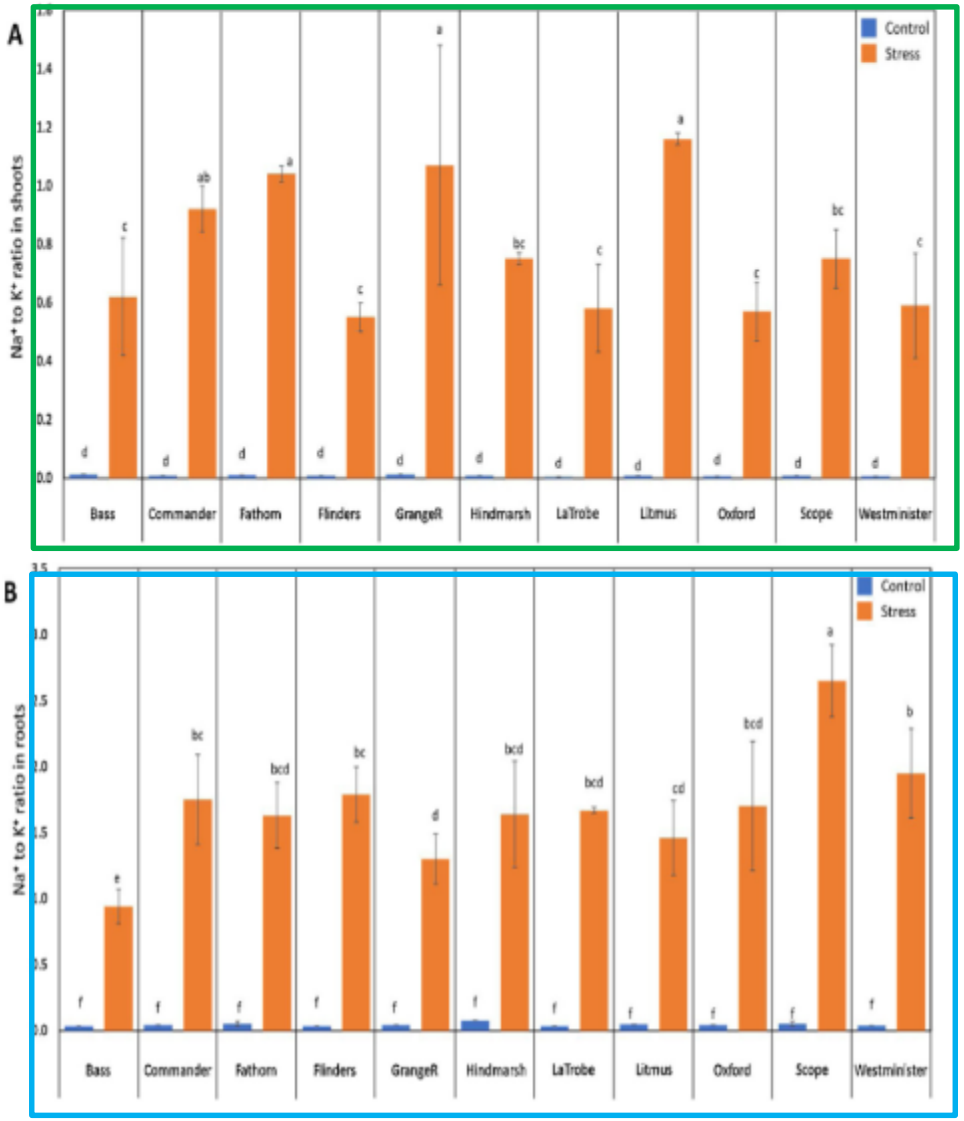


Figure 4: Effect of 150 mM NaCl stress on Na+:K+ratio of eleven barley cultivars. A. in shoots and B. in roots of barley lines.

- Under salt condition, both shoots and roots in all lines showed increases Na^+:K^+ ratio.
- Na+/K+ ratio in root are higher than shoot.
- The most tolerant line has the lowest shoot and root Na+/K+ ratios, while the most sensitive line has the highest Na+/K+ ratios.

Method 2: Measuring Na^+ and K^+ concentrations

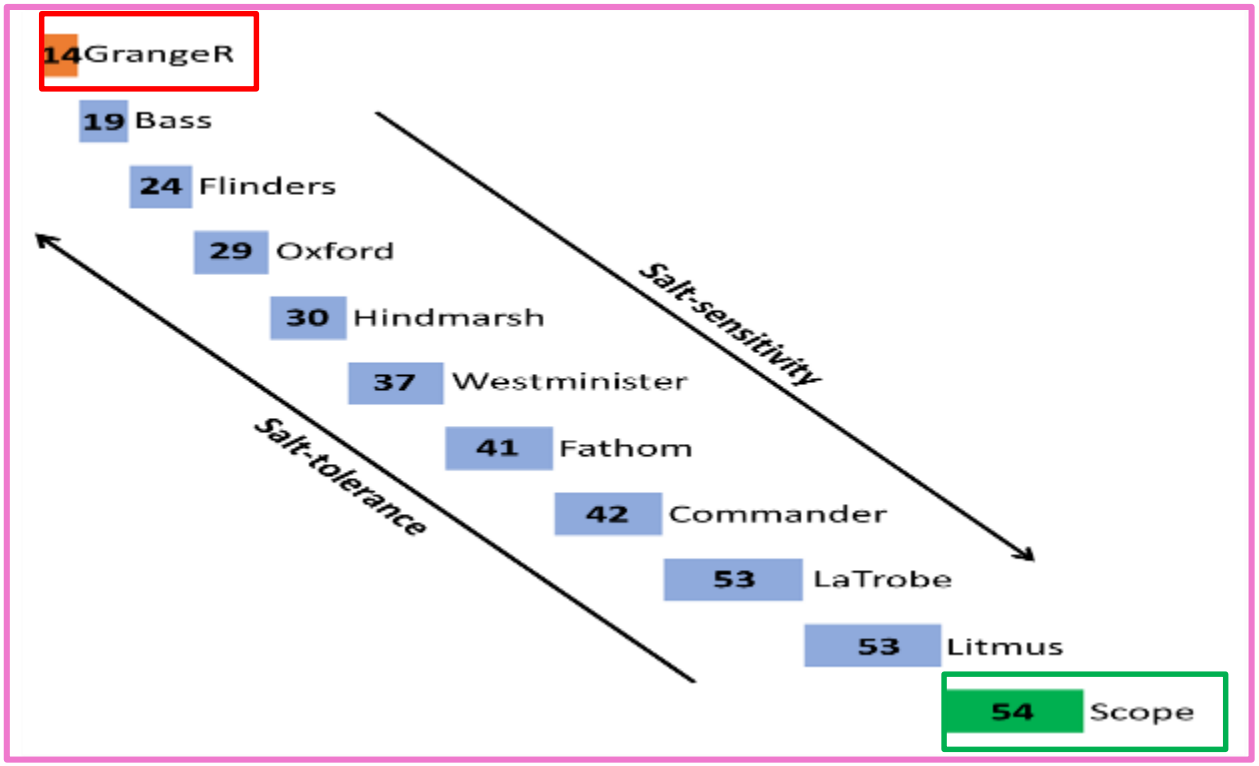
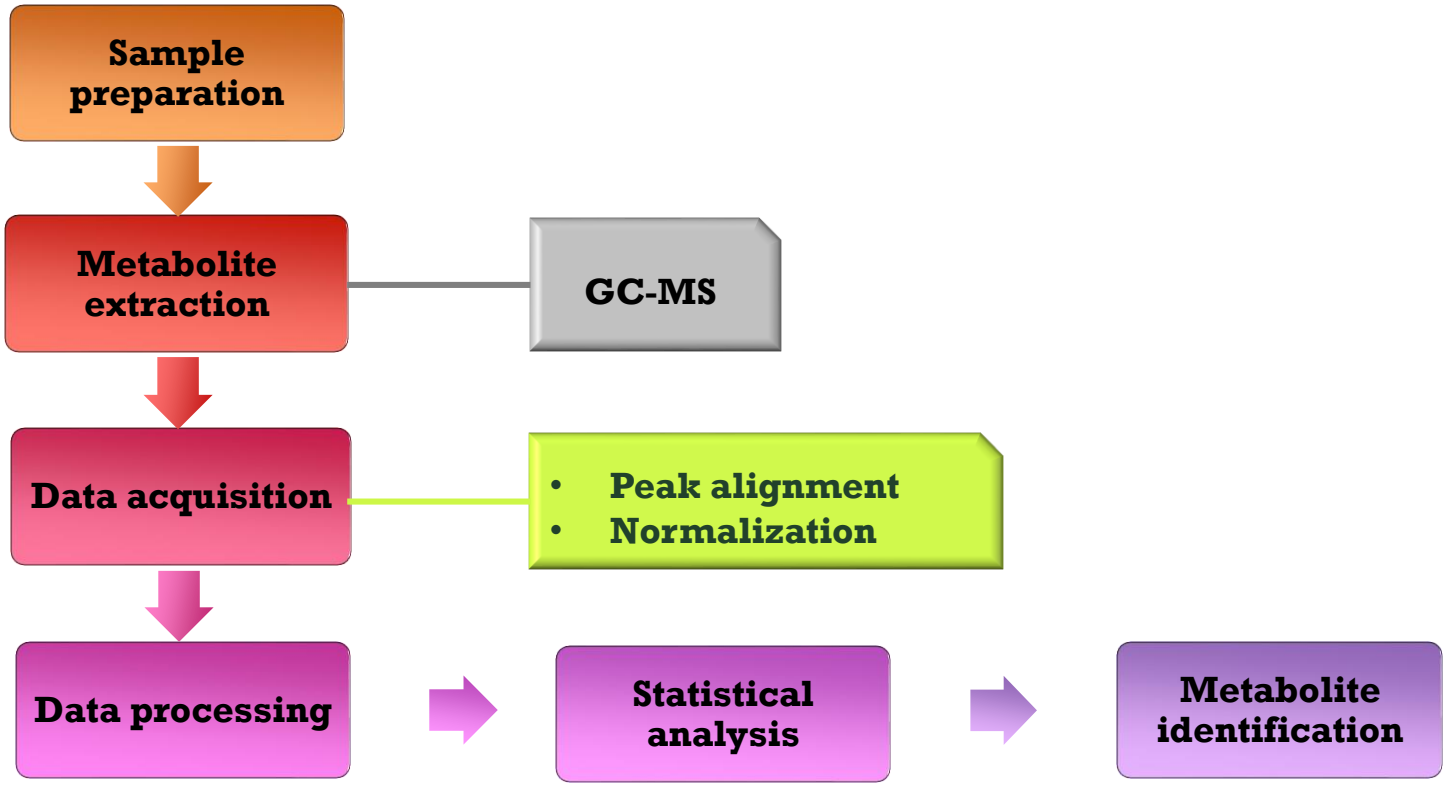


Figure 5: Salt-stress tolerance indicative ranking profile for barley lines.

- Both growth parameter and Na^+:K^+ ratio are added together to develop this overall tolerance ranking profile.
- GrangeR is identified as the most salt-tolerant line and Scope is identified as the most salt-sensitive line.

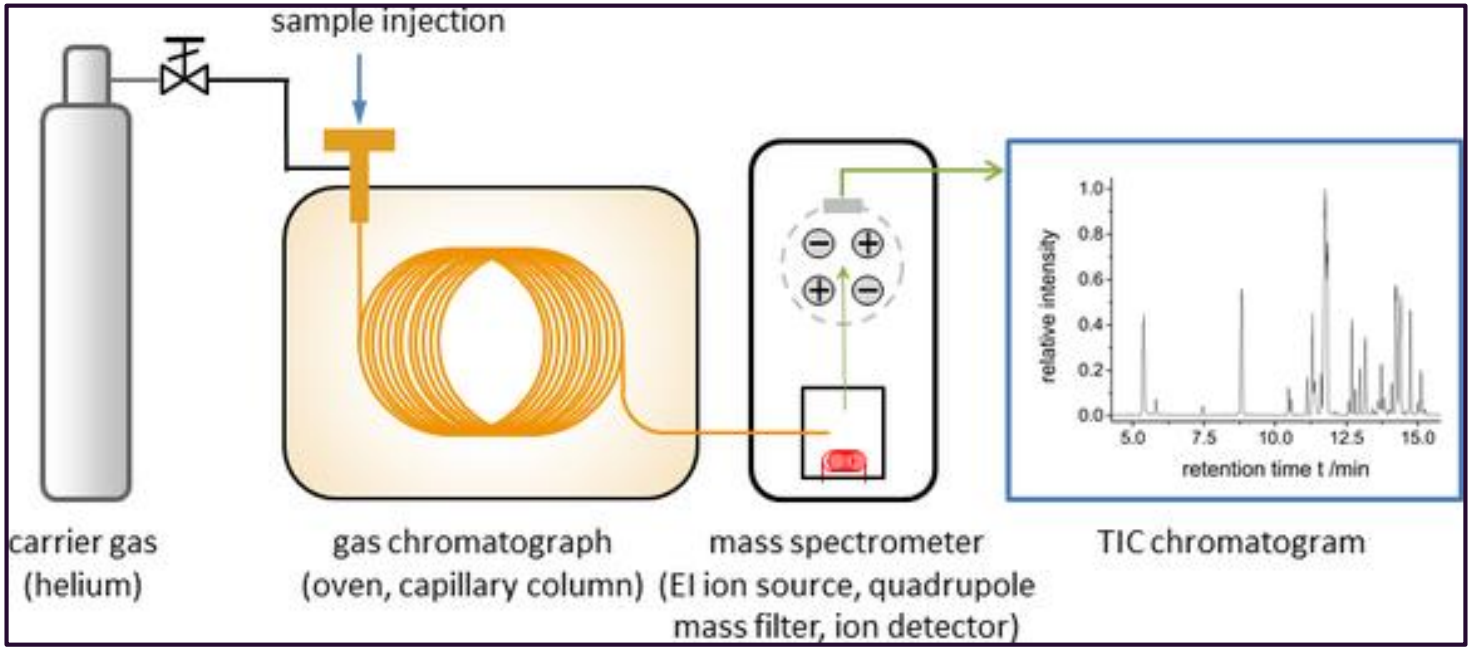
What are the techniques used and what type(s) of data generated?

- Method 3: Untargeted metabolomics



GC-MS

- **Principle of gas chromatography mass spectrometry (GC-MS)**



https://kavaforums.com/forum/threads/a-strange-table-comparison-of-what-gets-extracted-by-different-solvents.10574/

What is the expected outcome?

Let's brainstorm!

- Method 3: Untargeted metabolomics

Metabolomes

- Grain of two barley cultivars cv. GrangeR and Scope.
- Analysis by using GC-MS

Metabolite-candidate peaks

- A total of 62 peaks are identified based on Metabolomics Standards Initiative (MSI).
- The metabolite profile includes amino acids, sugars and sugar alcohols, fatty acids, organic acids, glycerol derivatives and other chemical classes

- Method 3: Untargeted metabolomics
  - Multivariate data analysis: PCA & PLS-DA
  - Univariate data analysis: FC & volcano plot

Principal component analysis (PCA)

- PCA is a multivariate data analysis to find the relation of those variables by combining multiple variables for analysis.

Partial least square discrimination analysis (PLS-DA)

- PLS-DA is used for classification or differentiate between variables

Volcano plot

- Volcano plot is used to identify differential metabolites from noisy metabolomics data based on *p*-values from a *t*-test and fold-change (FC) values.

- The metabolite profiles were analyzed in combination with multivariate and univariate analyzed to screen cultivar species that responses to salt stress.

- Method 3: Untargeted metabolomics
  - Discrimination between grains of barley cultivars analyzed by PCA

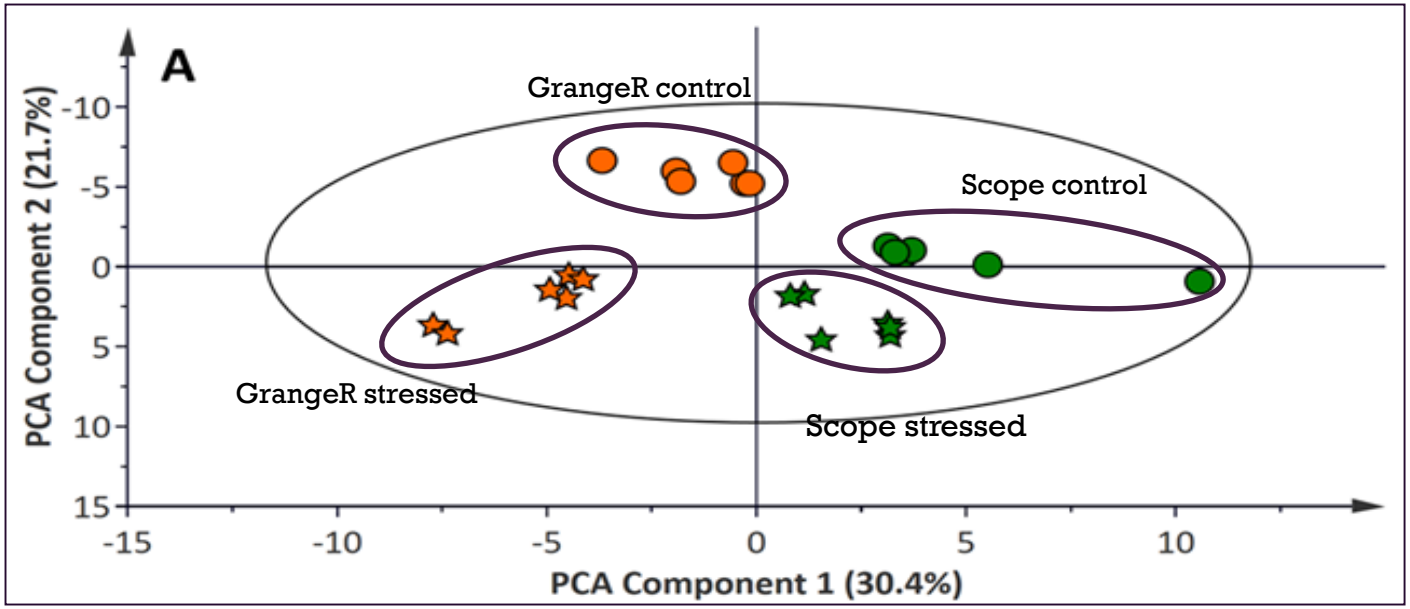


Figure 6: PCA plots of grain metabolomes of barley cultivars (n = 6). A. Score scatter plot. Symbols: Scope control ; Scope stressed ; Granger control ; Granger stressed ; Metabolites under consideration: .

- The results of the PCA showed good separation and no outliers of the samples, but the ability to predict the model remains low.
- Therefore, analysis by PLS-DA after PCA will increase the ability to predict the mode.

- Method 3: Untargeted metabolomics
  - Discrimination between grains of barley cultivars analyzed by PLS-DA

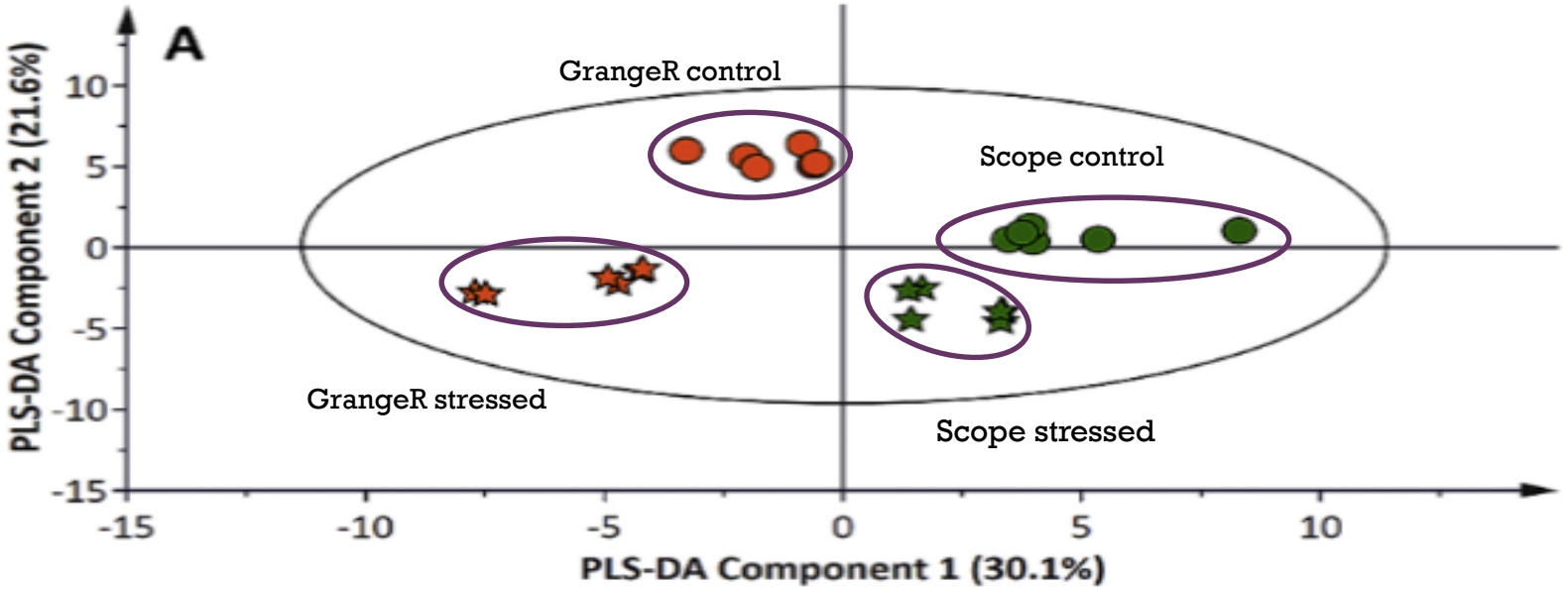


Figure 7: PLS-DA plots of grain metabolomes of barley cultivars (n = 6). A. Score scatter plot. control: ; Scope stressed: ; Granger control: ; Granger stressed: ; Metabolites under consideration: .

- The results of the PLS-DA display clear separation: GrangeR is separated from Scope along principal component 1, while the salt stress sample is isolated from the control sample along principal component 2.

- Method 3: Untargeted metabolomics
  - Metabolic variations in grains of barley cultivars by analyzed by PLS-DA and volcano plots

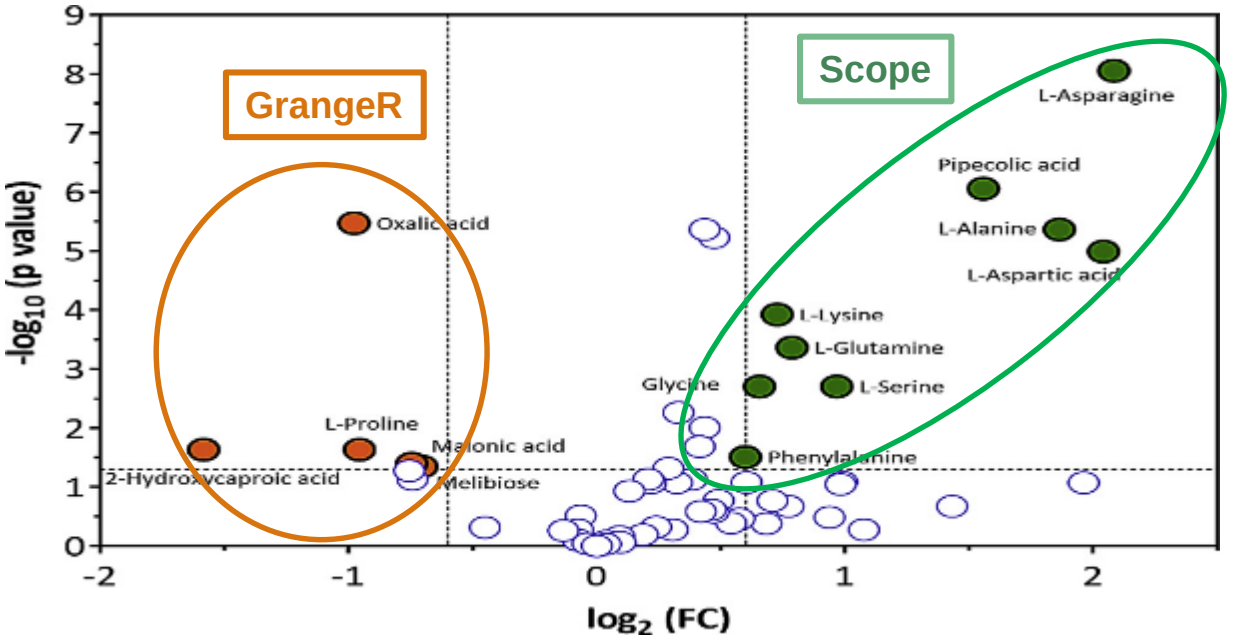


Figure 8: Volcano plot of grain metabolomes of barley cultivars (n = 6). Symbols: Significant metabolites in GrangeR: orange circles ; Significant metabolites in Scope: green circles ; Metabolites statistically not significant: white open circles .

- Of the 65 metabolites identify, 14 are significant biomarkers including 5 in GrangeR and 9 in Scope.
- Biomarker metabolites identified in Scope are all amino acids, while those found in GrangeR include organic acids, sugar and amino acid.

- Method 3: Untargeted metabolomics
  - Metabolic variations in grains of barley cultivars analyzed by PLS-DA and volcano plots

Scope **GrangeR**

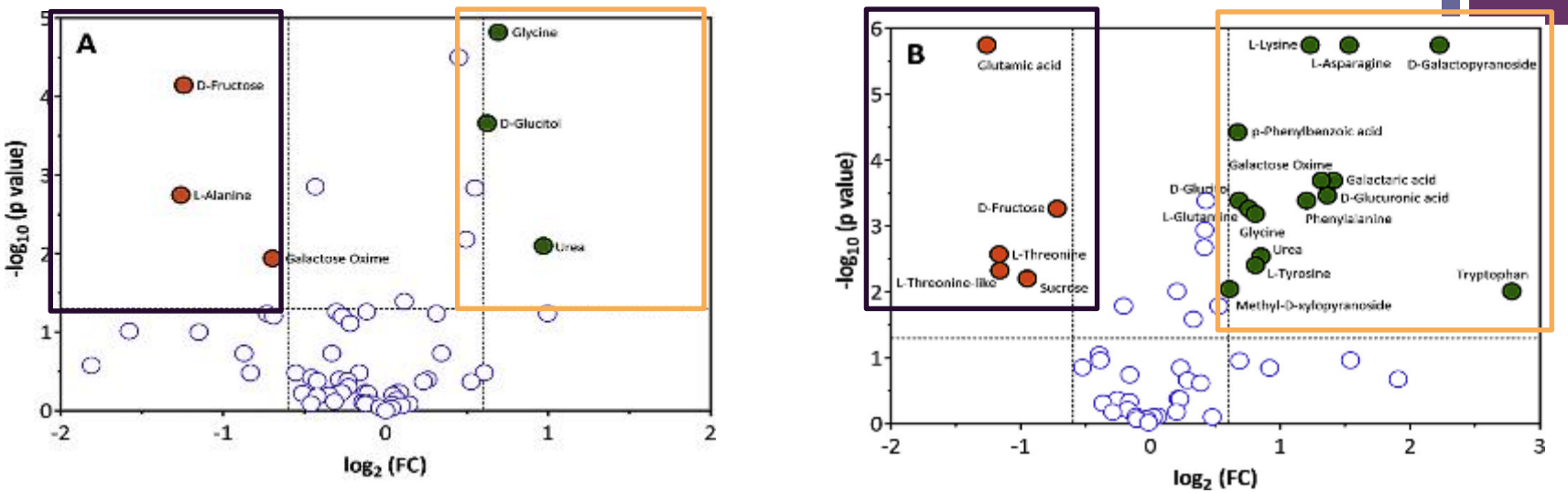


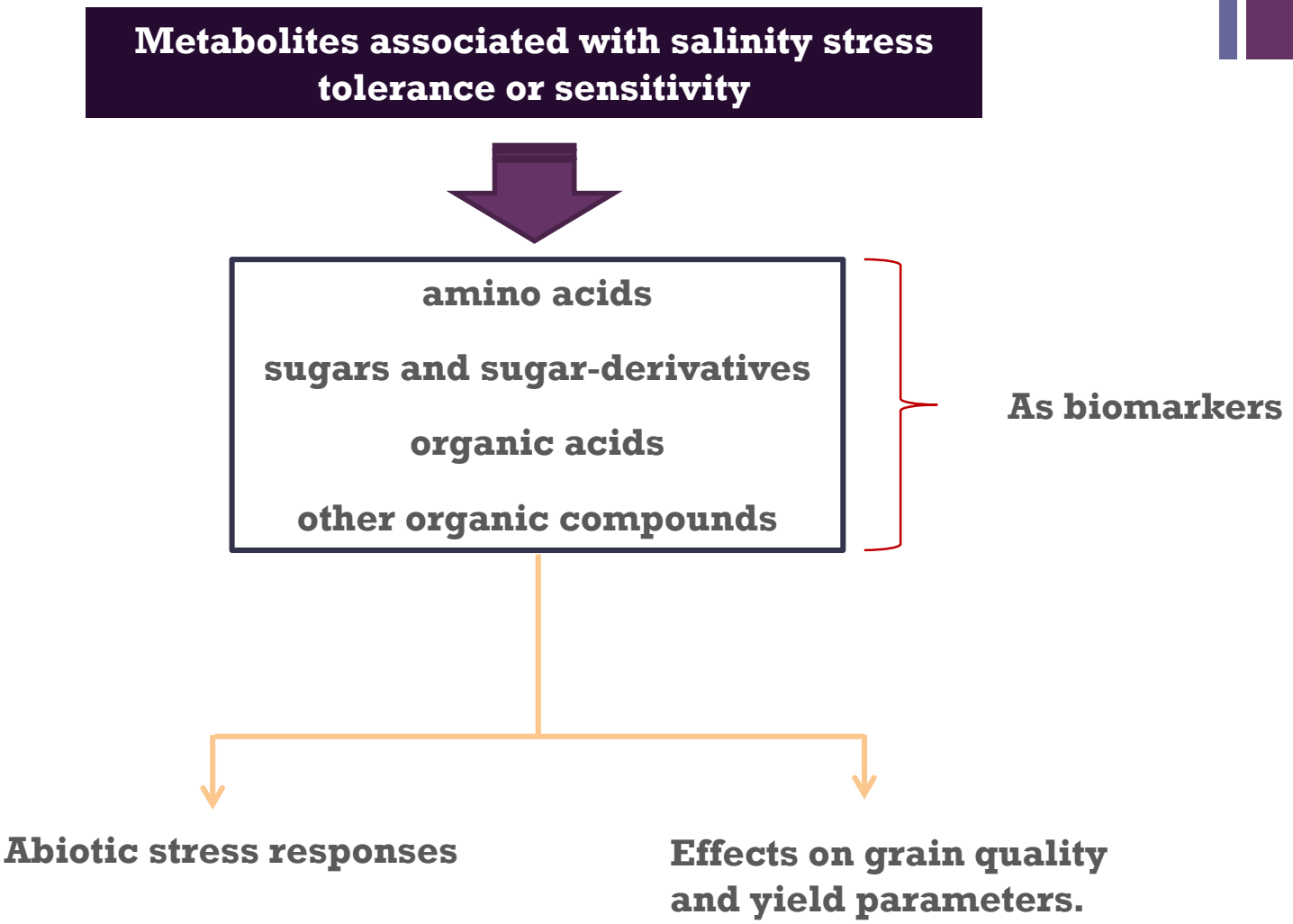
Figure 9 : Volcano plot of grain metabolomes of barley cultivars under salinity stress (n = 6). A. Scope. B. GrangeR Significant metabolites in control samples: orange circles 🟠 Significant metabolites in salt-stressed samples: green circles 🟢 .

- Under salinity stress, the metabolic profile of grains is greatly different when compared with control grain.
- Scope grain display 6 significant metabolites divided into 3 in control and 3 in salt stress, while GrangeR grain showed 20 significant metabolites divided into 5 in control and 15 in salt stress.
- Significant metabolite of GrangeR are higher than Scope, which could be because of it elevated GrangeR responses to salinity than Scope.

What are the key findings?

Let's brainstorm and write on the board!

Conclusion


Metabolites associated with salinity stress tolerance or sensitivity
amino acids
sugars and sugar-derivatives
organic acids
other organic compounds
As biomarkers
Abiotic stress responses
Effects on grain quality and yield parameters.

Is the experimental design/workflow suitable to prove the hypothesis?

Let's brainstorm!

Did the key findings address or prove the hypothesis?

Let's brainstorm!

What else can be done to further strengthen the work/study?

Let's brainstorm!

Q&A

ANY QUESTIONS?

15 mins to go…
Fill in the discussion assessment form!

- What is the main idea of the study?
- Summarize the findings and state whether the findings address the scientific and/or societal questions/problems.
- Do you like this article? Why?