Influence of salinity exposure on wheat root metabolism in the context of tissue tolerance and susceptibility

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Background

- Salinity is responsible for large yield losses of wide variety of crops.
- Wheat is one of major staple food crop, but it is particularly salt sensitive and its production is significantly affected by soil salinity.
- Salinity stress causes adverse effects on plant growth and development at both physiological and biochemical levels.
- Within the plant, root tissues are the most vulnerable to salinity as they are directly exposed to salt in the soil.
- To date, a number of studies have focused on plant metabolic responses to salinity in above ground tissues, however there is surprisingly little known about the metabolic responses of wheat roots exposed to salt.
- This project uses high throughput mass spectrometry based metabolomics and proteomic approaches to elucidate the metabolic and proteomic changes in wheat roots under salinity.
- Overall, this research will improve fundamental knowledge of metabolic responses in salt stressed wheat with a focus on root physiology.

Methodology

- Two wheat varieties Mocho (Mocho de Espiga Branca) and Gladius are currently being grown in glasshouse for bulking up seeds for future experiments.
- For proteomic and metabolomics experiments plants are grown in half strength Hoagland solution in a hydroponic system, salt stress is applied from 25 mM increments until it reaches final 150 mM NaCl concentration to prevent salt shock.
- Root tips are harvested after 3 days and 6 days of exposure to final salt stress.
- Root tip proteins are analysed post salinity treatment by MRM (Multiple Reaction Monitoring) Mass Spectrometry.
- Metabolome changes of root tips is analysed by GCMS.
- Ion analysis in root and shoot tissues are done using flame photometry and ICP-OES.

Roots tend to increase the accumulation of sugars and TCA cycle intermediates under salt stress

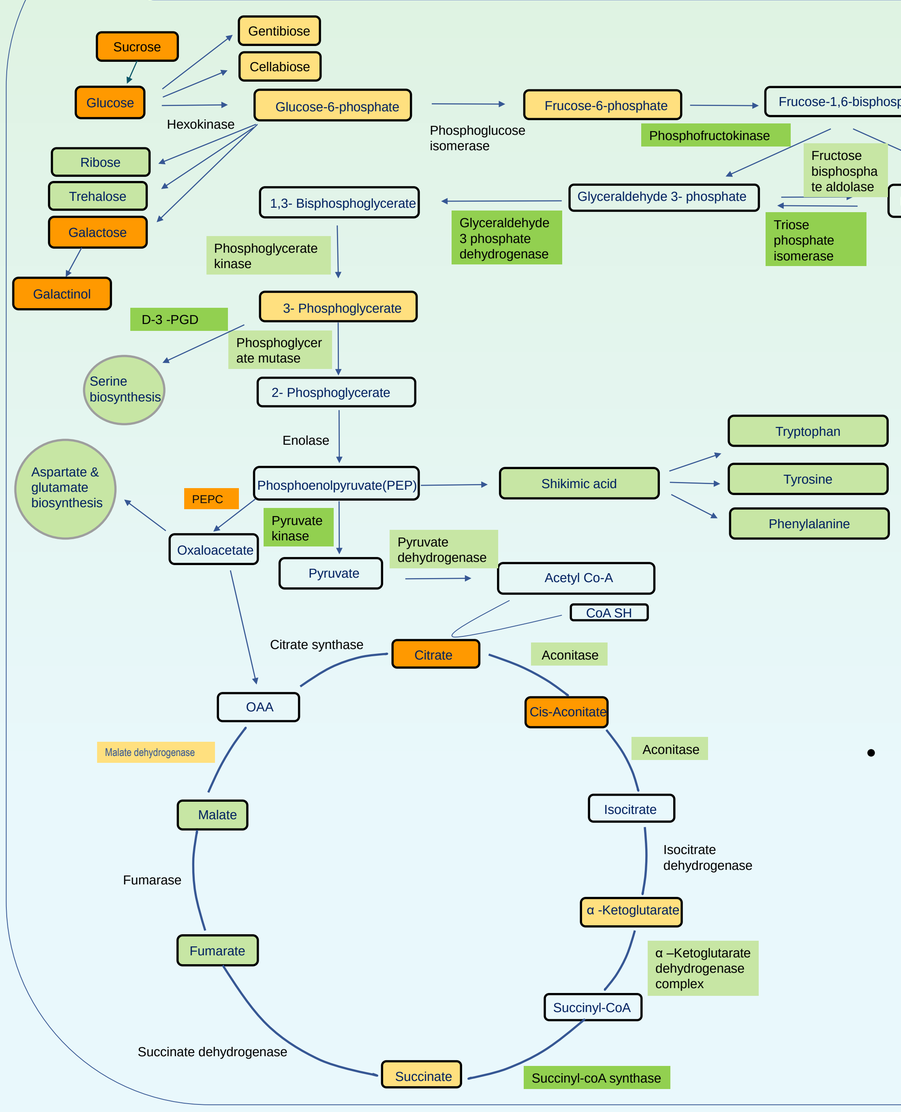

Fig 1. Schematic representation of the changes of metabolic pathways in roots after 3 days of exposure to 150 mM salt stress conditions (Proteins and metabolites in dark green represents significant downregulation in salt treated roots compared to controls ($p<0.05$, orange means significant up-regulation ($p<0.05$), light green means downregulation and light orange represents upregulation

- Roots tend to enhance the accumulation of sugars and some of the TCA cycle intermediates, but glycolysis and amino acid synthesis was inhibited under salt stress conditions.

Conclusion and future directions

- The results suggests the importance of accumulation of sugars and energy synthesis in roots to cope with the salt stress conditions.
- Sugars and organic acids could be acting as the potential components of maintaining osmotic potential within the roots under salt stress.
- A time course study of the levels of proteins and metabolites involved in the central carbon metabolism is needed to provide to provide broader understanding on molecular mechanisms operating under salt stress in roots.

Acknowledgements

Bhagya Dissanayake is a PhD candidate and a recipient of the GRDC – Plant Energy Biology Scholarship.

Salt stress significantly hampers the growth of wheat at both tissue and whole plant level

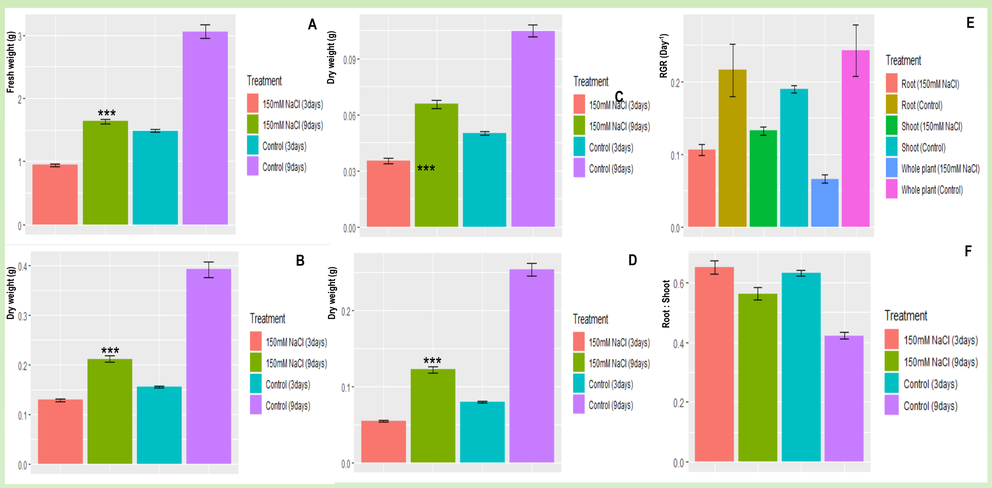

Fig 1. Whole Plant fresh weight (A), Whole Plant dry weight (B), Shoot dry weight (C), Root dry weight (D), Relative growth rate (E), Root to shoot ratio of control and salt treated plants (F), (n=4,*** $p<0.001$)

- Scepter showed significant reduction in fresh weight and dry weight at the whole plant level and organ level (roots and shoots) after 3 days of treatment with 150 mM NaCl.
- The relative growth rate was reduced at both tissue and whole plant level.
- An increase of root : shoot ratio was observed after 3 and 9 days of 150 mM NaCl treatment (Fig 1F), which is considered to be a common response of plants increase the proportion of roots in order to accumulate more Na^+ ions within the root level.

External addition of GABA overcomes the hampering of the root respiratory rate under salt stress and salt shock treatments

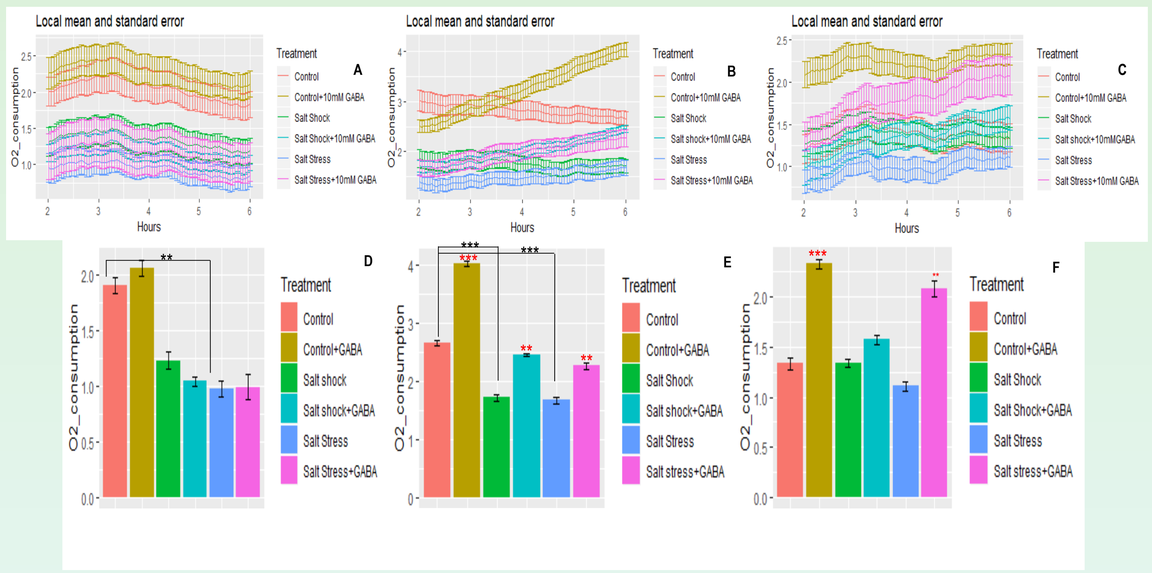

Fig 2. Rate of O_2 consumption of roots after 3 days (A), 6 days (B), and 9 days of treatments (C), O_2 consumption of roots at 6h of measurement at 3 days (D), 6 days (E) and 9 days (F) of treatments, (n=4, $p<$**0.05, ***$p<0.001$).

- The rate of O_2 consumption of salt stressed and salt shocked roots are lower than that of the control
- Treatment with GABA can significantly increase the O_2 consumption rate of control, salt stressed and salt shocked roots
- The results indicates that that capacity of roots to use GABA under salt stress to drive respiration increases with salt exposure

Under salt stress wheat tends to accumulates more Na^+ within the root level?

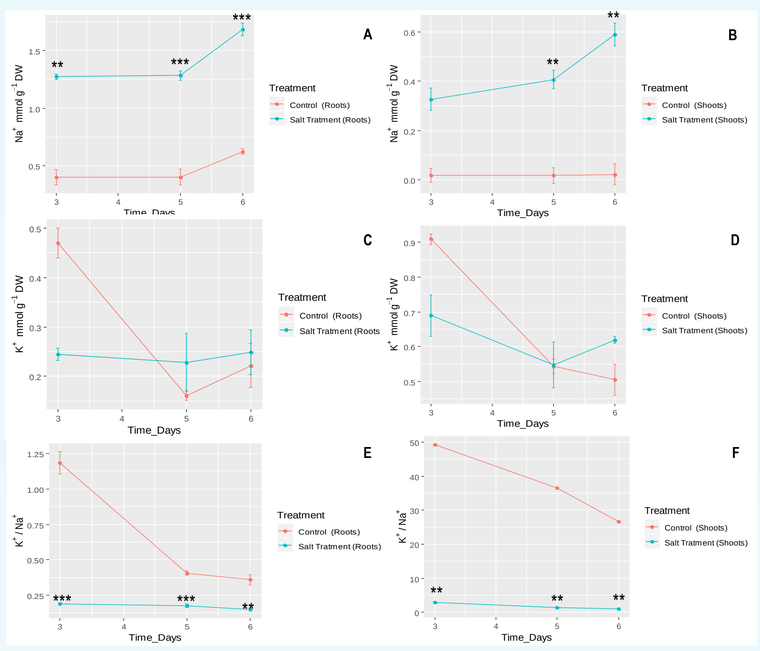

Fig 3. Amount of Na^+ in roots after 3, 5 and 6 days of 150mM NaCl (A), Amount of Na^+ in shoots after 3, 5 and 6 days of 150mM NaCl (B), Amount of K^+ in roots after 3, 5 and 6 days of 150mM NaCl (C), Amount of K^+ in shoots after 3, 5 and 6 days of 150mM NaCl (D), K^+/Na^+ in roots after 3, 5 and 6 days of 150mM NaCl (E), K^+/Na^+ in roots after 3, 5 and 6 days of 150 mM NaCl (F), (n=4, $p<$**0.05, ***$p<0.001$).

- Significant increase of Na^+ was observed in both roots and shoots after 3, 5 and 6 days of 150 mM NaCl treatment.
- Salt treated roots have lower K^+ content than controls at day 3, but maintains K^+ stably over controls in day 5 and day 6.
- Having high K^+/Na^+ ratio shoots perform better than roots under salt stress