Engineering Salt Tolerance in Plants

Nathan Ramsay (UG) | 140170850; N.Ramsay1@ncl.ac.uk | Biology BSc Honours (C100)
School of Biology
Supervised by: Dr. Tahar Taybi (PhD, MSc, BSc)
Assisted by: Lilian Izuegbunam (MSc, BSc); Tracey Stevenson (MSc, BSc)

Aims

- Ultimately, to develop a new approach of engineering salt tolerance in plants by modifying their DNA
- To successfully transfer a particular segment of DNA (a gene) from a salt-tolerant plant to a salt-sensitive plant
- To grow the newly transformed plants and compare them, at both the genetic level and physiological level, to the untransformed plants to see if the transformation was successful

Introduction & Background

It is estimated that **food production must increase by 70%** in the next 40 years[1] in order to adequately sustain the projected human population.

By the year 2050, there will be over **2.3 billion more mouths to feed**[2] than there are today.

However, **$27 billion dollars**[3] worth of crops are lost each year due to the adverse effects of salinity (high salt levels).

This is a result of over 20% of global farmland being contaminated with high levels of salt. **This is an area the size of France** and is set to increase even further in the forthcoming decades.

While some plants are naturally resistant to salt-stress and can grow under high levels of salinity, others, including many crop plants, **die quickly when exposed to high salt levels**.

Extensive work has been carried out over the past 20 years to help understand how and why these plants are able to not only survive, but **thrive in highly saline environments.**

The key mechanisms behind salt-tolerance are now clear, and it is also clear that these mechanisms are expressed (but regulated differently) by both salt-tolerant and salt-sensitive plants.

What is not clear, however, are the reasons behind the differential expressions in different plants.

In this project, salt-sensitive plants (*Arabidopsis thaliana),* which had been transformed with DNA from salt-tolerant plants (*Thellungiella salsuginea*), were grown and studied in order to determine if salt tolerance can be engineered in salt-intolerant plants.

Materials & Methods

- Wild type *A. thaliana* transformed with P5CS1 gene (from *T. salsuginea*) by inoculating with bacteria containing the plasmid (circular DNA)
- P5CS1 is an important gene, in both *A. thaliana* and *T. salsuginea* (**Fig. 1**), involved in salt-tolerance by regulating production of proline (a solute which regulates water loss/uptake)
- Plasmid also contained gene for kanamycin (antibiotic) resistance
- Plants grown; seeds harvested
- Next generation plated on agar containing kanamycin
- Seedlings grown in tubes; transformation confirmed via DNA isolation and extraction, amplification via PCR and visualization via gel electrophoresis

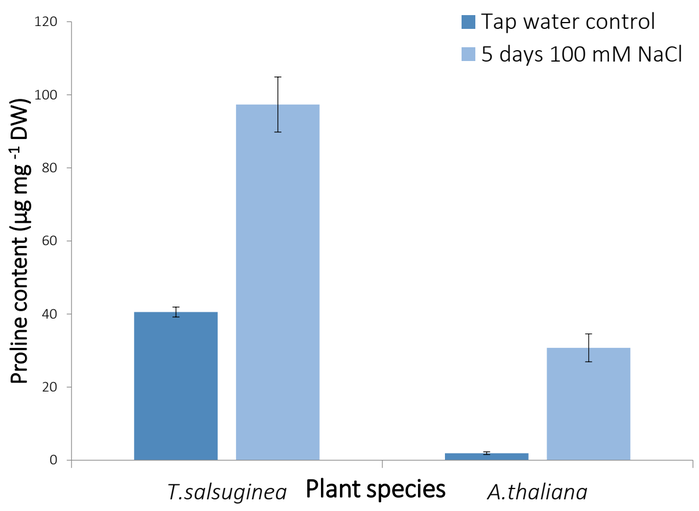

Fig. 1 Proline content in shoots of *A. thaliana* and *T. salsuginea* after either 5 days of tap water or salt-treatment with 100 mM NaCl. Both plants produced proline in response to salt-stress, but *T. salsuginea* produced more and could therefore tolerate salt better.

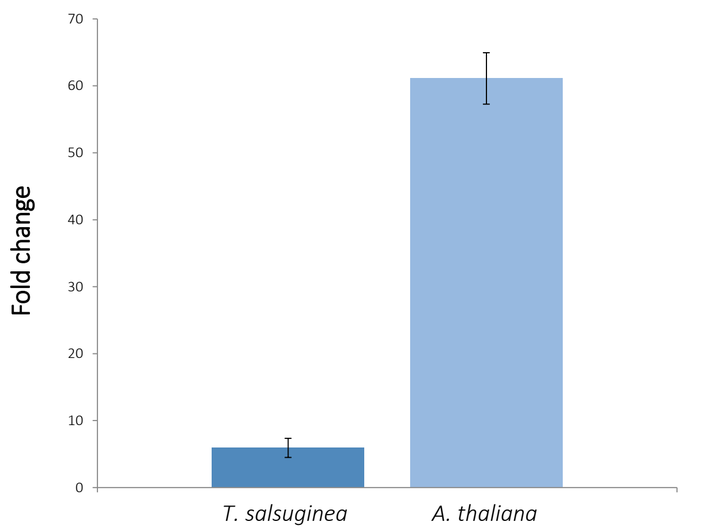

Fig. 2 Relative quantification of *P5CS1* transcript fold change in *T. salsuginea* and *A. thaliana* after 5 days treatment with 100 mM NaCl. Each point is the fold change comparatively to unstressed control plants. Despite a bigger fold-change in proline, *A. thaliana* does not produce enough initially to be able to tolerate salt-stress.

Results & Discussion

- The recipient plant was shown to have taken up and expressed the P5CS1 gene
- The expression of the transgene should have increased the capacity to resist salt-stress in the transformed plant
- The fact that the transformed seedlings grew and survived on agar containing kanamycin (**Fig. 3)** was an early clue that the transformation was successful

Fig. 3A Ten-day old transgenic *A. thaliana* seedlings on an agar plate containing kanamycin.

Fig. 3B Five-week old transgenic *A. thaliana* seedlings growing in a glass tube on agar containing 50 µg ml^{-1} kanamycin.

Although we did not have time to test the transformed plants' capacity to resist salt-stress (they took too long to mature), it is well established that proline accumulation is an important mechanism for resisting salt-stress[4] and plants expressing the P5CS1 gene have an increased capacity to do so.[5]

The next step would be to engage these methods on crop plants that are salt-sensitive. This would probably be more difficult than the work performed here: the plants used were closely related, and so successful transfer of genetic material between the two is, in comparison, easier to achieve.

Nonetheless, the results achieved here are a positive step in the right direction in terms of engineering salt-tolerance in plants.

Conclusions

- The P5CS1 gene was successfully transferred from a salt-tolerant plant to a salt-sensitive plant
- The gene was shown to have been expressed in the recipient plant through a variety of molecular and biochemical techniques, allowing comparison to the donor plant
- A new approach of engineering salt tolerance in plants by modifying their DNA was developed

References

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