

Salt levels in the soil are increasing for many reasons but include increasing drought reducing the levels of terrestrial water. This causes problems for crops, which are nearly all sensitive to salt, and also for wild plant species. To begin to characterise this, we will use the non-cereal plant genus, *Brachypodium*. Three species of this genus, *B. distachyon*, *B. stacei* and *B. hybridum* are important grass models, and representative lines of each have been genome sequenced. *B. hybridum* is thought to have arisen from the fusion of *B. distachyon* and *B. stacei* in a process called “allotetraploidisation”. In this project, we will use *Brachypodium* to describe how grasses, and therefore cereals which are domesticated grasses, can be tolerant to salt. It will also test if these mechanisms of salt tolerances differ within and between different wild *Brachypodium* species. To investigate these questions, the experiments are split into two work packages (WP). In WP1, we will examine the responses to salt in 12 established lines covering each species, with a particular focus on possible mechanisms of salt tolerance. The cell wall surrounding plant cells may play an important role in salt tolerance, but the exact mechanisms have not been fully established. We will characterise salt responses involving the cell wall and set these in the context of other known changes in ion channels, which transport salt around the plant and osmotic changes, which maintain the turgidity and function of the cell. We will also focus on roots as these are the first organs to experience salt stress. Our first experiments will assess the responses of the reference lines of each species to various concentrations of salt and also if salt is persistently applied (“salt stress”) or briefly applied as a “salt shock”. The responses to salt will be characterised microscopically and also by assessing the expression of all genes using transcriptomic approaches based on the “RNA-seq” technique. Changes in the cell wall will be measured by isolating the cell walls and determining changes in proteins that provide important functions. Other changes in the cell wall will be viewed *in situ* using labelled antibodies that target such components as pectins, arabinogalactan proteins (AGP), extensins and hemicelluloses. Together, these experiments will provide “molecular landmarks” for salt responses in *Brachypodium* that could be related to tolerance and may differ within and between species. To further characterise the role of important cell wall components, we will use existing mutants in fasciclin-like AGPs (FLA) and pectin methylesterases (PME) and generate new lines that over-express FLA and PME. These lines will be assessed for salt stress and shock responses and could unequivocally show the importance of these cell wall components. In WP2, we will explore how the salt landmarks can vary amongst the natural populations of each species. To do this, new collections of each species will be established based on areas with high and low salt in Southern Spain. The salt levels in the soil of the exact sampling site will be determined. The collections will also allow us to explore the hypothesis that salt tolerance could be a feature of the fitter *B. hybridum* species and could have influenced its speciation. It is expected that ~150 will be established, representing each species and site with different salt levels. The genetic diversity of the line will be characterised using 18 genetic markers known as simple sequence repeats to reveal intra- and interspecies variation. The responses to salt shock and stress will be assessed and compared. Subsequently, variation in the display of the salt responsive landmarks will be investigated in a representative subset (~60) of accessions. Genetic and salt responsive landmark variation will be related to ecological origins and the salt levels at the original sampling site. This innovative programme will yield data that will have implications for cereal breeding programmes and how variation in responses to salt can affect population structure.