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**Title:** Developing Phenotypic and Genomic Tools to Study Salt-Tolerance in Seashore Paspalum

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**Summary Text:**

Seashore paspalum is becoming a prominent turfgrass for salt affected areas. A driving force in the development of paspalum has been the set of resources generated by breeders such as the world- wide germplasm collection gathered by Dr. Ron Duncan. To maintain this rapid pace of development, we seek to build upon and update these resources in three key ways.

First, while the current germplasm collection is widespread geographically, it may not represent the full breadth of phenotypic and genotypic variation present in the species due to its focus on golf courses and “turf-type” ecotypes. To increase available diversity, we have collected 20 phenotypically diverse individuals from wild populations during a June 2016 collection trip along the southeast coast of the United States (Image 1). Over the next year, we plan to extend our collection efforts both within the US and internationally.

Second, we are developing genomic resources for seashore paspalum. An early draft of the seashore paspalum reference genome has been completed and made available to us. As a pilot study we have resequenced the genomes of six genetically diverse accessions available from the USDA GRIN collection, and have mapped our new sequences to the reference genome sequence for SNP calling and further downstream analyses. These bioinformatic steps are currently ongoing, but preliminary observations suggest that read quality is high and that there is sufficient nucleotide diversity for downstream analyses including population genetics and marker-assisted breeding. A long-term goal is to expand our sequencing efforts to more accessions to identify genomic regions that may contain genes relevant for turfgrass improvement. These sequences and results will be of immense and immediate use to breeders attempting quantitative genetics studies in this system.

Third, as sequencing costs continue to plummet, phenotyping methods are quickly becoming the bottleneck for large-scale quantitative genetics studies. With this in mind we are developing a high- throughput system for scoring salt-tolerance and other phenotypes in seashore paspalum. We combine measurements of the concentration of 20 ions (“Ionomics”) with analysis of images from plants grown at varying salinity levels (Image 2). We have completed a small pilot project of this method in which we collected tissue from each plant for ionomics analysis on two separate weeks. The macronutrients (Na, K, Ca, Mg) show large treatment effects (Figure 1), but genotypic differences remain unclear among the small sample of accessions studied.

We see potential genotypic variation in tissue Na<sup>+</sup> concentration in the second week of our middle salt treatment (EC<sub>w</sub> = 30); however, whether this is due to genotypic differences or chance is uncertain. In contrast, we notice strong genotypic differences in heavy metal accumulation that are consistent across treatments and time points (Figure 2). The patterns of accumulation are also consistent across heavy metal ions (i.e., genotypes that have high Cd

concentrations tend to also have high Co, Zn, and Cu concentrations). Image analysis is ongoing. A second pilot is currently underway using a modified methodology aimed to improve our ability to detect genotypic differences in salt tolerance.

By increasing diversity in the germplasm collection, improving the genomic resources for the species, and developing high throughput phenotyping methodologies, we have taken the first vital steps toward developing seashore paspalum as a tractable system for large-scale genetic studies.

### Summary Points:

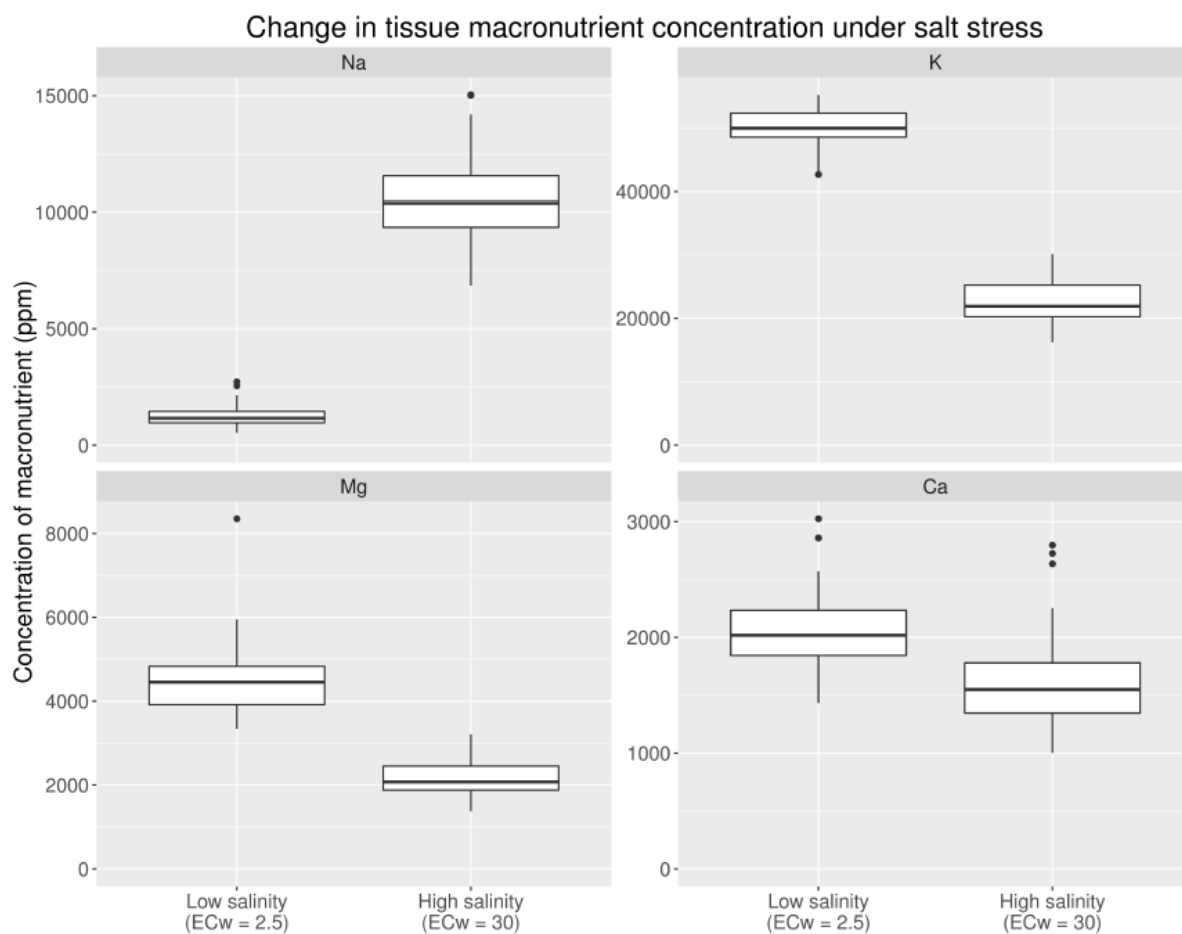
- We have added 20 wild accessions to our collection with further collection trips planned.
- We performed whole genome re-sequencing on six accessions at 30x coverage. Bioinformatic analysis is ongoing. Preliminary results indicate abundant nucleotide diversity for further analysis. More accessions will be sequenced in the future.
- A high throughput phenotyping system is in development. A second pilot with improved methods is underway.
- Ionomics analyses suggest that salt tolerance is heavily influenced by the environment (treatment) for this small set of accessions.
- Heavy metal accumulation appears to be under genetic control and to vary among genotypes.



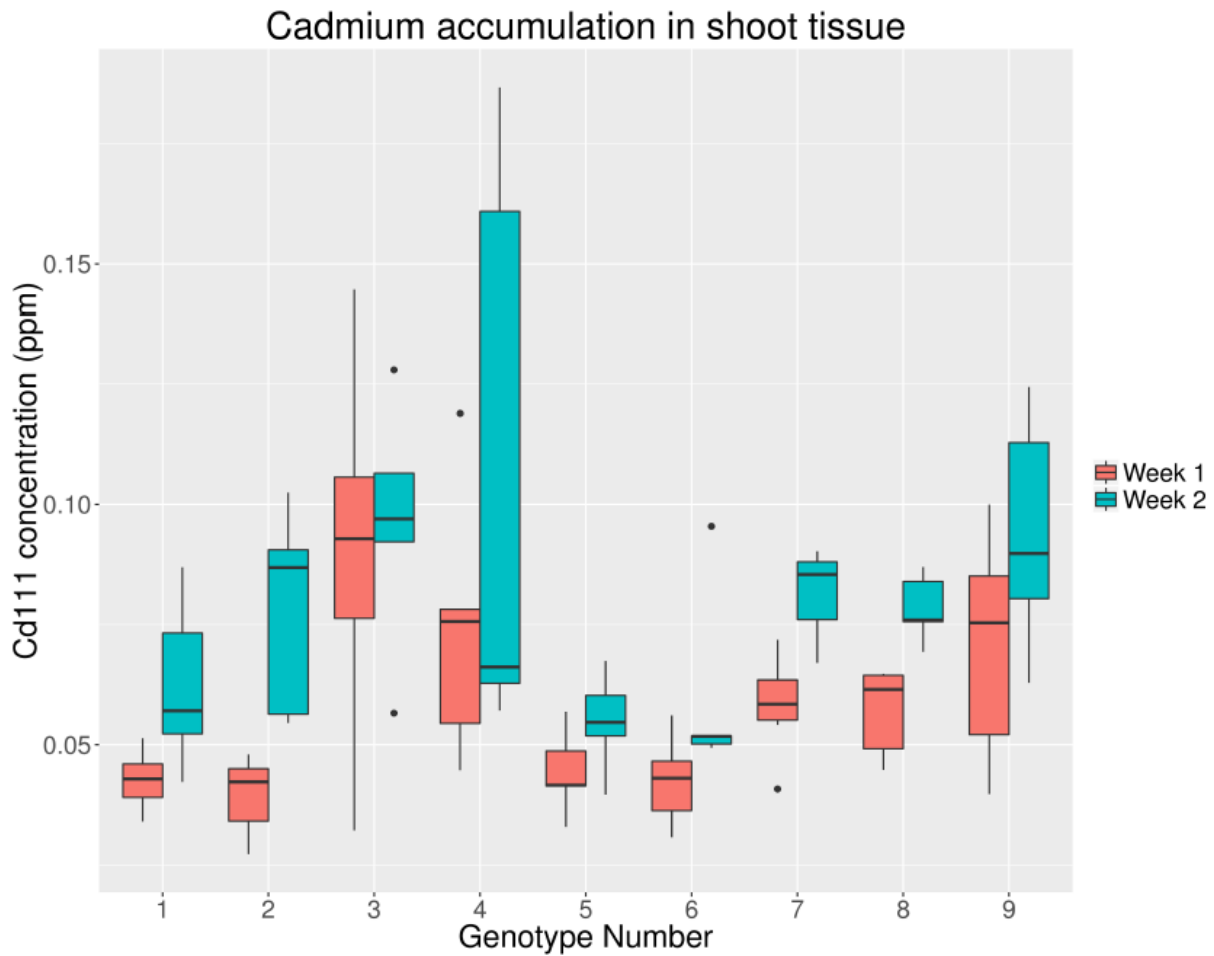
**Image 1.** A patch of wild seashore paspalum in Georgia. Individuals were collected from this site and others like it along the coast of the South Eastern United States during our June 2016 collection trip.



**Image 2.** Seashore paspalum accessions being subjected to three salinity treatments in the first pilot of our phenotyping methods.



**Figure 1.** Changes in macronutrient concentration when plants are subjected to salt stress. Data shown are from the first week of tissue collection.



**Figure 2.** Differences in Cadmium accumulation between genotypes. Tissue was collected from plants in the low salt ( $EC_w = 2.5$ ) treatment on two occasions. A similar pattern is seen in the concentration of other heavy metals.