

### **RNA sequencing approach to identifying genes associated with aluminum toxicity tolerance in cowpea (*Vigna unguiculata* L. Walp.)**

Cowpea is an excellent crop to grow on marginal lands prevalent throughout the southeast, but a thorough understanding of its ability to tolerate aluminum toxicity is lacking. This study characterized the transcriptome of two cowpea varieties that exhibit significant differences in aluminum toxicity tolerance using RNA sequencing. The transcriptomes of the tolerant Mississippi Pinkeye 2 Purple Hull (MSP2PH) and sensitive White Acre (WA) were evaluated 6, 24, and 48 hours after treatment with 50 $\mu$ M AlCl<sub>3</sub>. Total RNA quality was high with all RNA integrity number (RIN) scores of all samples being above 9.0, and the total number of reads from each sample ranged from about 20,000,000 – 60,000,000. The transcript profiles of the samples clustered by variety, treatment, and time point after principal component analysis and hierarchical clustering indicate high sample consistency. A moderately strict threshold of 1 log-fold change (logFC) and a false-positive discovery rate of  $p \leq 0.01$  was used to identify differentially expressed genes (DEGs) for all comparisons. The total number of DEGs between plants of the same variety under different conditions at 6, 24, and 48 hours after treatment was 1806, 2067, and 1111 for MSP2PH and 2344, 2577, and 1918 for WA. The number of DEGs unique to the aluminum-toxic conditions that were not differentially expressed under control conditions between MSP2PH and WA at the 6, 24, and 48 hour time points were 390, 607, and 633, respectively. Additionally, 60 of these DEGs were identified across all three time points, and a total of 14 from this subset were up-regulated in MSP2PH. These 14 DEGs, which include trans-membrane and metal-binding proteins known for their involvement in metal toxicity tolerance in other species, are good candidate genes for future characterization and offer a deeper insight into the mechanisms of aluminum toxicity tolerance in cowpea.