

**Phenotypic Responses and Gene Expression of Southern Pea (*Vigna unguiculata* L. Walp.) to Aluminum Toxicity.**

Aluminum toxicity is a limiting factor to cowpea production in acidic soils across the world. This study characterized the response of cowpea roots to aluminum-toxic conditions using a high-throughput phenotyping method. A total of 22 cultivars of commercially available cowpea from the United States were screened under hydroponic conditions using two nutrient solutions (0.5mM  $\text{CaCl}_2$  with 0 or 50 $\mu\text{M}$   $\text{AlCl}_3$  at pH 4.3) and data was collected for seven root traits: primary root growth rate, primary root length, total root length, number of root tips, root surface area, root volume, and average diameter. The experiment was repeated four times in a climate-controlled greenhouse at the Tennessee State University Research and Extension Center in Nashville, Tennessee. The primary indication for aluminum toxicity tolerance for each genotype was assessed using relative primary root growth rate, as this characteristic is used as the standard for identifying aluminum toxicity tolerant varieties across many species. Other traits were also considered especially in relation to relative primary root growth rate. Top Pick Brown Crowder and Mississippi Pinkeye 2 Purple Hull showed the highest level of aluminum toxicity tolerance, while White Acre and Iron and Clay showed the lowest levels of tolerance. Mississippi Pinkeye 2 Purple Hull and White Acre were chosen for RNA sequencing at 6, 24, and 48 hours after treatment due to their significantly different relative primary root growth rates. Quality control was performed using FastQC software before and after trimming of low-quality reads, adapter sequences, and polyA/T tail sequences. HiSAT2 and bowtie2 were used to align clean reads and rRNA to the reference genome, respectively, and BUSCO was used to evaluate alignment quality. Principal component analysis was also performed for the evaluated traits, and broad-sense heritability for all traits was calculated. All traits had heritability values of at least 0.5. Two traits, relative primary root length and relative number of root tips, showed significant correlations with primary root growth rate. Causal genes identified in this experiment may be used in the future for varietal improvement of aluminum toxicity tolerance, while validation in the field would be another step to confirm the effectiveness of these genes.