Induction of Root Proteins in Near Isogenic Sorghum Lines Tolerant and Susceptible To Toxic Levels of Exchangeable Aluminum

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The soils of the acid savannas or "Cerrado" of Brazil are commonly characterized by low pH, low phosphorus availability, high P fixation, low fertility, and toxic aluminum. Plant cultivars with tolerance to Al toxicity are essential for sustainable production in these acid savannas. The development of improved cultivars for these conditions is dependent on adaptive mechanisms genetically transmitted. These adaptive mechanisms are related to factors that impede the entrance of toxic Al into the root cells and the interaction of Al with polypeptide root exudates. The objective of this research was to identify proteins in the root tips induced as a result of Al stress. Seeds of a near-isogenic pair of sorghum lines were germinated for three days in water and placed in a nutrient solution with zero and 60 µM Al for 96 hours. Root tips (1.5 mm) were excised, ground with 6.8 pH buffer extraction solution in a 1:1 proportion and centrifuged at 120,000g. The pellet was resuspended in sample buffer. The material was run on an SDS-PAGE gel electrophoresis. The results indicate formation of a protein band at approximately 95 KD in the root tips with Al stress. The band was not observed in the root tips without Al stress. These results indicated that proteins are induced in the microsomal membrane fraction of the root tip under Al stress. Preliminary results indicated that the Al-tolerant line produced a larger quantity of this protein and may be a factor contributing to Al tolerance.

Regeneration of Plants from and Transient Gene Expression in Mesophyll Protoplasts of Sorghum

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The development of efficient and reproducible techniques for regeneration of fertile plants from protoplasts opens up opportunities for genetic transformation by direct DNA uptake. It also facilitates the production of somatic hybrids between sexually incompatible species. Xu and Wei (1993) reported success in regenerating plants from protoplasts isolated from the inflorescence-derived calli of two sorghum cultivars. However, the leaf is the most suitable source of plant protoplasts because it allows isolation of a large number of relatively uniform protoplasts without destroying the plant.

We have developed a protocol for regeneration of plants from mesophyll protoplasts of sorghum seed parent 296B. The sixth leaf (with ligule fully emerged) from 18-day old plants (grown in dark for 2 days before harvesting) proved to be the most suitable source of viable protoplasts. The protoplasts regenerated a cell wall within 24 hours of embedding in KM8 agarose medium. The first division was observed after 6 days after plating, and the second after 10 days. Microcolonies were visible after 15-20 days, which resulted in microcalli after 25-30 days. Plants were obtained after 4-5 weeks of culture of the microcalli on MS medium supplemented with 0.2 mg l^{-1} kinetin and 2 mg l^{-1}