DEVELOPMENT OF SALT TOLERANT CULTIVARS IN *Sorghum bicolor* (L) Moench BY TISSUE CULTURE METHOD.

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ABSTRACT

Sorghum bicolor (L) Moench is generally sensitive to salt and acid (high aluminium) soil stresses. As with any stress phenomenon, intra-specific variability exists within the genus. *In Vitro* cell selection and somaclonal variation offer an alternative to traditional breeding methodology for generating improved breeeding lines for hybrid development. Tissue culture method was developed for induction and maintenance of embryogenic calli established from young embryo explants of three *Sorghum bicolor* cultivars namely Mtama 1, el Gardam and Seredo. Embryogenic calli were obtained by culturing young embryos on Linsmaier and Skoog's (LS) medium containing 2mg/l 2,4 dichlorophenoxyacetic acid (2,4D) and 0.5 mg/l Kinetin. Calli were subjected to 50 mM, 100 mM, 150 mM and 200 mM NaCl to screen them for salinity tolerance. Calli subjected to 0.0 mM NaCl served as controls. Calli with a tolerance to salinity stress had a higher activity of succinate dehydrogenase which reduced trimethyl tetrazolium chloride (TTC) to formazon. The amount of formazon was measured spectrophotometrically and a graph of NaCl concentration against absorbance was plotted. From the TTC test (viability test) 100 mM NaCl was selected as the optimum concentration which was incorporated into LS media to initiate tolerance in sorghum calli.

Sorghum shoots were regenerated from embryogenic calli of both NaCl treated and the controls cultured on LS medium supplemented with 1.0 mg/l Indole Acetic Acid (IAA) and 0.5 mg/l benzyl adenine (BA). Rooting was achieved by supplementing LS medium with 3 mg/l Indole Butyric Acid (IBA).

By using Random amplified polymorphic DNA (RAPDs) technique it was established that there was variation between NaCl treated plants and the controls. This was both in the individual and pooled DNA samples. A genetic distant matrix was calculated and used to construct a dendrograme as a measure of relatedness which led to the conclusion that somaclonal intra cultivar variation had resulted in some of the cell lines becoming tolerant to salinity.

To establish whether salinity tolerance had been achieved in plants regenerated from NaCl treated calli some parameters conforming to C_4 photosynthetic pathway were measured. These included CO_2 assimilation rate, titratable acidity, malate content and water potential. CO_2 assimilation rate was measured along with Photosynthetic Active Radiation (PAR), stomata conductance (SC) and Transpiration Rate (TR) using infra red gas analyser (IRGA). The CO_2 assimilation rate was synchronized with SC and TR. The CO_2 assimilation rate started increasing in the morning reached a peak at noon and decreased as the afternoon progressed following

decrease in PAR. This pattern was observed in all the 3 cultivars throughout the sampling period. The CO_2 assimilation rate in the controls followed the same pattern except the mean values were significantly lower ($P \leq 0.05$).

The treated plants accumulated significantly ($P \leq 0.05$) higher levels of titratable acidity and malate than the controls in all the 3 cultivars. The two parameters had a day light pattern of increasing in the morning reached a peak at noon in parallel with PAR and CO_2 assimilation rate and decreased as the afternoon progressed.

High positive regression correlations ($r^2 = 0.7$ and above) existed between titratable acidity and malate content; malate and CO_2 assimilation rate ($r^2 = 0.6$ and above) illustrating that these parameters were interdependent.

Water potential (ψ) was significantly lower ($P \leq 0.05$) in the treated plants than in the controls. Although low water potential indicated physiological drought in the treated plants, they did not show decline in CO_2 assimilation rate which accounted for high TR and SC at high PAR. This might have indicated that they had become salinity tolerant.

No change in ploidy level was achieved by intercultivar or intracultivar protoplast fusion because internuclear fusion failed due to nuclei repulsion. However, cytoplasmic hybrids (cybrids) were obtained but no plants were regenerated from the cybrids due to failure to resynthesize cytoskeletons.

Phosphoenol phyruvate carboxylase (PEPC) enzyme was successfully purified by ammonium sulphate precipitation and DEAE - sepharose 6B gel filtration using sorghum and Kalanchoe extracts. It showed two subunits of molecular weight 95 kDa and 50 kDa common in both sorghum and Kalanchoe. The enzyme activity amongst the 3 sorghum cultivars; between the treated and controls, between sorghum and Kalanchoe was not significant. This might have indicated that the enzyme from the leaves of the two plant species was similar and therefore, had same properties. Therefore, detection of polymorphism using PEPC protein as a marker in measurement of relatedness was not successful.