

***In Vitro* Selection for Water Stress and Salinity Tolerance in Two Kenyan maize (*Zea
mays* L.) Genotypes**

Matheka Mutie Jonathan

**A thesis submitted in partial fulfilment of the requirements for the award of the
Degree of Master of Science (Biochemistry) in Jomo Kenyatta University of
Agriculture and Technology.**

2007

ABSTRACT

In vitro cell selection is widely used to obtain salinity and drought tolerance in cereals, including maize. The objective of this study was to obtain tolerance to salt and water stress in a local dryland hybrid (PH01) and an open pollinated variety (KAT) by applying *in vitro* selection and regeneration procedures. Embryogenic calli were initiated from immature zygotic embryos of KAT and PH01 R1 plants. The growth of calli decreased as the concentration of NaCl, PEG or mannitol increased in the callus induction media (CIM). After growing on CIM for 3 months to induce somaclonal variations, calli were screened for tolerance to salt or osmotic stress for 4 ½ months using a stepwise selection procedure. In this procedure calli were exposed to NaCl (0, 0.6, 0.75%), PEG (0, 18, 20%) or mannitol (0, 3.28, 5.28%). Selected calli had consistently lower values of all the recorded parameters compared to unselected (control) calli. However, survival and regeneration increased as salt or water stress increased in culture. After four and half months in selection, 52% of KAT and 62.78% of PH01 calli exposed to 0.75% NaCl survived and of these 40% KAT and 94% PH01 regenerated shoots. Salt selected PH01 regenerated shoots at a higher frequency (53%) than KAT (22%). Out of the calli treated to 20% PEG, 64% PH01 and 45% KAT survived, and of these 74% PH01 and 22% KAT regenerated shoots. PEG-selected PH01 calli regenerated shoots at a frequency of 13%. Of the calli selected on 5.28% mannitol, 30% PH01 and 29 % KAT survived, and of these 54% PH01 and 17% KAT regenerated shoots with a shoot regeneration frequency of 53 and 4% respectively. 5.28% mannitol selected calli exposed to 7.28% mannitol had a survival and regeneration capacity of 100%, and regenerated shoots at 100% frequency. None of the KAT shoots regenerated from calli exposed to 0.75% NaCl or 20% PEG survived the greenhouse conditions (hardening).

Putative tolerant plants surviving hardening process were transferred to soil in the screen house and were watered till maturity. Maturing plants were observed to have one or more variations in height, leaf surface appearance, and in male and female flower traits compared to control plants. The most common phenotypic aberrations in plants regenerated from stress-selected calli were dwarfism (85%) and lack of tassel (55%). Others include barren stalk (5%). But perhaps glossy, rolled and yellow striped leaves were the most interesting observations on plantlets derived from mannitol-selected cells. Despite these abnormalities, plants reached physiological maturity and set viable seeds.