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Progress in transformation and regeneration of tropical inbred maize lines in Kenya

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Tropical inbred maize lines have a reputation of being difficult to transform, mainly as a result of their inherent limitations associated with resistance to *Agrobacterium* infection and their recalcitrance to in vitro regeneration. To enhance the capacity for public sector maize transformation, the Plant Transformation Facility at Kenyatta University, Kenya, embarked on a program to improve transformation of diverse tropical inbred maize lines using *Agrobacterium tumefaciens*. We evaluated both N6 (Frame et al., Plant Physiol. 129:13-22, 2002) versus LS (Negrotto et al., Plant Cell Rep.19:798-803, 2000) media with different hormone regimes and optimized transformation and regeneration protocol for tropical inbred maize lines. Using immature embryos as explants, four Kenyan tropical inbred lines TL21, TL22, TL23 and TL18; two Sudanese inbred lines IL1, IL2 and CIMMYT inbred lines CML 216 and CML 244 have been investigated for their tenability to transformation and regeneration. Transformation frequencies (callus resistant events over total explants) and efficiencies (plantlet regenerating events over total explants) for the recovered events were used to evaluate successful transformation (Figures 1 and 2).

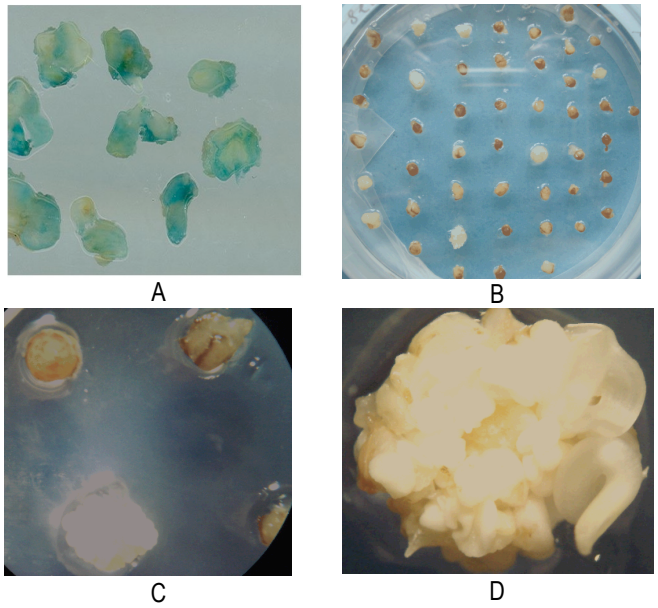

Figure 1. Selection and regeneration of transformed tropical maize inbred lines. A: Positive *Gus* staining calli after four weeks on selection II. The immature embryos were transformed with EHA101 *Agrobacterium* strain harboring pTF102 vector containing the p35S-GUS gene, described in Frame et al. (2002). B, C and D: Dying and surviving calli after four weeks on selection II medium (3mg/L Bialaphos). (For full color, see p. 33.)

Transformation frequency as high as 70% was recorded, transformation efficiency was lower and ranged between 20-30% for all the genotypes. There were no regenerants from non-transformed control plates.

The success in transformation and regeneration has led to the application of approaches to reduce dehydration stress in tropical inbred lines. A novel artificial miRNA (amiRNA) approach, as an alternative to RNAi, has been used to negatively regulate the ZmPARP1 gene in tropical maize genotypes, thereby enhancing energy use efficiency in the transgenic lines. As an adaptive mechanism to regulate growth in response to drought stress by integrating stimuli to alter transcriptional activity, one of the Elongator components has been overexpressed in tropical maize and is being tested for its ability to increase stress tolerance.

The same maize inbred lines are being considered for genetic upgrading to combat maize streak virus (MSV) disease. Several peptide aptamers have been isolated that specifically interact with viral replication proteins using yeast two hybrid assays. The best interacting peptide aptamer candidates are being evaluated in maize suspension cells to confirm interference with the function of the viral replication proteins. The aptamer genes will be introduced into the maize germplasm via *Agrobacterium tumefaciens*, and the transgenic lines evaluated for resistance to the disease.

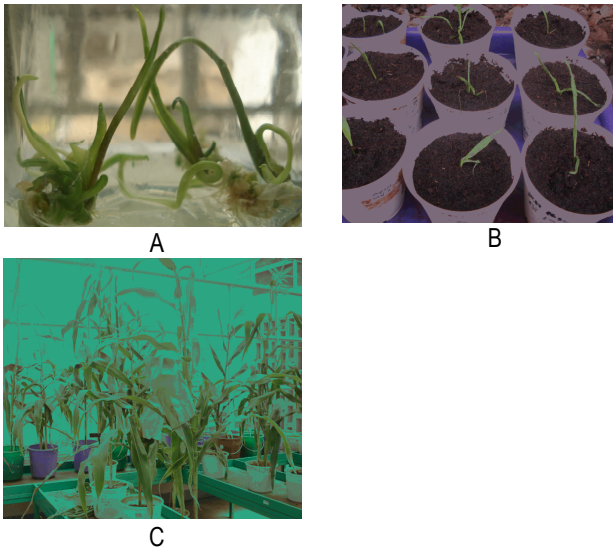

Figure 2. Putative transformed T1 tropical maize plantlets. A: Plantlets growing after one week in regeneration medium. B: Acclimatization of putatively transformed plantlets in the growth chamber. C: Selfing of T1 plantlets growing in the greenhouse. (For full color, see p. 33.)

In conclusion, the transformation of tropical inbred lines has progressed to a level at which agronomically useful genes can now be introduced into the tropical maize genome employing *Agrobacterium* as a vehicle of DNA delivery, suggesting a remarkable improvement in extending *Agrobacterium*-mediated transformation systems to elite cultivars of economically important tropical Kenyan maize. The application of this technology has the potential to significantly impact maize production systems that experience drought stress in Sub-Saharan Africa.