MAIZE TRUST REPORT DECEMBER 2005

DEVELOPMENT OF DROUGHT TOLERANT MAIZE BY GENETIC MODIFICATION

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1. *Agrobacterium*-mediated transformation

We have been using the "gene gun" to transform maize. However transformation using the bacterium *Agrobacterium tumefaciens* is more efficient and has recently become available in the public domain by researchers at Iowa State University for use in maize. We are therefore developing this technique in collaboration with Dr Jesse Machuka of Kenyatta University, via our joint PhD student, Richard Okoth, and Drs. Rachel Chikwamba and Blessed Okole of the CSIR in Pretoria. Jesse has transformed the maize line A188, one of the inbred parents of HiII, the readily transformable maize line. Both of these lines were those published by Iowa State University when they put their *Agrobacterium*-mediated transformation methodology into the public domain. However, Jesse has also transformed some local Kenyan lines developed i.a. by CIMMYT and KARI. I saw the transformed maize plants when I visited Jesse on 11th November 2005 and they looked very healthy. He has assayed the transgenic plants for the presence of the GUS reporter protein but will send material to our lab for us to do the Southern blots to confirm the transgenic nature of the plants. He is currently pollinating the putative transgenic plants to obtain seed for further testing (see below).

Dr Blessed Okole has supplied us with immature embryos of A188. We have transformed them with the constructs I will describe below. We have also derived callus tissue from the embryos and have also transformed this. These regenerants look, if anything, healthier than the regenerants from the immature embryos. As we cannot set maize seed in our UCT facilities I personally transported the putative transgenic plants to the CSIR on 25th November when I attended a meeting in Pretoria. Blessed and Rachel will oversee the pollination of these plants and then return the seed to UCT for further testing.

2. Constructs being used for *Agrobacterium*-mediated transformation at UCT

a) Promoters

It is essential to clone genes for drought tolerance downstream of stress-inducible promoters. That is to prevent the gene from being expressed throughout the life of the plant, causing growth problems. Therefore the promoter of the *XvSAP1* gene, encoding a stress-responsive signalling membrane protein (see below) has been fused to the luciferase marker gene. However, we have made two deletions to determine the minimum size of this drought-inducible promoter. We thus have three promoter-luciferase constructs that we will be testing in cell cultures of both black Mexican sweetcorn and HiII to determine relative efficiencies.

It is this (and other promoters still to be worked on) that is the subject of our patent with the Maize Trust. Rosemary Wohlson, UCT's IP expert is working on this patent and we will be forwarding it to the Maize Trust early in 2006.

Concurrently PhD student Richard Okoth and post-doctoral fellow Revel Iyer are fusing the three promoters to three genes of interest for *Agrobacterium*-mediated transformation.

b) Genes of interest being cloned downstream of the three promoter constructs

XvSAP1, isolated by PhD graduate Dahlia Garwe, codes for a stress-responsive signalling membrane protein that confers on transgenic tobacco and *Arabidopsis* tolerance to dehydration, salinity and high temperature

XvPrx2, isolated by PhD student Kershini Govender, codes for an antioxidant of the peroxiredoxin group of proteins. A general result of abiotic stress is oxidative stress. XvPrx2 belongs to a recently discovered group of peroxiredoxins viz. Type II peroxiredoxin. The protein has been demonstrated to be active against a wide range of reactive oxygen species, and we postulate it could alleviate oxidative stress.

XvAld1, being worked on by PhD student Alice Maredza, codes for an aldose reductase that converts glucose to the osmoprotectant, sorbitol. Transgenic *A. thaliana* plants show tolerance to dehydration and salinity.

3. Constructs to be used for *Agrobacterium*-mediated transformation at UCT and Kenyatta University

As soon as all the constructs are completed in early December 2005 Revel and our tissue culture technical officer, Marian Bezuidenhout, will transform them into A188. In addition the phenotypes will be determined in cell cultures of HiII and black Mexican sweetcorn.

In addition Richard will take them to Kenyatta University when he returns there in December 2005 for a six-month period, where he will transform them both into A188 and local Kenyan varieties. Thus we will have two concurrent, but complementary exercises, being carried out on *Agrobacterium*-mediated transformation of maize.

4. Future analysis of transgenic maize plants

As soon as we receive seed from the CSIR after fertilization of our putative transgenic plants some will be sent to Jesse and Richard for growing in the biosafety approved glasshouse facilities at Kenyatta University to test for tolerance to dehydration and other abiotic stresses. Some of the seeds will be used to establish T_2 generation plants for further testing both physiologically and at the molecular genetic level at UCT.

Other seeds will be retained at UCT for growth and analysis of the transgenic maize plants. This will involve in-depth physiological testing of dehydration responses as well as molecular genetic analyses. The former will include water efficiency use eg. stomatal conductance, photosynthesis efficiency, respiration and seed setting. The latter will include PCR, Southern, northern and western blot analyses, the last

requiring us to produce antibodies to XvPrx2 – we have antibodies to XvSap1 and XvAld1. Important molecular genetic aspects to be determined include transgene copy number and gene stability over generations, which will involve the CSIR and Kenyatta University producing T_2 and T_3 plants.

Glasshouse trials of the transgenic plants can be carried out at UCT, CSIR and Kenyatta University. A suitable site for field trials could be Pannar Seeds in KwaZulu-Natal as they have already performed field trials on transgenic maize imported from the USA. We have a close working relationship with them as they are our partners in the development of maize resistant to *Maize streak virus*. Pannar is currently applying to field test these plants.

Funding and project duration

The funding has been adequate for the work done to date and we expect to start field trials in 2008.