The Maize Trust Report – July 2006

Development of Drought Tolerant Maize by Genetic Modification

a) Project Leader: Prof Jennifer A Thomson

b) Actions Taken, Progress Made and Results Achieved:

In my project proposal I emphasised the importance of transforming maize using Agrobacterium instead of particle bombardment, and to introduce the genes for drought tolerance under the control of a stress-inducible promoter. To this end nine gene constructs have been made. We have the full-length stress-inducible *XvSAP1* promoter (2 kb) as well as two deletions resulting in 1.5 kb and 1 kb promoters as we wish to use the shortest one that is still inducible. These have been cloned upstream of the firefly luciferase gene, *luc*, (as a marker), *XvSAP1*, encoding a membrane protein known to protect transgenic plants from abiotic stresses, and *XvPrx1*, encoding a very promising antioxidant that protects DNA from reactive oxygen species generated during abiotic stresses. The following transgenic plants have been generated:

1. Tobacco carrying all three promoters linked to the *luc* gene
2. Maize carrying all three promoters linked to *XvSap1* and *XvPrx1*

We are waiting for all these transgenic plants to set seed before testing the T_1 generation for tolerance to abiotic stress. This is being done by PhD student Richard Okoth in the laboratory of Prof Jesse Machuka at Kenyatta University in Nairobi. Richard is doing a sandwich PhD under the UCT USHEPiA programme which allows him to do half his work at UCT and half at Kenyatta University. Prof Machuka's lab is superbly equipped for Agrobacterium transformation of maize. Richard has succeeded in producing between 2 and 13 transgenic plants for each of the six constructs. This is an excellent achievement. Once the seeds have been set Richard will return to UCT to do the analysis of the promoters and stress resistance in the transgenic maize.

As maize transformation is slow we are also testing the above nine constructs in Arabidopsis, a model plant which can be transformed fairly easily and rapidly. This work only began a few months ago so we do not have any results yet.

We are still testing a third *Xerophyta viscosa* gene, *XvAld1*, encoding an aldose reductase, that converts glucose to sorbitol (an osmoprotectant), for its ability to protect transgenic Arabidopsis against abiotic stresses. The gene is expressed to highest levels in response to dehydration stress. We have also made transgenic *Digitaria sanguinalis* (a model monocot) and they are tolerant to 50 and 100 mM NaCl. As a result of these positive data we are now linking this gene to the three promoters for transformation into maize.

We are working actively on the patent involving the abiotic stress-inducible promoter. The background work has been completed and we are just waiting on the proof of concept data to complete the application. As Rosemary Wohlson, who has been

helping us with the patent, has left UCT to join the CSIR, Piet Barnard, who is also familiar with the patent, has taken over.

c) Problems Encountered:
We have still not developed a suitable system for setting seed from transgenic maize plants at UCT. We tried working with the CSIR but they were not successful. Fortunately our partner Prof Jesse Machuka from Kenyatta University has been extremely successful in doing this. His success is largely due to the fact that his lab is dedicated to maize transformation using Agrobacterium and also that, as it is so close to the equator, maize growth is very strong. As we infect immature embryos with Agrobacterium it is important to have a steady supply of these. One of the reasons for Prof Machuka's success is that these embryos are available all year round. However, we would also like to be able to set seed at UCT. To this end I have bought a plant growth chamber with sufficient light intensity to allow this. The cost is approximately R750 000, of which I have received R250 000 from UCT and the rest from various other grants. It should be installed before the end of the year.

d) Milestones that have not been Achieved: None

e) Adequacy of the Funding: We have received R360,000 being 80% of the first year's funding (July 2005-July 2006). We have spent R319,038.88 and thus have R40,961.12 left. This funding has been adequate for our first year's expenses. We are also grateful for the special equipment grant of R79,313.26 which has enabled us to purchase a much needed refrigerated/heated shaking incubator for our maize tissue culture work and 30 highly accurate Gilson pipettes for general laboratory use.

f) Duration of the Project: Three years