**The Maize Trust Report – April 2008**

a) Project Leader: Prof Jennifer A Thomson

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b) Actions Taken, Progress Made and Results Achieved

As stated in my 2007 report PhD student Richard Okoth produced a number of seeds from transgenic maize plants transformed with the various promoter constructs driving two *Xerophyta viscosa* genes, *XvPrx2* (encoding an antioxidant) and *XvSap1* (encoding a membrane signalling protein) as well as a reporter gene, *lux* (encoding luciferase). During the course of 2007 we took delivery of a new plant growth chamber (costing about R1 million including installation). We are now finally in a position to grow maize plants to maturity and set seed! Richard's plants are currently growing in this chamber, they will be allowed to set seed and the resulting plants will be tested for dehydration tolerance. This is a major milestone. In the meantime Richard has been testing the promoter constructs in other plant systems as outlined below.

Any successful development of transgenic dehydration tolerant plants requires that the introduced gene(s) are under the control of a stress-inducible promoter. If the gene(s) are switched on continuously by a constitutive promoter, normal growth is hampered often resulting in dwarfing of the transformed plants. We are therefore pursuing the promoter, XvPsap1, which originates from the upstream region of *XvSap1*. This gene confers tolerance to dehydration, high temperatures and salinity in model plants. The promoter is 2 kb long, which is rather long for introduction into transgenic plants. Therefore two shorter derivatives of 1.5 and 1 kb were also tested as it is possible that smaller constructs will be more efficient. In order to test the three promoters they were cloned upstream of the reporter gene, *lux*, and transformed into tobacco and black Mexican sweetcorn (BMS) in tissue culture. The transgenic tobacco plants were tested to ensure that the correct constructs were integrated into the plant's genome. The transgenic plants were phenotypically normal. They were then

subjected to dehydration and the levels of lux activity measured. The 2 kb promoter proved to be the most efficient, followed by 1.5 kb which was approximately half as effective. Both constructs showed a peak of activity after 3 days of dehydration and activity declined thereafter. This is very promising as it suggests that the activity of the two *X. viscosa* genes should peak shortly after transgenic maize plants are subjected to lack of water. The activity of the 1 kb promoter was considerably lower and the levels only peaked after 6 days. The transgenic BMS cells are currently being tested.

In order to test the promoters in transgenic maize, HiII maize has been transformed with the above constructs using Agrobacterium transformation. Positive transformants have been obtained and the plants will be subjected to dehydration stress.

Another milestone was the filing of our patent on the stress inducible promoter. This has taken a great deal of work by Richard as well as two post-doctoral fellows, Revel Iyer and Thokozile Lewanika. In order to submit the patent a thorough analysis of the promoter needed to be undertaken.

Bioinformatic analyses of XvPsap1 (~2kb) promoter and its truncated fragments XvPsap2 (~1.5kb) and XvPsap3 (~1kb) were performed. The promoter sequences were blasted into the NCBI database to probe for sequence homology with existing plant promoters. No complete similarity was observed suggesting the novelty of the promoter sequences. Identification of sequence motifs in comparison with other plant promoters was conducted using the PLACE and PLANTCARE software. Various *cis*-acting elements including those involved in drought stress-inducibility were identified. These include dehydration and heat stress, abscisic acid, jasmonic acid, light and low temperature responsiveness. Additionally, common *cis*-acting elements in both the promoter and enhancer regions were observed. These included meristem specific activation, endosperm expression, auxin-responsive and circadian control elements. These motifs serve as necessary guidelines on the possible stress experiments to be performed on the putative transgenic plants transformed with gene constructs harbouring XvPsap promoters driving reporter and *X. viscosa* genes.

The aldose reductase gene, the third *X. viscosa* gene of interest, has also been cloned downstream of the XvPsap promoters. These constructs were bombarded into BMS cells and conferred tolerance to 200 mM salt and 200 mM sorbitol. These results are extremely promising and transgenic maize plants will be generated with these constructs during 2008.

c) Problems encountered:

None of any significance, especially since the new growth chamber was installed.

d) Milestones that have not been achieved:

None

e) Adequacy of funding:

Adequate – refer to financial report submitted with this report.

d) Duration of the project:

Three years after the end of 2008