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Mr Leon du Plessis
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Dear Mr du Plessis

Please find enclosed our first annual report on the research funded by the Maize Trust. I hope you will find it in order and thank you for your continued support.

Yours sincerely

Jennifer A Thomson
Professor of Microbiology

REPORT TO THE MAIZE TRUST – OCTOBER 2002

DEVELOPMENT OF DROUGHT TOLERANT CROPS BY GENETIC MODIFICATION

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In our project proposal to the Maize Trust, we reported that we had isolated a number of genes from the resurrection plant, *Xerophyta viscosa*, with potential for protecting transgenic plants against drought and other abiotic stresses. We then proposed proceeding with one of these genes, namely that coding for aldose reductase (Mundree *et al.*, 2000), whose product converts glucose to the osmoprotectant, sorbitol. While we have continued to work on this, we decided to continue to work in parallel on a number of the other genes. We therefore report on progress on all of these genes and their products.

Our approach is as follows:

- Determine the expression, at the level of transcription, of each gene in response to a variety of abiotic stresses in *X. viscosa* leaves.
- Do the same for expression at the level of translation. This will often require the production of specific antibodies.
- Determine the mode of action of the protein in response to abiotic stresses.
- Introduce the gene into our model monocot grass, *Digitaria sanguinalis*. We use this as an intermediate step as transformation of maize is a long and costly process and we only wish to transform those genes which show significant abiotic stress protection in transgenic *D. sanguinalis*.
- Introduce the genes into maize and test for abiotic stress tolerance in greenhouse trials.

1. *XVSAP1* (Ms Dahlia Garwe, PhD student)

The sequence of this gene showed it to code for a highly hydrophobic protein with six transmembrane regions. The deduced amino acid sequence showed 49% identity to a low temperature regulated protein from wheat. It also showed 56% identity to proteins that may be cold responsive in *Arabidopsis thaliana*. However, analysis of gene expression in *X. viscosa* leaves using semi-quantitative RT-PCR, indicated that *XVSAP1* is induced by dehydration, salt stress, both low and high temperature, and high light treatment. The gene was cloned into a dicot plant transformation vector and introduced into *A. thaliana* and tobacco using *Agrobacterium tumefaciens*. (The reason we introduced it into dicots before monocots is that this PhD student has been seconded to us by the Zimbabwe Tobacco Research Institute).

Transgenic plants showed greater tolerance than vector transformed and untransformed control plants under the following conditions:

- Treatment at 42°C for 12 hours followed by growth at normal temperatures for 2 weeks
- Growth in media containing 50 mM and 100 mM NaCl (*A. thaliana*) and 200 mM NaCl (tobacco)
- Growth in media containing 150 mM mannitol

These results suggest that XVSAP1 may confer tolerance to a variety of abiotic stresses in transgenic plants.

To prove that XVSAP1 is a membrane-associated protein, attempts were made to express it in *Escherichia coli* in order to prepare antibodies. Unfortunately, probably due to its membrane location, expression proved toxic to the bacteria. We have since had a synthetic peptide made and the antibodies have been shown to be specific to XVSAP1. Immunogold electron microscopy is underway.

GARWE, D, THOMSON, J.A. and MUNDREE, S.G. (2002) Molecular characterization of *XVSAP1*, a stress responsive gene from the resurrection plant *Xerophyta viscosa* Baker. **J. Exp. Bot**. (in press)

2. **Aldose reductase** (Dr. Sam Muyanga, post-doctoral fellow, Alice Maredza, PhD student)

The *X. viscosa* aldose reductase converts glucose to the osmoprotectant, sorbitol. Progress on this gene has been disappointingly slow. It appeared that some of the data presented by the previous post-doctoral fellow working on the project in 2001 were fraudulent. Although some of his early data showing abiotic stress tolerance in transgenic plants are probably correct, we decided to re-clone the gene and re-introduce it into *D. sanguinalis*. This has been done and tests are underway to determine their effects on the transgenic plants subjected to a variety of abiotic stresses.

3. **Peroxiredoxin** (Ms Shaheen Mowla, PhD student)

One of the cDNAs isolated from the *X. viscosa* library, *XvPer1*, showed high homology to recently identified antioxidant enzymes from *A. thaliana*, *Oryza sativa* and *Hordeum vulgare*, 1-Cys peroxiredoxin, at both the DNA and deduced amino acid levels. The gene was shown to be induced when *X. viscosa* leaves were subjected to dehydration (with decreased expression during rehydration), heat (42°C), high light intensity and salinity (100 mM).

It is proposed that peroxiredoxins act by protecting DNA from damage during abiotic stress (Stacy et al., 1996). Therefore we cloned *XvPer1* into the *E. coli* expression vector, pProEXHTa and used the purified protein to raise antibodies. Western blot analysis was carried out on proteins isolated from stressed *X. viscosa* leaves. The results confirmed the pattern of expression of the *XvPer1* transcripts. Immunofluorescence studies revealed that XvPer1 is localized in the nucleus of dehydrated *X. viscosa* leaf cells. This work has been accepted for publication (Mowla et al., 2002).

This gene has been cloned into a monocot plant expression vector and and is being introduced into *D. sanguinalis*.

MOWLA, S.B., THOMSON, J.A., FARRANT, J.M. and MUNDREE, S.G. (2002) A novel stress-inducible antioxidant enzyme identified from the resurrection plant *Xeropyta viscosa*. **Planta 215**: 716-726

4. **Dehydration Response Element binding protein, DREB1A** (Ms Kershini Govender, PhD student and Dr Bienyameen Baker, post-doctoral fellow)

Many genes that are induced in response to dehydration carry dehydration response elements (DRE) within their promoters. DRE-specific binding proteins have been isolated from *A. thaliana* and expression of *Dreb1A* under the control of the CaMV promoter in transgenic plants has been demonstrated to result in strong constitutive expression of the stress-inducible genes. As a result the plants exhibited increased tolerance to freezing, salt and dehydration stresses (Liu et al. 1998).

A truncated *Dreb1A* cDNA isolated from the *X. viscosa* library and attempts are being made to clone the complete gene. Probing dehydrated *X. viscosa* leaves with the 450-bp fragment, however, showed that expression occurs under cold stress. In order to identify possible DRE binding sites in both the *Dreb1A* and *Xvper* promoter regions, a genomic library of *X. viscosa* was prepared and screened. Clones representing both genes have been isolated and the positions of the genes within the inserts are being determined. The ultimate aim of this work is to investigate the effects of making transgenic plants expressing both the *X. viscosa Dreb1A* gene, under the control of its own DRE, and *Xvper* gene also under DRE control. This could minimise any damage to transgenic plants expressing drought-responsive genes constitutively.

5. **Galactinol synthase, GolS** (Mr Shaun Peters, MSc student)

The raffinose family (RFO) of oligosaccharides is unique to plants and is known to occur in large amounts in seeds. Increases in raffinose and stachyose have been reported in plants exposed to dehydration and cold stress. Galactinol synthase, GolS, is the first committed enzyme in the RFO pathway. The *XVGolS* gene was isolated from the *X. viscosa* library and shown to be induced by dehydration of *X. viscosa* leaves. The gene has been cloned into a monocot plant expression vector, transformed into *D. sanguinalis* and transgenic plants are being analysed. XVGolS shows identity of between 65 and 72% to GolS sequences from *Brassica napus*, *Pius sativum*, *A. thaliana* and *O. sativum* (to which it is most closely related).

6. **Vacuolar H+-ATPase subunit c** (Mr Saberi Marais, MSc student)

Vacuolar ATPases have been implicated in response of plants to salinity stress. Subunit c is integral to V-ATPase function, and its c-DNAs have been identified and characterised in a range of angiosperm species. Subunit c's steady-state transcript levels display a 2 to 4-fold increase in response to salinity stress. As a result, the abundance of V-ATPase subunits increases and assembles to form functional V-ATPase heteromultimers. The resultant V-ATPases acidify intracellular compartments, including the vacuolar lumen, and contributes to a proton motive force capable of driving the secondary transport of ions and metabolites across membranes. A gene for the c subunit, *vatp1xv*, was isolated from the *X. viscosa* library and shown to be induced when *X. viscosa* leaves were subjected to 150 mM NaCl and dehydration. Further characterization of this gene and its protein are underway.

7. **Heat shock protein, Hsp90** (Ms Sally-Anne Walford, completed MSc)

The deduced amino acid sequence of the cloned *XVHsp90* gene showed ca. 85% similarity to Hsp90s from plants and it contains the endoplasmic reticulum targeting and retention signals. Western blot analysis showed that the XVHsp90 concentration increased significantly in response to heat and dehydration. We will probably not continue working with this gene. We have submitted a paper on this work but it is still in the review process.

Future work

1. Completion of the tests of *XVSAP1* and aldose reductase transgenic plants subjected to a variety of abiotic stresses.

2. Make transgenic *D. sanguinalis* carrying *XVSAP1* and *XvPer1* and test plants as above.

3. Clone the complete *Dreb1A* cDNA and make transgenic plants to be tested as above.

4. Continue the work on the DRE of both *Dreb1A* and *XVPer1* as outlined above.

5. Continue the characterization of GolS and the ATPase subunit c and their expression in *X. viscosa* in response to stresses. Clone into plant expression vectors in preparation for introduction into plants.

References

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