The effect of enhance proline synthesis in soybean, with special reference to drought stress, comparing antisense and sense technology

JA de Ronde, RN Laurie

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PROTEIN RESEARCH TRUST (PRT)

1. **PROJECT TITLE:** The effect of enhance proline synthesis in soybean, with special reference to drought stress, comparing antisense and sense technology

2. **PROJECT TEAM:**

Division Biotechnology: Abiotic stress

Kobie de Ronde	-	Senior Researcher
Robert Laurie	-	Researcher
Tania Caetano	-	Technician
Helen Phasa	-	General Assistant
Ria Geyling	-	Senior Technician

3. **MANAGERS SUMMARY:**

3.1 Aim

The aim was to test the hypothesis that transgenics containing the *P5CR* gene in an antisense direction will be sensitive to drought stress and that transgenics containing it in a sense direction will be more tolerant to drought stress. The transgenics were compared with different physiological and morphological mechanisms that may be influenced by drought stress. The transgenic plants were validated for the presence of the *P5CR* gene.

3.2. Results

During 2002 an application was filed at the Department of Agriculture for an intended field trial. Permission was granted in January 2003 for 2 seasons of field trials under the rain out shelter to evaluate the transgenic plants against drought. Due to the late approval the trials were not planted in 2002, but will be in October 2003. The genotypes that will be planted in the field trial, were selected on their drought performance at the greenhouse

experiments in 2001/2002. Seed from these plants were analyzed via molecular techniques to verify the presence of the *P5CR* gene in the genome by means of Southern blotting, copy number detection and PCR. DNA analyses were performed on germinated seed to be used for all the experiments. The PCR analyses demonstrated that the kanamycin marker gene (*NPTII*) and *P5CR* gene were amplified in all the transgenic plants. A heat shock primer, specifically manufactured to detected the presence of the heat shock promoter in the plant's genome, also proved the transgenic nature of the plants.

Two methods for Southern blotting and copy number detection, namely radioactive and DIG labeling were tested. The efficiency of the labeling with the DIG method was much higher as with the radioactive method. For the visualization of copy numbers and the detection of the *P5CR* gene in the genomic DNA a *P5CR* gene product was manufactured by means of PCR. Control genomic DNA was spiked with the *P5CR* gene in 1, 3 and 5 copies to visualize the copy numbers of the different genotypes. In order to visualize copy numbers of a specific gene from plant genomic DNA it must be known which restriction enzyme sites borders the gene of interest and which single digest sites are available inside the gene itself. Thus, the gene was cut on the borders with restriction enzyme (Bgl II) and is shown as a single band on a x-ray film. The intensity of the amplified band of the genotype compares well with the 5 copies of the spiked control, indicating that the transgenic genotypes contained at least 5 copies of the *P5CR* gene. Detection of the amount of events that the gene integrated the genome was made possible by a Pst1 digestion that cuts the DNA inside the *P5CR* gene. Three bands were displayed in all the genotypes that indicated that at least three integrations took place in the transgenic genome. Positive transformation were thus confirmed of the soybean plants that will be used for the field trials.

3.3 Importance of results

If the sense Ibis plants are proven (in the field trials) more drought tolerant than the control plants, the gene can be used in other crops in enhancing drought tolerance. Other genes can be transferred to soybean in render it more tolerant to e.g. insect damage. The

techniques sorted out on soybean can be used for screening of other soybean cultivars for environmental stresses.

3.4 Problems with the research

The research is one year behind schedule due to the late GMO field trial permission.

3.5 Possible future research

- The transgenic plants will be tested in field trials if we are successful with innovation fund grant.
- The transgenic soybean will be included in the breeding program at Potchefstroom.
- The effect of the *P5CR* on cold stress can be tested.
- The transformation process of soybean can facilitate the transfer of any gene of interest to any soybean cultivar resulted in a better soybean.
- The soybean breeding lines can evaluated for drought tolerance using the screening techniques

3.6 Technology transfer

Presentations

De Ronde, JA, T Caetano, MM Greyling, 2002. The effect of enhance proline synthesis in soybean. Sojakundigheidsforum, Caesars, Johannesburg 6 November

De Ronde, JA, Kok C, 2003. Commercialization of the first genetically modified South African drought tolerant soybean, NRF innovation fund forum, Pretoria, 24 February

Publications

De Ronde Jacoba A., Cress William A., Krüger Gert H.J. , Strasser Reto J. and Van Staden Johannes Photosynthetic response of transgenic soybean plants containing the *P5CR* gene during heat and drought stress *J Plant Physiol submitted*

De Ronde JA., Cress William A., and Van Staden J. Phenotypic evaluation for drought tolerance of transgenic soybean. *SAJ Plant Soil submitted*