TOWARDS GENETIC MODIFICATION OF SUNFLOWER

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INTRODUCTION

Classical sunflower breeding has made great achievements in the past decades. This is illustrated by the fact that between 1980 and 1990 yield per hectare has doubled in the EEC (Statistiques des oléagineux et protéagineux, Prolea, France, 1992). Despite these achievements classical breeding has its limitations. New germplasm may only be found within the genus. Interesting traits beyond the genus-boundary are not available for classical sunflower breeding techniques.

Genetic modification overcomes this boundary. Genes from virtually any organism may be introduced into and expressed in sunflower. In order to be able to do so one needs an efficient transformation system and an efficient regeneration system. With the transformation system DNA containing the foreign genes of interest is introduced into sunflower cells present in an explant; these cells then are 'transformed'. With the regeneration system plants are regenerated from the explant, optionally with positive selection for regenerates developing from transformed cells. Resulting plants should have the introduced genes in the germlines, in order to produce transformed, genetically modified progeny plants, which have the new DNA present in each and every cell.

As a DNA vector we use *Agrobacterium tumefaciens* strain LBA4404 (courtesy Dr. R.A. Schilperoort; Gene 7:15, 1982) containing a BIN19 (Bevan; Nucl. Acids. Res. 12:8711, 1984) derived plasmid with an NPT.II gene as a selection marker and GUS.INT as a non-selection marker (vector: MOGEN Int., The Netherlands; GUS.INT: courtesy Dr. L. Willmitzer; Mol. Gen. Genet. 220:245, 1990). The strain also contained the Vir-helper plasmid pToK47 (courtesy Dr. M.P. Gordon; J. Bacteriol. 169:4417, 1987).

In the transformation/regeneration systems VANDERHAVE is working on, four types of explants are used: immature embryos (3 mm) for indirect regeneration; half immature embryos (7 mm) for direct regeneration, cotyledons from young seedlings (5 days) and finally cotyledon nodes from young seedlings.
The regeneration media are based on the medium published by Paterson and Everett (Pl. Sci. 42:125, 1985): MS medium with 0.5 g/l casein amino acids, 40 mg/l adenine sulphate, 5 g/l KNO_3, 100 mg/l inositol, 0.1 mg/l GA_3 and 0.5-1.0 mg/l BAP.

RESULTS AND DISCUSSION

For both immature embryo systems an average of about 20 shoots per regenerating embryo (sh/rem) are obtained, with maxima above 50 sh/rem. For the other systems about 10 shoots per successful cotyledon are obtained and 5 shoots per cotyl node. In terms of seedsetting plants this would be 6, 3, 2 and 1 per explant respectively.

Transformation efficiency is tested using assays with X-Gluc, a compound which is converted into a blue dye in a reaction catalyzed by the gene product encoded by the GUS.INT gene. The blue colour represents transformed sunflower tissue, since GUS.INT is not expressed in *Agrobacterium*.

Extensive experimenting resulted in a very efficient transformation procedure: routinely large surface areas of the explants are stained blue after an X-Gluc assay.

At present we have protocols for:

1) a very efficient transfer of T-DNA into sunflower cells and
2) a high frequency regeneration system from several sunflower explant types.

These developed techniques as such can compete with the best for other crops. For most other crops this would fulfil the conditions for a routine transformation/regeneration protocol. Indeed rather frequently 'blue' shoot primordia were observed in early stages of regeneration development. The frequencies with which mature transgenic regenerates were found however, did not correspond with the early frequencies. Despite many experiments with thousands of explants and the production of thousands of putative transformants, only one fully transformed, yet not viable shoot was obtained as well as a number of chimeras.

Apparently the sunflower explants used harbour ample cells competent for transformation and ample cells competent for regeneration. For some reason it appears that the coincidence of both phenomena (transformation plus regeneration from the same cell) is very low. This might be due to for instance inadequate cell competence, or poor T-DNA gene expression in differentiated tissue. In the future the protocols will be optimized. Using different phytohormonal regimes it will be investigated whether cell competence can be influenced. Furthermore experiments will be done to optimize gene expression, using for instance anti-methylating compounds (DNA methylation has been shown to cause temporal gene shut down in transformation procedures (Peerbolte *et al.*; Plant. Mol. Biol. 6:285, 1986)).