EXPLORATION OF GENE FUNCTION AND ORGANISATION DURING DEVELOPMENTAL EVENTS (SUBPROJECT 2)

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Keywords: gene organisation, regulatory elements, molecular biology, developmental genetics, transcription factors.

Introduction

As the development of biological organisms is controlled by the modulation of expression of genes, in depth understanding of the elements that control their expression is also necessary to facilitate successful control of gene expression in a transgenic plant. For the effective examination of these elements a model easily transformed transgenic system is required. This will accelerate the transfer of the multiple gene constructs used to identify enhancer elements. Two tissue specific stylar promoters (Budelier et al. 1990; Harikrishna et al. 1996) and a plant viral promoter will be examined to identify enhancer and silencer elements. Deletion constructs of these promoter elements when fused to a reporter system such as GUS will enable the identification of DNS sequences that contain silencer and enhancer elements. These promoter deletions will be transferred into a yeast one-hybrid system to facilitate identification of genes encoding transcription factors that bind to these sequences. Synthetic oligonucleotide sequences of the putative binding sites in the promoter will be used to confirm the yeast one-hybrid results. These isolated genes will be further characterised by sequence and expression analysis. Computer analysis of the sequence will be used to identify motifs that facilitate dimerization or binding with DNA (helix-loop-helix). The interaction between the cloned transcription factors to enhance or to reduce DNA binding capability will be examined. Protein engineering techniques may be also used to modify the transcription factors to enhance or to reduce DNA binding capability. These constructs will be used in an *in vivo* transcription assay system to enable the study of protein structure changes on DNA binding and hence gene expression. The objectives of the study were: (1) To identify enhancer and silencer elements from a tissue specific promoter; (2) to identify and clone genes encoding transcription factors that bind to the above elements; and (3) to characterise these cloned genes.

Materials and Methods

A tissue culture regeneration system for a local variety of tomato, Mardi MT 1 or 11 was developed. This system was used in conjunction with several strains of *Agrobacterium* to develop an efficient system of transformation for tomato MT 1 or 11. Promoter GUS fusions of both ChiP and pMON 9612 were subjected to exonuclease III digestion to produce deletion constructs of various sizes. An Agrobacterium transformation vector was modified to facilitate insertion of the promoter deletion fragments in the form of an expression cassette. This was used in transformation experiments to generate transgenic tomato plants. Analysis of floral tissues of these transgenic plants by GUS staining facilitated the identification of enhancer and silencer elements in the full-length promoter. A cDNA library of mRNA derived from mature pistils of tomato was constructed using the yeast one-hybrid matchmaker kit (Clontech). The yeast one-hybrid cDNA library were transferred into yeast strains containing the putative promoter binding sites fused to the His3 gene of the matchmaker kit. Yeast colonies that grow in histidine deficient media contain putative transcription factors that bind to the fused DNA elements. The cDNA clones containing putative transcription factors were isolated, purified and characterised by sequencing and northern blotting. Fusion protein expressed *in vitro* will be used in mobility shift assays to support the data obtained from binding assays (yeast one-hybrid). Putative binding sites in the promoter will be identified by a combination of mobility shift assays and sequence analysis followed by computer algorithm motif searches.

Results and Discussion

An improved tissue culture regeneration system for local tomato has been developed. However, Agrobacterium mediated transformation of local tomato has so far not been successful. Modifications to the protocol have been made and transformation is underway. Concurrently a transformation system for tobacco (a closely related species) has been set up. Promoter deletions have successfully been cloned as a fusion driving a GUS reporter gene in an Agrobacterium transformation vector. Putative transformed tobacco plants containing 1 deletion construct has been obtained. Promoter deletions have also been produced from the 1.4Kb full-length promoter, each deletion being 200 bp shorter than the other. Full sequence information has been obtained and a comparison with the promoter sequence database made. Several putative transcription factor binding sites were identified and cloned into a yeast one-hybrid matchmaker His vector.

Conclusions

An improved regeneration system for two local tomato cultivars has been developed. A tobacco transformation system has also been successfully developed and several putative transgenic plants produced. Chi2;1 promoter deletion have been constructed and fully sequenced. Sequence comparison with the promoter sequence database allowed the identification of several putative transcription factor binding sites. These have been cloned into a yeast one-hybrid vector system. Deleted promoter fragments have been fused with GUS in an *Agrobacterium* transformation vector and are being transformed into tomato and tobacco.

References

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Supported by IRPA Grant 29-02-03-0001