Agrobacterium *MEDIATED TRANSIENT GENE EXPRESSION IN LEAVES OF* Phaseolus vulgaris

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Introduction

Transient gene expression (TGE) assays have two important advantages as compared to stable transformation; gene expression can be measured very shortly after DNA delivery and is not biased by position effects. These assays are almost exclusively applicable to protoplasts. However, expression in isolated protoplasts is not necessarily similar to expression in the intact tissues and organs from which protoplasts are derived. Transient assays in intact tissues should largely overcome this problem and could therefore, be a valuable alternative for stable transformation, especially in recalcitrant species. We developed a TGE assay system for complete leaves of *Phaseolus vulgaris* via *Agrobacterium* transformation.

Experimental Procedure, Results and Discussion

A. tumefaciens strain C58CI Rif^R (pGV2260) harbouring the binary vector pTJK136 was grown overnight in the presence of antibiotics and acetosyringone. The vector pTJK136 contains a *gus* gene with intron, under the control of 35S promoter and 3' nos. This vector is derived from vector pTHW136 (obtained from Plant Genetic Systems, Gent, Belgium) by changing the promoter and 3' end controlling the *gus-intron* gene. The bacteria were concentrated to O.D.2.4 and first leaves of *P. vulgaris* seedlings were vacuuminfiltrated with this concentrated suspension. The infiltrated leaves were rinsed with water and placed on thoroughly wet sterile filter papers. Different parameters affecting the transformation efficiency in this system have been compared using histological assays. *Agrobacterium* growth conditions (i.e. growth at pH 5,6 and presence of acetosyringone) and vacuum infiltration increased the transformation efficiency significantly. With optimal transformation conditions, histochemical GUS-staining revealed large GUS-expressing sectors and up to 50 % of the leaf area was stained. Light and electron microscopy studies revealed that infiltration led to a high number of bacteria being infiltrated evenly into intercellular spaces of all cell layers of the leaf tissue. A large group of bacteria were present near the stomatal openings. It is thus suggested that preincubation of the bacteria in acetosyringone-containing medium at acidic pH induces the *vir* machinery and the presence of bacteria in the intercellular spaces could further lead to enhanced efficiency of transformation.

Our results showed that the GUS expression from pTJK136 in tobacco protoplasts was much higher than from the plasmid pTHW136, which contains the original *p35S-gus-intron-3'35S* cassette from p35SGUSINT (1). GUS-expression from two vectors, pTJK136 and pTHW136, also have been compared in intact bean leaves using this TGE assay system, supporting the earlier results from electroporation in tobacco protoplast.

The described assay for intact tissues is very simple and a number of samples can be treated simultaneously and with ease. This technique can be useful in analysis of any parameter important for efficient T-DNA transfer, structural and functional analysis of promoters, study of normal and mutated gene function etc. Hence it is suggested that this technique could be an alternative to particle bombardment or electroporation mediated transient expression systems in intact tissues.

1.Vancanneyt *et al.* Mol. Gen. Genet 1990;220:245-250.