TRANSGENE STABILITY - INHERITANCE AND EXPRESSION IN *BRASSICA NAPUS*.

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ABSTRACT

Several constructs have been introduced into oilseed rape (*Brassica napus*) by *Agrobacterium* mediated transformation. The plants produced have been fully characterised by molecular and biochemical analysis. Maintenance of transgene expression, in transgenic lines with different T-DNA insertions, has been assessed both histochemically and quantitatively and the stability of transgene expression and inheritence over successive generations is being determined.

INTRODUCTION

The successful commercialisation of genetically modified crops will depend on the level of transgene expression and the stability of the foreign gene through successive generations. Consequently, any knowledge of transgene instability and its causes could be of considerable benefit for the registration of transgenic varieties. Transgene instability has been observed by many companies involved in the commercialisation of transgenic crops (Finnegan and Elroy, 1994). Oilseed rape will be one of the first crop plants to have registered transgenic varieties for widespread use, yet little data has been published about the level of transgene stability in this species.

EXPERIMENTAL

Plant transformation

Six constructs, based on the pSLJ binary plasmid (Jones *et al.*, 1992), containing the constitutive promoter 35S, regulating the *gus* reporter gene and the *bar* gene (conferring resistance to the

herbicide Basta); the nopaline synthase promoter (nos) regulating the *gus* reporter gene and the *bar* gene; the embryo specific promoters cruciferin and oleosin from *B. napus* driving the *gus* reporter gene (Keddie *et al.*, 1994) have been introduced into a doubled haploid line of Westar via *Agrobacterium* mediated transformation (Radke *et al.*, 1992). Primary transformants were characterised biochemically, to determine levels of expression of the transgene and any alteration in tissue specificity and at the molecular level to determine transgene copy number. Two lines containing constructs with embryo specific promoters showed some alteration in tissue specificity, expressing both in parts of the flowers and in the leaves. Analysis by PCR showed the promoter to be complete in both cases.

Stability in successive generations

Primary transformants containing either one or two copies of the transgene, were both self pollinated and back crossed onto the untransformed Westar line. The F_1 progeny were then analysed for Mendelian segregation of transgene expression. The F_1 lines have been classed according to the type of instability observed (Table1).

TABLE 1. Description of the classes of stability observed.

Stability Class	Description
I	Stable expression
II	Physical loss of the transgene
III	Loss of expression with transgene still present
IV	Alteration in tissue expression patterns
V	Gross alteration in expression levels over generations

Instabilities II and III have been observed for each promoter. In addition, primary lines containing the embryo specific promoters showed class IV instability. This atypical tissue expression pattern has been shown to be stably inherited to the F_1 generation.

We are currently screening further generations of the characterized lines for the occurrence of any instability. The significance of any instability observed during this study and possible epigenetic effects will be considered, with reference to their potential effect on the successful commercial release of transgenic crops.

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