INTRODUCTION OF THE MAIZE TRANSPOSABLE ELEMENTS Ac AND Ds, INTO *BRASSICA NAPUS.*

N.MCKENZIE, P.J.DALE.

Brassica and Oilseeds Research Department, John Innes Centre, Colney Lane, Norwich, NR4 7UJ, UK.

ABSTRACT

The two maize transposable elements, Ac and Ds, have been introduced into *Brassica napus* on different transgene constructs. The Ds element is associated with an excision marker gene and is expected to transpose only under the influence of transposase encoded from the Ac construct. The behaviour of the elements is being tested in this heterologous genetic background to determine the feasibility of using a two element transposon system for the isolation of genes from *Brassica*.

INTRODUCTION

The well characterised maize transposable elements Activator (Ac) and Dissociator (Ds), have been found to transpose in heterologous plant species, for example tobacco, *Arabidopsis*, tomato, potato, petunia and rice (for review see Chasan, 1993).

The Ds element does not contain a functional transposase gene and therefore cannot induce its own transposition (non-autonomous). It transposes only under the influence of transposase produced by an Ac element within the same plant. A two-element system using Ds mobilised by the presence of an Ac element, which is itself unable to transpose because of a deletion within its 3' terminus (stable Ac or sAc), is found to be effective in transposon tagging experiments in *Aradidopsis thaliana* (Bancroft *et al.*,1992). Ds is used as an insertional mutagen, and it inactivates the function of any gene that it inserts into. The mutation can then be stabilised by selecting for segregants in the progeny without the tranposase source (sAc), to prevent further excision events. The Ds element can then be used as a tag to identify the flanking sequences (ie. the gene) by molecular analysis.

In this study we aim to introduce a two-element transposon system into *Brassica napus*, to determine the behaviour of the transposons and to investigate their potential for use in gene tagging experiments to isolate plant genes from this agriculturally important plant.

EXPERIMENTAL

Ds and sAc constructs have been introduced into *Brassica napus* var. Westar by *Agrobacterium* mediated transfer based on the methods of Radke *et al.* (1992).

The constructs used are based on the pSLJ binary plasmid system (Jones *et al.*, 1992). The Ds construct contains, the streptomycin phosphotransferase gene (*spt*) as an excision marker. The Ds element has been cloned into the 5' untranslated leader of the marker gene, where it inactivates gene function, until the Ds element has excised. This has been shown to provide a reliable method to monitor excision events in tobacco (Jones *et al.*,1989). In addition, the Ds element itself contains phosphinothrycin acetyl transferase (confers resistance to the herbicide Basta) as a re-insertion marker gene, to select plants in which there has been re-insertion back into the genome.

The sAc construct has the transposase gene driven by the cauliflower mosaic virus 35S promoter. This promoter has been found to increase levels of transposase and increase excision frequencies of Ds in heterologous host plants (Scofield *et al.*,1992). The reporter gene *gus* (β-glucuronidase) provides a reliable marker gene to select for the presence of the sAc construct, so that progeny not expressing *gus* activity are expected to contain the stabilised mutation caused by insertion of Ds into a tagged gene.

Initial transformant plants have been characterised at the molecular level by PCR to detect the presence of the transgenes, then by Southern blot analysis to determine the number of copies of both left and right borders. In 93 transformed plants containing sAc, and 13 containing the Ds construct, copy number varies from 1 to 8. However there seems to be a high degree of truncation (63% of plants), exhibited by variation in the numbers of left and right border fragments for the same transgenic plant. So far we have crossed single copy Ds plants with both one and two copy sAc plants. When transposition is established in *Brassica napus*, we will perform further crosses with higher copy number plants, particularly Ds, to increase the chance of obtaining mutant phenotypes when screening progeny for tagged genes.

We are currently analysing the F2 generation seed by sowing onto media containing streptomycin to detect evidence of excision events. Excision is expected to restore *spt* gene function, resulting in full green (germinal excision events) or partially green (somatic excision) seedlings; the non-excised element is expected to produce seedlings that are bleached white. Green seedlings will be analyzed at the molecular level by both PCR and Southern blotting for further confirmation of Ds activity and behaviour.

We are also investigating Ac/Ds behaviour in other species of *Brassica*, and have recently confirmed transposon activity with a similar two-element system in *Brassica oleracea.* (Wen, personal communication).

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