Caspase-resistant VirD2 protein provides enhanced gene delivery and expression in plants

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Abstract

Mutagenesis of two aspartic acid residues in the caspase cleavage sites of the Agrobacterium tumefaciens VirD2 protein makes the protein resistant to cleavage by a plant caspase-like activity.

This resistance of the VirD2 protein to caspase cleavage increases the efficiency of gene transfer and expression after Agrobacterium inoculation of various plant species.

Introduction

Agrobacterium-mediated transformation can be successfully practiced only with some plants, and the transformation efficiency is extremely low with many economically important crops such as cereals.

Agrobacterium tumefaciens VirD2 protein is a key element in the transfer of T-DNA from the Agrobacterium tumour inducing plasmid into the nucleus of plant cells and its integration into the host genome. The VirD2 protein is a substrate for a plant caspase-like protease activity (PCLP) in tobacco.

We demonstrate here that mutagenesis of the VirD2 protein to prevent cleavage by PCLP increases the efficiency of reporter gene transfer and expression. These results indicate that PCLP cleavage of the Agrobacterium VirD2 protein acts to limit the effectiveness of T-DNA transfer and is a novel resistance mechanism that plants use to combat Agrobacterium infection.

Results

Fig 1 Schematic representation of the Agrobacterium tumefaciens VirD2 protein.
The endonuclease domain is represented by the blue shaded area and the two nuclear localisation signal domains (NLS 1 and 2) by the red areas. The omega (ω) sequence is located at the C-terminus next to the second NLS domain. The positions of the two caspase cleavage sites are indicated.

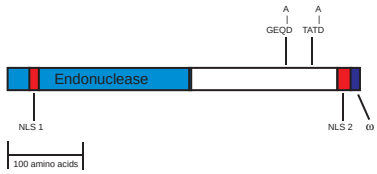


Fig. 2 Increase in efficiency of GFP expression in plant tissues agroinfiltrated with A. tumefaciens encoding a PCLP-resistant VirD2 protein. Plant tissues were agroinfiltrated with A. tumefaciens carrying a GFP gene along with either a wt VirD2 gene (left hand panels) or a mutated, PCLP-resistant VirD2 gene (mut VirD2, right hand panels) and fluorescence was observed by confocal laser scanning microscopy. The type of plant is indicated on the right.
Bars, 100 µm

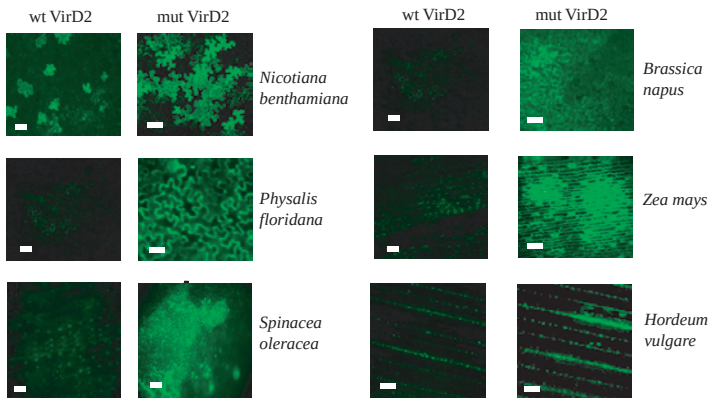
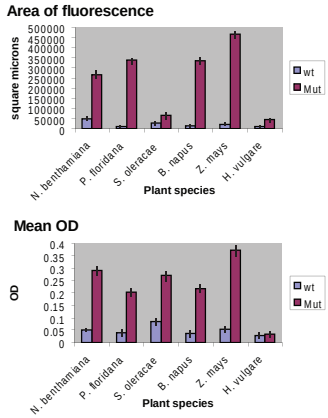


Fig 3. Typical results showing the area of fluorescence in a field of view with a size of 640,000 lm², and optical density (OD) for tissues agroinfiltrated with A. tumefaciens containing wild type (wt) or mutant (mut) virD2, and mutant/wt ratios. Data are mean ± SD from three independent experiments with three replicates in each. Agroinfiltrated sites were about 1 cm in diameter and contained several patches of fluorescence.



Conclusions

Modification of two PCLP cleavage sites TATD and GEQD in the VirD2 protein can significantly increase the efficiency of gene transfer and expression suggesting that PCLP-mediated cleavage of the VirD2 protein also occurs during Agrobacteriuminfection and limits the efficiency of T-DNA transfer and subsequent levels of expression of genes contained within the T-DNA.

Protection of the VirD2 protein from PCLP constitutes a powerful approach to increase the efficiency of transient gene expression systems and potentially also of stable plant transformation.

For further technical detail see Chichkova et al., The Plant Cell (2004) 16, 157-171 and Reavy et al., Plant Cell Reports (2007) 26, 1215 - 1219