Towards plant gene targeting - Enhancing homologous recombination frequency in tobacco



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Introduction

Homologous recombination (HR) is one of the principle forces creating the genetic diversity that drives evolution and is the fundamental instrument underlying most crop breeding programs. In some lower eukaryotes and a few animal systems the precision of HR has been harnessed to develop gene targeting (GT) technologies. By comparison, it has so far proved impossible to establish a feasible GT system for plants. The development of efficient GT protocols for a wider range of higher eukaryotes would have massive impact in agriculture, medicine and biological research. GT is a technique that can be used to replace, modify or repair an endogenous gene, via HR, with a copy that has either been modified *in vitro* or one that is a naturally occurring allelic variant. The difficulty in achieving routine GT in higher plants centre on the fact that exogenous DNA integrates into plant genomes very readily by illegitimate recombination (IR) while HR frequencies are extremely low.

In this project, we have managed to stimulate (up to a 1000 fold) HR by over-expressing multiple recombinases in tobacco plants. We have also developed new GT vectors that will allow efficient selection of rare GT events.



Impact of recombinases on plant development



Gene targeting ingredients



Frequency of ICR in pollen and seedlings

While ICR stimulation in seedlings remains modeste, some of our transgenes have induced recombination in pollen by up to a 1000 fold.



RecG-RuvC

DMC1 DMC1 DMC1 DMC1 DMC1 DMC1

Rad51 Rad51



Conclusions

RecG

* Homologous recombination is a rare event and we have succeeded in inducing ICR in pollen of tobacco plants by up to a 1000 fold.

* Will this very high stimulation of ICR increase the GT efficiency?

* New GT vectors have been designed to address this question.

References: 1) Trends in Genetics (2005) 21, 172-181. 2) Plant Physiology (2004) 135, 16-24 3) The EMBO J. (1994) 13, 484-489.

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