ROTHAMSTED RESEARCH

Rothamsted Research Annual Report 2002-2003

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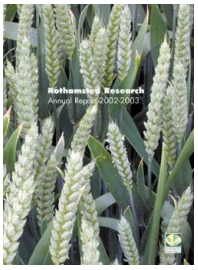

Transformation and Promoter Tagging in Wheat

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Genetic transformation is the integration of recombinant nucleic acid molecules into the genome of a target cell. It is a powerful tool in plant biology and is commonly used to study gene function, by over-expression or targeted silencing of specific sequences. However, a range of other applications is possible, including identification of genes and promoters by tagging. Unlike *Arabidopsis*, for which germ-line transformation is available, wheat can only be transformed via delicate tissue culture procedures involving the regeneration of plants via somatic embryogenesis and until recently has proved recalcitrant to transformation by *Agrobacterium*.

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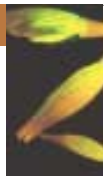


Genetic transformation and promoter tagging in wheat

Huw D Jones

DNA-delivery via biolistics or *Agrobacterium*

Since the first reports of wheat transformation in the early 1990s, robust protocols using biolistics to introduce DNA into regenerable scutella tissues have been developed and are now routine in several laboratories world-wide. We have used optimised protocols based on biolistics to produce over six hundred transgenic lines in thirty wheat genotypes, including elite cultivars grown in the UK. These lines have proved to be uniquely useful in the characterisation of several important promoters and in the study of the role of a wide range of proteins including a suite of high molecular weight glutenin subunits and the gibberellin 2oxidase enzyme. However, plants produced by this method tend to have a high transgene copy number, occasionally with multiple rearrangements, and in some cases this can complicate the analysis of transgene expression. The soil bacterium *Agrobacterium tumefaciens* (the causative agent of crown gall disease) is known to deliver low copy number T-DNA insertions and is now the transformation method of choice for most other plant species. Wheat is normally outside the host range of *Agrobacterium*, but we have successfully optimised a range of variables affecting T-DNA delivery and regeneration, and produced transgenic wheat plants using this approach. Initial analysis of copy number and transgene segregation in these lines indicates that the majority display simple, low copy integration patterns and a 3:1 inheritance ratio (Figure 1).

Wheat florets expressing GFP (green fluorescent protein).(left)

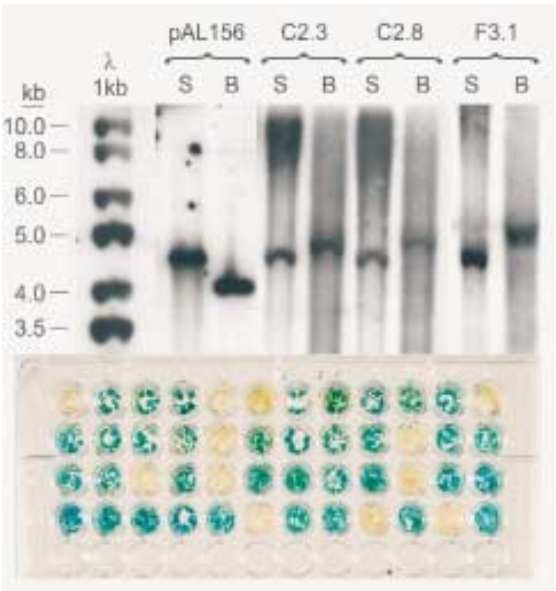


Figure 1. Southern blot of three plants (C2.3, C2.8 & F3.1) transformed via agrobacterium showing low copy number, simple integration patterns (top). GUS assay of T_1 progeny plants showing a 3:1 segregation of transgene expression (bottom)

Transgene integration

The precise order of the molecular events leading to the stable integration of a transgene into the host plant genome is unclear. However, it appears that regardless of the method of DNA-delivery, genetic transformation involves double-stranded illegitimate recombination at one or more loci, utilising the cell's nuclear repair machinery. The moment of integration probably coincides with a DNA metabolic event such as replication or transcription. Some *in situ* hybridisation data suggest that physical integration occurs at random within and between plant chromosomes. However other analyses demonstrate a preference for distal chromosomal locations and actively transcribed regions of the plant genome with the possibility that native plant genes are disrupted in the process. We have used fluorescence *in situ* hybridisation (FISH) to study the distribution of transgene insertions in wheat. Initial analysis reveals no preference for particular wheat chromosomes but a bias towards the telomers (Figure 2).

Promoter tagging

Although the nuclear genome of all cells in a genetically modified plant will contain transgene copies, the regulatory promoter sequences upstream of the coding region will dictate when and where in the plant the transgene will be expressed. One of the bottlenecks to the application of plant transformation technologies is the lack of well-characterised tissue-specific, developmentally regulated and environmentally induced promoters that can be matched with specific coding sequences to drive expression in particular tissues or developmental stages. We are using two approaches to overcome this bottleneck. Firstly to use markers such as green fluorescent protein (GFP) or beta-glucuronidase (GUS) to

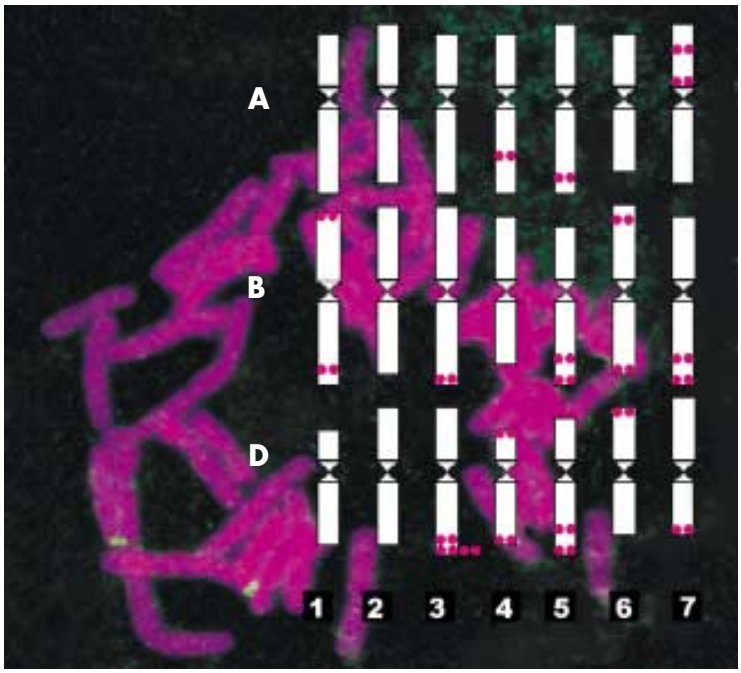
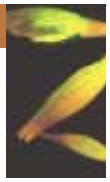


Figure 2. Fluorescence *in situ* hybridisation (FISH) of transgenes in wheat chromosomes (background). Representation of integration sites from over 20 independent lines analysed by FISH showing bias towards telomers (foreground).

(Work done by Jean Jacquet, a BBSRC-CASE student in collaboration with Mark Tester, Cambridge University and Peter Jacks, Monsanto UK)



characterise known promoter sequences in transgenic wheat. More than ten constitutive or tissue-specific promoter sequences are currently being analysed in wheat transgenics (Figure 3). In a second approach, we are using promoter tagging to identify novel regulatory sequences. We have shown previously that transforming with promoterless marker genes can generate novel, specific, heritable expression patterns (Figure 4). However, the difficulty in generating sufficiently high numbers of independent transgenic lines in wheat limits this approach. In collaboration with Christine Foyer and Gabriela Pastori we have exploited the ability of the maize Activator/Dissociation (AcDs) transposon to jump a promoterless marker gene around the wheat genome. By generating a limited number of Ac and Ds parental lines and crossing them, we have made a large number of plants each containing both the Ac and Ds insertions. We have already demonstrated by sequencing that the Ds elements in progeny plants of several Ac x Ds crosses do undergo transposition and we believe that transposon-mediated promoter tagging in wheat is feasible.

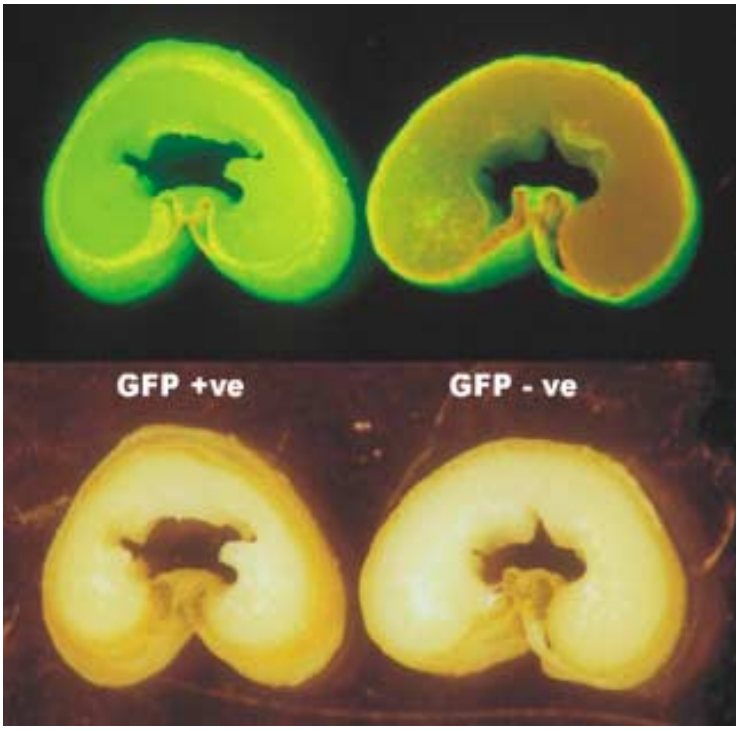


Figure 3. GFP expression in immature wheat seeds driven by the rice actin promoter visualised under UV light (top). Same seeds under white light (bottom)

Exploitation

The output from whole genome and expressed gene (EST) sequencing projects continues to increase exponentially but annotation to ascribe gene function lags significantly behind. Genetic transformation is already one of the key methods used to investigate or validate gene function and, together with associated high-throughput techniques such as transient expression, is set to be an important tool for functional genomics. The hexaploid status of bread wheat and the fact that it has many unique structural and physiological features complicates the use of model species to accurately predict gene function in this important food and feed crop. Thus, the ability to over-express, knockout and tag specific gene sequences directly in a specific wheat variety is a compelling technology in many areas of wheat research. We are currently the only publicly funded laboratory in the UK capable of agrobacterium-mediated wheat transformation and Rothamsted Research is in a unique position to exploit this technology for research to benefit UK and world agriculture.

Figure 4. Random insertion of a promoterless GUS gene results in anther-specific expression patterns in immature inflorescence