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# A transgenic approach to understanding gene expression in cereals

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**SUMMARY –** As methods for the genetic transformation and regeneration of cereals become increasingly facile and efficient, other limitations in the use of this approach become more obvious. For some transgenic applications, strong constitutive over expression is required while for others, finely targeted or inducible expression is preferable. However, there is a lack of well-defined constitutive, tissue-specific or inducible promoters for cereals. Here, we describe our work to characterize promoter sequences that drive defined patterns of expression in transgenic wheat using the GUS reporter gene.

## Introduction

Genetic transformation underpins a range of specific research methods for identifying genes and studying their function *in planta*. Knowledge gained in this way can inform and speed-up conventional breeding strategies. Genetic transformation also allows the direct manipulation of specific traits via introduction of novel genes into locally-adapted germplasm. A range of research strategies that incorporate transformation as a component are in common use. In model plant species, populations tagged with T-DNAs or heterologous transposons are proving uniquely useful for identifying genes and promoters [see recent reviews (An *et al.*, 2005; Radhamony *et al.*, 2005)]. The availability of strongly constitutive, tissue-specific or inducible promoter sequences and siRNA technology is facilitating highly targeted over-expression and precise down-regulation of candidate genes (reviewed by Jones, 2005). In addition, fluoro- or colorimetric reporter genes, matrix attachment regions, epitope tags or targeting sequences are increasingly incorporated into transgene cassettes to study gene expression, organelle morphology or protein trafficking. Wheat has many unique biologically and commercially important traits, including aspects of development, end-use quality and disease resistance, that cannot easily be accessed via model species. Thus there is an increasing demand from the wheat research community for access to these transformation-based tools and technologies.

## Development of a transformation platform

The major cereal crops were all first transformed using the direct transfer of naked DNA via a biolistic device (reviewed by Barcelo *et al.*, 2001). Subsequently, reports for the transformation of each of these species (except oat and millet) via *Agrobacterium* (Cheng *et al.*, 2004) have also been reported, summarised in Fig. 1.

Although wheat poses more challenges than maize or rice, several laboratories around the world are now routinely transforming one or more wheat genotypes using either *Agrobacterium* or biolistic DNA delivery. Rothamsted Research in the UK currently use both a biolistic method (Sparks and Jones, 2004) and an *Agrobacterium* method (Wu *et al.*, 2003; Jones *et al.*, 2005) to produce approximately 500 transgenic lines per year and now offers a cost-recovery, wheat transformation service to academic researchers. Detailed analysis of T-DNA insertions at a genetic and molecular level revealed 30% of all lines transformed by *Agrobacterium* possessed a single copy of the transgene but that two thirds also contained vector backbone sequence (Wu *et al.*, 2006).

## Control of transgene expression

The initiation of transcription and translation are important components of the complex regulatory mechanisms that determine the dynamic, spatial and temporal distribution of proteins in a plant.

Alongside transcriptomic approaches, the use of transgenic plants, often incorporating reporter gene fusions encoding luciferase, GUS or GFP, have been widely used to study the role of specific promoter and enhancer elements (reviewed by Venter and Botha, 2004). In addition to basic research into the control of transcription *per se*, transgenic approaches to improve crop phenotypes requires the incorporation of regulatory elements around the coding sequence to give the desired transcriptional control of the transgene. There is a conspicuous shortage of well characterised constitutive, tissue-specific, developmentally-regulated or inducible promoters to drive candidate genes in transgenic plants. Less than twenty promoters have been used in transgenic wheat (listed in Jones, 2005). Approximately half of these are considered broadly constitutive and half show some tissue-specificity however, many are not well characterised or are simple variants of each other (for example, with or without an intron sequence).

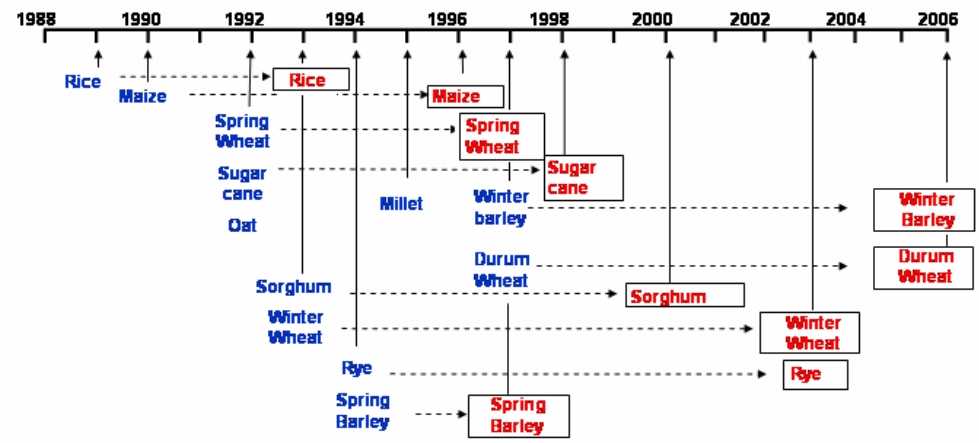


Fig. 1. Time-line of first reports for the transformation of cereal crops. DNA-delivery by biolistics (unboxed) and *Agrobacterium*-mediated transformation (boxed).

To provide promoters for our in-house transformation constructs, we have begun to validate specific promoter sequences in wheat transgenic plants using transcriptional GUS fusions. To date, we have focussed on sequences giving constitutive or seed-specific expression but have also tested one inducible promoter. Some of the promoters used are listed in Table 1. Histochemical and fluorometric GUS analysis have revealed qualitative and quantitative differences in temporal and spatial patterns of expression.

Table 1. Summary of promoter:GUS studies in transgenic wheat

| Promoter | Source | No. transgenic lines made | Localisation of expression | Timing of expression |
|---|---|---|---|---|
| Ubiquitin | Maize | >750 | "Constitutive" | |
| Actin | Rice | 43 | "Constitutive" | |
| Rice Tungro Bacilliform Virus | Viral | 6 | "Constitutive" (but not in roots or pollen) | |
| HMW Glutenin subunit 1Dx5 | Wheat | 23 | Endosperm specific | 10 dpa |
| HMW Glutenin subunit Bx17 | Wheat | 13 | Endosperm specific | 11 dpa |
| LMW Glutenin subunit | Wheat | 20 | Endosperm specific | 13 dpa |
| Globulin 1 | Maize | 10 | Transfer cells of aleurone & scutellum | 12-13 dpa 20-21 dpa |
| Heat Shock (Hvhsp17) | Barley | 27 | Seeds & leaves | Heat inducible |
| Globulin 7S | Wheat | In progress | Aleurone? | ? |
| Lipid transfer protein | Wheat | In progress | Aleurone? | ? |

A comparison of temporal and spatial GUS expression driven by three broadly constitutive promoters revealed strong blue staining in most tissues. Exceptions were pollen and roots where no staining was visible with the Rice Tungro Bacilliform Virus promoter (Fig. 2A). Seed-specific promoters which possess activity in regions of the starchy endosperm or transfer aleurone and that are first detectable at 7-10 days post anthesis are being studied (Fig. 2B) (see also Lamacchia *et al.*, 2001). In addition, a promoter from the barley *Hvhsp17* gene (Raho *et al.*, 1995) shows a heat-inducible phenotype in the organs studied so far (seeds and leaves) (see paper by Freeman *et al*. in this meeting).

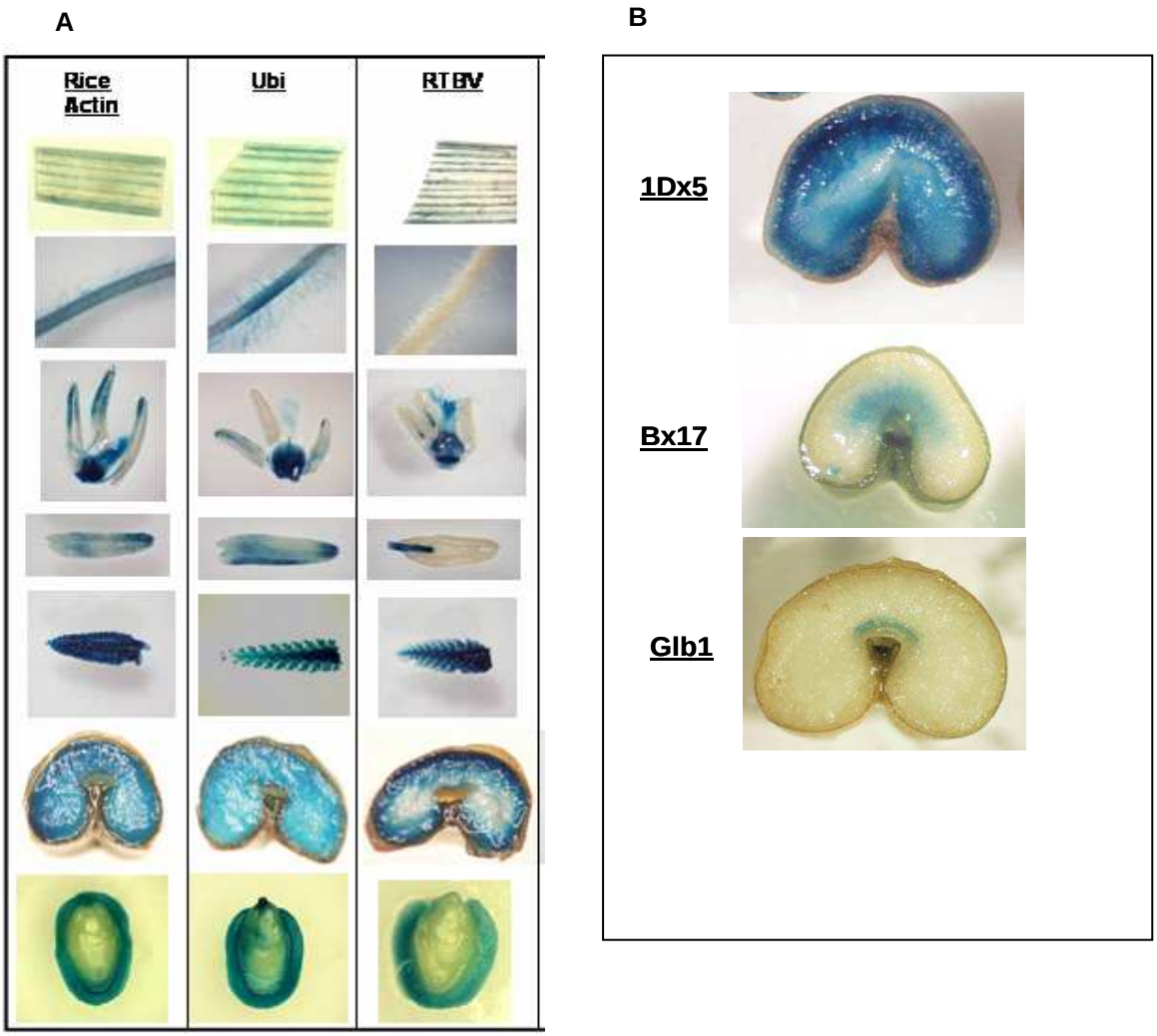


Fig. 2. Patterns of staining observed in transgenic wheat plants with various promoter:GUS fusions. A. broadly constitutive promoters, B. seed-specific promoters.

## Summary and future plans

To facilitate transgenic approaches to study gene function and to provide a route to using genetic engineering for crop improvement, we aim to provide a robust transformation platform at Rothamsted Research and to provide a catalogue of transformation vectors containing well-characterised promoters with pre-defined expression patterns. The first of these aims is now achieved and we offer wheat transformation as a service for research scientists, from within Rothamsted Research and elsewhere, facilitated by the BBSRC MONOGRAM programme. We also aim to isolate and characterise promoter sequences for driving specific or inducible patterns of expression in transgenic wheat. We have already described the expression patterns of several promoters and we intend to use genomic sequence data from rice and Brachypodium to isolate further promoters and regulatory

elements with specific expression patterns for use in cereal genetic improvement. Further information can be found at www.bract.org and www.rothamsted.bbsrc.ac.uk/cpi/wdi/hj.html.

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