Identification Of Novel Promoters Useful For Crop Biotechnology

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Need for Research:

1

There is public concern about the safety of genetically engineered crops.

Research Goal:

To create a "toolkit" of useful promoters for precisely controlling transgene expression in crop plants.

These promoters will allow researchers to engineer crops that express transgenes only when and where they are needed, thus minimizing unintended effects on plant physiology and the environment.

Research Objective:

2

To identify and characterize cereal crop promoters with useful and reliable expression patterns.

Types of gene expression patterns being pursued:

- Tissue/organ-specific expression
- Developmental stage-specific expression
- Expression everywhere but the seed

Approach:

We are using global gene expression profiling to identify candidate rice and barley genes with organ-specific expression patterns.

Gene Expression Analysis:

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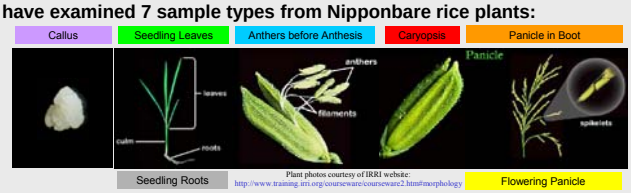
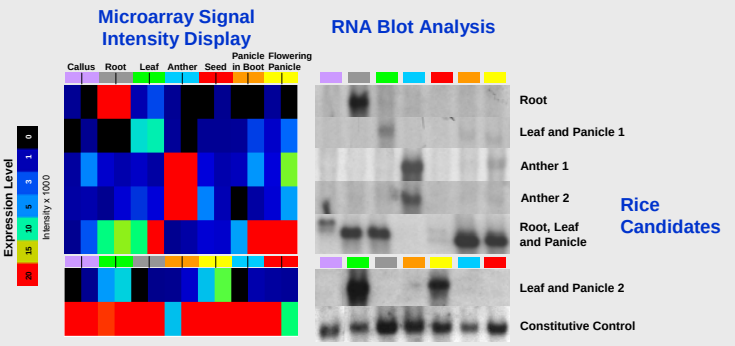
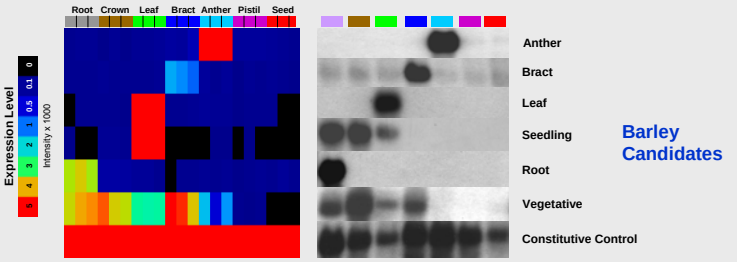
We have examined 7 sample types from Nipponbare rice plants:



Two or more independent biological replicates for each sample type were harvested and used for analysis with a 15,753 element rice cDNA microarray developed by Hans Bohnert's group at the University of Illinois. The microarray results were globally normalized and 6 genes with organ-specific patterns of expression were selected; their expression data is shown below:



We have also mined a large barley gene expression dataset created by Arnis Druka, Robbie Waugh and colleagues (Druka *et al*. submitted). They examined 8 developmental stages and 15 tissue types of Morex barley using the 22,700 element Affymetrix Barley1 GeneChip. The expression data for 6 selected candidates is shown:



Promoter Isolation:

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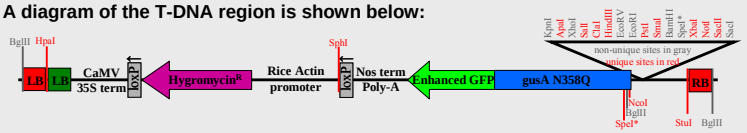
The 5' upstream promoter sequence (up to 2.5kb) of 6 rice candidates has been PCR amplified from genomic DNA. Promoter sequences of the barley candidates are being identified from a Morex barley BAC library using gene specific probes.

Promoter Testing Vector Construction:

The currently available promoter testing binary vectors typically contain elements (e.g. the CaMV 35*S* promoter) that may promiscuously alter the expression of the nearby candidate promoters being examined (Yoo *et al. Planta* 4:523-30 2005) We have constructed a novel pGreen (www.pgreen.ac.uk/) binary vector that avoids some of these problems and is well suited for monocot promoter characterization. A diagram of the T-DNA region is shown below:



The constitutive rice actin promoter + intron drives the expression of *hptII* for hygromycin resistance. LoxP attachment sites (gray boxes) for the Cre recombinase flank this promoter-selectable marker expression cassette. This will allow the removal of this portion of the T-DNA from the transgenic plants if desired. An extra copy of the left border sequence (green box) has been incorporated to decrease the occurrence of *Agrobacterium* transferring not only the T-DNA, but the backbone binary vector sequences. The *gusA* reporter gene was translationally fused to the enhanced version of Green Fluorescent Protein. Promoter reporter fusions to this combination reporter gene will allow the detection of promoter activity in both live transgenic plant cells by visualizing eGFP fluorescence or through the histochemical staining of *GUS*-encoded β-glucuronidase activity.

Rice Transformation:

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We are transforming our promoter constructs into Nipponbare rice using *Agrobacterium*-mediated transformation as previously described by Sallaud *et al*., *Theor. Appl. Genet.* 106:1296-1408, 2003 with modifications incorporated from Yang *et al*., *Plant Sci.* 167: 281-288, 2004.

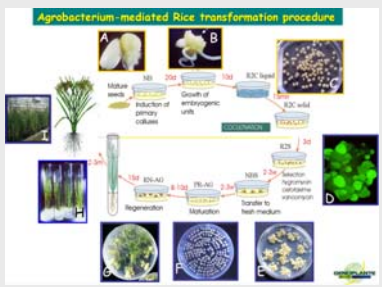
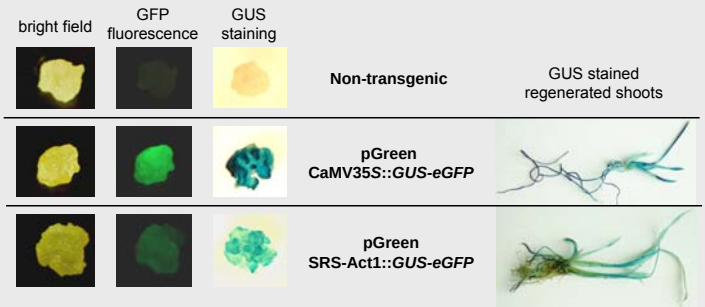


Image courtesy of Genoplante website
http://genoplante-info.infobiogen.fr/OryzaTagLine/project.htm

Reporter gene detection in hygromycin resistant calli and regenerated shoots containing our novel promoter testing vector is shown below:



Future Plans:

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We will characterize the expression conferred by each candidate promoter using transgenic rice, barley and/or wheat plants.

Tools developed from this project including the promoters, vector constructs and transgenic plants, and resources from a related research project* will be made publicly available.

*see Poster 940 and a presentation in the Plant Transgene Genetics Workshop "Recombinases For Controlled Eukaryotic Genome Manipulation" James Thomson, Yuan-Yeu Yau & David Ow

If you have suggestions for types of promoters that would be useful in your own research, please contact Roger Thilmony at thilmony@pw.usda.gov or (510) 559-5761