Identification Of Novel Promoters Useful For Crop Biotechnology

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Need for Research:
There is public concern about the safety of genetically engineered crops. We have also mined a large barley gene expression dataset created by Arnis Druka, Hans Bohnert’s group at the University of Illinois. We are using global gene expression profiling to identify candidate rice and barley genes with organ-specific expression patterns.

Gene Expression Analysis:
We have examined 7 sample types from Nipponbare rice plants: Leaf, Root, Callus, Seedling Leaves, Seedling Roots, Anther and Panicle. 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:

Promoter Isolation:
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 35S promoter) that may promiscuously alter the expression of the nearby candidate promoters being examined. 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:

Rice Transformation:

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Future Plans:
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.ars.usda.gov or (510) 559-5761.