2008

Analysis of heterologous promoters in transgenic cereals

Agnelo Furtado
Southern Cross University

Robert J. Henry
Southern Cross University

Alessandro Pellegrineschi

Fumio Takaiwa
National Institute of Agrobiological Sciences, Japan

Publication details

Furtado, A, Henry, RJ, Pellegrineschi, A & Takaiwa, F 2008, 'Analysis of heterologous promoters in transgenic cereals', paper presented to 58th Australian Cereal Chemistry Conference, Gold Coast, Qld., 31 August - 4 September.

ANALYSIS OF HETEROLOGOUS PROMOTERS IN TRANSGENIC RICE

A Furtado[1], RJ Henry[1], F Takaiwa [2]

[1]Cooperative Research Centre for Molecular Plant Breeding, Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW 2480, Australia.
[2]Plant Biotechnology Department, National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki, Japan

INTRODUCTION

The genomes of cereals are one of the many that humans have remodelled through careful selection and breeding-criteria such as high yield, disease tolerance or environmental adaptability. Biotechnology offers another avenue for modification of genomes by transferring one or more transgenes for end-use quality applications or the use of plants as biofactories for the manufacture of various products. The economics of using cereal grain for the production of bio-molecules would depend on value of the bio-molecule and the amount synthesised in the grain. Several reports demonstrate that rice grains can be used for end-use quality or as a production platform for seed-based edible vaccines. Beta-carotene was produced in the rice grain with the aim of alleviating Vitamin A deficiency in human populations (Paine *et al.*, 2005), and rice grains have also been used as a platform for the production of vaccines (Takaiwa *et al.*, 2007; Wu *et al.*, 2007). To maximise the benefits of this technology it would be highly desirable to generate transgenic rice with multi-traits (gene stacking) conferred by many genes under control of as many promoters. However, use of the same promoter to drive more than one transgene, can result in impaired expression of one or more transgenes through a process of homologous sequence-dependent gene silencing (transcriptional silencing) (Butaye *et al.*, 2005; Jorgensen, 1992; Rocha *et al.*, 2005). The need to stack more than one trait in the same seed tissue of rice will require the use of homologous or heterologous promoters with the same tissue specificities but with little homology.

The main objective of this study was to investigate if barley and wheat seed-specific promoters from storage protein and non-storage protein genes can direct seed- or endosperm-specific reporter gene expression in transgenic rice. The other objective was to determine the levels of GFP expression under various barley and wheat promoters in rice seeds and compare them to those of rice storage protein promoters (the 1350 bp *Glutelin B-1* and the *1007 bp α-Globulin* promoters).

MATERIALS AND METHODS

Plant material and transformation of rice

Seeds of rice (*Oryza sativa* L.) c.v. Jarrah were obtained from Plant Industry, CSIRO, Canberra, Australia. The binary construct pEvec202N*nos* (Furtado and Henry, 2005) was used to prepare all the constructs used for rice transformation. The promoter-*gfp* fragments were directionally cloned into the *Hind* 111 and *Apa* I sites of the pEvec202N*nos* construct to generate the relevant binary promoter constructs. The promoters linked to the *gfp* gene are the 1007 bp *Globulin* (*Glob*) (Qu and Takaiwa, 2004) and the 1350 bp *Glutelin-B1* (*GluB-1*) (Takaiwa *et al.*, 1991) gene promoters from rice, the 433 bp *D-hordein* (*D-Hor*) (Sorensen *et al.*, 1996) the 549 bp *B-hordein* (*B-Hor*) (Sorensen *et al.*, 1996)gene promoters from barley, and the 425 bp High Molecular Weight-glutenin (*HMW-Glu*) (Thompson *et al.*, 1985)gene

promoter from wheat. The Cauliflower Mosaic viral promoter (*CaMV35S*) (Guilley *et al.*, 1982) was also included as a positive control for constitutive expression. Transformation of rice was carried out as published elsewhere (Furtado and Henry, 2005).

Fluorescence microscopy for detection of GFP fluorescence
Detection of green fluorescence from GFP was carried out using a compound microscope equipped with an attachment for fluorescence as published elsewhere (Furtado and Henry, 2005)

Selection of immature seeds for detection and measurement of GFP expression
Developing seeds corresponding to T1 and T2-generation from all transgenic plants were analysed by fluorescence microscopy and all images are representative images of corresponding transgenic events. Measurement of GFP in developing seeds was carried out by monoclonal antibody-based ELISA on T2-generation seeds at 20 days after flowering (DAF).

RESULTS AND DISCUSSION

The 1007 bp *Globulin (Glob)* and the 1350 bp *Glutelin-B1* (*GluB-1*) gene promoters from rice, directed *gfp* expression in developing seed tissue but not in the floral, leaf or root tissue (Table 1). Unlike the storage protein promoters from rice (*Glob* and the *GluB-1),* the *B-Hor* and the *D-Hor* storage protein promoters from barley directed *gfp* expression in both seed and non-seed tissues (Table-1), where expression of GFP was observed in the glumes, lemma, palea, lodicules and possibly the anther filaments of floral tissues, and in the midrib, veins and stomata of leaf tissue. In seed tissue, the *B-Hor* and the *D-Hor* promoters did not direct expression of GFP in the embryo, but did in the pericarp and throughout the endosperm (including the aleurone). The *HMW-Glu* promoter-directed expression of GFP was not observed in the floral tissue but was observed in the leaf tissue and in root tissue (Table 1). In developing seeds the *HMW-Glu* promoter directed differential *gfp* expression, with no expression observed in the embryo but was observed in the vascular parenchyma, pericarp, and endosperm including the aleurone (Table 1).

We obtained a range of GFP expression levels in T2-seeds, and this variation corresponds to differences in transgene copy number and position effect from different transgene events (Figure 1). In our study, GFP expression levels under the *Glob* promoter from rice was higher than all other promoters tested, and this is in agreement with other studies (Hwang *et al.*, 2002; Qu and Takaiwa, 2004; Wu *et al.*, 1998). GFP-expression levels under the *B-Hor* and *D-Hor* promoters were similar (Figure 1), although there were more kernels with high *D-hor*-directed GFP than with *B-hor*-directed GFP. In case of the *HMW-glut* promoter, GFP-expression levels were lower than the other storage protein promoters even though it shares high homology to the barley *D-Hor* and the rice *Glob* promoters. T2-generation Kernels with the highest GFP concentration relative to total soluble seed protein for the *α-Glob*, *GluB-1*, *B-hor*, *D-hor* and the *HMW-Glu* promoters were 2.2%, 0.06%, 2.4%, 0.96% and 0.4% respectively.

We have demonstrated that storage protein seed-specific promoters from barley (*B-hor* and *D-hor*) and wheat (*HMW-Glu*) failed to direct seed-specific *gfp* expression in transgenic rice, as expression of GFP was observed in the leaf and root tissue, and in maternal seed tissues. These results reflect that the regulatory mechanisms in rice that control endosperm-specific expression of rice storage protein genes may not exert similar spatial control on storage protein promoters from other cereals. Our results also indicate that promoters operating in

heterologous systems do not always mimic the control of gene expression which they exhibit in their native species. Promoters from heterologous systems can direct gene expression in the endosperm of rice and at high levels as is the case for the *B-hor* promoter from barley. Leakiness of heterologous storage promoters in rice indicates that their utility, at least in rice, may be limited if highly endosperm-specific expression of transgenes is desired.

Table 1: Spatial and temporal control of *gfp* expression under various promoters in transgenic rice.

Promoter used/ size in bp	Species / Septicity	Floral tissues	Leaf tissue					Leaf tissue	Root tissues
			Pericarp	Endosperm	Aleurone	Embryo	Vascular Parenchyma		
Camv35S / 298	Cauliflower Mosaic Virus / Constitutive in plants	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes +
Glutelin-B1 / 1350	Endosperm / rice	No	No	Yes	Yes	No	No	No	No -
Globulin / 1007	Endosperm / rice	No	No	Yes	Yes	No	Yes	No	No -
B-Hordein / 549	Endosperm / barley	Yes+1	Yes	Yes	Yes	No	Yes	Yes+2	Yes
D-Hordein / 433	Endosperm / barley	Yes+1	Yes	Yes	Yes	No	Yes	Yes+2	Yes
HMW-Glutelin / 425	Endosperm / wheat	No	Yes	Yes	Yes	No	Yes	Yes	Yes

Numbers after + or – represent fluorescence levels when compared to similar tissues from CaMV35S plants

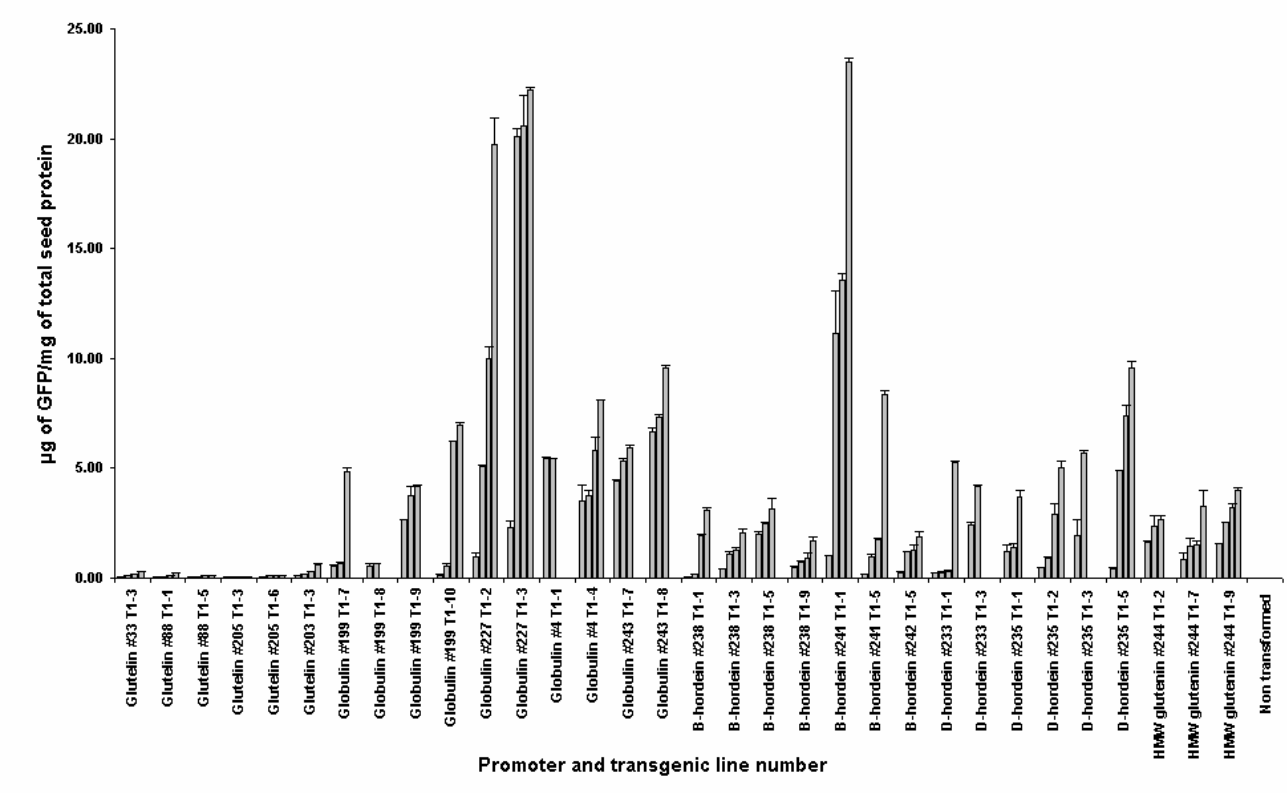

Figure 1: Expression of *gfp* in individual T2-generation rice seeds under control of storage protein promoters from rice, barley and wheat. The *Globulin* promoter directs higher *gfp* expression than all the promoters including the *Glutelin-B1* promoter. Concentration of GFP was measured by ELISA in single seeds (up to 4) and expressed as concentration per milligram of total soluble seed protein. Data represents values of 2 independent ELISA measurements.

References

Butaye, K.M.J., Cammue, B.P.A., Delaure, S.L. and De Bolle, M.F.C. (2005) *Mol. Breed.*, **16**, 79-91.

Furtado, A. and Henry, R.J. (2005) *Plant Biotechnol. J.*, **3**, 421-434.

Guilley, H., Dudley, R.K., Jonard, G., Balazs, E. and Richards, K.E. (1982) *Cell*, **30**, 763-770.

Hwang, Y.-S., Yang, D., McCullar, C., Wu, L., Chen, L., Pham, P., Nandi, S. and Huang, N. (2002) *Plant Cell Rep.*, **20**, 842-847.

Jorgensen, R. (1992) *Agbiotech News and Information.*, **4**, 265-273.

Paine, J.A., Shipton, C.A., Chaggar, S., Howells, R.M., Kennedy, M.J., Vernon, G., Wright, S.Y., Hinchliffe, E., Adams, J.L., Silverstone, A.L. et al. (2005) *Nature Biotechnology*, **23**, 482-487.

Qu, L.Q. and Takaiwa, F. (2004) *Plant Biotechnol. J.*, **2**, 113-125.

Rocha, P.S.C.F., Sheikh, M., Melchiorre, R., Fagard, M., Boutet, S., Loach, R., Moffatt, B., Wagner, C., Vaucheret, H. and Furner, I. (2005) *Plant Cell*, **17**, 404-417.

Sorensen, M.B., Muller, M., Skerritt, J. and Simpson, D. (1996) *Mol. Gen. Genet.*, **250**, 750-760.

Takaiwa, F., Oono, K., Wing, D. and Kato, A. (1991) *Plant Mol. Biol.*, **17**, 875-885.

Takaiwa, F., Takagi, H., Hirose, S. and Wakasa, Y. (2007) *Plant Biotechnol J*, **5**, 84-92.

Thompson, R.D., Bartels, D. and Harberd, N.P. (1985) *Nucleic Acids Res.*, **13**, 6833-6846.

Wu, C.Y., Adachi, T., Hatano, T., Washida, H., Suzuki, A. and Takaiwa, F. (1998) *Plant and Cell Physiol.*, **39**, 885-889.

Wu, J., Yu, L., Li, L., Hu, J., Zhou, J. and Zhou, X. (2007) *Plant Biotechnol. J.*, **5**, 570-578.