Screening of tissue- and/or stress-specific promoter from T-DNA insertion rice mutants

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ABSTRACT

Using transfer DNA (T-DNA) with functions of gene trap and gene knockout and activation tagging, a mutant population containing approximate to 60,000 lines was generated by Dr. Yu's lab, Academic Sinica. The materials contain a promoterless GUS gene next to the right border. GUS activity screening facilitated identification of genes responsive to various stresses and those regulated temporally and spatially in whole plant. Gene expression patterns and physiological function were further studied. Leaves, roots, and panicles were cut and treated with salt, sorbitol, 4℃, and 45℃ for 24hr, respectively, before GUS staining. At present, 80 lines (~4.5%) had been obtained from 1800 mutants screening. Among the 80 lines we selected, 13 lines showed constitutive expression, 47 lines showed increased GUS activity while 20 showed less. In addition, we also found some lines showed root- or leaf- or spike- specific expression pattern. One of them further selected and characterized from TRIM database searched. The *OsAR* gene, encoding a NADPH-dependent aldose reductase, was inducible by ABA, salt, and sorbitol stresses. These results suggested that the T-DNA tagging lines are useful to obtain the stress-responsive and tissue specific promoters and genes in rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops in the world as well as an excellent model cereal crop for genomics research. In comparison to other cereal crops, it has the smallest genome size (430 Mb) and much molecular and genetic information (ESTs, markers, genetic and physical maps, etc.) is available. Besides, *Agrobacterium tumefaciens* -mediated rice transformation system have well established on a routine basis. Transfer DNA (T-DNA) has the advantage that the inserted element acts as a tag for gene identification. Gene knockout and activated by insertion of *Agrobacterium* derived T-DNA has been developed for tagging genes in Arabidopsis, petunia, periwinkle, and rice. A mutant population pool containing approximate to 60,000 lines was generated by Dr. S. M. Yu, Academic Sinica, using T-DNA insertion strategy. These materials also contain a promoterless GUS gene next to the right border. Here, we described the preliminary analysis of the expression pattern of GUS under different abiotic stress and in different organ using the materials mentioned above.

Table 1 GUS expression in pTag4 tagged lines in response to abiotic stresses

Experiment	No. of lines	No. of GUS positive lines	No. of stress-independent lines	No. of stress-responsive lines	No. of stress responsive lines							
					Sorbitol		NaCl		4 ℃		45 ℃	
					Up	Down	Up	Down	Up	Down	Up	Down
1	450	19 (4.2)	6 (1.3)	13 (2.8)	6	4	3	3	4	3	3	4
2	486	19 (3.9)	3 (0.6)	16 (3.2)	12	4	5	0	1	2	0	4
3	564	36 (6.4)	4 (0.7)	32 (5.6)	14	7	22	3	6	4	2	5
4	300	6 (2.0)	0(0)	6 (2.0)	5	1	4	1	1	3	1	4
Total	1800	80 (4.5)	13 (0.7)	67 (3.7)	37 (2.1)	16 (0.9)	34 (1.9)	7 (0.4)	12 (0.7)	12 (0.7)	6 (0.3)	17 (0.9)

Numbers in brackets indicate % of total lines.

Materials and Methods

Rice T2 seeds from T-DNA insertion mutants

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Plant cultured by Hydroponics

Leaf
Root
spike

↓

± stress treatment

salt
sorbitol
chilling
heat

↓

GUS staining

↓

Bioinformatic analysis (TRIM database)

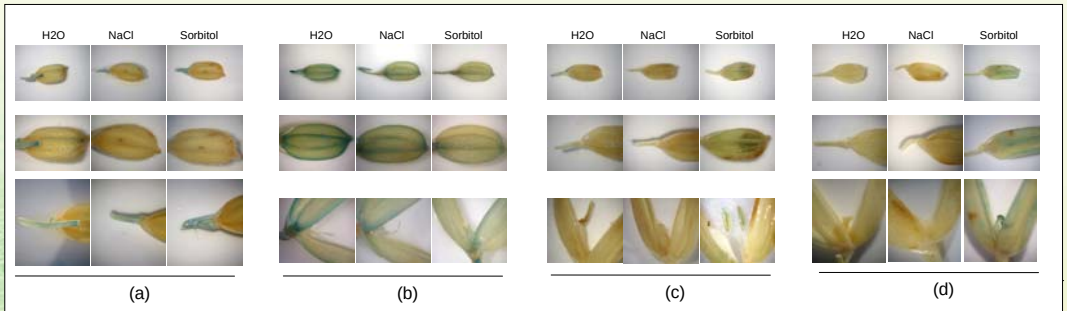

Fig. 2. GUS staining identifies stress-regulated promoter from T-DNA rice spikes. (a) putative constitutive promoter (b) putative stress down-regulated promoter (c) putative anther-specific promoter (d) putative stress up-regulated promoter

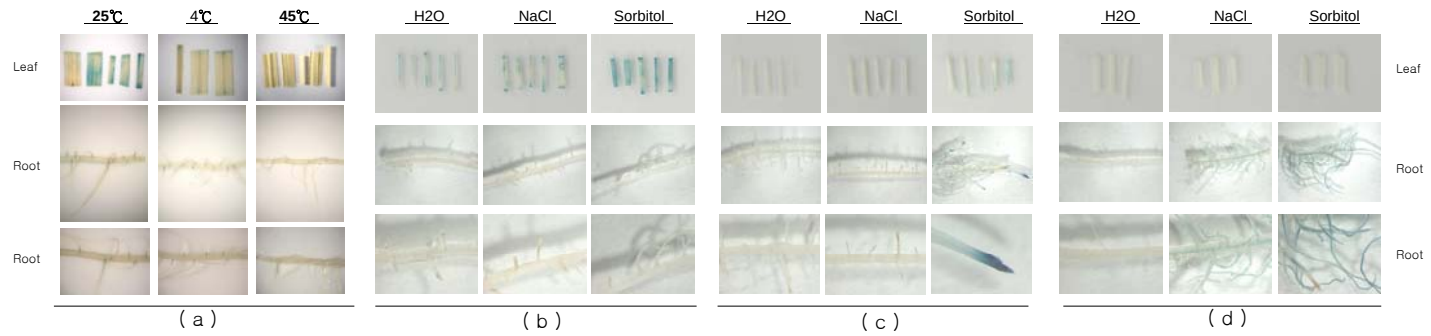

Fig. 1. GUS staining identifies stress-regulated promoter from T-DNA rice leaves and roots. (a) Putative temperature stress down-regulated promoter (b)- (d) putative stress up-regulated promoter (c) putative root tip specific expression promoter (d) putative vascular tissue specific expression promoter

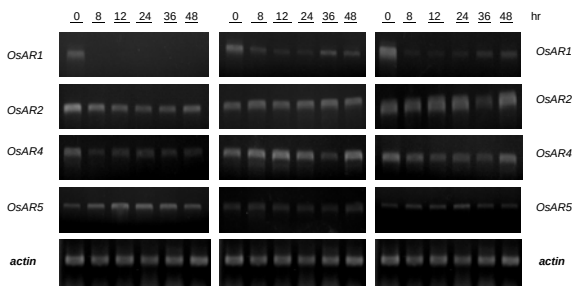

Fig. 3. Time course expression of aldose reductase isogenes in TNG67 suspension cell under different stress treatment. ABA, 20 μM; NaCl, 200 mM; Sorbitol, 300 mM.

RESULTS and DISCUSSIONS

1. At present, 80 lines showed GUS positive response (~4.5%) had been obtained from 1800 mutant lines screening (Table 1).
2. Among the 80 lines we selected, 13 lines showed constitutive expression, 47 lines showed increased GUS activity while 20 showed less. (Fig.1-2)。
3. Differential GUS expression was detected in various tissue and various stress treatment. Some lines showed sorbitol-specific expression (ex. Fig. 1c, Fig. 2c, 2d), some lines showed leaf specific (Fig. 1b), or root dominant expression (Fig. 1c, 1d).
4. To obtain the flanking sequence through TRIM database search, we identified the *OsAR* gene, encoding a NADPH-depentent aldose reductase would be related to sugar alcohol biosynthesis, were induced by ABA, salt, and sorbitol treatment (Fig.3). Further investigations were underway.

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