

MATERIALS AND METHODS

We selected 20 T₁ progenies from the R6 line, in which *bar* sequence mutations were not detected but PAT protein expression was disrupted. PCR was performed by using the specific primers 35S-F1-5'-CCACTGAATCAAAGGCCATGGAGTCAA-3' and BAR-R2-5'-TAGCGCTCCCGCAGACGGAC-3' to produce a single band of 718 bp (including 548 bp of the 35S promoter and 170 bp of *bar*). The PCR products were separately gel purified followed A-tailing and cloned into the pGEM-T vector. Five positive clones were sequenced.

RESULTS

As shown in Supplementary Fig. 1, four T₁ lines were found to have one deletion, four were found to have an SNP, and one was found to have multiple nucleotide polymorphism (MNP) mutations when compared to the corresponding Ba15 PCR sequence.

	10	20	30	40	50
▶ Translate ▶ Consensus	AGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
p35-bar-1_1.seq(1>701)	→ AGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-9-3-T7promoter.ab1(11>1142)	→ ◀AGACTGGCGAACCGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-14-2-T7promoter.ab1(14>998)	→ ◀AGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-14-4-T7promoter.ab1(7>1026)	→ ◀-----TGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-35-2-T7promoter.ab1(11>1141)	→ ◀AGACTGGCGAACAGTTCACACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-35-4-T7promoter.ab1(8>1076)	→ ◀AGACTGGCGAACAGTACATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
▶ 6-30-2-T7promoter.ab1(10>1078)	← ◀AGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAA				
	480	49	500	510	520
▶ Translate ▶ Consensus	TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
p35-bar-1_1.seq(1>701)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-9-3-T7promoter.ab1(11>1142)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-14-2-T7promoter.ab1(14>998)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-14-4-T7promoter.ab1(7>1026)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-35-2-T7promoter.ab1(11>1141)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-35-4-T7promoter.ab1(8>1076)	→ TTGGAGAGAACACGGGGGACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				
▶ 6-30-2-T7promoter.ab1(10>1078)	← TTGGAGAGAACACGGGAAACTCTAGGGGGATCTACCATGAGCCCAGAACGACCGC				

Supplementary Fig. 1. Sequence mutations of targeted CaMV 35S promoter of T₁ progenies from R6

Supplementary Table 1. TALEN-mediated *bar* mutations of T₀ transgenic lines

Line no. ^z	Target site (56bp)	Mutation clones/ Total clones	Target bar gene DNA mutation			Target gene amino acid mutation			
			Mutation types	Mutations	Mutation location	Amino acids	Mutation types	Mutation location	PAT expression
R1	×	1/3	Deletion	-1	1bp	183	Frame shift	-	PAT +
R2	×	2/5	SNP	C to T	213bp	184	No change	-	PAT +
R3	×	1/3	Insertion	+1	3bp	184	Frame shift	-	PAT +
R6	×	2/4	Deletion/SNP	-3/-1,A to G	1-4bp	183	Substitution,Frame shift	2, E to G	PAT +/-
R7	×	1/6	Deletion	-100	1-100bp	150	Deletion, Frame shift	1~34	PAT -
R9	×	2/4	Deletion	-1/-1	1bp, 551bp~	183	Frame shift	-	PAT +
R14	×	1/3	Deletion	-9	544bp~	181	Deletion	182-184 (stop codon)	PAT +
R15	×	-	-	-	-	184	-	-	PAT -
R16	×	1/3	Deletion	-5	548bp~	182	Deletion	183-184 (stop codon)	PAT +/-
R17	×	3/4	Deletion	-1	1bp	183	Frame shift	-	PAT -
R20	×	-	-	-	-	184	-	-	PAT -
R21	×	-	-	-	-	184	-	-	PAT -
R24	×	1/3	Deletion	-5	548bp~	182	Deletion	183-184 (stop codon)	PAT +/-
R26	×	-	-	-	-	184	-	-	PAT -
R28	×	1/6	SNP	G to A	145bp	184	Substitution	49, D to N	PAT +/-
R29	×	-	-	-	-	184	-	-	PAT -
R31	×	-	-	-	-	184	-	-	PAT -
R32	×	1/5	SNP	G to A	437bp	184	Substitution	146, G to D	PAT -
R33	×	1/1	Deletion, SNP	-2, G to A	1bp / 26bp	183	Frame shift	-	PAT +
R35	×	-	-	-	-	184	-	-	PAT -
R37	×	-	-	-	-	184	-	-	PAT -
R41	×	1/4	deletion	-3	550bp~	183	Deletion	184 (stop codon)	PAT +

^z Eight T₀ rice plants of R15, R20, R21, R26, R29, R31, R35 and R37 showed inactive protein expression of PAT but without *bar* sequence mutation.