Omonrice 18: 1-10 (2011)

DEVELOPMENT OF TRANSGENIC RICE LINES RESISTANT TO INSECT PESTS USING *Agrobacterium tumefaciens*- MEDIATED TRANSFORMATION AND MANNOSE SELECTION SYSTEM

Tran Thi Cuc Hoa and Ho Thi Huynh Nhu

ABSTRACT

Insect pests including stemborer caused severe loss in rice production worldwide. Genetic transformation to integrate insect resistant genes like Bt genes in the rice genome has been shown to be efficient in developing varieties resistant to insect pests. In our study, we constructed two new vectors, pUBB-Man containing the genes cry1Ab and pmi and pUBC-Man containing the genes cry1Ac and pmi. The gene pmi is a selectable marker which allows using mannose for selection. The use of mannose to replace antibiotics/herbicides for selection helped avoiding the concerns on biosafety of the transgenic plants. Transformation was done by using Agrobacterium tumefaciens strain LBA 4404 to transfer the transgenes to embryogenic calli of the two indica varieties, IR64 and Mot Bui. The frequency of transformation ranged from 1.00-5.83% indicating the mannose selection system we applied was efficient in rice transformation. The transgenic lines were confirmed by Southern blot analysis showing the integration of cry1Ab or cry1Ac gene in the rice genome. In bioassay tests for resistance to yellow stemborer in the laboratory and in the net house, it was recorded that a large number of transgenic lines were highly resistance. The promising homozygous transgenic lines (F8) have been selected.

Key words: *Agrobacterium tumefaciens***,** insect pests, insect resistance, mannose, rice, stemborer

INTRODUCTION

Stemborer is one of the insect pests causing heavy loss to rice production. To control this pest, the conventional breeding method is not much effective because no resistant donors in the rice germplasm have been identified although extensive screening was done at the International Rice Research Institute and elsewhere for the last 30 years. Therefore, to breed rice varieties resistant to stem borer using genetic transformation technique is the most promising approach, this would also help in reducing the use of insecticides and the cost of production for farmers.

A few authors have reported the development of some rice transgenic lines of japonica and indica types resistant to pink stem borer, stripped stem borer, yellow stem borer and leaf folder. (Nayak et al. 1997; Datta et al. 1998, Chen et al. 2005). Wu et al. (2002) reported the development Japonica transgenic lines carrying c*ry1Ab* showing stable resistance to stem borer under field testing. Breitler et al. (2004) reported that seven homozygous transgenic lines harboring the *cry1B* or *cry1Aa* genes were tested in the field in Spain showing high resistance to striped stem borer. Most recently, it was reported that Bt rice was approved to apply in China (ISAAA Brief 41-2009). However, there were no other reports on the adoption of resistant transgenic lines derived from varieties of improved genetic background, particularly indica varieties. Moreover, so far in the procedures of transformation, the normal selection methods based on using marker genes conferring tolerance antibiotics or herbicides were used; this caused the concerns on the adverse effects of GM plants on environment and public health.

In this study, efforts have been made to transform two indica varieties, IR64 and Mot Bui with the *cry1Ac* or *cry1Ab* gene to develop transgenic lines of improved genetic background resistant to stem borer. This was achieved by using *Agrobacterium* method for transformation and mannose selection system.

MATERIALS AND METHODS

Construction of vectors carrying *cry1Ab* or *cry1Ac* and *pmi* as marker gene

To serve the purpose of this study, we constructed the new vectors, namely pUBC-Man and pUBB-Man (Figure 1). *Vector pUBB-Man*: was constructed by using the vector pCaCar (Hoa et al. 2003) but the genes, *crtI* and *psy* on this vector were removed by the restriction enzymes- *Hin*d III + *Bam*HI and were replaced by the ubiquitin promoter plus the *cry1Ab* gene. To achieve this objective, the ubiquitin-*cry1Ab* from the vector pUBB (supplied by Dr. Altosaar) was isolated by the restriction enzyme- *Hin*dIII + *Spe*I and the DNA fragment of 2.2 kb carrying the ubiquitin promoter was selected. The vector pUBB was also cut with *Spe*I + *Bam*HI to select the DNA fragment of 1.9 kb carrying the *cry1Ab* gene. These two DNA fragments of 2.2 kb and 1.9 kb were inserted simultaneously at the corresponding positions of *Hin*dIII and *Bam*HI on the vector-pCaCar. The resulted vector- pUBB-Man was transferred to the competent cells of *A. tumefaciens* strain LBA 4404 (Hoekema et al., 1984). *Vector pUBC-Man*: was constructed by using vector pCaCar (Hoa et al., 2003) but the genes, *crtI* and *psy* on this vector were removed by *Hin*dIII + *Bam*HI and were replaced by ubiquitin-*cry1Ac* isolated from the vector pUBC (supplied by Dr. Altosaar). The steps on cloning were performed as in the construction of pUBB-Man vector described above. The resulted pUBC-Man was transferred to the competent cells of *A. tumefaciens* strain LBA 4404.

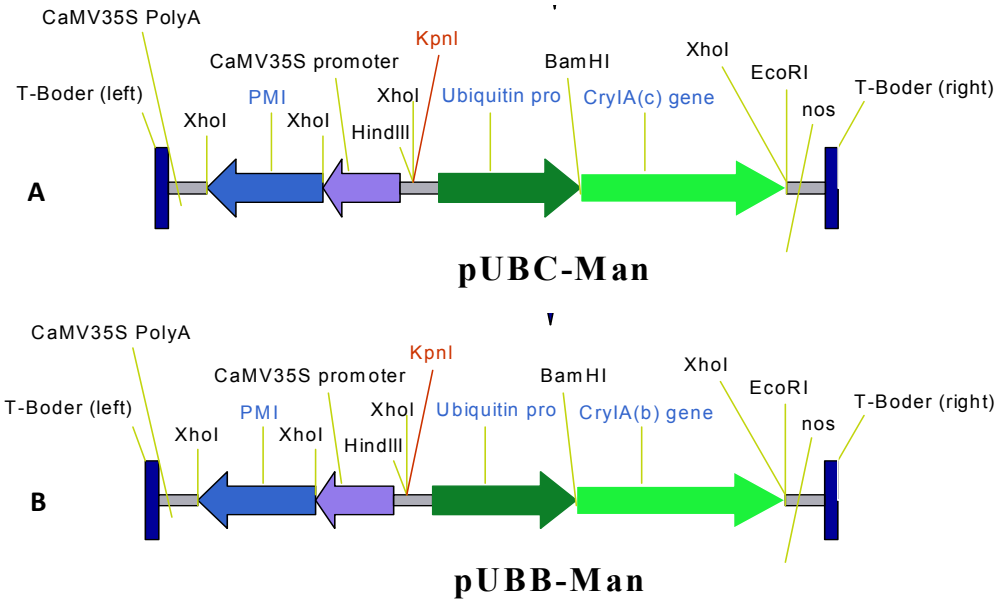

Figure 1. Diagram of the vector pUBC-Man (A) containing the gene *cry1Ac* and the vector pUBB-Man (A) containing the gene *cry1Ab*. Both the two vectors contain the selectable marker gene *pmi*.

Rice varieties and transformation methods

Two indica varieties, IR64 and Mot Bui were used for transformation. The immature embryos or mature dehulled grains from these varieties were cultured for inducing callus formation. The resulting embryogenic calli were then inoculated with *A. tumefaciens* strain LBA 4404. Method of transformation described by Hoa and Bong (2003) was followed. Selections were done at 2 week intervals on MS medium (Murashige and Skoog 1962) containing 30 g/l sucrose and 25 g/l mannose at the first round of selection, 15 g/l sucrose and 25 g/l mannose at the second round and 5 g/l sucrose and 35 g/l mannose at the third round. The plants resistant to mannose were

confirmed by chlorophenol red (CR) assay (Hoa and Bong 2003) before transferring to soil.

DNA isolation and Southern analysis

DNA was isolated from leaves following the procedures of McCouch et al. (1988). Ten micrograms of DNA were cut with *Eco*RI and *Bam*HI (two cuts) to identify the presence of *cry1Ab* in the lines transformed with pUBB-Man and was cut with *Kpn*I (one cut) to determine the integration pattern and number of copy. For the transgenic lines transformed with pUBC-Man, genomic DNA was digested with *Xho*I (two cuts) to identify the presence of *cry1Ac* and cut with *Kpn*I (one cut) to determine the integration pattern and number of copy. The restricted DNA was fractionated through 0.8% agarose gel by electrophoresis at 50 Voltage for 12 hr, prior to capillary transfer to immobilization on nylon membranes (Hybond-N^+, Amersham). The *cry1Ab* or *cry1Ac* genes were labeled by DIG and used as a probe). Hybridization, washing and detection were performed following the descriptions of Wünn et al. (1996).

Segregation of transgenic lines

The self-pollinated T_1 seeds of the T_0 transgenic plants were cultured in the medium containing ½ MS + 20g/l mannose. The resistant plants were evaluated after 2 weeks in culture and transferred to soil in the net house. Leaf samples were taken for Southern analysis.

Bioassay of transgenic lines for yellow stem borer (*Tryporyza incertulas*) resistance

Rice transgenic lines

Fifteen day old seedlings of Bt rice lines after mannose test and check varieties were transplanted in plastic pots of 15 cm diameter. Test plants were laid out in randomized complete block design (RCBD) with three replications in 3 metal trays of 1.2 x 2.4 x 0.2 m. Assays were carried out at 30 days after transplanting.

Raising stemborer

Female moths of yellow stem borer were collected under light trap and reared for laying eggs in tubular mylar cages containing growing rice plants. The moths laid eggs after 1- 2 days. Eggs masses were collected 2 days before hatching and placed in plastic containers lined with moist filter papers. Eggs hatched after 5 -7 days.

Petri dish assay

A single stem section about 7 cm long was cut from each plant. Three stems were taken from each line (3 replications/line) and each stem was placed into petri-dish lined with moistened filter paper and released 5 neonatal larvae. The petri-dishes were sealed with parafilm to prevent the escape of larvae and kept in laboratory temperature of 25 ± 2^OC. Stem sections were dissected to observe larval mortality, growth stage at 5 days after infestation.

Whole plant assay

Test plants were uniformly maintained for number of tillers (10 tillers/plant), there were three replications per line. Each plant was infested with 10 neonatal yellow stem borer larvae (1 larva/tiller) and covered individually by tubular mylar cage to prevent larval dispersal.

Percentage of dead shoots were recorded at 5, 10, 15 and 20 days after insect inoculation. Performance of Bt lines against yellow stem borer were classified depending on percentage of dead shoots as below:

Scale 0: 0%
Scale 1: from 1 to 10%.
Scale 3: from 11 to 20%.
Scale 5: from 21 to 30%.
Scale 7: from 31 to 60%.
Scale 9: above 60%.

Plants were dissected to record number of dead and live larvae, larval development stage and weight. The survival of insect was recorded at 25 days after insect inoculation: the number of insect survived and the weight of insect survived.

RESULTS AND DISCUSSION

Development of transgenic lines carrying the cry1Ac or cry1Ab genes

For rice transformation, so far the use of the selective marker genes conferring either resistance to antibiotics or resistance to herbicides was widely applied (Christou 1997; Hei et al. 1997). However the use of these selective marker genes

has caused widespread public concerns because of inadequate knowledge of their effects on the environment and on human health. To overcome this constraint, alternative selection systems have been developed. Recently, the system using mannose selection based on *pmi* - a gene derived from *Escherichia coli* encoding phosphomannose isomerase (Miles and Guest 1984) as selectable marker was tested. It was reported that, using this selection system, transgenic sugar beet (Joersbo et al. 1998), maize and wheat (Wright et al. 2001), japonica rice cv. Taipei 309 (Lucca et al. 2001) and indica rice cv. IR 64, MTL 250 (Hoa et al. 2003) were successfully developed.

This selection system is based on the conversion of mannose to mannose-6-phosphate by endogenous hexokinase which accumulates in the cells resulting in severe growth inhibition. The transgenic *pmi*-expressing cells have the ability to convert mannose-6-phosphate, an unusable carbon source for most plant cells, into fructose-6-phosphate- a carbohydrate source that can be utilized by the plant tissue. Only the transgenic plants (expressing the *pmi* gene) were able to metabolize mannose into a usable source of carbon resulting in normal growth on media containing mannose, while non-transformed tissues either stops growing or dies due to starvation (Hansen and Wright 1999).

Two weeks after in the rooting medium, roots of the putative transformants were tested for phosphomannose isomerase activity using the chlorophenol-red (CR) assay. In this assay, plant tissues able to metabolize mannose acidified the medium and turned it from red to yellow, a consequence of a decrease of pH from 6.0 to 5.0. Wells that contained tissues unable to utilize the mannose remained red (basic), indicating that either they were not transformed or the selectable gene did not express. The lines that were tested for the CR assay were further analyzed for the presence of the *pmi* gene by Southern blotting. The presence of the *pmi* gene was observed in the lines showing color changing in CR assay, but not in the lines without color change in the CR assay. Therefore, the mannose selection system associating with CR assay was a rapid and sensitive method to screen putative transformed plants resistant to mannose. The transgenic events can be identified by using CR assay as early as 75 days after the start of transformation.

In our study, we constructed two vectors, each had the Bt gene conferring resistance to stem borer (*cry1Ab* or *cry1Ac*) plus the *pmi* gene as selectable marker. Each vector was transformed into two Vietnamese varieties having improved agronomic traits, IR64 and Mot Bui by *Agrobacterium*-mediated transformation method using the mannose selection system as described above. The results of transformation experiments were presented in Table 3. In total, 1,424 calli were inoculated and after selection on medium containing mannose, 93 plants were regenerated and survived to grow. These plants were taken for Southern blot analysis to confirm the presence of the *cry1Ac* or *cry1Ab* genes in the T_0 plants. Figure 2 showed the presence of the *cry1Ac* gene in some T_0 lines developed from IR64 and Mot Bui. The T_0 plants showing positive in Southern blot analysis were also positive in CR test. Among 93 plants, 51 were positive in Southern blot analysis and CR test, or a transformation efficiency of 3.58% was obtained on the average.

For transformation with the vector pUBB-Man the transformation efficiency in IR64 and Mot Bui varied from 1.80-4.78% and 5.53-5.80 %, respectively (Table 1). For transformation with the vector pUBC-Man, the efficiency in IR64 and Mot Bui ranged respectively from 1.00-2.40% and 3.07-5.71% (Table 1). In our transformation experiments, the efficiency of transformation was quite satisfactory. Aldermita and Hodges (1996) transformed Indica cultivars by *Agrobacterium* method and using hygromycin selection, and they obtained a transformation efficiency of 1-4%. Our results indicated that the transformation method that we used was efficient to produce transgenic plants from indica improved varieties.

From transgenic T_0 plants, 30 T_1 lines were selected based on Southern blot analysis. Figure 3 showed the results of Southern blot analysis of 7 T_1 plants developed from T_0 line (E2-2/pUBC-Man/IR64) with the presence of the *cry1Ac* gene. From T_1 lines showing resistance to stem borer, 36 T_2 lines were selected.

Table 1. Efficiency of transformation using *A. tumefaciens* LBA 4404

Vector/ Variety	Experiment	No. of inoculated callus (A)	No. of resistant calli	No. of plant regenerated	No. of T_0 transgenic plant (confirmed by CR+ and Southern blot+) (B)	Effici-ency (B/A)%
pUBC-Man						
IR64	1	230	60	25	11	4.78
	2	110	10	5	2	1.80
	3	100	9	4	2	2.00
Mot Bui	1	120	10	8	5	4.16
	2	140	16	12	8	5.71
	3	130	14	10	4	3.07
pUBB-Man						
IR64	1	125	15	6	3	2.40
	2	140	10	5	2	1.42
	3	100	6	3	1	1.00
Mot Bui	1	109	10	7	6	5.50
	2	120	10	8	7	5.83
Grand total		**1424**	**170**	**93**	**51**	**3.58**

Results on bioassay of resistance to stem borer of transgenic lines T1 (IR64) transformed with *cry1Ab* and *cry1Ac*

Bioassay of T_1 transgenic lines

The resistance of 30 T_1 lines to yellow stem borer was tested in the net house, using the W1263 as resistant check and IR29 as susceptible check. The evaluation of resistance scale based on the percentage of died shoots and converted to scale 1-9. The resistant check had scale of 5 and rated to be moderately susceptible, the susceptible check had scale of 7 and rated to be susceptible. A large number of the transgenic T_1 lines had the scale of 1-3 and were rated to be resistant- moderately resistant. But a few transgenic lines had the scale of 5-7 (Table 2).

The classification of transgenic lines based on scale of resistance at 20 days after insect inoculation is summarized in Table 3. Out of 30 transgenic lines tested, 13 lines or 40.62% were rated resistant (scale 1) and 10 lines or 31.25 were rated moderately resistant (scale 3). The data obtained in bioassay of the resistance to stem borer obviously indicated that the expression of the transgene in most of the transgenic lines was quite satisfactory to confer the resistance to yellow stemborer.

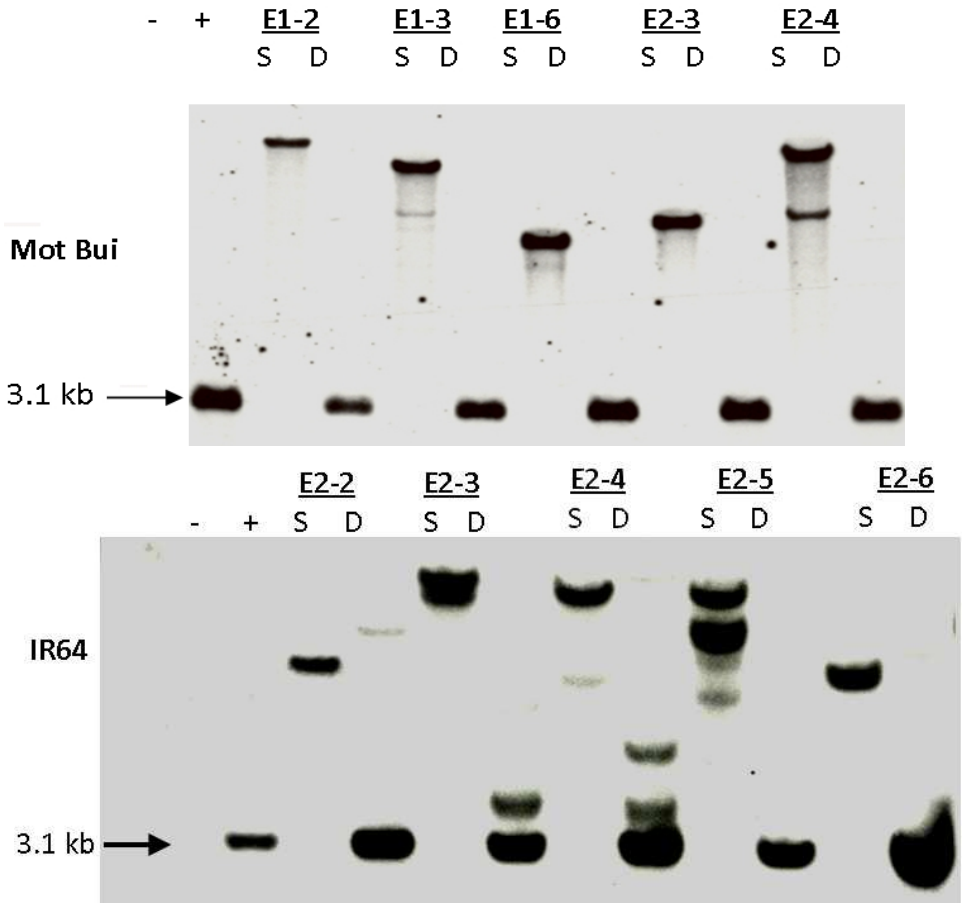

Figure 2. Southern blot analysis of T_0 lines from Mot Bui and IR64 transformed by pUBC-Man. DNA was digested by *kpn*I (S: single cut) and (D: double cut) by *Xho*I and hybridized with the probe carrying *cry1Ac* gene. The arrow indicates the DNA band (3.1 kb) contained *cry1Ac* gene.
M: 1kb Marker; -: non transformed check; +: plasmid DNA

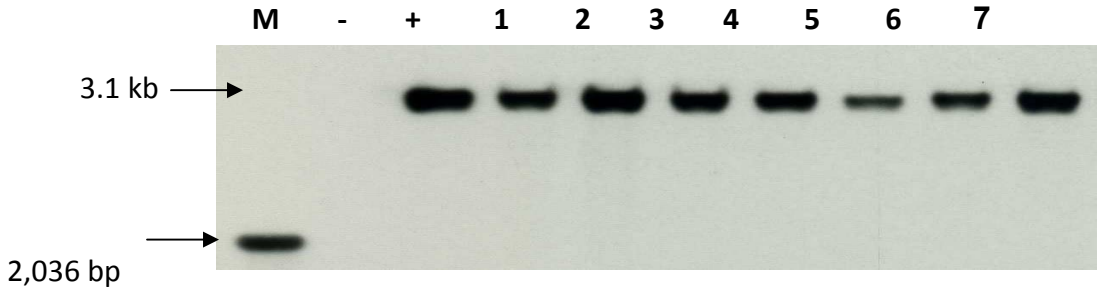

Figure 3. Southern blot analysis T_1 plant (1 to 7) developed from T_0 line (E2-2/pUBC-Man/IR64).
DNA digested with *Xho*I and probe with *cry1Ac*. The arrow indicates DNA band (3.1 kb) containing *cry1Ac* gene. M: 1kb Marker; - : untransformed plant; +: plasmid DNA

Table 2. Scale of resistance and reaction of T_1 (IR64) lines to yellow stem borer at 20 days after inoculation.

S. No.	Line	Scale	Reaction	S. No.	Line	Scale	Reaction
1	E2-1-1/*cry1Ac*	1	R	17	E2-3-3/*cry1Ac*	3	MR
2	E2-1-2/*cry1Ac*	1	R	18	E2-3-4/*cry1Ac*	3	MR
3	E2-1-3/*cry1Ac*	1	R	19	E2-4-1/*cry1Ac*	5	MS
4	E2-1-4/*cry1Ac*	1	R	20	E2-4-2/*cry1Ac*	7	S
5	E2-1-5/*cry1Ac*	1	R	21	E2-4-3/*cry1Ac*	7	S
6	E2-2-1/*cry1Ac*	3	MR	22	E2-4-4/*cry1Ac*	7	S
7	E2-2-2/*cry1Ac*	3	MR	23	E2-4-5/*cry1Ac*	5	MS
8	E2-2-3/*cry1Ac*	1	R	24	E2-4-7/*cry1Ac*	1	R
9	E2-2-4/*cry1Ac*	1	R	25	E2-5-1/*cry1Ac*	3	MR
10	E2-2-5/*cry1Ac*	3	MR	26	E2-5-2/*cry1Ac*	1	R
11	E2-2-6/*cry1Ac*	5	MS	27	E2-5-4/*cry1Ac*	3	MR
12	E2-2-7/*cry1Ac*	3	MR	28	E2-5-5/*cry1Ac*	3	MR
13	E2-2-8/*cry1Ac*	1	R	29	E2-5-6/*cry1Ac*	1	R
14	E2-2-9/*cry1Ac*	1	R	30	E2-6-1/*cry1Ac*	7	S
15	E2-3-1/*cry1Ac*	3	MR	31	IR 29 (SC)	7	S
16	E2-3-2/*cry1Ac*	1	R	32	W 1263 (RC)	5	MS

R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible; SC: Susceptible check; RC: Resistant check

Table 3. Classification of transgenic lines based on scale of resistance at 20 days after insect inoculation.

Scale	Reaction	No of lines	Percentage of lines (%)
0	Highly resistant	0	0
1	Resistant	13	40.62
3	Moderately resistant	10	31.25
5	Moderately susceptible	4	12.50
7	Susceptible	4	12.50

Bioassay of T_2 lines

The T_2 transgenic lines were evaluated for the resistance to stem borer by petri disk test. Data were recorded for the number of alive larvae, number of dead larvae and the percentage of dead larvae (Table 4).

Table 4. Percentage of dead larvae on T_2 (IR64) transgenic lines.

S. No.	Transgenic line (T3)	Countable larvae 5 days after inoculation			Percentage of dead larvae (%)
		Total (no.)	Alive larvae (no.)	Dead larvae (no.)	
1	E2-11-1-1/*cry1Ac*	14	0	14	100.00
2	E2-11-2-7/*cry1Ac*	13	0	13	100.00
3	E2-11-4-4/*cry1Ac*	12	0	12	100.00
4	E2-12-1-1/*cry1Ac*	14	0	14	100.00
5	E2-12-3-4/*cry1Ac*	15	0	15	100.00
6	E2-12-5-3/*cry1Ac*	9	0	9	100.00
7	E2-12-7-7/*cry1Ac*	15	0	15	100.00
8	E2-12-8-5/*cry1Ac*	10	0	10	100.00
9	E2-12-9-5/*cry1Ac*	12	0	12	100.00
10	E2-12-10-7/*cry1Ac*	12	0	12	100.00
11	E2-13-1-8/*cry1Ac*	11	0	11	100.00
12	E2-13-8-4/*cry1Ac*	13	0	13	100.00
13	E2-15-6-7/*cry1Ac*	14	0	14	100.00
14	E2-15-7-2/*cry1Ac*	15	0	15	100.00
15	E2-15-8-4/*cry1Ac*	15	0	15	100.00
16	E2-16-1-1/*cry1Ac*	14	0	14	100.00
17	E2-16-2-6/*cry1Ac*	13	0	13	100.00
18	E2-16-3-3/*cry1Ac*	14	0	14	100.00
19	E2-16-5-7/*cry1Ac*	14	0	14	100.00
20	E2-16-6-1/*cry1Ac*	12	0	12	100.00
21	E2-16-7-5/*cry1Ac*	14	0	14	100.00
22	E2-16-8-7/*cry1Ac*	13	0	13	100.00
23	E2-16-9-1/*cry1Ac*	14	0	14	100.00
24	E2-18-1-9/*cry1Ac*	14	0	14	100.00
25	E2-18-2-2/*cry1Ac*	11	0	11	100.00
26	E2-18-7-4/*cry1Ac*	14	0	14	100.00
27	E2-18-8-9/*cry1Ac*	14	0	14	100.00
28	E2-13-7-2/*cry1Ac*	12	1	11	91.67
29	E1-2-4-1/*cry1Ab*	10	1	9	90.00
30	E2-13-2-8/*cry1Ac*	12	3	9	75.00
31	E2-9-3-1/*cry1Ac*	11	3	8	72.72
32	E2-13-3-5/*cry1Ac*	11	3	8	72.72
33	E2-15-4-6/*cry1Ac*	13	5	8	61.53
34	E2-13-4-2/*cry1Ac*	13	6	7	53.84
35	E2-9-5-5/*cry1Ac*	12	7	5	41.67
36	E2-9-1-4/*cry1Ac*	12	9	3	25.00
37	**W 1263 (RC)**	**14**	**7**	**7**	**50.00**
38	**IR 29 (SC)**	**14**	**14**	**0**	**0.00**

SC: Susceptible check; RC: Resistant check

It was shown that among 39 lines tested, 27 lines containing the *cry1Ac* gene had 100% of the larvae found to be dead. One line containing the *cry1Ab* gene (No. 29) had 90% of the dead larvae. The percentage of dead larve found in the resistant check variety (W1263) were 50% and in the susceptible check (IR29), all larvae were found to be alive. These results indicated that the resistant *cry1Ac* gene expressed effectively to confer resistance in the transgenic lines. To date, we have obtained T_8 transgenic lines of IR64 grown in the net house showing resistant to yellow stemborer (*Tryporyza incertulas*).

CONCLUSIONS

In development of Bt rice, we constructed the new vectors to apply mannose selection system and were successful to create transgenic lines from two indica varieties, IR64 and Mot Bui harboring *cry1Ac* or *cry1Ab* genes. In bio-assay test, the transgenic Bt lines (T1, T2) from IR64 showed high resistance to stem borer. We have obtained homozygous Bt lines (F8) from IR64 ready for further testing.

ACKNOWLEDGEMENT

The authors wish to thank the Rockefeller Foundation for financial support.

REFERENCES

Aldermita RR and TK Hodges. 1996. *Agrobacterium tumefaciens*-mediated transformation of japonica indica rice varieties. Planta. 199: 612-617.

Breitler JC, JM Vassal, MM Catala, D Meynard, V Marfà, E Melé, M Royer, I Murillo, BS Segundo, E Guiderdoni and J Messegue. 2004. *Bt* rice harbouring *cry* genes controlled by a constitutive or wound-inducible promoter: protection and transgene expression under Mediterranean field conditions. Plant Bio J 2(5):417 – 430.

Chen H, Tang W, Xu C, Li X, LinY and Zhang Q. 2005. Transgenic indica rice plants harboring a synthetic *cry2A** gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran rice pests. Theor Appl Genet 111(7): 1330-1337.

Christou P. 1997. Rice transformation: bombardment. Plant Mol. Biol. 35: 197-203.

Datta K, Z Vasquez, J Tu, I Tonizo, MF Alam, N Oliva, E Abugo, GS Khush and SK Datta. 1998. Constitutive and tissue specific differential expression of the *cryI(A)b* gene in transgenic rice plants conferring resistance to rice insect pests. Theor Appl Genet. 97: 20-30.

Hansen G and M Wright. 1999. Recent advances in the transformation of plants. Trends Plant Sci 4: 226-231.

Hei Y, T Komari and T Kubo. 1997. Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Mol. Biol. 35: 205-218.

Hoa TTC and BB Bong. 2003. Efficient *Agrobacterium* -mediated transformation of indica rice (*Oryza sativa* L.) using mannose selection system. Vietnam Journal of Agriculture & Rural Development 1+2: 60-63.

Hoa TTC, S AlBabili, P Schaub, I Potrykus and P Beyer. 2003. Golden indica japonica rice lines amemable to registration. Plant Physiology 133: 161-169.

Hoekema A, PW Roelvin, PJJ Hooykaas and RA Schilperoort. 1984. Delivery of T-DNA from the *Agrobacterium tumefaciens* chromosome into plant cells. EMBO J. 3: 2485-2490.

ISAAA. 2009. Brief 41-2009: Global Status of Commercialized Biotech/GM Crops (http://www.isaaa.org/resources/publications/briefs/41).

Joersbo M, I Donaldson, J Kreiberg, SG Peterson, J Brunstedt and FT Okkels. 1998. Analysis of mannose selection used for transformation of sugar beet. Mol Breed 4: 111-117.

Lucca P, Ye Xu and I Potrykus. 2001. Efficient selection and regeneration of transgenic rice plants with mannose as selective agent. Mol Breeding 7: 43-49.

McCouch SR, G Kochehert, ZH Yu, ZY Wang, GS Khush, WR Coffman and SD Tanksley.

1988. Molecular mapping of rice chromosomes. Theor Appl Genet 78: 815–829.

Miles JS and JR Guest. 1984. Nucleotide sequence and transcriptional start point of the phosphomannose isomerase gene of Escherichia coli. Gene 32: 41-48.

Murashige T, F Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 18: 473-497.

Nayak P, D Basu, S Das, A Basy, D Ghosh, NA Ramakrishnan, M Ghosh, SK Sen. 1997. Transgenic elite indica rice plants expressing CryIAc delta endotoxin of *Bacillus thuringiensis* are resistant again yellow stemborer (*Scirpophaga incertulas*). Proc Natl Acad Sci USA 94: 2111-2116.

Wright W, J Dawson, E Dunder, J Suittie, J Reed, C Kramer, Y Chang, R Novitzky, H Wang, L Artim-Moore. 2001. Efficient biolistic transformation of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) using the phosphomannose isomerase gene, pmi, as the selectable marker. Plant Cell Rep. 20: 429-436.

Wiinn J, A Klotti, PK Burhardt, GC Ghosh Biswas, K Launis, VA Iglesias, I Potrykus. 1996. Transgenic indica rice breeding line IR58 expressing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* provides effective pest control. Bio/Technology 14: 171-176.

Wu G, H Cui, G Ye, Y Xia, R Sardana, X Cheng, Y Li, I Altosaar, Q Shu. 2002. Inheritance and expression of the *cry1Ab* gene in Bt (*Bacillus thuringiensis*) transgenic rice. Theo Appl Genet 104 (4) 727-734.

Chọn tạo dòng lúa biến đổi gen kháng sâu bằng phưởng pháp chuyển gen qua *Agrobacterium tumefaciens* và chọn lọc bằng mannose

Các sâu hại lúa bao gồm sâu đục thân gây tổn thất nặng cho sản xuất lúa trên thế giới. Chuyển nạp gen để đưa các gen kháng sâu như các gen *Bt* vào cây lúa là giải pháp hữu hiệu để tạo ra các giống lúa có tính kháng sâu cao. Trong nghiên cứu này, chúng tôi đã thiết kế hai véc-tơ mới, pUBB-Man mang gen *cry1Ab* và gen *pmi* và pUBC-Man mang gen *cry1Ac* và *pmi.* Gen *pmi* là gen đánh dấu chọn lọc được dùng để sử dụng mannose là tác nhân chọn lọc. Việc sử dụng mannose thay cho hệ thống chọn lọc dùng chất kháng sinh hoặc chất kháng thuốc trừ cỏ nhằm khắc phục sự lo ngại về tính an toàn sinh học của cây trồng biến đổi gen. Việc chuyển nạp gen được thực hiện bằng phương pháp *Agrobacterium tumefaciens* chủng LBA 4404 để chuyển gen vào mô sẹo tạo phôi của 2 giống lúa indica, IR64 và Một Bụi. Hiệu quả chuyển nạp gen được ghi nhận từ 1,00 - 5,83% cho thấy phương pháp chọn lọc mannose chúng tôi sử dụng tỏ ra hữu hiệu đối với chuyển nạp gen ở lúa. Các dòng lúa biến đổi gen được xác định bằng phân tích Southern cho thấy sự hiện diện của gen *cry1Ab* hoặc *cry1Ac* đã được gắn vào bộ gen cây lúa. Kết quả thử nghiệm tính kháng sâu đục thân màu vàng trong phòng thí nghiệm và trong nhà lưới cho thấy một số dòng biến đổi gen có tính kháng sâu cao. Một số dòng triển vọng thuần (T_8) đã được tuyển chọn.

Từ khóa: *Agrobacterium tumefaciens***,** kháng sâu, lúa, mannose, sâu hại, sâu đục thân