Field Release and Biosafety Assessment of Transgenic Plants

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Abstract

As population increases at a rapid rate and arable land decreases dramatically in China, the demand for yield increase in agriculture has become one of the first priorities. Biotechnology, especially plant genetic engineering, has been playing a key role in improving agriculture in China. We have obtained transgenic tomato, tobacco, sweet pepper and potato plants with viral coat protein genes and/or *B.t.* or spider toxic genes against viruses and/or insects. We also transformed PG and EFE genes (antisense) in tomato to control its maturation. Our transgenic plants have been tested in the field since 1989. In collaboration with the Dandong Academy of Agriculture Sciences, Beijing Agriculture University and the Jiangsu Academy of Agriculture Sciences, we have performed field tests at 11 locations nationwide. The genetic stability and performance of the foreign gene, and the quality and yield of the transgenic plants have also been studied. We paid attention to biosafety issues, too. A group of European scientists who visited several sites where transgenic plants were released concluded that the biosafety level was high in China. We anticipate that many more transgenic crops will reach the stage of farmer release within 3-5 years.

Plant genetic engineering techniques have been routinely applied to improve crop quality and to increase crop yield in China (Chen and Gu, 1993). Scientists applied these modern techniques to obtain transgenic plant lines with favorable traits such as resistance to viruses (Abel *et al.*, 1986), insects (Barton *et al.*, 1987), fungi (Broglie *et al.*, 1991), etc. Our group has produced various transgenic plants, including virus-resistant tobacco, tomato and sweet pepper. During the last several years of field trials in collaboration with the Dandong Academy of Agricultural Research, Beijing Agricultural University, and the Jiangsu Academy of Agricultural Sciences, we have analyzed various important genetic as well as agronomic traits of those plants. Here we summarize the results of our field releases.

PK873

PK873 is an oriental tobacco variety containing the coat protein gene of tobacco mosaic virus (TMV-cp, Chinese isolate) cloned in our laboratory. It was first released in Liaoning Province, Northeast China, in 1988. To date, more than 3,000 hectares of PK873 have been harvested and some of the tobacco plants processed into cigarettes for quality analysis. After inoculation with tobacco mosaic virus, T6 generation of PK873

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showed a high resistance to virus infection and the antiviral characteristics were stably inherited (Table 1). Nopaline analysis and PCR amplification of the transgene from samples of R1 generation (1988 plants) to T6 generation (1993 plants) revealed that the target gene had been maintained in more than 90% of the plants tested (Table 2). No deleterious effects were recorded based on various quality assays (Zhu et al., 1994).

PK863

PK863 is a hybrid between the transgenic tobacco variety 90082T and MSG28 transformed with the cucumber mosaic virus coat protein gene cloned from CMV-infected tobacco plants in China (Hu *et al.*, 1990). PK863 was released to the field in Liaoning Province in 1992 whereas 90082T and MSG28 were released to the field in 1990 and 1991, respectively. In the field, the yield of PK863 (2377.0kg/acre) was obviously higher than that of untransformed 90082 (1134.3kg/acre) or G28 (964.4kg/acre). Resistance to CMV infection of three varieties is summarized in Table 3. Kanamycin selection and PCR assay of different PK863 lines are listed in Table 4. Analysis by Zhengzhou Tobacco Research Institute indicated that PK863 shows similar quality and gas chromatogram to those of several tobacco varieties used in the China tobacco industry.

PK893

Transgenic tobacco plant line PK893 with CMV-cp gene plus a modified *Bacillus thuringiensis* δ-endotoxin (*Bt* toxin) gene was released to the field in Liaoning Province in 1992. Transgenic tobacco plants expressing both CMV-cp and *Bt* toxin genes were protected from CMV infection (data not shown) and feeding damage of *Manduca sexta* (tobacco hornworm) larvae (Table 5).

8805T

Transgenic tomato plant line 8805T was found to be protected from CMV infection to a certain degree (Table 6), and its yield and total sugar content were similar to those of untransformed control plants (data not shown).

PK05C

Sweet pepper (*Capsicum annuum* cultivar *Zhongjiao* 2) was transformed with CMV-cp gene and the transgenic plants inoculated with CMV showed a high resistance to virus infection comparing with untransformed control plants in the greenhouse. Line PK05C derived from these transgenic plants was also resistant against virus infection when it was released in the field (data not shown) in 1992; and meanwhile, it still maintains good agronomical characteristics, including high yield, favorable taste and suitable fruit shape.

Transgenic rice lines

The coding region of the eighth largest segment (S8) of rice dwarf virus (RDV) was cloned from a RDV Fujian isolate and transferred into the rice (*Oryza sativa* var. *japon-*

ica) cultivars Zhonghua 8 and Zhonghua 10, respectively. Total DNA from T_1 seedlings of transgenic rice plants was analyzed by PCR assay. In case of one plant line T0-3, 29 out of 40 T_1 seedlings showed the expected size of the amplified DNA fragment. χ^2 tests indicated a good agreement with the segregation ratio of 3:1. In another case, line T0-2, 16 T_1 individuals were positive in PCR assay out of 31 seedlings and showed a 1:1 segregation ratio. These results have been confirmed by Southern analysis (Zheng *et al.,* in press). Further work will involve challenging the transgenic rice plants with RDV and trying to obtain RDV-resistant transgenic rice plants.

In order to monitor the possible transmission of transgenes into untransformed plants, seeds of untransformed tobacco plants were collected at different distances from the PK863 plot in the field. By using kanamycin selection and PCR assay, only two plants were found to be positive among 66 untransformed tobacco seedlings taken at 100 meters from the PK863 plot. Others showed negative results (Table 7). At the same time, seedlings of eggplant, tomato, cucumber and maize taken at different distances from PK863 were also analyzed by PCR assay and no positive results were found. In addition to our survey, a group of seven scientists from European countries visited China to assess the situation with regard to large scale release of transgenic plants with viral sequence and transgenic microbes in 1995. They found that no sign of adverse effect of large scale release of transgenic tobacco could be detected (Report of European Biosafety Experts Mission to China, 1995, unpublished).

In summary, we have released transgenic tobacco, tomato and sweet pepper plants into the field and carried out large scale trials. We plan to carry out field trials for transgenic rice plants in the near future. Data collected so far from the field are both interesting and encouraging. In our experiments the transgene was stably inherited from generation to generation and was not detected in other untransformed species growing near the transgenic plants. Together with our other work on gene transformation and plant regeneration (Bao *et al.,* 1993; Zhou *et al.,* 1994), it is reasonable to expect that biotechnology has a promising future in molecular breeding for crop improvement.

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Table 1 Analysis of virus infection using PK-873 (R6) and non-transgenic control plants*

Varieties	Days after infection	% Infection	Degree of inoculation	Resistance
T-1	20	93.1±4.4	34.6±3.8	4
	40	96.0±5.8	47.9±4.1	
NC89	20	95.8±4.2	40.1±3.2	5
	40	100	55.2±3.6	
PK-873	20	12.9±2.3	4.8±1.4	1
	40	37.6±6.0	9.4±1.5	
Greek-1	20	92.8±5.1	33.0±3.5	3
	40	94.7±6.8	45.5±4.1	
B-536	20	90.3±6.1	30.1±3.1	2
	40	94.8±5.2	42.5±3.2	
T-2	20	97.4±5.6	42.7±5.1	6
	40	100	58.1±6.4	

*0.5 μ g/ml TMV was manually inoculated onto tobacco leaves. Data were collected from plots of 20 tobacco plants Nine plots each were used for the statistics. All the varieties, except for PK-873, were major cultivars used in the Chinese tobacco industry.

Table 2 Analysis of the segregation patterns of nopaline production and PCR assay in transgenic tobacco plants of PK873

	Line	Total no. of plants	Nopaline-positive	PCR-positive
1988	216	29	22	-
	847	17	13	-
	1202	22	17	-
	1619	32	25	-
1989	251	35	32	32
	305	44	42	40
	324	27	26	-
	325	16	16	16
1990*	18	40	40	16
	58	22	22	16
	66	53	52	15
	91	46	46	16
1993*	1	33	33	14
	2	19	19	16
	4	54	52	16
	5	30	29	16

* In 1990 and 1993, only 16 nopaline-positive seedlings were taken for the PCR assay.

Table 3 Analysis of virus infection on PK863 in 1995

	Infection degree	% Infection
90082 (Untransformed)	65.1±14.3	93.1±4.0
G28 (Untransformed)	75.3±12.8	90.5±6.7
90082T	4.2±3.8	23.5±5.4
MSG28	37.5±15.7	10.6±8.4
PK863	3.7±2.2	10.7±4.9

The extracts of CMV-infected leaves were diluted 200 times and then inoculated onto PK863 leaves manually. Data were collected from 30 plants at 30 days after inoculation.

Table 4 Three-week seedlings of PK863 selected with 200mg/L kanamycin

Plot	Total no.of plants	Green shoots			Yellow shoots		
		no.	root	PCR-positive	no.	root	PCR-positive
1	36	33	+	32	0	-	-
2	54	54	+	53	0	-	-
3	48	40	+	40	2	-	0
5	27	24	+	21	0	-	-
9	60	53	+	49	0	-	-

Table 5 Toxicity of transgenic tobacco line PK893 to insects

Plot	Total no.of plants	Total no.of insects	Insects/ Plate	1 day			3 day			5day			7 day			Total number of dead insects
				RL (mg)	WI (mg)	NDI	RL (mg)	WI (mg)	NDI	RL (mg)	WI (mg)	NDI	RL (mg)	WI (mg)	NDI	
1	10	100	10	10	71	3	26	81	2	50	100	11	25	105	16	32
2	12	120	10	10	69	2	30	90	6	55	98	8	32	110	13	29
3	10	100	10	11	72	5	28	84	4	43	102	4	40	104	12	25
control	10	100	10	31	81	0	150	150	0	415	270	2	458	298	1	3

RL: Reduction of tobacco leaves; WI: Weight of insects; NDI: Number of dead insects
RL and WI are mean values.

Table 6 Analysis of virus infection to 8805T

Generation	Total no of plants	Degree of infection						% Infection	Resistance index
		0	1	3	5	7	9		
8805T (R1)	118	50		42	16	10		57.9	25.9
8805	98		12	18	20	35	7	100	57.2
8805T (R2)	170	110		16	44			34.4	16.7
8805	58		6	21	14		17	100	56.0
8805T (R3)	123	101		16				15.0	4.98
8805	58			15	20	18	5	100	60.5

Table 7 Three-week seedlings of untransformed tobacco plants taken at different distances from PK863 plot selected with 200mg/L kanamycin

Distance (meter)	Total no. of plants	Green shoots			Yellow shoots		
		no.	root	PCR positive	no.	root	PCR positive
50 (East)	60	0	0	0	44	0	0
50 (South)	55	0	0	0	50	0	0
100 (South)	66	3	2	2	43	0	0
300 (South)	80	0	0	0	55	0	0