13. Safety Assessment of Transgenic Tomato Plants Expressing a Coat Protein Gene of Cucumber Mosaic Virus

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Introduction

Introgression of transgenes into weeds or crops by outcrossing, and unintentional intake of these crops as foods are thought to be the main problems of field cultivation of transgenic plants.

Here, we studied the extent to which outcrossing of transgenic tomatoes harboring a coat protein gene of cucumber mosaic virus (CMV) would occur.

Materials and Methods

Transgenic tomato lines No.405 and No.707, which are homozygous for the kanamycin resistance gene and a coat protein gene of CMV, were used in this study. Outcrossing rates of transgenic tomatoes were determined using an electric fan in a closed greenhouse, with insects (honeybee (*Apis mellifera*) and bumblebee (*Bombus terrestris*)) as pollinators in a netted greenhouse, and under natural conditions in an isolated field. An anthocyanin-less tomato mutant (*ah*) was also used as a control for outcrossing of non-transgenic tomato plants.

Results

1) No difference in pollen fertility was detected between transgenic and non-transgenic tomato plants.
2) No outcrossing of transgenic tomatoes by pollen dispersal was observed under windy conditions created by an electric fan in a closed greenhouse (Fig. 1, 2).
3) The outcrossing rate of transgenic tomatoes using insects in a netted greenhouse was very low (1.5%). This rate was almost the same as the natural outcrossing rate (Table 1).
4) No outcrossing of transgenic and non-transgenic tomatoes was observed as an index of kanamycin resistance and the anthocyanin-less trait under natural conditions in an isolated field (Tables 2,3).

Conclusion

As shown here, the almost completely self-pollinating nature of tomato was fully

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retained after transformation. Moreover, since almost all the seeds for tomato production in Japan, including backyard tomato production, are purchased from commercial seed companies, the probability of cultivating a new generation carrying transgenes and of uptake of transgenic tomato unintentionally is considered to be very low even if the pollen of a transgenic tomato plant is crossed with non-transgenic tomatoes under field conditions.

Since tomatoes cannot winter in Japan because of the low temperature, transgenic tomatoes cannot become weeds, and disturb the natural or weed vegetation in Japan. It is thus considered that the cultivation of transgenic tomatoes in fields is unlikely to pose any problems for the environment.

References

1) Sato,T., Fukumoto,F., Hanada,K., Terami,F., Imai,T. and Hirai, M. (1994) : Cucumber mosaic virus resistance in transgenic tomato plants expressing the cucumber mosaic virus coat protein gene. XXIV International Horticultural Congress, Kyoto. Japan. Abstracts: p.171.

2) Sato,T., Fukumoto,F., Hanada,K., Terami,F., Imai,T. and Hirai, M. (1995) : Resistance to cucumber mosaic virus in transgenic tomato plants expressing the cucumber mosaic virus coat protein gene. 1 st International Symposium on Solanaceae for fresh Market, Malaga, Spain, Abstracts: p.139.

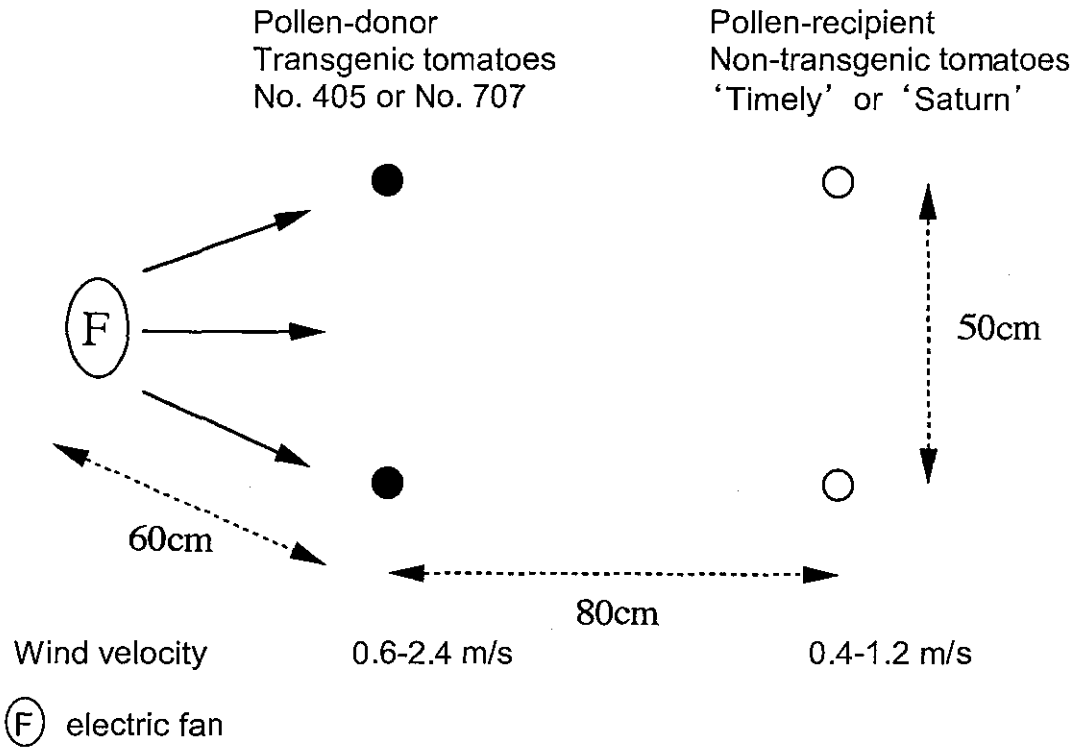

Fig. 1 Pollen dispersal based on fruit set using an electric fan in a closed greenhouse

Table 1 Outcrossing rate of transgenic tomatoes with the CMV-CP gene by insects in a netted greenhouse

Pollen-donor	Pollen-recipient	No. of seedlings tested	No. of seedlings derived from		Outcrossing rate (%)
			outcrossing	selfing	
Experiment 1 (honeybee (*Apis mellifera*))					
No. 405	'Timely'	188	0	188	0
No. 707	'Saturn'	324	25	299	7.7
Experiment 2 (bumblebee (*Bombus terrestris*))					
No. 405	'Timely'	173	0	173	0
No. 707	'Saturn'	28	0	28	0
Experiment 3 (bumblebee (*Bombus terrestris*))					
No. 405 or No. 707	'Timely'*	399	2	397	0.5
No. 405 or No. 707	'Saturn'*	85	0	85	0
wild type tomato	*ah* tomato**	545	0	545	0
Total		1742	7	1715	1.5%

*: As kanamycin resistance of the seedlings is used as an index of outcrossing rate, the pollen donor cannot be defined.

**: Outcrossing can be detected because the anthocyaninless trait is recessive. However the pollen donor cannot be defined.

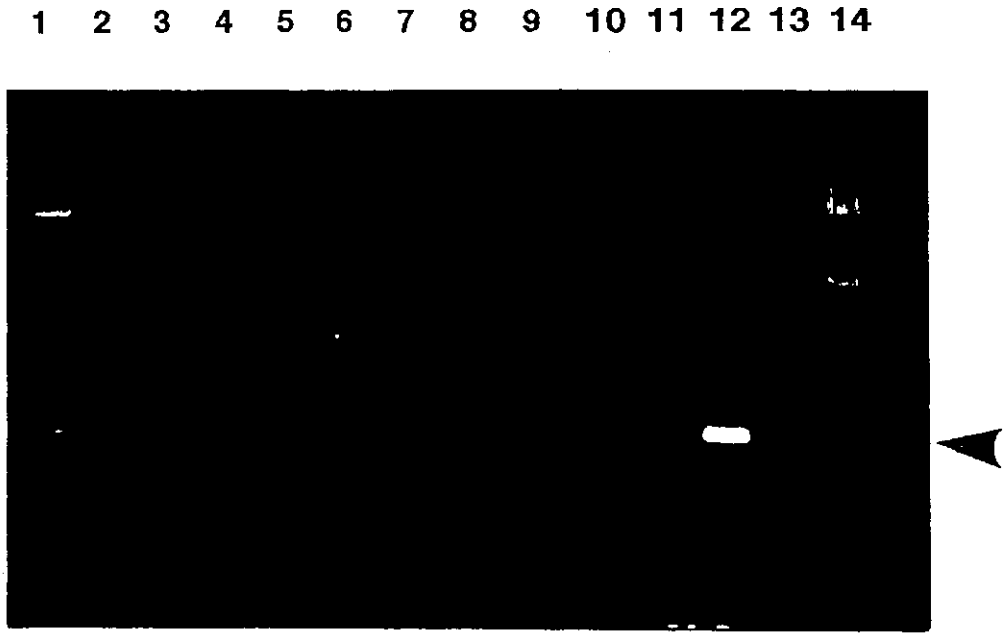

Fig. 2 Confirmation of CMV-CP gene by PCR

1,14: Molecular size marker (λ Eco R I and Hind III)

2-11: Seedlings of recipient tomatoes (non-transgenic tomatoes)

12: Transgenic tomato (No. 405)

13: Non-transgenic tomato

Table 2 Gene flow from transgenic tomatoes in an isolated field indexed by kanamycin resistance

'Saturn'	Distance (cm) from transgenic tomato (No. 707)									
Direction	50	100	150	200	250	300	350	400	450	500
East	0/75*	0/73	--	0/65	--	0/77	--	0/76	--	--
West	0/80	0/77	--	0/73	--	0/69	--	0/76	--	--
South	0/67	0/78	--	0/79	--	0/69	--	0/55	--	0/75
North	0/68	0/73	--	0/63	--	0/77	--	0/76	--	--
'Timely'	Distance (cm) from transgenic tomato (No. 405)									
Direction	50	100	150	200	250	300	350	400	450	500
East	0/58	0/75	--	--	0/69	0/79	0/76	0/72	--	--
West	0/68	0/62	--	0/51	--	0/62	--	0/57	--	--
South	0/49	0/73	--	0/77	--	0/78	--	0/80	--	0/69
North	0/62	0/53	--	0/59	--	0/68	--	0/64	--	0/79

*: No. of kanamycin-resistant seeds/ No. of seeds sown

--: Not tested or no seed obtained.

Table 3 Gene flow from transgenic tomatoes in an isolated field indexed by the anthocyaninless trait

	Distance (cm) from transgenic tomato (No. 707)									
Direction	50	100	150	200	250	300	350	400	450	500
East	0/100*	0/97	0/98	0/101	0/94	0/93	0/96	0/111	--	--
West	0/100	0/101	0/89	0/107	0/100	0/84	0/74	0/94	--	--
South	0/94	0/125	0/117	0/105	0/111	0/120	0/129	0/118	0/129	0/129
North	0/103	0/110	0/107	0/116	0/117	0/101	0/132	0/128	0/113	0/143
	Distance (cm) from transgenic tomato (No. 405)									
Direction	50	100	150	200	250	300	350	400	450	500
East	0/58	0/59	0/79	0/105	0/105	0/24	0/98	0/77	--	--
West	0/74	0/70	0/98	0/85	0/126	0/176	0/112	0/121	--	--
South	0/80	**	0/87	0/62	0/90	0/97	0/70	0/123	0/103	0/116
North	0/91	0/80	0/97	0/89	0/98	0/89	0/107	0/141	0/101	0/115

*: No. of seedlings with anthocyanin at hypocotyl/ No. of seeds sown

**: No seed obtained.

--: Not tested.