15. Environmental Risk Evaluation of Transgenic Melon Plants with Introduced Coat Protein Gene of Cucumber Mosaic Virus (CMV) in an Isolated Field

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One line (M5) of transgenic melon plants that harbors the coat protein gene of CMV (Yoshioka *et al.*, 1993) and non-transgenic melon plants derived from untransformed regenerated plants were used as experimental plant materials. In the experiment on pollen dispersal, Fusarium-resistant melon cv. 'Ooi' (old pure-bred line in Japan) was used as control for non-transgenic melon plants. The layout of the isolated field and greenhouse is depicted in Fig. 1. The results are summarized as follows (Table 1).

Development of virus disease: Twenty-two plants each of transgenic and non-transgenic melon plants were transplanted into plot No. 7 field in the isolated field (Fig.1). Only one transgenic plant and one non-transgenic plant developed symptoms of the disease. The ELISA analysis revealed that the transgenic plant was infected with PRSV while the disease of the non-transgenic plants was caused by CMV. Then recombination of coat protein gene of CMV with that of PRSV isolated from transgenic melon plants was analyzed by ELISA. The results obtained showed that proteins extracted from PRSV reacted only to the antibody of PRSV, indicating that the coat protein gene of CMV failed to recombine with PRSV coat protein gene.

Pollen dispersal by insects: Recipient melon plants which did not harbor the kanamycin resistance gene (NPT-Ⅱ) and were not resistant to Fusarium wilt were planted around donor (transgenic and/or non-transgenic Fusarium wilt resistant cv. 'Ooi') melon plants (Fig. 1).

Fusarium-resistant progenies of recipients were observed at a distance of 15 m from the donor, while progenies harboring the kanamycin resistance gene were observed at a distance of 10m from the donor. Since cv. 'Ooi' harbors the homozygous Fusarium wilt resistance gene and transgenic melon harbors heterozygous NPT-Ⅱ gene, more progenies exhibiting Fusarium wilt resistance were observed than progenies harboring the NPT-Ⅱ gene in No. 1, No. 2 and No. 3 areas. However progenies resistant to *Fusarium* wilt and progenies with the NPT-Ⅱ gene were not detected at a distance of 25 m from the donor (Table 2). These results indicated that the degree of pollen dispersal was not different between transgenic melon plants (M5) and non-transgenic melon plants (cv. Ooi). When a large number of melon plants was cultivated, pollen was not dispersed over a long distance by insects.

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Degree of self-fertilization: In this study, 16 and 21 female (bisexual) flowers from the transgenic and non-transgenic melon plants respectively, were covered by paper bags to prevent contact with visiting insects. None of the bagged flowers developed fruits. Previously, we demonstrated that wind was not an agent of pollination of melon (Tabei *et al.,* 1994). The present results confirmed that cross-pollination by insects is the main method of pollination in melon.

Overwintering of melon : Since the greenhouse was not equipped with any heating facility, all the transgenic and non-transgenic plants in greenhouse were killed as a result of the low temperature conditions by the end of December. Fruits obtained by artificial pollination were either placed on the ground or buried under the earth of the isolated field. Germination of seeds was observed from fruits left on the ground following decomposition of the fruits. However, these seedlings were killed under the low temperature conditions before they could bear any fruit. Although germinated seedlings were not observed from fruits buried under the ground during autumn, seedlings emerged from these fruits in the following spring. This observation suggested that if seeds of melon fruits are buried under the ground they could overwinter unlike the whole plants.

Conclusion

The results obtained indicated that there were no differences between transgenic melon plants and non-transgenic melon plants in terms of pollen dispersal, degree of self-fertilization and overwintering.

References

1) Tabei, Y., Oosawa, K., Nishimura, S., Watanabe, S., Tsuchiya, K., Yoshioka, K., Fujisawa, I. and Nakajima, K. (1994) : Breeding Science **44**: 101-105.
2) Yoshioka, K., Hanada, K., Harada, T., Minobe, Y. and Oosawa, K. (1993) : Japan. J. Breed. **43**: 629-634.

Table 1 Items for environmental risk evaluation of transgenic melon plants in an isolated field

Evaluation items	Results	
	Transgenic melon	Non-transgenic melon
1. Virus disease		
1) Virus disease observed	PRSV[1]	CMV[2]
2) Recombination between coat protein gene of CMV and PRSV	None	
2. Reproductive characteristics		
1) Degree of pollination[3]	No difference (No pollination)	
2) Pollen dispersal by insect	10m[4]	15m[4]
3. Possibility of overwintering		
Plant	Impossible	
Fruits put on the ground	Impossible	
Fruits buried under the ground	possible	
4. Influence on soil microflora	No difference	

1) Papaya Ring Spotted Virus, 2) Cucumber Mosaic Virus
3) Degree of pollination when female flowers were covered by paper bag
4) Pollen dispersal by insect of transgenic and non-transgenic melon plants were detected by PCR analysis of kanamycin resistant gene (NPT-Ⅱ) and Fusarium wilt resistance, respectively

Table 2 Comparison of pollen dispersal between transgenic and non-transgenic melon plants

	Distance from donor[1] (m)	Number of progenies with NPT-Ⅱ gene	Number of progenies exhibiting resistance to Fusarium wilt
No. 1[2]	5	5/100[3]	12/195[4]
No. 2	10	3/100	7/192
No. 3	15	0/100	2/198
No. 4	25	0/100	0/194
No. 5	25	0/100	0/197
No. 6	25	0/100	0/199
No. 8	30	0/100	0/198
No. 9	40	0/100	0/195
cont. 1[5]		0/30	0/197
cont. 2[6]		12/30	0/196
cont. 3[7]		0/32	30/30

1) Transgenic melon and/or Fusarium-resistant non-trangenic melon plants
2) No. of area (No. 1-9, Fig. 1) cultivated recipient (non-transgenic and Fusarium-suceptible) melon plant.
3) Number of progenies with NPT-Ⅱ gene / total number of progenies examined
4) Number of progenies exhibiting resistance to Fusarium wilt / total number of progenies examined
5) Progenies of recipient melon plants by self-pollination
6) Progenies of transgenic melon (M5) plants by self-pollination
7) Progenies of transgenic melon (cv. Ooi) plants by self-pollination

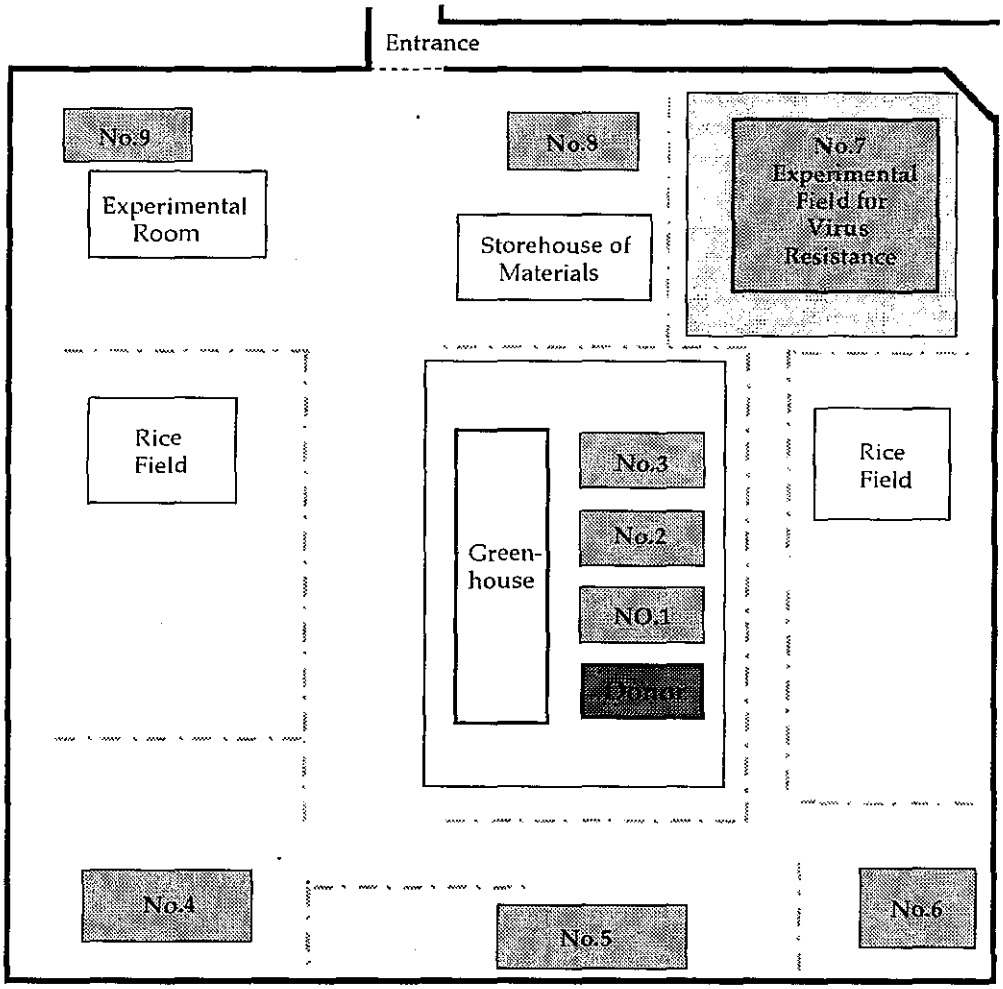

Fig. 1 Isolated field for environmental risk evaluation of transgenic melon

No. 1-3, 4-6, 8, 9: Recipient plant of dispersed pollen

No.7 Experimental field for virus resistance

Donor Transgenic melon plants and Fusarium resistant non-transgenic melon plants

The area cultivated with pumpkin to avoid pollen dispersal

Wind break