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Resistance to Viruses in Transgenic Tobacco Plants Introduced Mammalian 2, 5-Oligoadenylate Synthetase cDNA

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Summary

A human 2, 5-oligoadenylate synthetase cDNA was introduced into tobacco (*Nicotiana tabacum* cv. Samsun NN) plants by *Agrobacterium*-mediated transformation. The transgenic tobacco plants expressing 2, 5-oligoadenylate synthetase cDNA showed resistance to cucumber mosaic virus and tobacco mosaic virus.

Introduction

It is well known that interferons, a family of small proteins, are synthesized in animal cells following a virus infection. They enhance the resistance to many animal viruses. Interferons lead to an antiviral activity stimulating the production of 2, 5-oligoadenylate (2, 5-A) synthetase and protein kinase. The production of these two enzymes is stimulated by double stranded RNA molecules. As a reason for antiviral activities of interferons, it has been expected that 2, 5-A synthetase, activated by interferon and double stranded RNA, catalyzes the formation of 2, 5-A. 2, 5-A then activates an endoribonuclease called RNase L, thus inactivating virus RNA.

Sela *et al.* reported that an antiviral factor (AVF) is produced in N gene-carrying tobacco, which produces necrotic local lesions resulting from a hypersensitive reaction due to tobacco mosaic virus (TMV) infection, and that AVF seems to be a type of interferons (9). A series of their results has suggested that animal interferon-like systems are induced in hypersensitively virus-infected plants (1-4, 6-9). However, we could not detect animal interferon-like systems such as productions of interferon-like proteins, 2, 5-A synthetase and 2, 5-A in cowpea and

tobacco plants infected hypersensitively with cucumber mosaic virus (CMV) and TMV respectively (5, 10, 11)

In this experiment, we introduced a mammalian 2, 5-A synthetase cDNA into tobacco plants and examined antivirus activity of the transgenic plants.

Materials and Methods

Viruses and Plants: The ordinary (O) and yellow (Y) strains of CMV and TMV were used. Nontransgenic and transgenic tobacco (*Nicotiana tabacum* cv. Samsun NN) plants were grown in a growth chamber maintained at 26 C during a 12 hr day (light intensity, 8,000 lux) and at 25 C for a 12 hr night. Samsun NN tobacco plants infect hypersensitively with TMV and produce necrotic local lesions, but are infected systemically with CMV.

Construction of plasmid and plant transformation: Schematic diagram of the plasmid construction of the human 2, 5-A synthetase cDNA is shown in Fig. 1. The cDNA clone, pHE 25AS, carrying the human 2, 5-A synthetase cDNA was kindly provided from Dr. Y. Sokawa, Kyoto Institute of Technology. The human 2, 5-A synthetase cDNA removed from pHE 25AS was inserted between the *Bam* HI and *Sac* I restriction sites of pBI 121, a commercial plant transformation vector (Clontech). This resulting construct, named pBI-HE25AS, was introduced into *Agrobacterium iumefaciens* strain LBA4404 and used to transform tobacco plants. Tobacco plants transformed were grown as usual (13) and the primary transformed plants (Ro generation) were used for antiviral tests.

Results and Discussion

Tobacco plants transformed with a human 2, 5-A synthetase cDNA and nontransformed tobacco plants as controls were grown to about 30 cm height. The expression of 2, 5-A synthetase mRNA in the transgenic plant leaves was confirmed by Northern blot analysis, using a human 2, 5-A synthetase cDNA probe, but not in control leaves. Fully expanded third, forth and fifth leaves were cut at the petioles, inoculated with TMV (0.1 μg/ml) and floated on water in large petri dishes for 4 days in a growth chamber described in Materials and Methods, and numbers of local lesions formed on leaves were counted. As shown in Table 1, numbers of local lesions on transgenic tobacco leaves were 10-40% to the controls.

When the third leaves of nontransgenic and transgenic tobacco plants were inoculated with CMV-O (1 μg/ml), the mosaic symptoms appearing on top leaves in transgenic plants were very slight in comparison with those on nontransgenic plants (Fig. 2). Furthermore, virus activity in the top leaves of transgenic plants was smaller than that in nontransgenic plants. CMV-Y, in contrast to CMV-O,

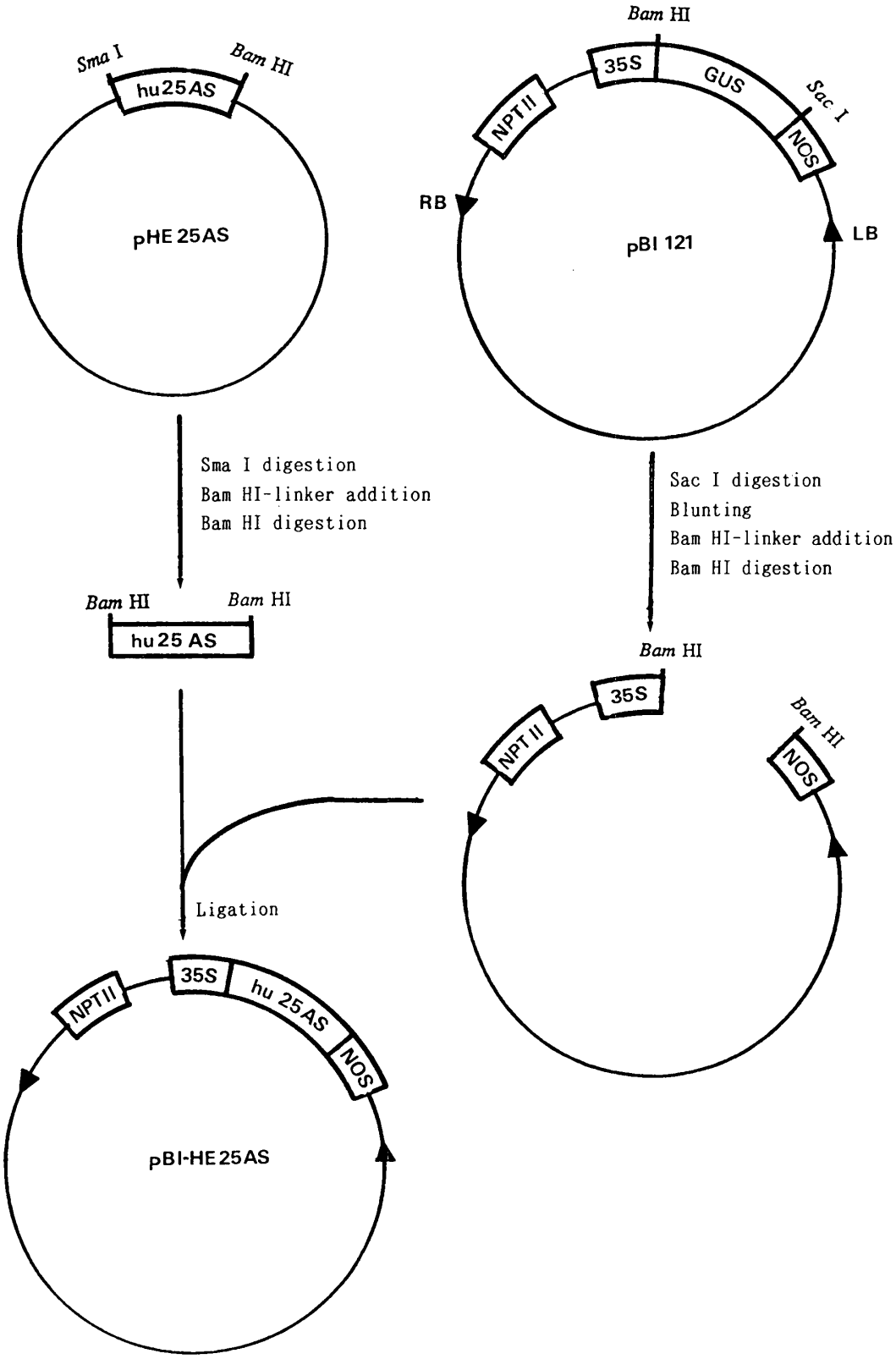

FIG. 1. Schematic diagram of construction of the plasmid pBI-HE25AS.

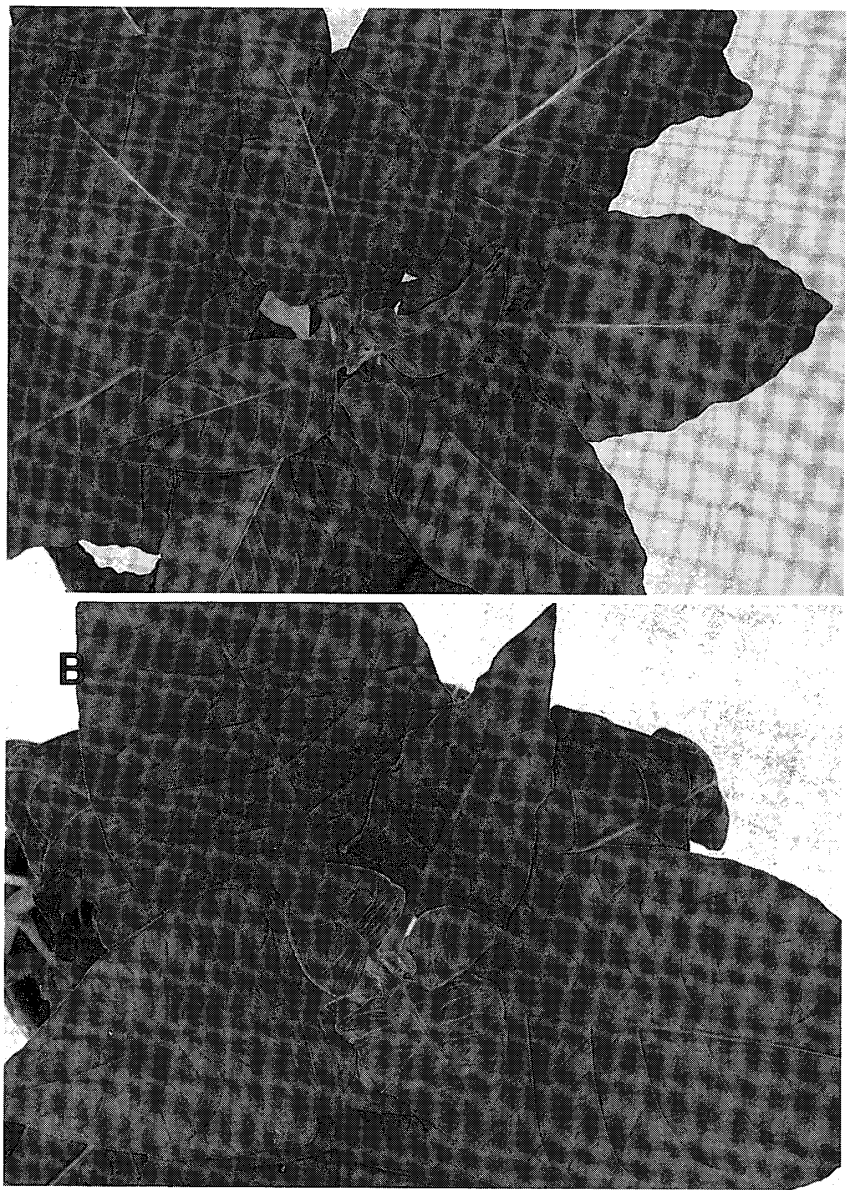

FIG. 2. Mosaic symptoms on nontransgenic control and transgenic tobacco plants infected with CMV-0. A : a control ; B : a transgenic tobacco plant expressing mammalian 2, 5-A synthetase cDNA. Mosaic symptoms are very slight.

usually causes very severe mosaic symptoms in developing leaves in infected tobacco. When transgenic tobacco plants were inoculated with CMV-Y (15 μg/ml), symptom appearance was delayed 1 or 2 days compared to controls, however, symptom severity of the transgenic plants was similar to that of nontransgenic plants.

In 1993, Truve *et al.* reported that transgenic potato plants expressing mammalian 2, 5-A synthetase are protected from potato virus X infection under field conditions, and in infected transgenic potato plants, virus concentration in

TABLE 1. *Number of necrotic local lesions produced on nontransgenic control and transgenic tobacco (Nicotiana tabacum* Samsun NN*) plants infected with TMV.*

Plant	Control		Plant	Transformant	
	Leaf position	Number of lesions per leaf		Leaf position	Number of lesions per leaf
C-I	a	341	T-I	a	81
	b	313		b	31
	c	516		c	23
C-II	a	405	T-II	a	18
	b	331		b	13
	c	421		c	10
C-III	a	364	T-III	a	111
	b	248		b	107
	c	283		c	89

a, b, c : Leaf position (a : third, b : fourth, c : fifth) from the lowest leaf to up.

leaves and in tubers were significantly lower than in nontransgenic controls (12). These results suggest that plants expressing 2, 5-A synthetase cDNA could show resistance to some extent against RNA viruses. The detailed mechanism of resistance remains to be clarified.

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