Satellite-Mediated Protection of Tomato Against Cucumber Mosaic Virus: II. Field Test Under Natural Epidemic Conditions in Southern Italy

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ABSTRACT

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Tomato necrosis devastated the tomato crop in the Basilicata region of Italy in 1988. Several hundred tomato (*Lycopersicon esculentum*) seedlings representing the cultivars UC82B, Rutgers, Bandera, and Italpeel were preventively inoculated or "vaccinated" with cucumber mosaic virus (CMV) strain S containing the nonnecrogenic satellite S-CARNA 5 (CMV-associated RNA 5) and placed in a commercial tomato field in the Basilicata region of southern Italy to test for protection against tomato necrosis. When surveyed in July 1989, the protective effect of the vaccination was greater than 95%. The fruit yields from the protected plants were double those of the nonprotected controls. In the experimental field, the incidence of lethal necrosis in nonprotected control plants was 40%; however, in neighboring fields total destruction of the tomato crop occurred for the second consecutive year.

In the summer of 1988, large vegetable-growing areas of the three southern Italian regions of Campania, Basilicata, and Apulia were stricken by massive outbreaks of virus disease in tomato, pepper, melon, watermelon, cucumber, and squash (2–4). The losses in tomato production were estimated at 300,000 tons (Office of Agricultural Affairs, U.S. Embassy, Rome; *personal communication*), which is equivalent to 12,500 ha of tomato production fields. Two disease patterns characteristic for cucumber mosaic virus (CMV) in tomato were observed. The first was the so-called shoestring and fernleaf syndrome (6); the second was the lethal tomato necrosis disease, first described and associated with CMV infection in 1974 (7,8,11) following a massive tomato necrosis epidemic in France (14). The two prevailing diseases were geographically quite separated, with the fernleaf/shoestring syndrome primarily occurring in Campania and Apulia, while the tomato necrosis epidemic was mainly concentrated in a very large coastal area where Basilicata borders on Apulia. While both types of disease are equally disastrous to tomato production, tomato necrosis in the field, because of its lethality to the whole plant, was a particularly dramatic sight in Basilicata (Fig. 1).

Recently, we have provided direct evidence that the 1988 epidemic in Basilicata was related to a strain of CMV (designated CMV-PG) carrying a 334-ribonucleotide satellite, PG-CARNA 5 (CARNA 5 = CMV-associated RNA 5). This satellite was isolated from the affected fields and was shown to be directly responsible for the catastrophic tomato necrosis outbreak (5). In the expectation that there would be a recurrence of tomato necrosis in Basilicata in 1989 and in view of the apparent success of satellite-mediated protection of tomato against CMV shown in the preceding report (10), we decided to do a limited test of the efficacy of the method against a possible repetition of the epidemic.

We report the results of introducing several hundred tomato plants preventively inoculated or "vaccinated" with CMV-S carrying S-CARNA 5 (10) into the open field at a tomato farm in Basilicata in the spring of 1989.

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MATERIALS AND METHODS

Test plants. Tomato (*Lycopersicon esculentum* Mill.) seedlings of cvs. UC82B, Rutgers, Bandera, and Italpeel were used. At the time of preinoculation or vaccination, these plants were either at the cotyledon stage (experiments 1–6) or at the three-leaf stage (experiments 7–18).

Inoculum source and vaccination. CMV strain S carrying the nonnecrogenic S-CARNA 5 used for vaccination of seedlings was obtained from the same source and purified as described in the preceding report (10). However, in contrast to that work, where total RNA extracted from CMV-S was used, precautions had to be taken to prevent inactivation of the inoculum during transportation. This necessitated the use of intact CMV-S virions for mechanical inoculation at a concentration of 50 μg/ml in 0.03 M Na_2HPO_4 with Celite used as an abrasive. After inoculation, the seedlings were kept in a greenhouse for 20 days, after which they were moved to a screenhouse to condition them

Fig. 1. Almost total destruction in Basilicata tomato field attributable to recurring epidemic of lethal necrosis in 1989. No "vaccinated" plants were introduced in this field.

before transplanting them to the field 14 days later. No insecticide treatment was used before transplanting.

Design of experimental field. The experimental field in Basilicata was prepared in the same general area at one of the farms where massive destruction from tomato necrosis had been recorded in the 1988 epidemic (Fig. 1). Plants were transplanted in groups of 6, 12, or 24, and those vaccinated at the cotyledon stage (experimental groups 1–6, Table 1) were arranged in a random block design of two or three replicates. For experimental groups 7–18 (seedlings vaccinated at the three-leaf stage) the number of plants was insufficient for statistical analysis. All other plants bordering the experimental groups consisted of Italpeel hybrid plants and were supplied by the farm (Table 1, group B). Distances between plants, agronomic practices, and pesticide treatments were those commonly used at the farm.

CMV and CARNA 5 detection in CMV-S-inoculated plants and in weeds by dot-blot hybridization. Leaf tissue samples (0.1 g) were crushed in 300 μl of 50 mM NaOH-2.5 mM EDTA in an Eppendorf tube and incubated at 25 C for 10 min, and 10 μl of the slurry was applied to a nylon filter membrane. Filters were exposed to UV light for 5 min to cross-link the nucleic acid. They were probed for the presence of viral RNA with cDNA prepared by the random priming method (12) from CMV-PG genomic RNA that had been fractionated from PG-CARNA 5 after electrophoresis in low-temperature gelling agarose. Probes against CARNA 5 were prepared from D-CARNA 5 cDNA clones in pSP65 cut with Eco RI and Hind III, after which the insert was separated from the linearized plasmid in low-temperature gelling agarose electrophoresis. Gel pieces containing the CARNA 5 inserts were used directly in an oligonucleotide randomly primed labeling reaction (1). Prehybridization, hybridization, and washing were by established procedures (9).

RESULTS

Presence of CMV and CARNA 5 in the field before the transplanting of tomato seedlings. Four weeks before the tomato plants were transplanted, weeds growing in the experimental field were sampled at random and indexed for the presence of CMV and CARNA 5 by inoculation to *Chenopodium quinoa* Willd. and by dot-blot hybridization. Among 12 different weed species collected, CMV was detected in *Amaranthus* L. spp., *Beta vulgaris* L., and *Fumaria* L. spp. CARNA 5 was detected only in *Amaranthus*.

CMV and CARNA 5 in experimental plants. During a 5-wk observation period before transplanting to the experimental field, none of the CMV-S-inoculated plants developed conspicuous disease symptoms. Yet CMV and CARNA 5 were easily detected in all but one of the 65 plants sampled (about 10% of the experimental plants), clearly the result of a low-level CMV-S + S-CARNA 5 spreading infection (10,15). No hybridization signals were observed with any of the mock-inoculated or untreated plants.

Occurrence of tomato necrosis in the experimental field. Tomato plants in the field became heavily infested with aphid colonies about 25 days after transplanting. Following this first appearance, insecticide was applied by the farmers and further aphid infestation became very sporadic. Despite these preventive measures, the first symptoms of lethal necrosis in the field appeared 30 days later. However, as compared with the 1988 epidemic outbreak in the same area, the progression of the disease was slower, and lethal necrosis incidence in the experimental field did not exceed 40% among the nontreated plants supplied by the farm (Table 1, group B). This contrasted with a neighboring field, where no preinoculated or vaccinated plants were placed, and where the incidence of necrosis and destruction was between 80 and 100%, similar to that of 1988 (Fig. 1). A comparison of two typical plots of cv. UC82B tomato with and without vaccination is shown in Figures 2 and 3. Note the bare ground in Figure 3 representing areas where the plants have died of necrosis, the epinastic stems, and reduced leaf size of surviving plants. The data collected at the time of the last survey (25 July 1989) are shown in Table 1. They indicated that vaccination decreased the incidence of necrosis in the field to insignificant levels and thereby dramatically increased fruit yield. Fruit quality as judged by color, shape, and weight was much superior to that of the control plants (Fig. 4).

DISCUSSION

The recurrence in 1989 of tomato necrosis in Basilicata (Fig. 1) has provided the first direct evidence that preventive inoculation or vaccination of

Table 1. Satellite-mediated protection of Italian field tomato crop against lethal necrosis

Experimental group number	Tomato cultivar	Treatment[y]	Necrotic plants/tested plants (no.)	Necrosis (%)	Average fruit yield/plant (kg)
1	UC82B	V	5/142	4 b[z]	1.10
4		M	19/60	32 a	0.55
5		U	22/72	31 a	0.66
3	Rutgers	V	2/72	3 b	...
6		U	18/48	38 a	...
2	Italpeel	V	4/48	8 b	...
B		U	155/419	37	...
7	UC82B	V	0/12		1.40
11		M	5/12		0.65
15		U	3/11		0.70
9	Rutgers	V	0/12		...
13		M	2/6		...
17		U	2/6		...
8	Italpeel	V	1/12		...
12		M	6/12		...
16		U	4/12		...
10	Bandera	V	1/12		...
14		M	4/6		...
18		U	4/6		...

[y] V = plants vaccinated with CMV-S total RNA, M = mock-inoculated plants, U = untreated plants.

[z] Means with the same letter (T grouping) are not significantly different.

Fig. 2. Vaccinated UC82B tomato plants with no detectable lethal necrosis.

seedlings with a combination of a mild CMV strain and a nonnecrogenic CARNA 5 has the potential of developing into a viable technology to protect tomato against this devastating disease under field conditions. It should be stressed that for the second consecutive year, the tomato necrosis epidemic occurred following an extremely high aphid infestation. Insecticide treatments were ineffective in preventing the disease, although they essentially eliminated the aphid population from the field. The detection of CARNA 5 in *Amaranthus* in the experimental field by dot-blot hybridization cast some light on a possible link between this weed, which is usually heavily infested by crowded aphid colonies, and the appearance and spread of CARNA 5 in the field. Although there is no direct evidence that the CARNA 5 detected in *Amaranthus* was a necrogenic variant, its discovery gives enough circumstantial evidence to consider this genus as one of the key hosts in the epidemiology of CMV and its CARNA 5.

The apparent success of this vaccination experiment confirmed the more comprehensive field test carried out at the Beltsville Agricultural Research Center in the summer of 1988 under simulated epidemic conditions (10). Although the Beltsville field test showed that the method was equally effective against infection by satellite-free CMV, we decided to limit the trials solely to an area in southern Italy affected by tomato necrosis because of concerns about the possibility of necrogenic CARNA 5 emerging (10) in areas where it had not occurred before on an epidemic scale. However, in 1989 the regions of Campania and Apulia were severely affected for the third consecutive year by fernleaf/shoestring disease, which in 1988 was caused by CMV devoid of satellite (4). The apparent success and safety of the tests reported here could result in future trials in areas affected by CMV without satellite.

The occurrence of a much greater incidence of tomato necrosis in fields where no vaccination was attempted, near the experimental field (compare Figs. 1 and 2), may have important consequences for the distribution of vaccinated seedlings in fields earmarked for satellite protection. The phenomenon observed here may be related to past observations that viruliferous aphids lose their virus-transmitting ability after feeding on naturally tolerant plants (13). Alternatively, incoming aphids after feeding on vaccinated plants might have transmitted the vaccine virus to neighboring seedlings.

Fig. 3. About 30% of mock-inoculated UC82B tomato plants in experimental field have tomato necrosis.

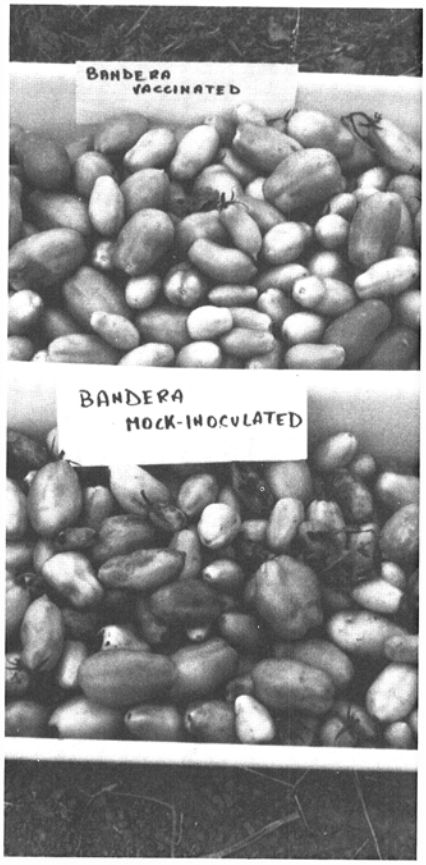

Fig. 4. Fruit produced by vaccinated and mock-inoculated tomato plants (cv. Bandera).

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