Tobacco Mosaic Virus Is Seedborne in Pimiento Peppers

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ABSTRACT

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Tobacco mosaic virus can be transmitted from one generation to the next on the seed of pimiento peppers. The virus was associated with the seed coats but rarely with the endosperm or embryo. More infected seedlings developed from freshly harvested seed than from seed stored for 9 mo. Disease incidence was greatest in transplanted seedlings; few if any plants became infected when seed was planted directly without transplanting. Observable systemic symptoms on seedlings took from 10 to 50 days after transplanting to develop. Treating seed with hydrochloric acid, calcium hypochlorite, sodium hypochlorite, or trisodium phosphate effectively controlled seedling infection.

Tobacco mosaic virus (TMV) commonly infects peppers (*Capsicum annuum* L.), with deleterious effects (2,10). Resistance to TMV has been incorporated into a number of commercial bell pepper types (5,6), but no TMV-resistant pimiento peppers are available.

The pimiento pepper cultivar Truhart Perfection or selections from it are widely grown in the $9 million raw-product pimiento pepper industry in northern Georgia and neighboring states. Truhart Perfection pepper was developed at the Georgia Experiment Station (4), where virus infection has plagued lines in breeding plots for the past 10 yr (J. W. Demski, *unpublished*). From 1975 to 1979, natural infection with TMV averaged 20% in field research stock and occasionally reached 100% in greenhouse production. Preliminary studies (8) indicated that seeds of both pimiento and pepper cultivar Avelar were the source of virus in the research greenhouse.

McKinney first reported seedborne TMV in pungent peppers in 1952 (15); however, little work has been done on how TMV is carried by the seed, especially for sweet pepper. Two comprehensive studies of TMV in tomato seeds have shown that the virus is associated with the inner and outer seed coat and sometimes the endosperm, but rarely if ever the embryo (3,16).

Because resistance to TMV in pimiento pepper is not known, the seedborne nature of the virus must be elucidated. The purpose of this study was to determine how TMV is carried from one generation to the next via the seed of sweet pepper and to find methods of control.

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MATERIALS AND METHODS

The virus isolate used in this study (if other than natural field infection) was obtained from a transplanted Avelar pepper seedling derived from seed from field increase material in Spalding County, GA. The virus source for this infected seedling was considered to be seedborne. The isolate was maintained in Avelar pepper or *Nicotiana tabacum* L. 'Samsun'. Mechanical inoculation with juice obtained by triturating about 1 g of infected tissue in 2 ml of 0.025 M potassium phosphate buffer (pH 7.2) containing 1% Celite was used to transfer the virus to other plants. The virus was increased in Samsun tobacco and purified by the polyethylene glycol method of Hebert (13).

Microprecipitin and ring interface serologic tests (1) were used for virus identification. *Lycopersicon esculentum* Mill. 'Marion' and 'Rutgers'; *N. glutinosa* L.; *N. tabacum* L. 'Burley 21', 'NC-95', and 'Samsun'; and *Chenopodium amaranticolor* Coste and Reyn. were used in host range studies. Seeds from mature fruit of Avelar or Truhart Perfection peppers were obtained from healthy and naturally infected plants in the field or mechanically inoculated plants in the field or greenhouse.

To determine how many seedlings were infected by seedborne virus and to prevent mechanical transmission, all plant material was handled under a protective cage on a greenhouse bench. The cage had plastic sides and a top of 1-in. wire mesh, which permitted watering from above but prevented the watering hose or workers from contacting the plants. Seeds from infected plants were placed in clay loam in germination flats under the cage. When the seedlings were 6–8 cm tall, they were lifted with sterile paper towels and transplanted individually into 10-cm clay pots. The pots were spaced to prevent contact among plants. In other tests, seeds from healthy plants were immersed in a suspension of purified TMV, dried on paper towels, and then planted in germination trays.

To determine the percentage of seeds with virus, individual seeds were removed from infected pods with alcohol-flamed, sterile forceps, triturated with mortar and pestle, and inoculated onto leaves of *N. glutinosa* plants.

Seeds were dissected with microforceps and scalpel under a dissecting microscope into three parts, the outer and inner seed coats and the embryo-endosperm. Individual seed parts were triturated in a drop of buffer between two glass spatulas, and the sap was rubbed onto a half-leaf of *N. glutinosa*. Some seed parts were surface-sterilized by being dipped in 9% HCl solution for 2 min.

Plants grown from healthy seed and identified as virusfree by indexing procedures were inoculated at three stages of growth: before flowering, at flowering, and when green fruits were set.

Six seed treatments were tested: 1) HCl; seed immersed for 30 min in 100 ml of a 9% hydrochloric acid solution with constant stirring, followed by a 1-hr rinse in tap water; 2) $Ca(OCl)_2$; seed immersed in 4.2% calcium hypochlorite solution for 15 min, followed by a 15-min tap water rinse; 3) NaOCl; seed immersed in a 2.63% sodium hypochlorite solution for 15 min, followed by a 15-min tap water rinse; 4) Na_3PO_4; seed immersed in a 10% solution of trisodium phosphate for 30 min, followed by a 1-hr rinse in tap water; 5) X-77; seed immersed in a 10% solution of the ionic wetting agent X-77 for 30 min, followed by five changes of tap water; and 6) control; untreated seed from an infected parent.

RESULTS

The virus most commonly isolated from naturally infected pimiento peppers produced very mild systemic symptoms in Samsun tobacco and did not infect Marion or Rutgers tomatoes systemically. This strain resembled the strain reported from the Southeast by McKinney (15) and Greenleaf et al (11).

Each of the 100 seeds from Truhart Perfection pimiento pepper plants naturally infected with TMV in the field and from TMV-inoculated plants grown in the greenhouse indexed positive on *N.*

glutinosa for the presence of virus. In similar tests with Avelar pepper, 98 of 100 seeds from field-grown plants and all 100 seeds from greenhouse-inoculated plants were contaminated with TMV. Time of infection has little effect on the recovery of TMV from individual seeds in mature pods. All 160 seeds from plants inoculated in the green fruit stage indexed positive for TMV.

Young seedlings (seed from infected Avelar parents) were removed from a germination flat and separated into roots and shoots. These plant parts were wiped with a moist cotton swab, and the swab was then used to rub leaves of *N. glutinosa*. Local lesions developed on *N. glutinosa* from 17 of 60 roots and two of 60 shoots.

Seed collected from infected Avelar and Truhart Perfection peppers and germinated in the greenhouse produced seedlings that were apparently not infected. More than 1,000 seedlings were inspected visually and 207 were assayed on *N. glutinosa* without detecting any viral infection. These plants were retained until the 12th-true-leaf stage. Direct seeding in sand, vermiculite, and a clay-loam soil did not induce development of systemic infection; however, when seedlings were transplanted, 5–16% and 10–41% of the Truhart Perfection and Avelar transplants, respectively, were infected (seeds came from two different sources for each cultivar). The lower percentages apply to seed stored for 9 mo, the higher percentages to freshly harvested seed. Of 1,036 Avelar seeds stored for 5 mo (typical winter storage), 258 produced infected seedlings after being transplanted.

Not all seedlings became systemically infected immediately after transplanting. In one test of 65 Avelar transplants, symptoms appeared on some seedlings within 10 days of transplanting, while others did not show systemic symptoms for 30 days. In another test, one plant did not show systemic symptoms until 50 days after it was transplanted.

Bioassays on *N. glutinosa* indicated that TMV was present on outer seed coats, inner seed coats, and embryo-endosperms of Avelar peppers. In 212 trials, assays of the outer seed coats averaged 57 lesions per half-leaf of *N. glutinosa*; those of the inner seed coats and embryo-endosperms averaged 26 and 21 lesions, respectively. With one exception, surface-sterilization (2 min in 9% HCl) of these seed parts prevented the isolation of TMV on *N. glutinosa*; two lesions developed from one of 156 surface-sterilized embryo-endosperms tested. When whole seeds were surface-sterilized, triturated, and assayed on *N. glutinosa*, TMV was recovered (11 lesions per half-leaf).

In three of four tests (60 plants per trial) with HCl, $Ca(OCl)_2$, NaOCl, and Na_3PO_4 seed treatments, no systemically infected seedlings developed after transplanting. In the fourth test, an infected seedling occasionally appeared in each of these treatments; however, seedborne infection did not exceed 5%, compared with an average of 12% in the wetting agent treatment and 21% in the control (no treatment).

Seeds from healthy plants (25 seeds per test) were immersed in 1.5 ml (14 mg/ml) of purified virus for 10 min with occasional stirring, dried on paper towels overnight, and sown in germination flats. The seedlings were transplanted into clay pots. Twenty-nine percent of the Avelar and 19% of the Truhart Perfection seedlings became infected. Control seed (no virus treatment) produced healthy seedlings.

DISCUSSION

Seedborne diseases can often be recognized by symptom development in plants grown in seedling beds or flats (12). Pimiento pepper seedlings, however, may not show symptoms of TMV infection in the seedling beds and indeed may not be infected systemically at this time. Many seedlings developing from seed of infected parents may be contaminated with TMV at this stage. Results of this study indicate that 28% of the roots and 3% of the shoots from infected seed carry enough TMV particles to give a positive bioassay. If these seedlings remain in the germinating media, systemic invasion may not take place because wound entry points are not available.

At the time of transplanting, enough TMV on the surface of the pepper seedlings may be introduced into wounds (on leaves, stems, or root hairs) to initiate infection. Many of the infections may be root infections instead of shoot infections, which further complicates the disease cycle. The virus may not always move rapidly from roots to shoots. McGuire (14) reported that systemic symptoms caused by tobacco ringspot virus appeared 13–25 days after infectious nematodes were placed in the root zone of cucumber; however, in 20% of the trials, the virus did not move systemically to the leaves. Infection of some pimiento and Avelar pepper plants (based on systemic shoot symptoms) was not detected for up to 50 days after transplanting, which could indicate a restrictive systemic shoot invasion after root infection. Nonetheless, these results indicate a more rapid systemic invasion of pepper than that found by Tosic et al (17), who reported that most *C. annuum* 'Neska sipka' plants did not become systemically infected with TMV until pod maturity.

Several observations indicate that virus particles on the external seed coat are mainly responsible for the carryover of TMV from seed to seedlings. First, seed treatments that effectively controlled the disease probably act only on virus particles on the external seed coat (alternatively, virus particles on the inner seed coat may not be significantly involved in contaminating the germinating seedling). Second, placing virus particles externally on known healthy seed resulted in seedling infection similar to that from naturally infected seed. Third, dissection studies found most of the virus on the external seed coat.

Treating pepper seed with calcium hypochlorite and sodium hypochlorite has been reported (7) to control *Xanthomonas campestris* pv. *vesicatoria* (9) (previously *Xanthomonas vesicatoria*). Results of this study indicate that these treatments are also effective against TMV. Thus, two control practices appear feasible for seedborne TMV on pimiento peppers: direct seeding to eliminate transplant handling, and the application of a seed treatment to inactivate the virus on the external seed coat.

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