Squash Mosaic Virus Variability: Nonreciprocal Cross-Protection Between Strains

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ABSTRACT

Interactions between strains IH and IIA of squash mosaic virus (SMV) in pumpkin (*Cucurbita pepo*) and cantaloupe (*Cucumis melo*) plants were studied. Complete reciprocal interference was observed in pumpkin. In cantaloupe, strain IH dominated in mixed infections, and was able to overcome initially suppressive effects from prior infection by IIA. The latter phenomenon is believed related to the fact that in cantaloupe the multiplication rate of strain IH is twice that of strain IIA. In pumpkin, the relative extent of multiplication of the two strains is equal.

The detection of interference, when the challenge strain is the milder of the two, was accomplished by utilizing host range differences and the serological intragel absorption technique.

When opposite cotyledons of pumpkins were inoculated simultaneously with equal concentrations of different strains of virus, each strain dominated in roughly 50 percent of the plants to the exclusion of the other. When the concentration of one strain was reduced in relation to the other, the number of plants in which it dominated was reduced proportionately. Identical experiments with cantaloupe resulted in more complete dominance of strain IH even when the concentration was much less than strain IIA.

Additional key words: Intragel absorption.

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SUMARIO (PORTUGUES)

Interações entre as raças IH e IIA do vírus SMV (squash mosaic virus) foram estudadas em plantas de jerimum (*Cucurbita pepo*) e melao cantaloupe (*Cucumis melo*). Completa e reciproca proteção foi observada em jerimum, enquanto em melão cantaloupe a raça IH mostrou-se capaz de vencer qualquer efeito supressivo inicial da raça IIA e apresentou evidente dominância após inoculações simultâneas com ambas as raças. Este fenômeno, observado em plantas de cantaloupe, pode ser explicado pelo fato da raça IH ter apresentado uma capacidade de multiplicãcao duplamente superior a da raça IIA. Por outro lado, ambas as raças apresentaram, aproximadamente, o mesmo grau relativo de multiplicação, quando inoculadas em plantas de jerimum.

O fenômeno da proteção ou pré-imunização, nas plantas primeiramente inoculadas com a raça mais severa do vírus, foi demonstrado utilizando-se diferenças nos circulos de hospedeiros e testes sorológicos, envolvendo estes a técnica de "absorção intragel". Com o uso da referida técnica sorológica, os resultados obtidos demonstraram que a mesma podera ser utilizada para detectar o fenômeno da proteção, quando os sintomas forem inadequados.

Quando as folhas cotiledonares de plantas de jerimum foram, simultaneamente, inoculadas com igual concentração de ambas as raças do vírus, cada uma predominou em, aproximadamente, 50 por cento das plantas inoculadas. Por outro lado, a medida em que a concentração de uma raça foi reduzida em relação a outra, reduzui-se, proporcionalmente, o número de plantas nas quais a mesma predominou. Semelhante procedimento com plantas de melão cantaloupe indicou uma maior dominância da raça IH, mesmo quando usada em concentracão mais baixa do que a da raça IIA.

The cross-protection, or interference, phenomenon is known to occur between many virus strains and is often used in establishing or demonstrating relatedness. When one virus isolate in a plant completely blocks the multiplication of a second, the two isolates generally are closely related. Such relationships usually have been confirmed by serology or other means. The most recent discussions of cross-protection and examination of possible mechanisms has been by Matthews (4) and Ross (7). Cross-protection studies have been done with squash mosaic virus (SMV) by Demski (1) and Lima (3).

This investigation deals with cross-protection between representatives of two serologically distinct squash mosaic virus strains (6).

MATERIALS AND METHODS.—In this investigation we used virus isolate H of serological group I and isolate A of serological group II (6). Strain IIA induces a severe reaction on pumpkin, a mild reaction on cantaloupe, and no reaction on watermelon. IH induces the reverse reaction on pumpkin, local lesions on watermelon, and a severe reaction on cantaloupe.

Small sugar pumpkin (*Cucurbita pepo* L.), powdery mildew-resistant 45 cantaloupe (*Cucumis melo* L.) and Florida Giant watermelon (*Citrullis vulgaris* Schrad.) were grown from seed in 10.2-cm (4-inch) diameter plastic pots containing a fertilized mixture of sand and peat moss (2:1, v/v). Experiments were conducted largely during summer months when greenhouse temperatures ranged from 25-32 C, night and day. During winter months experiments were conducted in growth chambers adjusted to approximate greenhouse summer temperature fluctuations, with illumination at 21,528 lux at plant height. All experiments were repeated at least twice.

Virus purification and inoculation.—The precipitation of plant protein by butyl alcohol and the precipitation of virus by polyethylene glycol 6,000 (PEG) (2) were used in the virus purification process.

Fifty to 100 g of infected plant material was homogenized in a blender with two volumes of 0.1 M phosphate buffer, pH 7.0. The resulting extract was strained through a double layer of cheesecloth and clarified by centrifugation for 20 minutes in a VRA rotor at 10,000 rpm in a Lourdes Model A-2 Beta-Fuge. The pellet was discarded and enough butyl alcohol added to the supernatant to make an 8% concentration. This mixture was stirred for at least 30 minutes at 4 C; coagulated green debris was removed by centrifugation for 20 minutes at 10,000 rpm in a VRA rotor. While stirring the liquid residue, PEG 6,000 and NaCl were added to a final concentration of 8 and 4% (w/v), respectively, to precipitate the virus. Stirring was continued for 30 to 40 minutes in the cold, after which the mixture was centrifuged for 30 minutes at 10,000 rpm in the Lourdes VRA rotor. The resulting pellet was resuspended in 0.1 M phosphate buffer pH 7.0 and clarified by centrifugation for 10 minutes at 10,000 rpm in the same rotor. The PEG precipitation was repeated two more times, using a Lourdes 9RA rotor, to concentrate the virus.

The concentration of purified virus was determined from the absorbance obtained at 260 nm and related to yield of virus per gram of fresh tissue. For all inoculations Carborundum was either added to purified virus suspensions or to crude sap. Inoculum was rubbed over the adaxial surfaces of cotyledons or of true leaves of the plants with a brush.

Cross-protection experiments.—Reciprocal cross-protection experiments were conducted in pumpkin and cantaloupe with strains IH and IIA. Each experiment utilized 60 test plants. Thirty were initially inoculated with one strain. Fifteen of these were reinoculated with the challenge strain after symptoms appeared along with an additional 15 not previously inoculated. Finally, 15 plants remained untouched throughout the experiment. Analysis of cross-protection or interference behavior was based, in part, on a comparison of symptoms of these variously treated plants. The following cross-protection experiments were run, here shown in a cryptic fashion (a) IH × IIA–pumpkin, (b) IIA × IH–pumpkin, (c) IIA × IH–cantaloupe, (d) IH × IIA/cantaloupe. In two of the tests indicated above the more severe strain is inoculated first. In this case a method other than symptomatology must be used in the analysis. In the IIA × IH–pumpkin experiment attempts to detect IH (milder strain in pumpkin) included intragel absorption (5) and attempted transfer to watermelon (local lesions are induced by strain IH, no infection occurs with strain IIA). In the IH × IIA–cantaloupe experiment intragel absorption only was used.

Where intragel absorption was used to detect replication of the challenge strain, virus purification and concentration procedures were first conducted with the doubly inoculated tissue. Antisera used in this test and intragel absorption techniques were those prepared and used by Nelson and Knuhtsen (6).

Strain dominance in simultaneously inoculated plants.—Two experiments were designed to detect possible interaction between the strains of SMV in pumpkin and cantaloupe plants when inoculations were made simultaneously. In each experiment, the IH and IIA strains were inoculated on opposite cotyledons of a group of 100 seedlings. Purified virus suspensions were used as inocula; final virus concentrations were adjusted to 0.1 mg/ml.

To observe the effect of inoculum concentration on strain dominance, experiments were conducted with both cantaloupe and pumpkin in which concentrations of one strain were varied from 0.1 mg/ml to 0.0001 mg/ml, while the concentration of the other strain was maintained constant at 0.1 mg/ml.

Strain concentration in pumpkin and cantaloupe.—This experiment was devised to determine if any correlation exists between the results obtained in interference studies and concentrations of IH and IIA in pumpkin and cantaloupe plants. Two groups, each with 20 plants, of pumpkin and cantaloupe, were used. One group of each cucurbit was inoculated with a suspension containing 0.5 mg/ml of the purified IH strain of SMV. The remaining plants were inoculated with an equal concentration of IIA. Ten days after inoculation, 50 g (wet weight) of infected leaves of each group of plants were separately harvested. Great care was taken to duplicate the purification procedure (previously described) in each group. Each final viral precipitate was resuspended in 20 ml of 0.1 M phosphate buffer, pH 7.0. After final clarification, optical densities were determined and concentrations estimated.

RESULTS.—*Cross-protection between strains of SMV in pumpkin and cantaloupe.*—The results of interference studies with strains of SMV in pumpkin and

TABLE 1. Dominance of one SMV strain over the other when different concentrations of IH and IIA strains are simultaneously inoculated into pumpkin and cantaloupe plants (both IH and IIA strains of SMV were simultaneously inoculated onto different cotyledons of each pumpkin and cantaloupe seedling)

Inoculum concentrations (mg/ml)[a]		Plants showing SMV-symptoms (%)			
		Pumpkin plants		Cantaloupe plants	
IH Strain	IIA Strain	IH symptom	IIA symptoms	IH symptom	IIA symptom
10^{-4}	10^{-1}	10	90	5	95
10^{-3}	10^{-1}	30	70	40	60
10^{-2}	10^{-1}	40	60	65	35
10^{-1}	10^{-1}	45	55	90	10
10^{-1}	10^{-2}	55	45	90	10
10^{-1}	10^{-3}	80	20	95	5
10^{-1}	10^{-4}	95	5	100	0

[a]Inoculum concentrations in mg ov virus per ml of purified virus suspensions.

cantaloupe plants differed. In pumpkin experiments, a complete reciprocal interference was observed; the interference in cantaloupe plants was unilateral. Leaves of pumpkin plants inoculated with strain IH showed a mild mottle 10 days after inoculation. When such leaves were rubbed with inoculum containing the IIA strain of SMV, (herein-after designated as IH × IIA–inoculated plants) no severe mosaic developed. Similar inoculations of healthy plants resulted in the appearance of typically severe symptoms of this strain.

Interference also was observed when pumpkin plants were first inoculated with IIA and later with IH (i.e., IIA × IH–inoculated plants). No evidence of IH in the doubly inoculated pumpkin plants was detected when extracts or purified virus preparations from these plants were bioassayed on watermelon. However, when cotyledons of watermelon plants were inoculated either with strain IH alone or a prepared mixture of IH and IIA strains, necrotic lesions were produced.

These results were confirmed by intragel absorption tests. Pumpkin tissue from the test IIA × IH was subjected to virus purification and concentration procedures. By use of this serological technique the antigen of IIA was readily detectable in this preparation but IH was not detected. Likewise in test IH × IIA–cantaloupe, only the IH antigen was detected when purified preparations from cantaloupe were subjected to intragel absorption.

Mildly mottled cantaloupe leaves infected with IIA strain of SMV, were not protected against subsequent infection by IH, since a severe green vein-banding symptom developed on IIA-infected plants as well as on the control plants inoculated with IH strain alone. In the doubly inoculated plants, the appearance of IH symptoms was delayed 3-4 days in relation to the inoculated controls. IH antigen was detected in these plants by intragel absorption test.

Squash mosaic virus strain dominance in simultaneously inoculated plants.—When pumpkin plants were simultaneously inoculated with equal concentrations of IH and IIA strains of SMV on opposite cotyledons, 52-55% of the plants developed severe mosaic symptoms and leaf distortions; only 45-48% showed a mild mottle. However, when the concentration of IIA was kept constant and the concentration of the IH inoculum was decreased, the number of plants showing IH symptoms decreased. Similarly, when the IH concentration was kept constant, the number of plants with severe mosaic (IIA symptom) decreased with decrease in IIA concentration (Table 1).

Experiments performed with cantaloupe produced different results. Plants simultaneously inoculated with the same concentrations of both strains on opposite cotyledons showed heavy dominance of the IH over the IIA strain. Ninety to 92% of the plants showed a green vein-banding and severe mottle (IH symptoms); only 8-10% showed a mild mottle (Table 1). Reduction of the concentration of IH to one-tenth that of IIA resulted in dominance of IH in 65% of such doubly inoculated plants. Those that showed IIA symptoms were presumed to be plants in which IH failed to become established since in all cases tested IH would multiply in cantaloupes previously infected with IIA. This phenomena was also demonstrated with strain IA (6).

TABLE 2. The relative concentrations of IH and IIA strains of squash mosaic virus in pumpkin and cantaloupe plants grown in the greenhouse and a growth chamber

Host	Virus concentrations (mg/gm wet weight)[a]			
	First Experiment		Second Experiment	
	IH strain	IIA strain	IH strain	IIA strain
Pumpkin	0.25	0.26	0.23	0.24
Cantaloupe	0.45	0.18	0.52[b]	0.27[b]

[a]Virus concentrations determined spectrophotometrically and converted to mg of virus per gm of fresh infected leaves.

[b]This experiment was conducted in the previously described growth chamber because of low night temperatures in the greenhouse.

Squash mosaic virus concentration.—Ten days after inoculation the concentration of both strains in pumpkin were approximately equal (Table 2). However, during the same incubation period twice as many IH particles developed in cantaloupe as did IIA particles (Table 2).

DISCUSSION.—The results of this investigation confirm earlier conclusions (6) that biotypes of strain II are less well adapted to *Cucumis* spp. than are strain I members. The prior conclusions were based on (i) lack of seed transmission of II in muskmelon (6), and (ii) lack of any records of isolation of strain II from field-grown muskmelons. Secondly, the in vivo demonstrated correlation of virus strain concentration with interference, or lack of it, suggests that the extent of virus strain replication in a host may be related to its ability to successfully compete with a different strain.

Several possible explanations for the failure of strain IIA to protect cantaloupe from infection by strain IH, as it did in pumpkin, are suggested by a recent review (7) of virus interactions in plants. Since the situation described here consists of two strains whose interactions differ in two systemic hosts, it seems clear that the virus-host interaction plays a crucial role in the explanation of this phenomenon. This is supported by the evidence that IIA multiplies in cantaloupe to only half the extent of IH. Interestingly enough the quantity of viral nucleoprotein extracted from IIA infected cantaloupe is approximately equal to that extracted from pumpkin infected by either strain singly. This suggests that there is certainly something special about the relationship between strain IH and cantaloupe. Since there is a definite difference in coat protein between these two strains as expressed in serological tests (6), it naturally follows that the RNAs will also differ. Thus, the replicase enzymes induced by the two presumably would be different in at least some respects. This difference apparently presents no advantage to either strain in pumpkin, but in cantaloupe it appears to give IH an advantage over IIA. This advantage could be better recognition between IH RNA and acceptor molecules in cantaloupe. It also could be due to the fact that IH is better able to use existing peptides in cantaloupe in the synthesis of its specific replicase. This further suggests the possibility that in cantaloupe at least, IIA is unable to use the IH replicase, while IH perhaps is able to use the IIA replicase. In any event, it can be definitely concluded that IH is better adapted to replication in cantaloupe than strain IIA, and therefore is able to dominate in mixed infections regardless of the sequence in which such infections arise.

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