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Methods for screening watermelon for resistance to papaya ringspot virus type-W

Nihat Guner[a], E. Bruton Strange[a], Todd C. Wehner[a,*], Zvezdana Pesic-VanEsbroeck[b]

[a]*Department of Horticultural Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695-7609, USA*

[b]*Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA*

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Abstract

Papaya ringspot virus-watermelon strain (PRSV-W) affects all agriculturally important species of the Cucurbitaceae, and is of economic interest because of its destructiveness. The objective of this study was to develop a consistent and reliable method to screen watermelon for resistance to PRSV-W. PRSV-W isolates 1637, 1870, 2030, 2038, 2040, 2052, 2169, 2201, 2207, and W-1A were maintained in 'Gray Zucchini' squash, and were used in the inoculations. Three experiments were run, a preliminary experiment to determine the important factors involved in disease development, a main experiment to quantify the effects of those factors, and a retest of three cultigens to determine test variability. The experiment was a split-plot treatment arrangement in a randomized complete block design with four replications. Whole plots were growth stage (cotyledon, first true leaf), subplots were pot size (55 or 100 mm), and sub-subplots were the 10 isolates. Plants were rated on a scale of 0–9 for each of three traits: leaf necrosis, mosaic symptoms, and leaf deformation. We found the best method for a screening of the watermelon germplasm collection for resistance to PRSV-W is to grow the seedlings in square, 100 mm diameter pots (or 55 mm diameter pots if uniform germination is expected) and inoculate plants at the first true leaf stage using PRSV-W isolate 2052 and the rub method. Significant differences were obtained (with LSD values of 0.6–1.5) using four replications of five plants per plot, but fewer replicates and plants may be adequate for a large germplasm screening experiment. The method can be used by researchers interested in screening for PRSV-W resistance in watermelon, verifying that resistance, studying its inheritance, and transferring it to elite cultivars.

Keywords: Cucurbitaceae; *Citrullus lanatus*; Mosaic; Disease; Pathology; Vegetable breeding

* Corresponding author. Tel.: +1-919-515-5363; fax: +1-919-515-2505.
E-mail address: todd_wehner@ncsu.edu (T.C. Wehner).

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1. Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a major crop in the southern United States. Around the world, over 10 viruses are known to be a problem in watermelon production (Provvidenti, 1986b). The most important virus diseases of watermelon in the United States are caused by papaya ringspot virus-watermelon strain (PRSV-W, formerly watermelon mosaic virus-1), watermelon mosaic virus (WMV), and zucchini yellow mosaic virus (ZYMV) (Adlerz and Crall, 1967). Virus diseases are destructive to the watermelon crop, and are difficult to control (Sherf and Macnab, 1986). The major control strategies involve insecticides to eliminate the insect vectors, herbicides to remove alternate hosts, or genetic resistance (Provvidenti, 1993).

Although resistance is generally pathogen-specific (Grumet, 1989), the most economical method for control of virus diseases is genetic resistance. Virus resistance in some cucurbits has been provided by virus coat proteins (Namba et al., 1992; Quemada et al., 1990). That technology may provide additional resistance if it can be used successfully in watermelon. However, natural resistance is often available to cucurbit breeders (Provvidenti, 1993). Already, the watermelon germplasm collection has been screened for resistance to some virus diseases. Boyhan et al. (1992) have identified PI accessions resistant to ZYMV, and Gillaspie and Wright (1993) have identified PI accessions resistant to WMV.

PRSV-W virus affects all agricultural species of the Cucurbitaceae, and is of great economic importance because of its destructiveness (Provvidenti, 1993). PRSV-W was known as watermelon mosaic virus-1 until it was shown that it was in fact a strain of papaya ringspot virus (Provvidenti, 1993). The virus is transmitted in a non-persistent manner by 24 species (15 genera) of aphid. Resistance to the virus has been identified in cucumber (*Cucumis sativus*), melon (*Cucumis melo*), squash (*Cucurbita* spp.), and gourds (*Lagenaria* spp. and *Luffa* spp.) (Provvidenti, 1993).

Watermelon has not been screened extensively for resistance to PRSV-W although there has been some preliminary research. Research using an unidentified isolate of PRSV-W demonstrated genetic differences among seven watermelon PI accessions (Munger et al., 1984). Hojo et al. (1991a) used an aggressive isolate, Ab-081, to screen watermelon for virus resistance. They identified one resistant accession, BT-8501, a wild, bitter-fruited watermelon from Africa (Hojo et al., 1991b). Additionally, there may be field tolerance available in some landraces of watermelon (Provvidenti, 1986a). As no screening of the watermelon germplasm has taken place, it is important to establish a screening procedure that minimizes the number of escapes and optimizes virus–plant interactions.

The objective of this study was to develop a consistent and reliable procedure to screen watermelon for resistance to PRSV-W. Important questions to be answered were the most consistent method of inoculation, the differences in virulence among isolates, and the effect of pot size and plant growth stage on viral infection, and variability of inbreds versus PI accessions.

2. Materials and methods

All experiments were run using the greenhouses of the Department of Plant Pathology, North Carolina State University, Raleigh, NC. Greenhouse temperatures

averaged 26–32 °C (day) and 13–19 °C (night) for the time the preliminary experiment was run. Greenhouse temperatures averaged 30–37 °C (day) and 13–24 °C (night) for the time the main experiment was run.

2.1. PRSV-W inoculum preparation

Ten isolates of PRSV-W were obtained from D.E. Purcifull of the University of Florida, Gainesville. The isolates used in this experiment were 1870, 2040, 2169, 2201, 2030, 1637, 2038, W-1A, 2052, and 2207 as described by Baker et al. (1991). The host plant used for virus multiplication and as a source of inoculum was 'Gray Zucchini' squash (*Cucurbita pepo* L.) from Seminis Vegetable Seeds (Woodland, CA).

Inoculum was prepared by grinding infected 'Gray Zucchini' squash leaves using mortar and pestle in 0.02 M phosphate buffer, pH 7.0. Leaf to buffer ratio was 1:5 (1 g infected leaf to 5 ml buffer) and was the same for both the rub and spray method of inoculation. All 'Gray Zucchini' squash plants for virus production were seeded in metromix 200 (Scotts-Sierra Horticultural Products Company, Marysville, OH) in 160 mm diameter (6 in., 1550 ml volume) clay pots. Plants were fertilized weekly with 150 mg kg^{-1} 20–20–20 N–P–K Peters Professional (Scotts-Sierra Horticultural Products Company, Marysville, OH).

2.2. Inoculation methods

The inoculation procedure used for increasing PRSV-W isolates in squash and for the main experiment was the rub method. The rub method consisted of dusting one leaf on each plant with carborundum (800 mesh, Fisher Scientific, Fair Lawn, NJ), then applying the inoculum to the leaf with a pestle rotated in a circular motion. After inoculation, carborundum was rinsed off of the leaves with water to prevent shading. The squash plants were kept in aphid-proof cages.

An alternate method of inoculation used in this experiment was the spray method. The spray method consisted of mixing the inoculum and 0.5 g of 800 mesh carborundum in a glass container connected to an airbrush (Model E300, Thayer and Chandler, Lake Bluff, IL). The pressure of the airbrush was 300 kPa (40 psi) driven by carbon dioxide gas. The inoculum was sprayed from a distance of approximately 10 mm onto the back side of the leaf where the mid-rib connected to a main vein. Inoculum was applied until there was visible laceration of the tissue. After inoculation, the leaf was rinsed with water.

2.3. Seedling tests

Two experiments were run: a preliminary experiment to determine the important factors involved in disease development, and a main experiment to quantify the effects of those factors. Plants were grown either in square 55 mm peat pots (55 mm × 55 mm × 60 mm, 825 ml volume, Jiffystrips, Jiffy Products, Shippagon, Canada) or square 100 mm plastic pots (100 mm × 100 mm × 91 mm, 600 ml volume, Kord, Lugoff, SC).

2.3.1. Preliminary experiment

The experiment was a single run with one pot size (55 mm), two growth stages (cotyledon and first true leaf), 10 isolates 1870, 2040, 2169, 2201, 2030, 1637, 2038,

W-1A, 2052, or 2207), and two inoculation methods (rub method using a pestle, and spray method using an airbrush) with all plants being kept in aphid-proof cages. The preliminary experiment was conducted during the fall of 1997.

2.3.2. Main experiment

The experiment was a split-plot treatment arrangement in a randomized complete block design with four replications. Whole plots were growth stage (cotyledon, first true leaf), subplots were pot size (55 or 100 mm), and sub-subplots were the 10 isolates 1870, 2040, 2169, 2201, 2030, 1637, 2038, W-1A, 2052, or 2207). Seeds were dusted with captan (*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide) as 50% wettable powder, Tomen Agro, San Francisco, CA) prior to planting to reduce the occurrence of damping off.

Two seeds of 'Charleston Gray' watermelon (known to be susceptible to PRSV-W) were planted per pot, with 150 pots of each size. Seedlings were thinned to one plant per pot using 110 pots of each size (200 test plants plus 20 controls). Twenty test plants were inoculated with one of 10 isolates of PRSV-W at one of two stages. In order to have plants at both stages at the time of inoculation, cotyledon stage plants were seeded 1 week after the first leaf stage plants. Uninoculated controls were used to provide a baseline for the rating system.

Chenopodium amaranticolor was used as a control to verify that each application of inoculum contained the PRSV-W virus. Many, but not all, isolates of PRSV-W induce necrotic local lesions in *C. amaranticolor* (Murant and Harrison, 1984). Each isolate of the virus was applied to three leaves of a separate *C. amaranticolor* plant prior to inoculating the appropriate watermelons. The inoculated leaves were marked at inoculation and checked for necrotic spots each time the watermelons were rated.

All inoculated plants were kept in a screened greenhouse containing no other cucurbits and no other virus experiments. Plants were rated three times, every 7 days for 21 days starting 14 days after inoculation. Replications 1 and 2 were conducted during the spring of 1998. Replications 3 and 4 were conducted during the fall of 1999.

2.3.3. Retest

The retest was part of a larger study run as a randomized complete block with nine replications and 74 cultigens. Plots consisted of three 100 mm × 100 mm square pots (600 ml volume, Kord, Lugoff, SC). Data were taken on only two of those plants to account for differences in germination. Data are presented here for three cultigens, since we were interested in partitioning test variability into that due to the method versus to the cultigen. The three cultigens were two accessions (PI 244017, PI 278027) and one inbred cultivar (Charleston Gray).

The cultigens were inoculated with four isolates of PRSV-W which were 2052, W-1A, 1870, and 2040. Plants that emerged properly were inoculated by using rub method at the first true leaf stage, and rated three times weekly on a 1–9 scale starting 2 weeks after inoculation.

2.4. Traits evaluated

Plants were rated on a scale of 0–9 for each of three traits: leaf necrosis, mosaic symptoms, and leaf deformation, where 0: none, 1–2: trace, 3–4: slight, 5–6: moderate,

7–8; severe, and 9: plant dead. There were no ratings of 9 for the mosaic or deformation traits. For each plot, the maximum, minimum, and mean rating among the five plants was recorded. An additional trait, average leaf damage, was calculated as the mean of the three traits for each rating date. Disease was detected visually for both experiments.

2.5. Data analysis

Data were summarized over plants in a plot, and were analyzed using the means, ANOVA, correlation, and GLM procedures of the SAS statistical package (SAS Institute, 1988). Range/LSD was used as an indicator of how well a test worked, or how useful a trait was. The number of LSD units separating the top and bottom treatment combinations is a good indicator of the usefulness of that test or trait.

3. Results and discussion

3.1. Preliminary experiment

For inoculation of the 10 isolates using two methods, we concluded that the rub method was superior to the spray method in that it was easier to use and the equipment was less cumbersome to transport (data not shown). The difficulties with the spray method were that each inoculated leaf had to be turned over before spraying, resulting in leaves being torn in the process. This was a problem, since plants inoculated at the cotyledon stage or the first true leaf stage have few leaves. Injuring a leaf in the process of inoculation opened up wounds for other pathogens, hindered the ability of the plant to produce energy, and created differences between injured and uninjured plants. Also, the carbon dioxide tank connected to the airbrush was heavy and difficult to carry around the confines of the greenhouse. Both methods resulted in 100% infection of inoculated plants. The 55 mm diameter pots were adequate for screening elite lines, although a larger size would permit slow germinating or late maturing accessions to develop more before inoculation.

Watermelon plants grown in cages had poor symptom expression in the fall season, probably as a result of shading from the screen. In addition, damping off occurred in the cool and low-light conditions of winter, suggesting fungicide seed treatment would be useful in those cases. Finally, watermelons grown in cages had poor growth in the winter, suggesting that seedlings should not be grown in cages when light limited growth.

3.2. Main experiment

The only treatment with significant differences for all traits and rating times was for isolate (*analysis of variance data not shown*). Isolates ranged from 2052 with severe foliage damage (7.3 at week 1) to 1637 with only slight foliage damage (3.7 at week 1), indicating a wide range in virulence among the 10 isolates (Table 1). The rating at week 2 had a higher range/LSD than the ratings at weeks 1 or 3, indicating the best separation of the isolates at that time. Isolate 2052 was the most virulent isolate for all traits and rating

Table 1
Resistance (damage, mosaic, necrosis, and deformation) of watermelon seedlings to 10 isolates of PRSV-W tested in the greenhouse using three rating dates (isolates in order by virulence)[a]

Isolate number	Damage			Mosaic			Necrosis			Deformation		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Main test												
2052	7.3	8.0	8.1	7.1	7.7	7.8	7.5	8.4	8.6	7.4	7.9	7.9
W-1A	6.4	7.1	7.7	6.2	7.1	7.5	6.2	6.9	8.0	6.9	7.3	7.6
2169	6.1	6.8	7.4	5.9	6.9	7.3	6.0	6.5	7.2	6.4	7.1	7.8
2201	6.5	7.0	7.6	6.5	6.9	7.6	6.0	6.8	7.6	6.9	7.4	7.6
2038	5.6	6.7	7.0	5.6	6.8	7.1	5.6	6.5	6.9	5.7	6.9	7.1
1870	5.4	6.2	6.9	5.6	6.4	6.9	4.6	5.4	6.5	5.9	6.9	7.2
2030	5.4	6.4	7.1	5.8	6.7	7.3	4.4	5.5	6.4	5.9	6.9	7.5
2040	4.8	6.0	7.1	5.1	6.0	6.9	4.1	5.4	7.0	5.1	6.4	7.3
2207	4.2	5.4	6.3	4.4	5.5	6.8	3.2	4.4	5.5	4.9	6.1	6.7
1637	3.7	4.4	5.4	3.9	4.9	5.7	2.6	3.3	4.6	4.6	5.1	5.9
LSD (5%)	1.1	0.9	0.7	1.0	0.8	0.6	1.5	1.4	1.2	1.1	0.7	0.6
Range/LSD	3.2	4.0	3.8	3.0	3.5	3.3	3.3	3.6	3.4	2.6	4.3	3.6
Retest (Charleston Gray)												
2052	7.9	8.8	9.0									
W-1A	7.6	8.3	8.9									
1870	7.9	8.5	9.0									
2040	7.9	8.6	8.9									
LSD (5%)	1.6	1.6	1.4									
Range/LSD	0.2	0.3	0.1									

[a] Data are means of two stages, two pot sizes, four replications, and five 'Charleston Gray' plants per plot. Plants were rated on a scale of 0–9 for each of three types of symptoms: necrosis, mosaic, and deformation, where 0: none, 1–2: trace, 3–4: slight, 5–6: moderate, 7–8: severe, and 9: plant dead (9 not used for mosaic or deformation). Damage trait is the mean of the three other traits, mosaic, necrosis, and deformation for the labeled week. Data for retest were collected only on the damage trait, since that was the most effective one in the main test.

times (Table 1), and that is the one we recommended for use in screening the watermelon germplasm collection.

There was no difference for pot sizes or plant stages, and pot size × plant stage interaction was not significant (data not shown). However, we recommended 100 mm diameter pot size for screening the watermelon germplasm collection. In this experiment, uninoculated controls and inoculated plants were not able to grow more than 250 mm in length in 55 mm pots. This restriction in plant size could affect the movement of the virus through the plant, as well as the ability of the plant to respond to the virus, giving either false negatives or false positives. On the other hand, plants grown in 100 mm pots were able to grow as long as 1000 mm. We would recommend 55 mm pots if uniform germination were expected in the test.

There were no significant differences among plant stages for foliage damage (Table 2), so a rapid test for virus resistance in a breeding program could be run using inoculation at the cotyledon stage. However, inoculation should be at the first true leaf stage for screening a wide diversity of germplasm such as the USDA collection, due to the inevitable differences in germination among PI accessions being tested. The lack of difference in foliage damage rating between stages indicates that the test should be reliable even if all accessions are not at the same stage during a test due to differences in growth speed.

Damage rating (the mean of mosaic, necrosis, and deformation ratings) was highly correlated with its component traits for the week in which they were rated, with all correlations being 0.89 or greater (Table 3). Therefore, plants could be rated for PRSV-W using any of the three component traits, or a combined rating that accounted for symptoms of mosaic, necrosis, and deformation. In a large germplasm screening test, it would be sufficient to make a single symptom rating at about week 2 for identification of PI accessions having a useful level of resistance to the virus.

Use of *C. amaranticolor* as a control to verify the virulence of the inoculum proved to be ineffective. Not all isolates induced necrotic spots on inoculated *C. amaranticolor* leaves, whereas all isolates induced viral symptoms on 'Charleston Gray' seedlings. These results are consistent with previous reports (Murant and Harrison, 1984). We recommend that susceptible controls be used for verification of virulent inoculum. A mixture of control plants inoculated with PRSV-W and uninoculated controls was used in this experiment for verification of infectious inoculum and as an indicator for other watermelon pathogens in the greenhouse, respectively. This method worked well, provided that knowledge of PRSV-W symptoms is adequate to distinguish between plants infected with PRSV-W, plants infected with other watermelon diseases, and healthy watermelon plants.

3.3. Retest experiment

The four isolates used in the retest were slightly more virulent than in the main test (Table 1), and much more similar than in the main test. When the recommended method was used to evaluate plant introduction accessions from the USDA germplasm collection, we observed variability over replications. We compared the variability of a typical PI accession with an inbred cultivar, and found them to be similar (Table 4). In fact, the susceptible PI accession PI 278027 was more uniform than the inbred cultivar Charleston Gray. On the other hand, the resistant PI accession PI 244017 was more variable than the

Table 2
Resistance (damage, mosaic, necrosis, and deformation) of watermelon seedlings tested in two pot sizes (55 and 100 mm) and at two growth stages (cotyledon and first true leaf) to PRSV-W tested in the greenhouse using three rating dates[a]

Pot size	Plant stage	Damage			Mosaic			Necrosis			Deformation		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Size × stage means													
55 mm	Cotyledon	5.8	6.4	7.0	6.0	6.6	6.3	5.1	5.9	6.8	6.9	6.8	7.3
	First true leaf	5.5	6.4	6.9	5.6	6.3	6.0	4.9	5.9	6.5	7.0	6.9	7.3
100 mm	Cotyledon	5.8	6.6	7.3	5.8	6.8	6.1	5.4	6.1	7.1	7.4	6.9	7.3
	First true leaf	5.1	6.3	7.0	5.1	6.3	5.5	4.8	5.7	7.0	7.1	6.8	7.1
Size means													
55 mm		5.6	6.4	7.0	5.0	5.9	6.6	5.8	6.4	6.9	6.1	6.9	7.3
100 mm		5.4	6.4	7.2	5.1	5.9	7.0	5.5	6.5	7.2	5.8	6.8	7.2
Stage means													
Cotyledon		5.8	6.5	7.1	5.2	6.7	7.2	6.2	6.0	7.0	5.9	6.8	7.3
First true leaf		5.3	6.3	7.0	4.9	6.3	7.0	5.7	5.8	6.7	5.3	6.8	7.2

[a] Data are means of 10 isolates, four replications, and five 'Charleston Gray' plants per plot. Plants were rated on a scale of 0–9 for each of three types of symptoms: necrosis, mosaic, and deformation, where 0: none, 1–2: trace, 3–4: slight, 5–6: moderate, 7–8: severe, and 9: plant dead (9 not used for mosaic or deformation). Damage trait is the mean of the three other traits, mosaic, necrosis, and deformation for the labeled week.

Table 3
Correlations of three traits and three rating dates for resistance of watermelon seedlings to PRSV-W in the greenhouse[a]

		Damage		Mosaic			Necrosis			Deformation		
		Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Damage	Week 1	0.92	0.83	0.95	0.88	0.82	0.90	0.87	0.71	0.89	0.85	0.72
	Week 2	–	0.89	0.93	0.96	0.89	0.77	0.94	0.78	0.77	0.91	0.76
	Week 3	–	–	0.86	0.89	0.95	0.68	0.83	0.92	0.67	0.76	0.89
Necrosis	Week 1	–	–	–	0.93	0.88	0.77	0.86	0.73	0.77	0.80	0.71
	Week 2	–	–	–	–	0.92	0.71	0.85	0.75	0.71	0.79	0.71
	Week 3	–	–	–	–	–	0.64	0.79	0.79	0.67	0.72	0.75
Mosaic	Week 1	–	–	–	–	–	–	0.77	0.61	0.75	0.76	0.64
	Week 2	–	–	–	–	–	–	–	0.75	0.73	0.88	0.76
	Week 3	–	–	–	–	–	–	–	–	0.57	0.68	0.85
Deformation	Week 1	–	–	–	–	–	–	–	–	–	0.78	0.60
	Week 2	–	–	–	–	–	–	–	–	–	–	0.72

[a] Data are over two stages, two pot sizes, 10 isolates, four replications, and five 'Charleston Gray' plants per plot. Plants were rated on a scale of 0–9 for each of three types of symptoms: necrosis, mosaic, and deformation, where 0: none, 1–2: trace, 3–4: slight, 5–6: moderate, 7–8: severe, and 9: plant dead (9 not used for mosaic or deformation). Damage trait is the mean of the three other traits, mosaic, necrosis, and deformation for the labeled week. Correlations above 0.7 are significant at the 1% level.

Table 4
Variability over replication for two PI accessions and one inbred cultivar differing in resistance to PRSV-W in the greenhouse[a]

Cultigen	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Mean	S.D.
PI 244017 (R)	5.0	3.0	2.0	3.0	4.0	3.0	3.4	2.6	2.8	3.2	0.9
PI 278027 (S)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	8.2	8.8	0.4
Charleston Gray (S)	8.3	7.0	9.0	9.0	8.3	9.0	9.0	9.0	9.0	8.6	0.7

[a] Plants were rated on a scale of 0–9 for disease, where 0: none, 1–2: trace, 3–4: slight, 5–6: moderate, 7–8: severe, and 9: plant dead. R indicates resistance and S indicates susceptibility to PRSV-W isolate 2052.

susceptible cultigens, with ratings of 2.0–5.0 in the nine replications. It is possible that some of the plants in the accession were more resistant, or that there was more variability in the test when the cultigen was resistant instead of susceptible. Unfortunately, no inbred cultivars exist that have resistance to PRSV-W, so it is not possible to determine which is the correct explanation. We concluded that the variability observed over replications was not due to accession variability, but to the test itself. The variability was not excessive, since we observed a range in disease rating from 7.0 to 9.0 (mean of 8.6) in 'Charleston Gray' over nine replications of greenhouse plots.

3.4. Summary

The best method for a screening of the watermelon germplasm collection for resistance to PRSV-W is to grow the seedlings in square, 100 mm diameter pots, inoculate plants at the first true leaf stage, use PRSV-W isolate 2052, and the rub method. Significant differences were obtained (with LSD values of 0.6–1.5) using four replications of five plants per plot, but fewer replications and plants may be adequate for a large germplasm screening study.

As noted by previous researchers, not all isolates of PRSV-W used in this study induced local lesions on *C. amaranticolor*. Others who have screened large numbers of accessions for resistance to WMV (Gillaspie and Wright, 1993) and ZYMV (Boyhan et al., 1992) noted the need to have a screening procedure that reduces the possibility of escapes. We have also found that to be important because escapes, as in the previous two studies, increase the number of lines that must be screened further. The possibility of escapes makes it necessary to increase the number of replications per accession and plants per replication in the test.

In this study we have developed a consistent and reliable method for screening watermelon for resistance to PRSV-W. The method can be used by researchers interested in screening for PRSV-W resistance in watermelon, verifying resistance, studying its inheritance, and transferring it to elite cultivars.

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References

Adlerz, W.C., Crall, J.M., 1967. Epidemiology of control of watermelon mosaic virus. Florida Agric. Exp. Stat. Ann. Rep. 403.

Baker, C.A., Lecoq, H., Purcifull, D.E., 1991. Serological and biological variability among papaya ringspot virus type-W isolates in Florida. Phytopathology 81, 722–728.

Boyhan, G., Norton, J.D., Jacobsen, B.J., Abrahams, B.R., 1992. Evaluation of watermelon and related germplasm for resistance to zucchini yellow mosaic virus. Plant Dis. 76, 251–252.

Gillaspie, A.G., Wright, J.M., 1993. Evaluation of *Citrullus* sp. germplasm for resistance to watermelon mosaic virus 2. Plant Dis. 77, 352–354.

Grumet, R., 1989. Genetically engineered plant virus resistance. HortScience 25 (5), 508–513.

Hojo, H., Pavan, M.A., Silva, N., 1991a. Aggressiveness of papaya ringspot virus-watermelon strain on watermelon cultivars. Summa Phytopathol. 17, 188–194.

Hojo, H., da Silva, N., Pavan, M.A., 1991b. Screening of watermelon cultivars and hybrids for resistance to papaya ringspot virus-watermelon strain. Summa Phytopathol. 17, 113–118.

Munger, H.M., More, T.A., Awni, S., 1984. A preliminary report on screening watermelons for resistance to watermelon mosaic viruses 1 and 2. Cucurbit Genet. Coop. Rep. 7, 61–62.

Murant, A.F., Harrison, B.D. (Eds.), 1984. CMI/ABB Descriptors of Plant Viruses, No. 292. Unwin Brothers, Old Woking, Surrey, England.

Namba, S., Ling, K., Gonsalves, C., Slightom, J.L., Gonsalves, D., 1992. Protection of transgenic plants expressing the coat protein gene of watermelon mosaic virus II or zucchini yellow mosaic virus against six potyviruses. Phytopathology 82, 940–946.

Provvidenti, R., 1986a. Reactions of PI accessions of *Citrullus colocynthis* to zucchini yellow mosaic virus and other viruses. Cucurbit Genet. Coop. Rep. 9, 82–83.

Provvidenti, R., 1986b. Viral disease of cucurbits and source of resistance. Food and Fertilizer Technology Center Technical Bulletin No. 93.

Provvidenti, R., 1993. In: Kyle, M.M. (Ed.), Resistance to Viral Diseases of Vegetables. Timber Press, Portland, OR.

Quemada, H., Sieu, L.C., Siemieniak, D.R., Gonsalves, D., Slightom, J.L., 1990. Watermelon mosaic virus II and zucchini yellow mosaic virus: cloning of 3′-terminal region, nucleotide sequences, and phytogenetic comparisons. J. Gen. Virol. 71, 1451–1460.

SAS Institute, 1988. SAS/STAT User's Guide, Release 6.03. SAS Institute, Cary, NC.

Sherf, A.I., Macnab, A.A., 1986. Vegetable Diseases and Their Control, 2nd Edition. Wiley, New York.