PLANT GENETIC RESOURCES

Screening the Watermelon Germplasm Collection for Resistance to Papaya Ringspot Virus Type-W

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ABSTRACT

Papaya ringspot virus watermelon strain (PRSV-W), formerly watermelon mosaic virus-1, is a major disease of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. The objectives of this study were (i) to screen the USDA watermelon germplasm collection for PRSV-W resistance, (ii) to verify the disease rating for the most resistant and most susceptible accessions, (iii) to determine the number of escapes on the basis of the retest of the germplasm screening test. The experiment was a randomized complete block with five replications and 1275 accessions. 'Charleston Gray' susceptible checks were used to verify that the PRSV-W inoculum was virulent. Enzyme-linked immunosorbent assay (ELISA) was performed after the last rating to determine whether the virus was in the plant tissue. The PI accessions with the highest resistance to PRSV-W that also had resistance to other watermelon viruses (ZYMV, zucchini yellow mosaic virus or WMV, watermelon mosaic virus, formerly watermelon mosaic virus-2) were PI 244018, PI 244019, PI 255137, and PI 482299. The first retest of the most resistant 21 PI accessions showed that there were some escapes that were not resistant to PRSV-W. Of the 21 PI accessions in the retest, seven PI accessions were identified for further testing. Of the 60 resistant PI accessions in the final retest, eight had resistance with a rating of 3.6 or less for the best, average, and maximum ratings: PI 244017 (best over all tests), PI 244019, PI 482342, PI 482318, PI 485583, PI 482379, PI 595203, and PI 244018.

Watermelon is a major crop in the southern USA. The most important virus diseases of watermelon in the USA are papaya ringspot virus-watermelon strain (PRSV-W, formerly watermelon mosaic virus-1), watermelon mosaic virus (WMV, formerly watermelon mosaic virus-2), and zucchini yellow mosaic (ZYMV) (Adlerz and Crall, 1967). Virus diseases are destructive to the watermelon crop and are difficult to control (Sherf and Macnab, 1986).

Major virus control strategies include the use of insecticides to eliminate virus vectors, herbicides to remove alternate hosts for the virus, and genetic resistance (Provvidenti, 1993), which is often pathogen-specific (Grumet, 1989). Of those controls, the most economical method is genetic resistance. Virus resistance may also be accomplished through virus coat proteins transferred into existing cultivars (Namba et al., 1992; Quemada et al., 1990), or by screening of germplasm collections. Either method might be used successfully in watermelon. The watermelon germplasm collection has been screened for resistance to several virus diseases. Boyhan et al. (1992) identified PI accessions resistant to ZYMV and Gillaspie and Wright (1993) identified PI accessions resistant to WMV. Researchers have screened other cucurbit species for resistance to PRSV-W and the inheritance of the resistance has been determined. Provvidenti and Gonsalves (1982) found in *Cucumis metuliferus* E. Meyer ex Naudin that accessions resistant to WMV were also resistant to PRSV and that the resistance was controlled by a single dominant gene.

PRSV-W virus infects all the agriculturally important species of the Cucurbitaceae (Provvidenti, 1993). PRSV-W was known as watermelon mosaic virus-1 until it was shown to be a strain of papaya ringspot virus (Provvidenti, 1993). This virus is transmitted in a nonpersistent manner by 24 species of aphid in 15 genera. Resistance to the virus has been identified in cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), squash (*Cucurbita* spp.), and gourds (*Lagenaria* spp. and *Luffa* spp.) (Provvidenti, 1993).

Previous research has demonstrated that screening watermelon for resistance to PRSV-W should be effective. Munger et al. (1984) used an unidentified isolate of PRSV-W to find genetic differences among seven watermelon PI accessions. Hojo et al. (1991a) used an aggressive Brazilian 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 land races of watermelon (Provvidenti, 1986).

The objectives of this study were (i) to screen the USDA watermelon germplasm collection for PRSV-W resistance; (ii) to verify the disease rating for the most resistant and most susceptible accessions; and (iii) to determine the number of escapes on the basis of the retest of the germplasm screening test.

MATERIALS AND METHODS

Three large experiments were performed: a germplasm screening, an early retest of the screening results, and a final retest. All experiments were performed in the North Carolina State University plant pathology greenhouses. Greenhouse temperatures ranged from 23 to 43°C (day) and 12 to 24°C

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Abbreviations: PRSV-W, papaya ringspot virus-watermelon strain; ZYMV, zucchini yellow mosaic virus; WMV, watermelon mosaic virus.

(night). The virus isolate was obtained from D.E. Purcifull, University of Florida, Gainesville. The PRSV-W isolate used was 2052 described by Baker et al. (1991) and was maintained on 'Gray Zucchini' squash (*Cucurbita pepo* L.) from Seminis Vegetable Seeds (Woodland, CA). All *Citrullus* Plant Introduction (PI) accessions were obtained from the Southern Regional Plant Introduction Station at Griffin, GA. PI accessions originated in 68 different countries, with 46 countries having fewer than 10 accessions each. Countries with the most accessions in the collection of 1275 were Turkey (296), Yugoslavia (163), Zimbabwe (122), India (120), Spain (71), Zambia (55), South Africa (36), Syria (28), Iran (27), China (26), and Nigeria (23).

Inoculum was produced by grinding infected Gray Zucchini squash leaves with 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). The inoculation procedure was the same for increasing on squash and for screening watermelon. Inoculation consisted of dusting one leaf on each 3-wk-old plant with an 800-mesh carborundum, then applying the inoculum to the leaf with a pestle which was rotated in a circular motion 8 to 10 times as if painting the leaf with inoculum. After inoculation, carborundum was rinsed off the leaves to improve light interception, and the plants were maintained in aphid-proof cages. All Gray Zucchini squash plants were seeded in metromix 200 (Scotts-Sierra Horticultural Products Company, Marysville, OH) in 160-mm diameter (1550-mL volume) clay pots. Plants were fertilized weekly with 150 mg kg^{-1} Peters Professional 20-20-20 N-P-K (Scotts-Sierra Horticultural Products Company, Marysville, OH).

The germplasm screening test was a randomized complete block with five replications of 1275 accessions. Each plot was a 100 × 100 mm square pot (600-mL volume) planted with two seeds and thinned to one plant before inoculation. In addition to the accessions tested, there were 50 check plants per replication of 'Charleston Gray' that were inoculated with the virus, and 50 plants of Charleston Gray that were not inoculated. The inoculated checks served as verification of viral infection and the uninoculated checks served as an indicator of other disease in the greenhouse that might confound symptom expression.

To screen large numbers of PI accessions for resistance to PRSV-W, a rating system was used that took into account the different growth habits and leaf morphologies of the different accessions. The rating system was general enough to allow for the differences in the PI accessions but specific enough to distinguish resistant plants. Plants were inoculated at the first true leaf stage, and rated weekly for 6 wk on a scale of 1 to 9 on the basis of severity of viral symptoms, where 1 = none, 2 = tendrils absent, 3 = tendrils absent, slightly stunted growth, 4 = mosaic patches and/or necrotic spots on leaves, 5 = leaves near apical meristem deformed, meristem yellow and reduced in size, 6 = apical meristem withered and brown, 7 = apical meristem dead with more basal leaves dying, 8 = most leaves dead, main stem green/yellow, 9 = plant dead. After the second rating, plants which had not emerged at the time of inoculation and those plants which were inoculated and had a rating under 4 were reinoculated to reduce the number of escapes. After the sixth rating, all plants that were not dead were tested by ELISA (Agdia Incorporated, Elkhart, IN) to determine if there was virus present in the leaf tissue. Tissue used for testing was taken from a sample of the top five leaves of the plant. Those plants which did not have virus in their system and which had a low rating were considered resistant.

Originally, we used *Chenopodium amaranticolor* Coste & A. Reynier to check inoculation efficacy. However, that was not a reliable indicator, so checks of Charleston Gray were inoculated and observed for viral symptoms. In the reinoculation stage, three check plants that were the same age as the test plants to be reinoculated and three 2-wk-old checks were inoculated periodically during the reinoculation to assure that the inoculum was virulent. All PI accessions were maintained in a screened greenhouse which contained no other cucurbits and in which no other viral experiments were being performed. The germplasm screening started in the summer of 1998 and ended in the fall of 1999.

Data were summarized as the average, the maximum, and the best of the six ratings. The best rating was the one with the greatest range over the 1275 cultigens, which was rating 3 in this experiment. Data were analyzed by means of the MEANS, ANOVA, CORRELATION, and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC). Data were based on ratings from single-plant plots, and each rating date was analyzed separately.

An early retest was performed after the completion of the first two replications of the germplasm screening to determine variability within the resistant accessions. This also provided a method for determining the number of escapes and errors in the germplasm screening before continuing with the next replications. Results were used to plan the next studies, and to begin seed increases of cultigens having resistance to PRSV-W. The early retest was performed with one replication of 21 plants per accession from the 21 most resistant accessions along with the susceptible check, Charleston Gray. Inoculation and rating procedures were the same as for the germplasm screening.

A final retest was performed after the completion of the germplasm screening to verify the reaction of the most resistant and susceptible accessions. We were also interested in measuring the variability over replications. The experiment was a randomized complete block with four replications of 72 cultigens along with two susceptible checks (Charleston Gray and 'Crimson Sweet'). Plots consisted of two 100- × 100-mm square pots (600-mL volume). Extra pots of each accession were planted to assure that all plots would have the same number of plants even with differences in germination. The cultigens were inoculated with four isolates of PRSV-W, which were 2052, W-1A, 1870, and 2040. Plants were inoculated by the rub method at the first true leaf stage, and rated three times weekly on a 1-to-9 scale starting 2 wk after inoculation.

RESULTS AND DISCUSSION

Germplasm Screening

Not all of the 1275 accessions germinated in all five replications, and data were obtained for 1248 accessions. The complete dataset was submitted to the Germplasm Resources Information Network (http://www.ars-grin.gov/, 5 Feb. 2002) for those interested in particular cultigens. The most resistant and most susceptible cultigens are presented here, along with checks and cultigens included in the retests (Table 1). The ANOVA indicated that there were highly significant differences ($P = 0.01$) among accessions for all rating dates. Since the best and average ratings were highly correlated ($r = 0.90$), and the maximum rating had a smaller F ratio than the other ratings, only the best rating was given in Table 1 to save space. In a study such as this, where most of the 1275 accessions were susceptible and had ratings of 8 to 9, there was the possibility that the few resistant accessions

Table 1. Best rating based on five replications of data for 1248 watermelon accessions inoculated with PRSV-W.

Rank	Accession or cultivar†	Country of origin	Best rating
Resistant			
1	PI 278005	Turkey	3.0
2	PI 277972	Turkey	3.0
3	PI 278009	Turkey	3.0
4	PI 244017	South Africa	3.4
5	PI 174104	Turkey	3.5
6	PI 164665	India	3.5
7	PI 164737	India	4.0
8	PI 244019	South Africa	4.0
9	PI 244018	South Africa	4.0
10	PI 278008	Turkey	4.0
11	PI 314655	Soviet Union	4.0
12	PI 277989	Turkey	4.0
13	PI 532667	Swaziland	4.0
14	PI 357752	Yugoslavia	4.0
15	PI 346082	Afghanistan	4.0
16	PI 482342	Zimbabwe	4.4
17	PI 277990	Turkey	4.5
18	PI 319212	Egypt	4.5
19	PI 234287	Portugal	4.8
20	PI 482303	Zimbabwe	4.8
21	PI 275628	Pakistan	5.0
22	PI 482261	Zimbabwe	5.0
23	PI 512364	Spain	5.0
24	PI 254742	Senegal	5.0
25	PI 177328	Turkey	5.0
26	PI 176489	Turkey	5.0
27	PI 271132	Tunisia	5.0
28	PI 485583	Botswana	5.2
29	PI 525086	Egypt	5.2
30	PI 195562	Ethiopia	5.3
31	PI 482322	Zimbabwe	5.3
32	PI 169232	Turkey	5.3
33	PI 169241	Turkey	5.3
34	PI 482318	Zimbabwe	5.4
35	PI 255137	South Africa	5.5
36	PI 525088	Egypt	5.6
37	PI 595203	US, GA	5.6
38	PI 278058	Turkey	5.6
39	PI 482379	Zimbabwe	5.7
40	PI 345543	Soviet Union	5.7
41	PI 172803	Turkey	5.7
42	PI 278026	Turkey	5.7
43	PI 169238	Turkey	5.7
44	PI 271986	Somalia	5.7
45	PI 482315	Zimbabwe	5.8
46	PI 534592	Syria	5.8
47	PI 526235	Zimbabwe	5.8
48	PI 482299	Zimbabwe	5.8
49	PI 482305	Zimbabwe	5.8
50	PI 482269	Zimbabwe	5.8
51	PI 482312	Zimbabwe	5.8
52	PI 246559	Senegal	5.8
Checks and Accessions Included in Retests			
54	PI 307609	Nigeria	6.0
55	PI 175663	Turkey	6.0
69	PI 482309	Zimbabwe	6.2
74	PI 357679	Yugoslavia	6.3
81	PI 534584	Syria	6.4
89	PI 502319	Uzbekistan	6.5
108	PI 482319	Zimbabwe	6.6
109	PI 537271	Pakistan	6.6
110	PI 512406	Spain	6.6
140	PI 169243	Turkey	6.8
159	PI 536450	Maldives	6.8
188	PI 490376	Mali	7.0
189	PI 254624	Sudan	7.0
191	PI 164687	India	7.0
193	PI 482254	Zimbabwe	7.0
194	PI 482317	Zimbabwe	7.0
195	PI 174105	Turkey	7.0
208	Grif 1729	PR China, Jiangsu	7.0
257	PI 177329	Turkey	7.2
338	PI 163203	India	7.4
358	Grif 1730	PR China, Jiangsu	7.4
400	PI 378617	Zaire	7.6
402	Grif 1733	PR China, Jiangsu	7.6
450	PI 505604	Zambia	7.8
487	Grif 1732	PR China, Jiangsu	7.8
558	Grif 5596	India	7.8
645	Grif 12335	PR China, Yunnan	8.0
650	Grif 12336	PR China, Yunnan	8.0
732	Grif 5597	India	8.0
781	Grif 5598	India	8.0
875	Charleston Gray	Check	8.2
915	Grif 1734	PR China, Jiangsu	8.4
923	Grif 5599	India	8.4
1012	Grif 1731	PR China, Jiangsu	8.8
Susceptible			
1106	PI 379245	Yugoslavia	9.0
1107	PI 178873	Turkey	9.0
1108	PI 279461	Japan	9.0
1109	PI 176490	Turkey	9.0
1110	PI 500309	Zambia	9.0
1112	PI 508442	South Korea	9.0
1117	Grif 1728	PR China, Jiangsu	9.0
1124	PI 357690	Yugoslavia	9.0
1128	PI 357735	Yugoslavia	9.0
1131	PI 278027	Turkey	9.0
1149	PI 532817	China, Shaanxi	9.0
1154	PI 176499	Turkey	9.0
1155	PI 500332	Zambia	9.0
1208	PI 254428	Lebanon	9.0
1209	PI 169245	Turkey	9.0
1210	PI 490382	Mali	9.0
1211	PI 378613	Zaire	9.0
1212	PI 211852	Iran	9.0
1213	PI 277991	Turkey	9.0
1214	PI 288522	India	9.0
1215	PI 381713	India, Rajasthan	9.0
1216	PI 172798	Turkey	9.0
1217	PI 183217	Egypt	9.0
1218	PI 296342	South Africa	9.0
1219	PI 357737	Yugoslavia	9.0
1220	PI 278018	Turkey	9.0
1241	PI 357680	Yugoslavia	9.0
1242	PI 279462	Japan	9.0
1243	PI 169263	Turkey	9.0
1244	PI 177323	Turkey	9.0
1245	PI 278003	Turkey	9.0
1246	PI 278038	Turkey	9.0
1247	PI 368524	Yugoslavia	9.0
1248	PI 532664	Swaziland	9.0
LSD (5%)			1.8
F ratio (all cultigens)			1.5
F ratio (52 res. and 52 sus. only)			9.3

Continued next column.

Table 1. Continued.

† Some countries listed as the origin of some accessions no longer exist as political units (Czechoslovakia, Soviet Union, Yugoslavia). Rank indicates the ranking of the cultigen for resistance to PRSV-W, based on best rating (as well as average and maximum ratings; data not shown).

identified were overwhelmed in the analysis of variance by the large number of susceptible accessions. To evaluate the importance of that effect, the ANOVA was rerun with only the most resistant 52 and the most susceptible 52 accessions. That effect was large in this study, with the F ratio changing from 1.5 for the 1248 cultigens to 9.3 for the 104 cultigens. Therefore, both analyses showed significant differences among PI accessions, but the smaller data set had a larger accession F ratio because of the smaller variances for replication and error, and the larger accession variance.

The criteria for identifying resistance was to use the best, average, and maximum ratings, along with ELISA results. There were 49 PI accessions that had a maximum rating less than 6.0, although it should be noted that

Table 2. Mean resistance ratings (best, average, and maximum) of the five PI accessions of watermelon (with 'Charleston Gray') having the highest resistance to PRSV-W and complete data (missing in no more than one replication).

Rank	Accession or cultivar	Country of origin	Virus rating†			Maximum rating				
			Best	Average	Max.	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1	PI 244017	South Africa	3.4	4.7	6.8	5.0−	9.0	6.0	9.0	5.0+
2	PI 244018	South Africa	4.0	5.5	8.0	5.0	9.0	9.0	9.0	–
3	PI 482342	Zimbabwe	4.4	5.3	7.2	9.0	9.0	9.0	5.0+	4.0+
4	PI 234287	Portugal	4.8	5.7	8.0	5.0	9.0	–	9.0	9.0
5	PI 482303	Zimbabwe	4.8	5.7	8.6	5.0−	9.0	9.0	9.0	9.0
875	Charleston Gray	Check, USA	8.2	8.0	8.9	8.3	7.0	9.0	9.0	8.3
LSD (5%)			1.8	1.1	0.6					

† Plants were rated on a scale of 1 to 9 on the basis of severity of viral symptoms, where 1 = none, 2 = tendrils absent, 3 = tendrils absent, slightly stunted growth, 4 = mosaic patches and/or necrotic spots on leaves, 5 = leaves near apical meristem deformed, meristem yellow and reduced in size, 6 = apical meristem withered and brown, 7 = apical meristem dead with more basal leaves dying, 8 = most leaves dead, main stem green/yellow, 9 = plant dead. Best is the average of third rating for the 5 replications. Maximum is the highest of six ratings for all of the replications. Average is the overall average of all the ratings for all the replications. Final rating refers to the sixth of six ratings for each replication. − and + indicate results from ELISA testing and refer to no virus detected in tissue and virus detected in tissue, respectively. Rank indicates the ranking of the cultigen for resistance to PRSV-W, based on best rating (as well as average and maximum ratings) for 1248 cultivars.

Table 3. Mean resistance ratings of 21 plants of 22 watermelon PI accessions found resistant to PRSV-W in the first and second replication of screening, including Charleston Gray as the check.

Accession or cultivar	Virus rating†		Rating date					
	Average	Maximum	1	2	3	4	5	6
PI 244018	6.1	8.5	5.0	5.3	4.7	5.3	7.8	8.4
PI 485583	7.1	9.0	5.7	5.7	6.3	7.1	8.6	9.0
PI 482303	7.2	8.9	5.5	6.1	7.0	7.7	8.0	8.9
PI 244017	7.3	8.8	5.5	6.3	7.2	7.2	8.5	8.8
PI 482319	7.6	8.7	6.7	7.5	7.0	7.2	8.5	8.5
PI 164687	7.6	8.9	5.9	6.9	7.6	7.7	8.7	8.9
PI 482317	7.7	9.0	5.6	6.5	8.0	8.2	9.0	9.0
PI 482254	7.8	8.9	5.7	7.1	8.0	8.2	8.8	8.8
PI 234287	7.9	9.0	6.1	7.1	7.8	8.4	9.0	9.0
PI 163203	8.0	9.0	6.4	7.1	8.0	8.3	9.0	9.0
PI 254624	8.0	9.0	6.1	7.3	8.2	8.2	9.0	9.0
PI 482299	8.0	9.0	7.0	7.2	7.7	8.0	9.0	9.0
PI 175663	8.1	9.0	7.1	7.7	7.6	7.9	9.0	9.0
PI 490376	8.2	9.0	6.7	7.7	8.2	8.6	9.0	9.0
PI 482309	8.3	9.0	6.7	7.9	8.4	8.5	9.0	9.0
PI 505604	8.3	9.0	7.5	8.1	7.9	8.3	9.0	9.0
PI 174105	8.4	9.0	6.9	7.8	8.8	8.9	9.0	9.0
PI 275628	8.4	9.0	6.7	7.9	9.0	9.0	9.0	9.0
PI 378617	8.5	9.0	7.4	7.9	8.4	9.0	9.0	9.0
Charleston Gray	8.6	9.0	7.4	8.3	9.0	9.0	9.0	9.0
PI 169243	8.7	9.0	7.5	8.5	9.0	9.0	9.0	9.0
PI 177329	8.9	9.0	8.3	9.0	9.0	9.0	9.0	9.0

† Analysis performed for means of each rating date plus the overall averages. Rating dates were at one-week intervals beginning two weeks after inoculation (first true-leaf stage). Plants were rated on a scale of 1 to 9 on the basis of severity of viral symptoms, where 1 = none, 2 = tendrils absent, 3 = tendrils absent, slightly stunted growth, 4 = mosaic patches and/or necrotic spots on leaves, 5 = leaves near apical meristem deformed, meristem yellow and reduced in size, 6 = apical meristem withered and brown, 7 = apical meristem dead with more basal leaves dying, 8 = most leaves dead, main stem green/yellow, 9 = plant dead. Average rating is the mean of the ratings of all 21 plants. Maximum ratings is the mean of the final rating of all 21 plants per PI accession. Rank indicates the ranking of the cultigen for resistance to PRSV-W, based on average and maximum rating.

all plants infected with the virus eventually died. That indicates that no immunity to the isolate used in this study was found. This contrasts with ELISA results, where we identified accessions that did not have virus in their tissues at the time of testing. This could be due to errors in the ELISA tests, but all positive and negative controls for all ELISA tests performed were accurate. It should be noted that no observations of watermelon PI accessions were taken beyond the sixth rating of virus symptoms. This study reports resistance to the most virulent PRSV-W isolate 2052 (collected in Florida). However, resistant accessions may not be resistant to other isolates in Florida or other regions where PRSV-W is found.

Our results paralleled that of previous researchers in that variation for virus resistance was identified in the watermelon germplasm collection. Boyhan et al. (1992) working with ZYMV, and Gillaspie and Wright (1993) working with WMV were able to identify resistant accessions in their watermelon research. PI 482299, PI 482261, PI 595203, and PI 255137 found to be resistant to ZYMV also had some resistance in this study to PRSV-W. Other accessions reported to have resistance to ZYMV were not tested in this study. One accession, PI 595202, reported to have ZYMV resistance did not show resistance to PRSV-W in this study. PI 244018 and PI 244019 found to be resistant to WMV by Gillaspie and Wright (1993), showed resistance to PRSV-W. Other accessions that were found to be resistant to WMV (PI 189316, PI 189317, and PI 248178) did not have resistance to PRSV-W. Provvidenti and Gonsalves (1982) worked with *C. metuliferus* and found that the accessions they identified as resistant to WMV were also resistant to PRSV. This was the case for some of the accessions screened in this study.

Gillaspie and Wright (1993) found there were plants

Table 4. Mean resistance ratings of the most resistant 60 and most susceptible and 12 cultigens (along with two checks) of watermelon for PRSV-W in the final retest.

			Virus rating†			Maximum rating			
Rank	Accession or cultivar	Country of origin	Best	Average	Max.	Rep 1	Rep 2	Rep 3	Rep 4
Resistant									
1	PI 244019	South Africa	2.5	2.4	3.1	3.6	3.6	2.5	2.6
2	PI 244017	South Africa	2.6	2.4	2.9	3.0	3.4	2.6	2.8
3	PI 482342	Zimbabwe	2.7	2.7	3.1	2.5	4.7	2.7	2.9
4	PI 482318	Zimbabwe	3.0	3.6	4.3	5.9	5.3	3.0	2.9
5	PI 485583	Botswana	3.1	2.7	3.5	3.1	4.5	3.1	3.2
6	PI 482379	Zimbabwe	3.4	3.5	4.1	4.1	5.6	3.4	3.3
7	PI 595203	United States	3.5	2.9	3.4	3.5	3.3	3.5	3.4
8	PI 512364	Spain	3.5	8.1	8.6	9.0	8.9	8.0	8.6
9	PI 244018	South Africa	3.6	3.0	3.5	3.6	3.1	3.6	3.6
10	PI 234287	Portugal	4.0	8.1	8.6	9.0	8.4	8.5	8.4
11	PI 482315	Zimbabwe	4.3	4.7	5.4	7.0	6.3	4.3	4.1
12	PI 482322	Zimbabwe	4.8	4.4	5.2	5.5	7.0	4.9	3.3
13	PI 255137	South Africa	5.8	5.3	6.0	5.0	7.8	5.8	5.5
14	PI 482299	Zimbabwe	5.9	5.2	6.1	5.0	7.8	5.9	5.9
Susceptible									
15	PI 169238	Turkey	6.3	8.1	8.6	9.0	8.3	8.6	8.6
16	PI 482312	Zimbabwe	6.8	6.1	6.8	5.5	8.1	6.8	6.9
17	PI 482261	Zimbabwe	7.0	4.8	5.6	4.1	4.3	7.0	6.9
18	PI 482303	Zimbabwe	7.0	6.4	7.1	7.4	6.8	7.0	7.1
19	PI 307609	Nigeria	7.0	7.0	7.6	8.8	7.6	7.0	7.3
20	PI 314655	Soviet Union	7.0	7.4	8.0	–	–	7.0	8.3
21	PI 526235	Zimbabwe	7.3	7.5	8.1	8.9	8.6	7.3	7.5
22	PI 357735	Yugoslavia	7.3	8.1	8.6	9.0	8.8	8.3	8.5
23	PI 164665	India	7.3	8.1	8.6	9.0	8.9	8.1	8.5
24	PI 345543	Soviet Union	7.4	7.2	8.0	8.8	8.9	7.4	7.0
25	PI 482305	Zimbabwe	7.5	7.1	7.7	8.5	7.0	7.5	7.9
26	PI 271132	Tunisia	7.5	7.3	7.9	7.9	9.0	7.5	7.4
27	PI 275628	Pakistan	7.5	7.9	8.3	9.0	8.8	7.5	8.1
28	PI 169232	Turkey	7.6	7.6	8.2	9.0	8.8	7.6	7.5
29	PI 278009	Turkey	7.6	7.6	8.2	9.0	8.1	7.6	8.0
30	PI 346082	Afghanistan	7.8	7.1	7.6	7.8	7.0	7.8	9.0
31	PI 195562	Ethiopia	7.8	7.6	8.1	9.0	8.0	7.8	7.8
32	PI 500332	Zambia	7.9	7.6	8.2	7.8	9.0	7.9	8.3
33	PI 174104	Turkey	7.9	7.7	8.3	9.0	8.1	7.9	8.3
34	PI 177328	Turkey	7.9	7.9	8.5	9.0	8.8	7.9	8.4
35	PI 254741	Senegal	7.9	8.0	8.5	9.0	8.8	7.9	8.3
36	PI 169241	Turkey	8.0	7.7	8.3	9.0	8.0	8.0	8.3
37	PI 534592	Syria	8.0	7.7	8.3	8.3	8.5	8.0	8.6
38	PI 482269	Zimbabwe	8.0	7.7	8.3	9.0	8.5	8.0	7.9
39	PI 525086	Egypt	8.0	7.8	8.3	9.0	8.4	8.0	8.0
40	PI 254742	Senegal	8.0	7.9	8.5	9.0	8.9	8.0	8.1
41	PI 278027	Turkey	8.0	8.2	8.6	9.0	9.0	8.0	8.2
42	PI 512406	Spain	8.1	7.7	8.3	8.3	8.6	8.1	8.3
43	PI 534584	Syria	8.1	7.8	8.5	8.5	9.0	8.1	8.5
44	PI 357690	Yugoslavia	8.1	7.9	8.4	9.0	7.9	8.1	8.6
45	PI 176489	Turkey	8.1	7.9	8.5	9.0	8.6	8.1	8.3
46	PI 172803	Turkey	8.1	7.9	8.6	9.0	8.6	8.1	8.3
47	PI 532817	China	8.1	7.9	8.4	9.0	8.0	8.1	8.5
48	PI 176499	Turkey	8.1	8.0	8.6	9.0	9.0	8.1	8.3
49	PI 319212	Egypt	8.3	7.8	8.6	9.0	8.8	8.3	8.3
50	PI 178873	Turkey	8.3	7.8	8.4	9.0	8.3	8.3	8.1
51	PI 502319	Uzbekistan	8.3	7.9	8.6	9.0	8.8	8.3	8.3
52	PI 279461	Japan	8.3	7.9	8.6	9.0	8.6	8.3	8.5
53	PI 379245	Yugoslavia	8.4	7.1	7.8	7.9	6.5	8.4	8.6
54	PI 278008	Turkey	8.4	7.5	8.1	8.0	7.3	8.4	8.6
55	PI 537271	Pakistan	8.4	7.7	8.3	8.3	8.1	8.4	8.5
56	PI 357679	Yugoslavia	8.4	7.8	8.3	9.0	7.5	8.4	8.4
57	PI 176490	Turkey	8.4	7.9	8.6	8.9	8.9	8.4	8.1
58	PI 246559	Senegal	8.4	8.0	8.6	9.0	8.6	8.4	8.4
59	PI 508442	South Korea	8.5	7.7	8.4	9.0	7.8	8.5	8.5
60	PI 277972	Turkey	8.5	7.9	8.5	8.4	8.6	8.5	8.5
61	PI 277989	Turkey	8.5	8.2	8.7	9.0	9.0	8.5	8.5
62	PI 164737	India	8.5	8.4	8.8	9.0	8.6	8.5	9.0
63	PI 525088	Egypt	8.6	7.8	8.5	8.9	8.3	8.6	8.4
64	PI 500309	Zambia	8.6	8.1	8.7	8.8	8.8	8.6	8.6
65	PI 357752	Yugoslavia	8.6	8.2	8.8	9.0	9.0	8.6	8.5
66	PI 278026	Turkey	8.6	8.3	8.7	9.0	8.5	8.6	8.6
67	PI 271986	Somalia	8.7	7.6	8.3	7.1	8.3	8.7	9.0
68	PI 532667	Switzerland	–	7.7	8.4	8.3	8.5	–	–
69	PI 536450	Maldives	–	8.0	8.7	9.0	–	–	8.4
70	PI 278058	Turkey	8.8	8.6	8.8	8.9	8.5	8.8	9.0
71	PI 278005	Turkey	8.9	8.1	8.7	8.8	8.5	8.9	8.5
72	PI 277990	Turkey	9.0	8.8	9.0	–	9.0	9.0	–
73	Charleston Gray	Check, USA	9.0	8.7	8.9	9.0	8.8	9.0	9.0
74	Crimson Sweet	Check, USA	9.0	8.8	9.0	9.0	8.9	9.0	9.0
LSD (5%)			1.4	1.5	1.4				

† Analysis performed for means of each rating date plus the overall averages. Plants were rated on a scale of 1 to 9 on the basis of severity of viral symptoms, where 1 = none, 2 = tendrils absent, 3 = tendrils absent, slightly stunted growth, 4 = mosaic patches and/or necrotic spots on leaves, 5 = leaves near apical meristem deformed, meristem yellow and reduced in size, 6 = apical meristem withered and brown, 7 = apical meristem dead with more basal leaves dying, 8 = most leaves dead, main stem green/yellow, 9 = plant dead. Average rating is the mean of the ratings of all 74 plants. Maximum ratings is the mean of the final rating of all 74 plants per PI accession. Rank indicates the ranking of the cultigen for resistance to PRSV-W, based on average and best rating (as well as average and maximum ratings; data not shown).

that tested negative for virus in ELISA that later tested positive. This is consistent with what we found. Thus, even though there were plants that tested negative for virus with ELISA, all plants eventually showed symptoms of PRSV-W and died. The issue of escapes should not be underestimated. Studies for resistance to WMV and ZYMV found, during retests, that some accessions which were initially rated resistant were actually just escapes. It is likely that some of the accessions reported to be resistant here are escapes.

The susceptible check used for this study was Charleston Gray, a widely available cultivar. However, we identified accessions having more susceptibility to PRSV-W. Those accessions would make excellent susceptible checks because they have high germination rates, and best ratings of 9.0 compared with Charleston Gray, which had a best rating of 8.2 (Table 1). Any of the following accessions could be used as susceptible checks: PI 379253, PI 512383, PI 169270, PI 178877, PI 169244, PI 560016, PI 368495, PI 357727, PI 381696, PI 502318, PI 176485, PI 176921, PI 368497, PI 368515, PI 379245, PI 178873, PI 279461, PI 176490, PI 500309, PI 508442, PI 357690, PI 357735, PI 278027, PI 507859, PI 195928, PI 532817, PI 176499, PI 500332, PI 512358, PI 379246, PI 357675, PI 512391, PI 512359, PI 296337, PI 169283, PI 179661, PI 295850, PI 179662, PI 169252, PI 183399, PI 357728, PI 357692, PI 512407, PI 169258, PI 234603, PI 357698, PI 357736, PI 169245, PI 490382, PI 378613, and PI 296342 (data in GRIN).

The PI accessions with the most resistance along with complete data (missing in no more than one replication) were PI 244017, PI 244018, PI 482342, PI 234287, and PI 482303 (Table 2). The PI accessions that showed resistance to other watermelon viruses in addition to resistance to PRSV-W in this study were PI 244018, PI 244019, PI 482226, PI 595203, PI 255137, and PI 482299.

Retests

The retest of the most resistant 21 PI resistant accessions (along with Charleston Gray check) determined on the basis of data from the first two replications showed that there were some escapes that were not resistant to PRSV-W (Table 3). Of the 462 plants tested, 58 plants representing 15 PI accessions were resistant to PRSV-W. Of those, nine plants from five accessions had a final rating under 9.0. Of the 21 PI accessions, the seven PI accessions having four or more plants out of 21 resistant were PI 164687, PI 244017, PI 244018, PI 482303, PI 482319, and PI 485583. PI 482319 had two plants alive at the time of the final rating in both the early retest and the germplasm screening. One accession, PI 244018, had 14 plants designated resistant, with three plants alive at the final rating. Also, it was reported resistant to WMV (Gillaspie and Wright, 1993). Another accession, PI 482299, had two plants resistant and was reported resistant to ZYMV (Provvidenti, 1991).

Three accessions considered to be resistant in the germplasm screening, PI 482254, PI 482317, and PI 505604, were mostly susceptible in the early retest with only one plant out of 21 per accession that showed resistance. It is likely that these PI accessions were escapes, especially since data for the test came from only the first two replications of the screening study. The remaining six PI accessions with no plants that met the criteria for resistance were probably also escapes (Table 3).

The final retest was conducted at the completion of the germplasm screening. The final retest results paralleled those of the early retest results, in that there were escapes that were not resistant to PRSV-W (Table 4). Of the 60 resistant PI accessions in the final retest, eight had resistance with a rating of 3.6 or less for the best, average and maximum ratings: PI 244019, PI 244017, PI 482342, PI 482318, PI 485583, PI 482379, PI 595203, and PI 244018. PI 244017 was given the highest resistance rating in both the germplasm screening and the final retest experiments. The 12 susceptible PI accessions had about the same level of susceptibility to PRSV-W as the Charleston Gray check.

Since the seed supply of many PI accessions was increased by open pollination or by sib pollination, there should be heterogeneity and heterozygosity within an accession. Thus, if resistance were quantitatively inherited, and accessions were segregating for resistance, the most resistant accession found in this study would not necessarily have the highest level of resistance possible. Resistance might be improved by intercrossing the most resistant accessions, or by crossing accessions with high vs. moderate resistance.

Further research is needed to determine whether other isolates of PRSV-W from different geographic regions react the same on the resistant and susceptible cultigens from this experiment. Additional research is needed to determine the inheritance of resistance to PRSV-W in the cultigens identified. The accessions with the highest resistance to PRSV-W should be used to develop inbreds with the highest possible resistance for use in developing resistant cultivars.

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