392291-VDR, a Watermelon Germplasm Line with Resistance to Squash vein yellowing virus-caused Watermelon Vine Decline

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392291-VDR (vine decline-resistant) is a watermelon [*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai] line with resistance to watermelon vine decline (WVD) caused by the whitefly-transmitted *Squash vein yellowing virus* (SqVYV). The line is derived from the U.S. PI 392291 after successive screening and pure line selection for six generations (S_6) in a greenhouse. The germplasm line 392291-VDR is not completely immune to infection by SqVYV because virus can be detected in the crowns of inoculated plants by tissue blot nucleic acid hybridization. However, 392291-VDR plants infected with SqVYV do not decline and die like plants of ‘Mickey Lee’, ‘Crimson Sweet’, and other seedless commercial watermelon cultivars. In addition, inoculated plants of 392291-VDR produce symptomless mature fruit unlike plants of WVD-susceptible commercial watermelon cultivars. In greenhouse tests (conducted in 2010 and 2011), inoculated plants of 392291-VDR were rated 1 to 2 on a 1 to 9 scale (1 = no symptoms and 9 = plants dead) compared with plants of ‘Crimson Sweet’ and ‘Mickey Lee’ that were all rated

9. Plants of 392291-VDR have a runner growth habit with vine length reaching 6 m. Leaves are lobed (lobate) and 19.3 cm long and 19.3 cm wide at the base. The line is monoecious with almost round fruit (18.4 cm diameter) that weigh ≈ 3.4 kg. Fruit rind pattern is medium-dark green with narrow dark green stripes. The color of fruit rind as determined using a Konica Minolta Chroma Meter (CR-400 with 8-mm aperture and 2° viewing angle) and the CIE $L^*a^*b^*$ color data software (CM-S100w SpectraMagic NX, Version 1.7; Konica Minolta) is medium-dark green with mean color coordinate readings of $L^* = 46.02$, $a^* = -15.3$, and $b^* = 23.5$. The narrow darker green stripes on the rind had mean color coordinate readings of $L^* = 37.8$, $a^* = -11.6$, and $b^* = 15.3$. In Charleston, SC, each plant produces approximately six fruits. Flesh color in mature fruit ranges from pale yellow to dark yellow with mean color coordinate readings of $L^* = 36.8$, $a^* = -1.7$, and $b^* = 15.2$. The average Brix value based on two tests conducted in Charleston, SC, and one in Fort Pierce, FL, was 6.7 (range, 4 to 9). Because commercial watermelon cultivars with resistance to SqVYV-caused WVD are not yet available, 392291-VDR may be a useful source of resistance in watermelon breeding, although its fruits are not commercially acceptable.

Origin

PI 392291 (FAO 35.599, other ID type) was collected from the coastal region in the Kilifi district of Kenya and was placed in the *Citrullus* germplasm collection of the USDA, Plant Genetic Resources Conservation Unit (PGRU), in Griffin, GA, in 1974. PI 392291 is part of the core subset of watermelon working germplasm collection being maintained by PGRU, Germplasm Resources Information Network (GRIN). The original seeds of PI 392291 used to develop 392291-VDR

were obtained from the GRIN station in Griffin, GA. Research conducted from 2006 to 2007 in Florida indicated that PI 392291 was resistant to SqVYV-caused WVD. Initially PI 392291 showed heterogeneity for resistance to SqVYV in greenhouse and field trials and therefore controlled self-pollinations were made to produce a line homogeneous for resistance. Screening, selection, and pollination of the plants that were most resistant to SqVYV were repeated for six successive generations using a pure line selection procedure to produce 392291-VDR. During each cycle, plants were inoculated mechanically as described before (Adkins et al., 2007; Kousik et al., 2009). The virus inoculum of SqVYV used for mechanical inoculations was maintained on squash plants (cv. Prelude-II) as described before (Kousik et al., 2009). Plants of PI 392291 and subsequent selections were inoculated two to four times on weekly intervals to prevent potential escapes.

Disease Resistance

WVD caused by SqVYV (genus: *Ipomovirus*, family: *Potyviridae*) is an emerging disease that has caused devastating losses for watermelon producers in southwest and west-central Florida (Adkins et al., 2007, 2008; Huber, 2006; Roberts et al., 2005). SqVYV is transmitted by whiteflies (*Bemisia tabaci*, biotype B) and can also be mechanically transmitted (Adkins et al., 2007). Monetary losses resulting from WVD in Florida were estimated at \$60 to \$70 million in 2004 (Huber, 2006). Symptoms of WVD typically occur at or just before harvest, when vines collapse rapidly (Adkins et al., 2007, 2011; Roberts et al., 2005). Although externally the fruits appear normal, rind necrosis and flesh degradation are often evident when fruits are cut, affecting fruit marketability (Adkins et al., 2007; Huber, 2006; Roberts et al., 2005). The known host range of SqVYV is limited to cucurbits, including several cucurbit weeds (Adkins et al., 2007, 2008; Webster et al., 2010). SqVYV is now widely distributed in cucurbits in southwest and west-central Florida (Adkins et al., 2007, 2008) and has been detected in Indiana (Egel and Adkins, 2007), Georgia (Webster and Adkins, 2012), and South Carolina (Kousik and Adkins, unpublished data). WVD has become endemic in Florida and has appeared to varying degrees in most seasons since it was first observed.

Management strategies for WVD and SqVYV in Florida include destruction of cucurbit weed and volunteer crop reservoirs of SqVYV and the management of whiteflies by insecticides and use of silver plastic mulch (Adkins et al., 2008, 2011; Kousik et al., 2008; Schuster et al., 2007; Webb et al., 2011). The complete destruction of virus reservoirs is difficult in practice and in recent years there has been a marked increase of insecticide resistant whitefly populations in Florida and other states, particularly to neonicotinoids (Dennehy et al., 2005; Schuster et al., 2006).

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Therefore, our program has worked on developing sources of resistance to SqVYV-caused WVD as a long-term solution. Furthermore, host resistance is considered the cornerstone of any integrated pest management strategy (IPM). The efforts leading to development of 392291-VDR were started in 2006 when we evaluated 218 PIs in the core watermelon collection (Kousik et al., 2009). PI 392291 was one of the most resistant PIs in these evaluations with a mean rating of 3.0 on a 1 to 9 scale compared with ‘Crimson Sweet’, which was rated 9.0 (Kousik et al., 2007). Most of the 218 PIs were highly susceptible to WVD. Further screening of PI 392291 in the field in 2006 and 2007 in Immokalee, FL, confirmed its resistance to WVD. Additional confirmation came from two greenhouse screens conducted in 2007 (Kousik et al., 2009). The disease evaluations for the development of 392291-VDR were done by mechanically inoculating single plant selections using a local Florida isolate of SqVYV (Adkins et al., 2007; Kousik et al., 2009). Because recent studies have indicated that minimal genetic diversity exists among SqVYV isolates collected from cucurbit crops and weeds in Florida, Indiana, and Georgia (Adkins et al., 2011; Webster and Adkins, 2012), it is likely that PI 392291-VDR will be resistant to WVD wherever SqVYV is found. Evaluations and selections of resistant plants from PI 392291 from 2008 to 2011 have shown a progressive improvement in development of the resistant line 392291-VDR (Table 1). The line can be easily crossed with cultivated watermelon to develop breeding populations. Preliminary evaluation of F₁ populations of a cross of 392291-VDR and ‘Mickey Lee’ indicated that the gene(s) controlling resistance to SqVYV are most likely not dominant. Further studies to determine the inheritance of resistance are in progress.

Figure 1 illustrates representative WVD resistance observed in 392291-VDR compared with the susceptible control cultivar Mickey Lee, which has declined. The inoculated leaf in 392291-VDR exhibits symptoms of petiole collapse, but typical symptoms of WVD do not spread throughout the plant as in ‘Mickey Lee’ or other watermelon cultivars such as ‘Crimson Sweet’ and ‘Tri-X 313’. Although inoculated leaves and petiole bearing these leaves generally show symptoms of SqVYV infection and frequently collapse and die, the symptoms do not spread beyond the node bearing the inoculated leaf. The new leaves and growth appear normal and symptomless and the plant produces fruit. Fruit of 392291-VDR also are free of WVD symptoms compared with fruit of ‘Mickey Lee’ or ‘Tri-X 313’ (Fig. 2). Although symptoms of SqVYV infection are absent in new plant growth and fruits, the virus is present and can be detected in symptomless crown tissue of inoculated 392291-VDR plants using tissue blot nucleic acid hybridization techniques (Turechek et al., 2010). Field studies conducted in 2010 and 2011 as a part of larger studies on using 392291-VDR and ‘Mickey Lee’ as pollenizers in seedless watermelon

Table 1. The reaction of PI 392291 and selections² thereof to watermelon vine decline (WVD) caused by *Squash vein yellowing virus* (SqVYV).

Yr PI/cultivar	Plants tested in select group	Mean rating (1–9) ³	Rating range	Plants rated ≤4 (%) ⁴
2008				
PI 392291	11	3.1	1–9	73
Crimson Sweet	13	6.3	1–9	33
2009				
PI 392291	10	2.0	1–7	70
Crimson Sweet	6	9.0	9–9	0
Mickey Lee	10	9.0	9–9	0
2010				
PI 392291	26	2.0	2–2	100
Crimson Sweet	20	9.0	9–9	0
Mickey Lee	20	9.0	9–9	0
2011				
392291-VDR	72	1.0	1–1	100
Mickey Lee	8	9.0	9–9	0
Tri-X-313	12	9.0	9–9	0
2012				
392291-VDR	16	1.0	1–1	100
Crimson Sweet	16	7.0	1–9	25
Mickey Lee	16	7.7	2–9	13
Tri-X-313	16	8.2	2–9	13

²In 2008 a total of 24 plants were tested. In 2009 a total of 90 plants derived from selections were tested. Data from the pure line selection with 10 plants that was fairly homogenous and led to development of 392291-VDR are presented. In 2010, 26 plants were evaluated. In 2011 a total of 204 plants were tested and data from 72 plants for the line being designated as 392291-VDR is presented. In 2012 16 plants designated 392291-VDR were evaluated.

³Individual plants were rated on a 1 to 9 scale where 1 = no symptoms and 9 = plant dead, as described previously by Kousik et al. (2009).

⁴Percentage of plants that were rated 4 or less on the 1 to 9 scale during the development of 392291-VDR.

Fig. 1. Infection by *Squash vein yellowing virus* (SqVYV) does not lead to typical watermelon vine decline symptoms in 392291-VDR. All the plants were mechanically inoculated with a local Florida isolate of SqVYV. The petiole and the inoculated leaf in 392291-VDR decline and collapse (left two plants); however, the rest of the plant continues to grow and produce fruit. In contrast, the entire commercial cultivar Mickey Lee plants decline and die (right two plants).

Fig. 2. Infection by *Squash vein yellowing virus* (SqVYV) does not lead to typical fruit symptoms of watermelon vine decline (WVD) in 392291-VDR. No internal rind discoloration or flesh degradation is visible in the yellow flesh of 392291-VDR fruit (left) in contrast to red flesh commercial cultivar Tri-X 313 fruit (right).

production further confirmed resistance in 392291-VDR to WVD (Kousik et al., 2012). In these studies, the mean adult whitefly counts were not significantly different between ‘Mickey Lee’ and 392291-VDR plots (Kousik et al., 2012). In another field study conducted in 2010, the mean rating for 392291-VDR was 3.8 with all plants rated less than 4 (100%) and significantly ($P = 0.05$) lower than ‘Mickey Lee’ that was rated 7.8 on the 1 to 9 scale with none of the plants rated less than 5. In our selection process, we inoculated the plants of 392291-VDR and the checks multiple times to prevent escapes and to mimic conditions that may be present in the field, that is, numerous viruleferous whiteflies infesting the plants. The plants of 392291-VDR survived multiple inoculations during the selection process, whereas the susceptible checks did not.

WVD has been a serious problem and production-limiting disease in Florida. Managing SqVYV-caused WVD will require an IPM approach using a combination of host resistance, insecticides, and other cultural practices. Therefore, even when resistance from 392291-VDR is incorporated into high-quality watermelon lines, an integrated approach should be used to manage WVD.

Other Diseases

PI 392291 was previously reported to be moderately susceptible to gummy stem blight (*Didymella bryoniae*) and was rated 4.5 on a 0 to 9 scale (Gusmini et al., 2005). It was found susceptible to bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) and was rated 8 on a 1 to 9 scale in experiments conducted by D. Hopkins at the University of Florida (USDA GRIN database, <<http://www.ars-grin.gov>>). In our experiments, 392291-VDR was found to be susceptible to powdery mildew caused by *Podosphaera xanthii* (Kousik et al., unpublished data).

Availability

Small amounts of seeds of 392291-VDR are available for distribution to interested research personnel and plant breeders. Address

all requests to Shaker Kousik, U.S. Vegetable Laboratory, USDA-ARS, 2700 Savannah Highway, Charleston, SC 29414 (e-mail: shaker.kousik@ars.usda.gov). Seeds of 392291-VDR will also be submitted to the National Plant Germplasm System where they will be available for research purposes, including the development and commercialization of new cultivars. It is requested that appropriate recognition of the source be given when this germplasm contributes to research or development of a new breeding line or cultivar.

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