Watermelon Germplasm Lines USVL608-PMR, USVL255-PMR, USVL313-PMR, and USVL585-PMR with Broad Resistance to Powdery Mildew

Chandrasekar S. Kousik¹ and Jennifer Ikerd

United States Department of Agriculture-Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC 29414

Mihir Mandal

ORISE participant sponsored by United States Department of Agriculture-Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC 29414

Scott Adkins and William W. Turechek

United States Department of Agriculture-Agricultural Research Service, U.S. Horticultural Research Laboratory, Fort Pierce, FL 34945

USVL608-powdery mildew resistant (PMR), USVL255-PMR, USVL313-PMR, and USVL585-PMR are watermelon [*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai] germplasm lines that exhibit high levels of resistance to powdery mildew (PM) incited by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (syn. *Sphaerotheca fuliginea*). Specifically, the hypocotyls, cotyledons, and true leaves of these four PMR lines are highly resistant to PM compared with the susceptible watermelon line USVL677-PMS (powdery mildew susceptible) or cultivar Mickey Lee on which severe PM and abundant development of conidia can be observed. The true leaves of these four PMR lines were also resistant to *P. xanthii* isolates from other states, including California, Florida, Georgia, New York, and South Carolina. Each of these four PMR lines are uniform for various growth characteristics, including fruit size, shape and color with red to pink flesh, and brix content

ranging from 6 to 8. Currently, commercial watermelon cultivars with PM resistance are rare. Hence, USVL608-PMR, USVL255-PMR, USVL313-PMR, and USVL585-PMR will be useful sources for incorporating resistance in commercially acceptable cultivars. These lines can easily be crossed with commercial watermelon cultivars to develop new breeding populations.

Origin

USVL608-PMR, USVL255-PMR, USVL313-PMR, and USVL585-PMR were derived from PI 307608, PI 482255, PI 482313, and PI 505585, respectively, which were obtained from the United States PI collection of watermelon maintained at the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Plant Genetic Resources and Conservation Unit (PGRU), in Griffin, GA. PI 307608 was originally collected from Nigeria, PI 482255 and PI 482313 were collected from Zimbabwe, and PI 505585 was collected from Zambia. Details of the individual PI used to develop these resistant germplasm lines can be obtained from the U.S. National Germplasm System website <https://npgsweb.ars-grin.gov/gringlobal/search.aspx>.

Disease Resistance

Powdery mildew of watermelon incited by *P. xanthii* is a major disease that can lead to significant yield reduction (Keinath, 2015; Keinath and DuBose, 2004; Zhang et al., 2011). The pathogen is known to infect the hypocotyl, cotyledons, true leaves, and fruit of watermelon (Ben-Naim and Cohen, 2015; Davis et al., 2007; Jahn et al., 2002; Keinath and DuBose, 2004; Kousik et al., 2011, 2018;

McGrath, 2017). Powdery mildew on watermelon has occurred with increasing frequency over the past decade (Davis et al., 2001; Keinath, 2015; Keinath and Rennberger, 2017; Kousik et al., 2011; Mercier et al., 2014). As a result, the Watermelon Research and Development Group listed PM as an important research priority (Kousik et al., 2016). Fungicides are used extensively to manage PM of watermelon, and many are effective and available to growers (Keinath, 2015; Keinath and DuBose, 2004; Keinath and Rennberger, 2017; McGrath, 2017). However, excessive use of fungicides is not economical and environmentally favorable. Therefore, alternative management strategies are needed. Host resistance offers that opportunity, but as stated previously, very few commercial watermelon varieties and pollenizers with resistance to PM are available (Kousik and Ikerd, 2016). Resistance has been identified in some watermelon PIs (Davis et al., 2007; Tetteh et al., 2010; Thomas et al., 2005), some of which have been used to develop red-fleshed breeding lines (Ben-Naim and Cohen, 2015). However, the presence of *P. xanthii* races across the United States and elsewhere complicates breeding efforts and creates a constant need for additional germplasm resources (Cohen et al., 2004; Jahn et al., 2002; Lebeda et al., 2011, 2016; McCreight, 2006; McGrath, 2017; Mercier et al., 2014; Pitrat et al., 1998).

Seven pathologically distinct *P. xanthii* races have been described based on melon (*Cucumis melo*) differentials, and extensive details of the race classification scheme have been described by various researchers (Cohen et al., 2004; Jahn et al., 2002; McGrath, 2017; Pitrat et al., 1998). Similarly, several other race classification schemes for melon have also been put forth (Lebeda et al., 2011, 2016; McCreight, 2006), including the possibility for existence of races based on watermelon (Kousik and Ikerd, 2014) and bitter melon (Dhillon et al., 2018). Distinct races of *P. xanthii* have not been classified for other cucurbit crops except melon because of the lack of highly resistant germplasm and the need for well-characterized differentials (Cohen et al., 2004).

We selected watermelon PIs that belonged to *C. lanatus* with red to pink flesh to develop PMR germplasm lines for use in breeding programs based on previous research (Davis et al., 2007; Tetteh et al., 2010). Individual plants of PI 307608, PI 482255, PI 482313, and PI 505585 exhibited varying levels of resistance in the original screen for PMR lines conducted in 2011 at the USDA, ARS, U.S. Vegetable Laboratory in Charleston, SC. Based on these findings, we used a pure line selection procedure to develop uniformly resistant lines for use in breeding programs. Similar heterogeneity in resistance among individual plants within each of these PIs from USDA GRIN (Germplasm Resources Information Network) for reaction to PM has also been noted by other researchers (Davis et al., 2007; Tetteh et al., 2010; Thomas et al.,

Received for publication 13 Feb. 2018. Accepted for publication 29 May 2018.

Financial support was provided in part by USDA NIFA SCRI CuCAP grant award 2015-51181-24285. We acknowledge the technical assistance of Jerry Johnson, Stephen Mayo, Carrie Vanderspool, and the numerous student workers over the years in conducting the greenhouse and field experiments. We appreciate the critical review of this manuscript by Phillip Wadl and Daniel Hasegawa before submission.

The use of trade, firm, or corporation names in this publication is for the convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agriculture Research Service of any product or service to the exclusion of others that may also be suitable.

¹Corresponding author. E-mail: shaker.kousik@ars.usda.gov.

2005). Heterogeneity in phenotypic reaction among plants within a given PI has also been noted for other diseases of watermelon (Guner et al., 2002; Kousik et al., 2009; Strange et al., 2002). This necessitates the need for developing individual lines with uniform levels of resistance by screening and selection for several generations for use in downstream studies and research. To develop resistant lines, 16 seedlings of each of the four PI were grown in a greenhouse in 6.4-cm square pots for four weeks and then inoculated by spraying 10^5 conidia/mL and rated for resistance to PM on a 0 to 10 scale described before (Kousik et al., 2018). We evaluated and selected plants expressing resistance to PM in hypocotyls, cotyledons, and true leaves to advance to the next generation. Resistant seedlings (rated ≤ 2 on the 0 to 10 scale) were transplanted into 11.4-L pots after phenotyping. The plants were allowed to grow in a greenhouse and were manually self-pollinated. Seeds harvested from each of the resistant plants were grown and screened in the next generation for resistance to PM. Sixteen seedlings were evaluated from each selection for each generation and the most resistant seedlings were advanced to the next generation. This process was continued for six successive generations to develop S_6 lines from which a single selection was designated as USVL608-PMR (PM resistant), USVL313-PMR, USVL255-PMR, and USVL585-PMR. During each screen, commercial susceptible cultivars Dixie Lee or Mickey Lee and a highly susceptible line USVL677-PMS (derived from PI 269677) were included as susceptible controls for PM resistance phenotyping. Very sparse to no development of PM was observed on the four PMR lines compared with USVL677-PMS on which severe PM with abundant conidia on the hypocotyls, cotyledons, and true leaves was observed during the selection process.

Field trials

Two field trials were conducted at the USDA, ARS, U.S. Vegetable Laboratory research farm in Charleston, SC, during Summer and Fall 2017 to confirm resistance in the USVL-PMR S_6 lines. Four-week-old transplants of the four PMR (USVL608-PMR, USVL255-PMR, USVL313-PMR, and USVL585-PMR) lines and the susceptible checks USVL677-PMS and 'Mickey Lee' were transplanted onto 91-cm-wide raised beds spaced 4.6 m apart and covered with white plastic mulch. A single drip tape placed ≈ 2.5 cm below the top of the beds and under the plastic was used to irrigate the plants on a weekly basis. A randomized complete block design with four replications for each entry or susceptible check was used to evaluate resistance to PM. Each PMR germplasm line or susceptible check plot was a single row of five plants spaced 46 cm apart. Spacing between plots was 2.7 m. Watermelon vines were turned once every week to prevent the plants from growing into neighboring plots. The row middles were

sprayed with Roundup Pro ($1.17 \text{ L}\cdot\text{ha}^{-1}$) and Dual Magnum ($1.17 \text{ L}\cdot\text{ha}^{-1}$) for weed management. During the season, weeds between beds were managed with two spot applications of Roundup and hand weeding.

Powdery mildew occurs naturally in the fields in Charleston, SC, and, therefore, plants in the field were not inoculated. Plant foliage for each plot was rated for PM using a 0 to 10 rating scale (Kousik et al., 2018) similar to the Horsfall and Barratt rating scale of increasing disease severity, where 0 = no visible PM, 1 = very sparse mycelial growth on leaves or cotyledons with very few to no visible conidia (1% to 3%), 2 = 4% to 6% of leaf area covered with PM and sparse development of conidia, 3 = 7% to 12%, 4 = 13% to 25%, 5 = 26% to 50%, 6 = 51% to 75%, 7 = 76% to 87% leaf area covered with PM and presence of abundant conidia, 8 = 88% to 94%, 9 = 95% to 97%, and 10 = 98% to 100% of leaf area covered with abundant conidia and leaf dying or dead. During each rating period in summer and fall, disease severity was recorded on lower leaves in the canopy. At least five lower leaves for each plot were observed to provide one rating for each plot. Three weekly ratings were made in the summer on 26 June, 6 July, and 10 July 2017. During the fall season, disease development was relatively slow and eight weekly

ratings from 22 Sept. to 7 Nov. were recorded. The ratings were converted to the mid percentage points for analysis. Data were analyzed using the PROC GLIMMIX procedure of SAS. Area under disease progress curves (AUDPC) was calculated for each plot using the disease severity data (Madden et al., 2007) and means were separated using the PDIF option ($\alpha = 0.05$). The disease severity percentage values for individual rating on 6 July during the summer and 7 Nov. during the fall season were arcsin transformed for analysis and means were separated using the PDIF option ($\alpha = 0.05$).

The four PMR lines were highly resistant to the local prevailing isolate of *P. xanthii* compared with USVL677-PMS and 'Mickey Lee' based on AUDPC over the summer and the fall seasons (Table 1). When PM pressure was high, the four PMR lines had $<2\%$ infection on their lower canopy leaves compared with $>70\%$ on USVL677-PMS and 'Mickey Lee' with the latter showing abundant PM conidia during the summer trial. Similar results were observed in the fall trial; however, the disease levels on 'Mickey Lee' were not as high as in the summer (Table 1).

Greenhouse trial

Two greenhouse trials were conducted to confirm resistance in hypocotyls, cotyledons,

Table 1. Powdery mildew [*PM (Podosphaera xanthii)*] severity on four powdery mildew-resistant (PMR) watermelon (*Citrullus lanatus* var. *lanatus*) germplasm lines in Summer and Fall field trials in 2017 at Charleston, SC.

Germplasm line	Summer trial AUDPC ^a	PM severity (%) ^b 7 June 2017	Fall trial AUDPC	PM severity (%) ^b 11 July 2017
USVL677-PMS	1,146 a ^x	82 a	1,433 a	60 a
Mickey Lee	931 b	75 a	598 b	28 b
USVL255-PMR	28 c	2 b	9 c	0 c
USVL313-PMR	28 c	2 b	0 c	0 c
USVL585-PMR	21 c	2 b	1 c	0 c
USVL608-PMR	21 c	2 b	14 c	0 c

^aAreas under disease progress curves (AUDPC) were calculated as described before (Madden et al., 2007). AUDPC for the summer trial is based on three weekly disease severity ratings on a 0 to 10 scale. For the fall trial, AUDPC is based on eight weekly disease ratings.

^bDisease severity was rated on a 0 to 10 scale of increasing disease severity, where 0 = no visible PM; 1 = very sparse mycelial growth on leaves or cotyledons with very few to no visible conidia (1% to 3%), 2 = 4% to 6%, 3 = 7% to 12%, 4 = 13% to 25%, 5 = 26% to 50%, 6 = 51% to 75%, 7 = 76% to 87%, 8 = 88% to 94%, 9 = 95% to 97%, and 10 = 98% to 100% of leaf or cotyledon area covered with abundant conidia and leaf dying or dead.

^xMeans followed by the same alphabet within a column are not significantly different ($P = 0.05$).

Table 2. Powdery mildew [*PM (Podosphaera xanthii)*] severity on four powdery mildew-resistant (PMR) watermelon (*Citrullus lanatus* var. *lanatus*) germplasm lines in two inoculated greenhouse trials in Charleston, SC.

USVL line	Powdery mildew severity (%) ^a Expt. I			Powdery mildew severity (%) ^a Expt. II		
	Hypocotyl	Cotyledon	True leaf	Hypocotyl	Cotyledon	True leaf
USVL677-PMS	93 a ^y	54 a	91 a	93 a	95 a	92 a
Mickey Lee	76 b	44 a	39 b	80 b	91 b	66 b
USVL255-PMR	3 c	0 b	1 c	10 c	1 c	2 c
USVL313-PMR	1 c	0 b	2 c	1 d	0 c	1 c
USVL585-PMR	2 c	0 b	1 c	7 c	0 c	1 c
USVL608-PMR	4 c	0 b	1 c	11 c	1 c	1 c

^aWatermelon seedlings were inoculated with a suspension of *P. xanthii* conidia (10^5 conidia/mL) when the first true leaf was fully expanded. Disease severity was rated on a 0 to 10 scale of increasing disease severity, where 0 = no visible PM, 1 = very sparse mycelial growth on leaves or cotyledons with very few to no visible conidia (1% to 3%), 2 = 4% to 6%, 3 = 7% to 12%, 4 = 13% to 25%, 5 = 26% to 50%, 6 = 51% to 75%, 7 = 76% to 87%, 8 = 88% to 94%, 9 = 95% to 97%, and 10 = 98% to 100% of leaf or cotyledon area covered with abundant conidia and leaf dying or dead (Kousik et al., 2018).

^yMeans followed by the same alphabet within a column are not significantly different ($P = 0.05$).

and true leaves on seedlings of the four PMR lines. Seedlings were grown in 7.6-cm square pots (3" Kord TRAD SQ Green; Kord Products, Toronto, Canada) filled with metro mix (Sun Gro Horticulture, Bellvue, WA). A randomized complete block design with four replications for each entry or susceptible check was used. Each replication had four seedlings per entry. Three-week-old seedlings were spray-inoculated with a local isolate of *P. xanthii* collected from watermelon plants in the greenhouse. Details of the local isolate called B108ML have been described previously (Kousik et al., 2018). The local *P. xanthii* isolate B108ML was routinely maintained in a growth chamber ($22 \pm 1^\circ\text{C}$) on "Early Prolific Straightneck (EPSN)" squash plants. For inoculation, seedlings were sprayed with a conidial suspension (10^5 conidia/mL in 0.02% tween 20) as described before (Kousik et al., 2011, 2018). Disease severity was recorded on the same 0 to 10 rating scale as described previously on hypocotyl, cotyledon, and true leaves. Disease severity rating data were converted to midpoint percentages and analyzed using the PROC GLIMMIX procedure of SAS. Mean separation was performed using the PDIF option ($\alpha = 0.05$).

In both greenhouse trials, the hypocotyls, cotyledons, and true leaves of the four USVL-PMR lines had significantly ($P \leq 0.05$) less PM than USVL677-PMS and 'Mickey Lee' (Table 2; Fig. 1). In both trials, the cotyledons and true leaves of the four PMR lines had less than 2% of the leaf area infected and no development of conidia was observed (Fig. 2). Conidia were sparse ($\leq 3\%$) on the hypocotyls of the four PMR lines compared with USVL677-PMS and 'Mickey Lee' in the first experiment. In the second experiment, more conidia was observed on hypocotyls of some of the PMR lines ($\leq 11\%$), but it was significantly less ($P \leq 0.0001$) than on the susceptible checks USVL677-PMS (93%) and 'Mickey Lee' (80%). The cotyledons and true leaves of

the PMR lines were highly resistant in the second experiment compared with USVL677-PMS and 'Mickey Lee' (Table 2).

Petri dish trials with PM isolates from other states

Resistance of the four PMR lines were evaluated against isolates of *P. xanthii* collected from different states, including Florida (isolates: GC1 and TRIXFRT), Georgia (WMGA), South Carolina (B108ML), New York (WMNY), and California (CADWM). Except for GC1 which was isolated from squash plants in Boynton Beach, FL, all the other isolates were collected from watermelon. The isolates were collected between the years 2008–17. The isolates were routinely maintained on EPSN squash cotyledons placed in 10-cm petri dish with blue blotter paper using modification of methods described by Bardin et al. (2007) as described in the following paragraphs. For maintenance of PM isolates, water agar plates ($8\text{ g}\cdot\text{L}^{-1}$) amended with sucrose ($6.8\text{ g}\cdot\text{L}^{-1}$), mannitol ($18.2\text{ g}\cdot\text{L}^{-1}$), and pimarin (400 $\mu\text{L}\cdot\text{L}^{-1}$ of 2.5% aqueous solution) were prepared. The agar surface was covered with a round sterile blue germination blotter paper (Anchor Paper, St. Paul, MN) with holes punched on its edges to insert the cotyledon petiole into the water agar (Fig. 3). The blue blotter paper on top of the water agar prevented excessive development of contaminating fungi and also prevented the leaf from sticking to the agar. Fully expanded cotyledons of EPSN squash plants were excised and the petiole was inserted into the water agar. The isolate was transferred onto the surface of the cotyledon using a sterile disposable plastic inoculating needle (1 μL). The plates were maintained in a growth chamber at $22 \pm 1^\circ\text{C}$. Generally abundant PM development with conidia was observed on EPSN cotyledons ≈ 10 –12 d after transfer (Fig. 3).

Plants of the four PMR lines and the susceptible check USVL677-PMS were grown in 7.6-cm square pots filled with Metro Mix in a PM-free room at room temperature ($25 \pm 2^\circ\text{C}$) under grow lights (12 h light/12 h dark, light intensity $120\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Larger water agar plates (14.5 cm) covered with blue blotter paper with holes punched on the edges were used for conducting these experiments.

Fully expanded true leaves were excised and the petioles were inserted into the water agar through the hole in the blotter paper as described previously. One true leaf from each of the four PMR lines and the susceptible check USVL677-PMS were placed in each disposable petri dish.

The true leaves from the four PMR lines and the susceptible check were inoculated as described in the following paragraphs. The EPSN squash cotyledons with abundant conidia from each isolate were placed in small 50-mL mist spray bottles (U.S. Plastic Corporation, Lima, OH) and 10 mL of water with 0.02% Tween 20 was added. The spray bottle was vortexed to dislodge the conidia and create a conidial suspension. The concentration of the conidial suspension was determined and a concentration of 10^5 conidia/mL was generally considered suitable and used for inoculation. The leaves in the petri dishes were inoculated by spraying $\approx 500\text{ }\mu\text{L}$ of the conidial suspension per plate. The petri dishes were then placed under fluorescent lights (12 h light/12 h dark) on wire shelves in the laboratory at room temperature ($25 \pm 2^\circ\text{C}$). Each PMR line and the susceptible check USVL677-PMS had four replications and the experiment was repeated three times. A randomized complete block design was used to arrange the plates on the shelves. Because the variances were similar, the data from the three experiments were combined and analyzed. Data on PM severity on each of the true leaves were recorded on the same 0 to 10 disease severity scale as described previously. Disease severity rating data were converted to midpoint percentages and analyzed using the PROC GLIMMIX procedure of SAS and means were separated using the PDIF option ($\alpha = 0.05$).

Severe PM with abundant development of conidia was observed on the true leaves of USVL677-PMS (Fig. 4) in all the experiments inoculated with the isolates GC1 (FL), B108ML (SC), WMNY (NY), CADWM (CA), and WMGA (GA). The isolate collected from watermelon fruit (TRIXFRT) was not as aggressive as the other five isolates (Table 3). The true leaves of all the four PMR lines had significantly less to no PM, confirming their broad resistance to *P. xanthii* isolates from different states (Table 3; Fig. 4).

Fig. 1. Powdery mildew-resistant (PMR) watermelon (*Citrullus lanatus* var. *lanatus*) germplasm line USVL608-PMR (on left) compared with susceptible line USVL677-PMS (right) in a greenhouse evaluation. The seedlings were inoculated with a local isolate (B108ML) of *Podosphaera xanthii* collected in Charleston, SC. Notice abundant *P. xanthii* conidia on USVL677-PMS compared with none on USVL608-PMR.

Fig. 2. Comparison of cotyledons of powdery mildew-resistant (PMR) watermelon (*Citrullus lanatus* var. *lanatus*) germplasm lines with susceptible line USVL677-PMS in a greenhouse trial. Seedlings were inoculated with a local isolate of *Podosphaera xanthii* (B108ML). Notice abundant *P. xanthii* conidia on cotyledon of USVL677-PMS (A) compared with the four PMR lines (B–E). (A) USVL677-PMS, (B) USVL608-PMR, (C) USVL255-PMR, (D) USVL313-PMR, and (E) USVL585-PMR.

In previous studies conducted by Ben-Naim and Cohen (2015) in Israel, PI 307608 from which we developed USVL608-PMR was considered as susceptible to PM. However, in trials conducted by Tetteh et al. (2010) in North Carolina, PI 307608 was considered resistant compared with the susceptible check PI 269677 and other commercial varieties. In our initial trials, plants within PI 307608 were

susceptible and, hence, we selected and developed a resistant source after six generations of phenotyping and selections. Further work is advised to evaluate these PMR lines in other countries and additional states within the United States to determine if the resistance is durable.

The PM pathogen, *P. xanthii*, has been known to evolve rapidly and develop resistance to fungicides and resistant hosts (McGrath, 2010). In addition, there exists the potential for *P. xanthii* races based on watermelon (Kousik and Ikerd, 2014). Therefore, even when commercial watermelon varieties with resistance to PM are developed from these or other germplasm sources, it will be prudent to use an integrated disease management strategy, including the use of fungicides to manage PM.

The four PI were selected for development of the PMR lines because of their pink to red flesh (Fig. 5) and the ability to easily cross with cultivated-type watermelon. Although these germplasm lines are red-pink flesh colored, they will not be accepted in the market because of their relatively low sugar content (6–8 °Brix). However, they will be useful sources for incorporating resistance into commercial cultivars. We are currently evaluating the genetics of resistance for each of these lines. Based on our preliminary experiments, resistance in USVL608-PMR is a dominant trait, and this line would be an appropriate candidate to transfer resistance

into commercial cultivars because of is uniformly red flesh (Fig. 5), decent °Brix, and high levels of resistance to PM. Details of each of these PMR lines are provided in the following paragraphs.

Characteristics of USVL608-PMR. USVL608-PMR was derived from PI 307608, which was originally collected in Nigeria sometime before 1965 and presented by the Botanist General, Ahmadu Bello University, Zaria, to the Agricultural Attache at the American Embassy in Lagos. It was deposited with PGRCU in Sept. 1965 (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1229921>). Plants of USVL608-PMR have a runner growth habit with lobed leaves. The line is monoecious with each plant producing one to two (mean 1.9) large oblong fruit (25.5 × 22.9 cm). Fruit generally weighs ≈6.9 kg (range 3.76–11.22 kg) based on field tests conducted at the U.S. Horticultural Research Laboratory (USHRL) farm in Fort Pierce, FL. Fruit rind color is solid dark green. The color of the 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 Americas, Ramsey, NJ) is dark green, with mean color coordinate readings of $L^* = 27.95$, $a^* = -5.81$, and $b^* = 7.26$. Fruit flesh is red colored with mean color coordinate readings of $L^* = 39.11$, $a^* = 23.34$, and $b^* = 16.46$. The seeds are generally black in color. The average brix value for flesh from the center of mature fruit is 7.7 °Brix and ranges from 5.9 to 9.3 °Brix. Plants of USVL608-PMR can easily be crossed with commercial cultivated-type watermelon to produce F₁ and F₂ seeds for breeding purposes.

Characteristics of USVL255-PMR. USVL255-PMR was derived from PI 482255 which was originally collected in 1982 from Bikita Do, Bikita district, Zimbabwe. It was deposited with PGRCU in 1983. According to PGRCU, PI 482255 is variable for rind pattern, fruit shape, and flesh color (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1377191>). In addition, in our original screen, it was variable for resistance to PM and, hence, we developed a uniformly resistant line for use in breeding programs. Plants of USVL255-PMR have a runner growth habit with lobed leaves with each plant producing two to three (mean 2.85) oblong fruit (21.1 × 17.8 cm). Fruit generally weigh ≈3.5 kg (range 2.48–4.52 kg) based on field tests conducted at the USHRL farm in Fort Pierce, FL. Fruit have light green-colored rind with very thin indistinguishable stripes. The mean color coordinate readings for the fruit rind is $L^* = 58.13$, $a^* = -15.28$, and $b^* = 30.36$. Fruit flesh is light pink in color with mean color coordinate readings of $L^* = 55.59$, $a^* = 9.17$, and $b^* = 8.44$. The average brix value for flesh from center of mature fruit is 6.6 °Brix and ranges from 4.4 to 7.9 °Brix. USVL255-PMR can easily be crossed with commercial cultivated-type watermelons to produce F₁ and F₂ seeds for breeding purposes.

Fig. 3. Maintenance of isolates of the causal agent of powdery mildew, *Podosphaera xanthii* on cotyledons of “Early Prolific Straightneck” (EPSN) squash (*Cucurbita pepo*) in a petri dish with water agar covered with a blue blotter paper. Notice abundant development of *P. xanthii* conidia on cotyledon of EPSN. Each isolate used in the study was maintained similarly in individual petri dishes.

Fig. 4. Reaction of powdery mildew-resistant (PMR) lines to *Podosphaera xanthii* isolates, panel 1 from California (CADWM) and Panel 2 from Florida (GC1). All the true leaves of the four USVL-PMR lines were resistant to isolates from different states in petri dish assays. Notice abundant *P. xanthii* conidia on the true leaves of USVL677-PMS (A) compared with the four PMR lines (B–E). (A) USVL677-PMS, (B) USVL608-PMR, (C) USVL585-PMR, (D) USVL313-PMR, and (E) USVL255-PMR.

Characteristics of USVL313-PMR. USVL313-PMR was derived from PI 482313 which was originally collected in Zimbabwe in 1982 near Man Yoni BC, at an elevation of 1100 m (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1377249>). It was deposited with PGRCU in 1983. The original PI 482313 was highly variable for resistance to PM, rind pattern, and flesh color. Hence, we developed a uniformly

resistant line for use in breeding programs. Plants of USVL313-PMR have a runner growth habit with lobed leaves with each plant producing two to four (mean 3.3) large oblong fruit (22.1 × 17.4 cm). Fruit generally weigh ≈3.5 kg (range 2.36–4.66 kg) based on field tests conducted at the USHRL farm in Fort Pierce, FL. Fruit are light green with dark green–striped rinds. The mean color coordinate readings for the light green areas of the fruit are $L^* = 67.74$, $a^* = -13.99$, and $b^* = 28.54$. The mean color coordinates for the darker green stripes are $L^* = 36.53$, $a^* = -10.37$, and $b^* = 13.98$. Fruit flesh is pink to red colored with mean color coordinate readings of $L^* = 62.195$, $a^* = 11.32$, and $b^* = 12.70$. The seeds are black in color. The average brix value for flesh from the center of mature fruit is 7.1 °Brix and ranges from 5.1 to 8.5 °Brix. USVL313-PMR can easily be crossed with commercial cultivated-type watermelons to produce F₁ and F₂ seeds for breeding purposes.

Characteristics of USVL585-PMR. USVL585-PMR was derived from PI 505585, which was originally collected in 1984 from Kampemba village, 9 km south of Mwinilunga to Kamapanda, North-West Province in

Table 3. Powdery mildew [PM (*Podosphaera xanthii*)] severity on one susceptible and four powdery mildew-resistant (PMR) watermelon (*Citrullus lanatus* var. *lanatus*) germplasm lines in three petri dish experiments inoculated with isolates collected from different states.

Isolate→	Powdery mildew severity (%) ²					
	GC1	B108ML	WMNY	CADWM	WMGA	TRIXFT
Germplasm line/state	FL	SC	NY	CA	GA	FL
USVL677-PMS	90 a ^y	88 ab	82 ab	78 b	62 c	30 d
USVL255-PMR	0	0	0	1	0	0
USVL313-PMR	0	0	1	3	1	0
USVL585-PMR	0	0	8	2	0	0
USVL608-PMR	0	0	1	1	1	0

²True leaves collected from each of the four germplasm lines were spray-inoculated with a suspension of *P. xanthii* conidia (10⁵ conidia/mL). Disease severity was rated on a 0 to 10 scale of increasing disease severity, where 0 = no visible PM, 1 = very sparse mycelial growth on leaves or cotyledons with very few to no visible conidia (1% to 3%), 2 = 4% to 6%, 3 = 7% to 12%, 4 = 13% to 25%, 5 = 26% to 50%, 6 = 51% to 75%, 7 = 76% to 87%, 8 = 88% to 94%, 9 = 95% to 97%, and 10 = 98% to 100% of leaf or cotyledon area covered with abundant conidia and leaf dying or dead (Kousik et al., 2018). The data presented are mean of three trials as the variances were homogeneous between the trials.

³Means followed by the same alphabet throughout the table are not significantly different ($P = 0.05$). Comparisons of disease severity on USVL677-PMS by different isolates can be made across the row. All the resistant lines had significantly lower to no disease compared with USVL677-PMS for all the isolates and would be followed by the alphabet e.

Fig. 5. Fruit characteristics of USVL developed powdery mildew-resistant (PMR) watermelon germplasm lines USVL608-PMR, USVL255-PMR, USVL313-PMR, and USVL585-PMR. These four PMR germplasm lines can be easily crossed with other watermelon lines to develop populations for breeding superior quality watermelon varieties with resistance.

Zambia and was considered as a primitive cultivar during the time of collection (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1400521>). It was deposited with PGRCU in 1985. Plants of USVL585-PMR have a runner growth habit with lobed leaves with each plant producing two to four (mean 3.15) oblong fruit (23.9 × 18.7 cm). Each fruit generally weighs ≈4.3 kg (range 2.8–5.2 kg) in field tests conducted at Fort Pierce, FL. Fruit have light green-colored rind. The mean color coordinate readings for the fruit rind is $L^* = 58.05$, $a^* = -114.11$, and $b^* = 28.81$. Fruit flesh is light pink in color with mean color coordinate readings of $L^* = 61.4$, $a^* = 9.37$, and $b^* = 8.64$. The seeds are black in color. The average brix value for flesh from the center of mature fruit was 6.1 °Brix and ranged from 4.7 to 7.7 °Brix. USVL585-PMR can easily be crossed with commercial cultivated-type watermelon to produce F₁ and F₂ seeds for breeding purposes.

Availability

Small amounts of seeds (≈25) of USVL608-PMR, USVL313-PMR, USVL255-PMR, and USVL585-PMR 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 the four PMR lines 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.

Literature Cited

- Bardin, M., M.E. Suliman, A-M. Sage-Palloix, and Y.F. Mohamed. Nicot, P.C. 2007. Inoculum production and long term conservation methods for cucurbits and tomato powdery mildews. *Mycol. Res.* 111:740–747.
- Ben-Naim, Y. and Y. Cohen. 2015. Inheritance of resistance to powdery mildew race 1W in watermelon. *Phytopathology* 105:1446–1457.
- Cohen, R., Y. Burger, and N. Katzir. 2004. Monitoring physiological races of *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), the causal agent of powdery mildew in cucurbits: Factors affecting race identification and the importance for research and commerce. *Phytoparasitica* 32:174–183.
- Davis, A.R., B.D. Bruton, S.D. Pair, and C.E. Thomas. 2001. Powdery mildew: An emerging disease of watermelon in the United States. *Cucurbit Genet. Coop.* 24:42–48.
- Davis, A.R., A. Levi, A. Tetteh, T.C. Wehner, V. Russo, and M. Pitrat. 2007. Evaluation of watermelon and related species for resistance to race 1W powdery mildew. *J. Amer. Soc. Hort. Sci.* 132:790–795.
- Dhillon, N.P.S., S. Sanguansil, S. Srimat, R. Schafleinter, B. Manjunath, P. Agarwal, Q. Xiang, M.A.T. Masud, T. Myint, N.T. Hanh, T.K. Cuong, C.H. Balatero, V. Salutam-Bautista, M. Pitrat, A. Lebeda, and J.D. McCright. 2018. Cucurbit powdery mildew-resistant bitter gourd breeding lines reveal four races of *Podosphaera xanthii* in Asia. *HortScience* 53:337.
- Guner, N., B.E. Strange, T.C. Wehner, and Z. Pesic-VanEsbroeck. 2002. Methods for screening watermelon for resistance to *Papaya ringspot virus* type-W. *Scientia Hort.* 94:297–307.
- Jahn, M., H.M. Munger, and J.D. McCright. 2002. Breeding cucurbit crops for powdery mildew resistance, p. 239–248. In: R.R. Belanger, W.R. Bushnell, A.J. Dik, and T.L.W. Carver (eds.). A comprehensive treatise, Chapter 15: The powdery mildews. APS Press, St. Paul, MN.
- Keinath, A.P. 2015. Efficacy of fungicides against powdery mildew on watermelon caused by *Podosphaera xanthii*. *Crop Protection* 75:70–76.
- Keinath, A.P. and V.B. DuBose. 2004. Evaluation of fungicides for prevention and management of powdery mildew on watermelon. *Crop Protection* 23:35–42.
- Keinath, A.P. and G. Rennberger. 2017. Powdery mildew on watermelon. *Clemson Coop. Ext. Publ. Fact Sheet. Hort.* 02. 12 Sept. 2017. <<https://www.clemson.edu/extension/publications/files/horticulture/HOR02%20Powdery%20Mildew%20on%20Watermelon.pdf>>.
- Kousik, C.S., S. Adkins, W.W. Turechek, and P.D. Roberts. 2009. Sources of resistance in U.S. plant introductions (PI) to watermelon vine decline caused by *Squash vein yellowing virus*. *HortScience* 44:256–262.
- Kousik, C.S., J. Brusca, and W.W. Turechek. 2016. Diseases and disease management strategies take top research priority in the watermelon research and development group members survey (2014–15). *Plant Health Prog.* 17:53–58.
- Kousik, C.S., R.S. Donahoo, C.G. Webster, W.W. Turechek, S.T. Adkins, and P.D. Roberts. 2011. Outbreak of cucurbit powdery mildew on watermelon fruit caused by *Podosphaera xanthii* in southwest Florida. *Plant Dis.* 95:1586.
- Kousik, C.S. and J.L. Ikerd. 2014. Evidence for cucurbit powdery mildew pathogen races based on watermelon differentials, p. 32–34. In: M. Havey, Y. Weng, B. Day, and R. Grumet (eds.). *Proceedings of cucurbitaceae 2014*. Amer. Soc. Hort. Sci., Alexandria, VA.
- Kousik, C.S. and J.L. Ikerd. 2016. Evaluation of watermelon varieties for tolerance to powdery mildew and *Phytophthora* fruit rot, 2014. *Plant Dis. Mgt. Rpt.* 10:V082.
- Kousik, C.S., M.K. Mandal, and R. Hassell. 2018. Powdery mildew resistant rootstocks that impart tolerance to grafted susceptible watermelon scion seedlings. *Plant Disease. First Look paper*. <<https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-09-17-1384-RE>>.
- Lebeda, A., E. Kristkova, and B. Sedlakova. 2011. Gaps and perspectives of pathotype and race determination in *Golovinomyces cichoracearum* and *Podosphaera xanthii*. *Mycoscience* 52:159–164.
- Lebeda, A., E. Kristkova, B. Sedlakova, J.D. McCright, and M.D. Coffey. 2016. Cucurbit powdery mildews: Methodology for objective determination and denomination of races. *Eur. J. Plant Pathol.* 144:399–410.
- Madden, L.V., G. Hughes, and F. Van den Bosch. 2007. The study of plant disease epidemics. Amer. Phytopathol. Soc. Press, St. Paul, MN.
- McCright, J.D. 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. *J. Amer. Soc. Hort. Sci.* 131:59–65.
- McGrath, M.T. 2010. Managing cucurbit powdery mildew organically. 7 Jan. 2018. <<http://www.extension.org/pages/30604/managing-cucurbit-powdery-mildew-organically>>.
- McGrath, M.T. 2017. Powdery mildew, p. 62–64. In: A.P. Keinath, W.M. Wintermantel, and T.A. Zitter (eds.). *Compendium of cucurbit diseases and pests*. 2nd ed. APS Press, St. Paul, MN.
- Mercier, J., M.J. Muscara, and A.R. Davis. 2014. First report of *Podosphaera xanthii* race 1W causing powdery mildew of watermelon in California. *Plant Dis.* 98:158.
- Pitrat, M., C. Dogimont, and M. Bardin. 1998. Resistance to fungal diseases of foliage in melon, p. 167–173. In: J.D. McCright (ed.). *Cucurbitaceae '98*. ASHS Press, Alexandria, VA.
- Strange, B.E., N. Guner, Z. Pesic-VanEsbroeck, and T.C. Wehner. 2002. Screening the watermelon germplasm collection for resistance to *Papaya ringspot virus* type-W. *Crop Sci.* 42:1324–1330.
- Tetteh, A.Y., T.C. Wehner, and A.R. Davis. 2010. Identifying resistance to powdery mildew race 2W in the USDA-ARS watermelon germplasm collection. *Crop Sci.* 50:933–939.
- Thomas, C.E., A. Levi, and E. Caniglia. 2005. Evaluation of U.S. plant introductions of watermelon for resistance to powdery mildew. *HortScience* 40:154–156.
- Zhang, H., S. Guo, G. Gong, Y. Ren, A.R. Davis, and Y. Xu. 2011. Sources of resistance to race 2WF powdery mildew in US watermelon plant introductions. *HortScience* 46:1349–1352.