USVL-220, a Novel Watermelon Breeding Line

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USVL-220 is a novel watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] breeding line containing the nuclear genome of watermelon cultivars (*Citrullus lanatus* var. *lanatus*) and cytoplasmic background (chloroplast and mitochondrial genomes) derived from the desert species *Citrullus colocynthis* (L.) Schrad. USVL-220 was developed at the USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC.

Development of USVL-220 began in 1999 with the greenhouse observations of F1 plants derived from reciprocal crosses between the U.S. PI 386015 (*C. colocynthis*) and the watermelon cultivars New Hampshire Midget (NHM), Extra Early Sugar Baby, Allsweet, or Charleston Gray (*C. lanatus* var. *lanatus*). In general, F1 plants derived from a cross in which the *C. colocynthis* (PI 386015) was the maternal parent produced a female flower for every two to three male flowers. Conversely, in the reciprocal cross, in which a watermelon cultivar was used as the maternal parent, the F1 plants produced a female flower for every four to six (NHM) or five to seven ('Allsweet' or 'Charleston Gray') male flowers (Levi et al., 2006). Like with most plant species, the chloroplast and mitochondria are inherited maternally in watermelon (Havey et al., 1998; Levi and Thomas, 2005). Several studies have indicated that interaction between nuclear and maternally inherited (chloroplast or mitochondrial) genes affect flower morphogenesis in plant species (Ehlers et al., 2005; Kheyr-Pour, 1980; Ross, 1978; Ross and Gregourius, 1985; Van der Hulst et al., 2004; Wade and McCauley, 2005). Diolez et al. (1986) indicated that mitochondria play a role in the biosynthesis of endogenous ethylene in plant tissues, whereas Salman et al. (2008)

suggested that ethylene reduces the formation of female flowers in watermelon.

A wide genetic diversity exists between watermelon cultivars (*C. lanatus* var. *lanatus*) and U.S. PIs of *C. lanatus* var. *citroides* and *C. colocynthis*, indicating that a large number of exotic alleles may have been excluded during many years of cultivation and selection for watermelon with desirable fruit qualities (Levi et al., 2001). Our primary objective here was to enhance watermelon cultivars (*C. lanatus* var. *lanatus*) with the exotic chloroplast and mitochondrial genomes of the desert watermelon *C. colocynthis* as has been shown in other important crop plants (Burke and Stewart, 2003; Stewart, 1990; Tao et al., 2004). Such breeding lines should be valuable in the development of cytoplasmic substitution lines, which have the nuclear genome of a watermelon cultivar but the cytoplasm of *C. colocynthis* versus that of cultivated watermelon (*C. lanatus* var. *lanatus*) and could be useful in examining if any nuclear–cytoplasmic gene interaction affects female flower production in watermelon.

The *C. colocynthis* exists in the hot deserts of North Africa, the Middle East, and South and Central Asia. As a desert plant, *C. colocynthis* can tolerate drought, intense sun exposure, and high day and low night temper-

atures better than the cultivated watermelon (Althawadi and Grace, 1986; Schafferman et al., 1998). Notable differences exist between the chloroplast genome of *C. lanatus* var. *lanatus* and that of *C. colocynthis* (Dane and Lang, 2004). Introducing the chloroplast and mitochondrial genomes of *C. colocynthis* into watermelon cultivars may improve their ability to survive drought conditions. Maternal effect on drought tolerance has been indicated in other crop plants, including the pondweed *Potamogeton anguillanus* (Iida et al., 2007) or Moss *Tortula ruralis* (Oliver et al., 2010). Cytoplasmic substitution lines containing the *C. colocynthis* cytoplasm versus that of cultivated watermelon should be useful for studying if the cytoplasm plays any role in drought tolerance.

Origin

USVL-220 was produced by first crossing a F1 hybrid [NHM (*C. lanatus* var. *lanatus*) × Griffin 14113 (*C. lanatus* var. *citroides*)] with *C. colocynthis* PI 386015 (used as the maternal parent). Then, most of the nuclear genes of this interspecific hybrid plant [PI 386015 × (NHM × Griffin 14113)] were replaced with nuclear genes from three watermelon cultivars (including Allsweet, Extra Early Sugar Baby, and Charleston Gray) through a series of outcrosses in which these watermelon cultivars were used as the male (pollinator) parents (Fig. 1). An F1 plant derived from the final outcross, in which an 'Allsweet' plant was used as the male parent, was self-pollinated and an F2 plant was selected. This F2 plant, which had elongated oval fruits with green dappled rind (rind thickness of 0.7 to 0.8 inch), red flesh, and sweet flavor (soluble solid content of 8.5% to 11.0%), was selected and self-pollinated. Progenies were selected using the same fruit quality criteria for eight successive generations to produce F10 seeds. These F10 seeds were designated as USVL-220 (Fig. 1).

Fig. 1. Pedigree of USVL-220 showing the processes of replacing most of the nuclear genes of *C. colocynthis* with those of watermelon cultivars (*Citrullus lanatus* var. *lanatus*) while retaining the maternally inherited cytoplasmic background (chloroplast and mitochondrial genomes).

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Fig. 2. USVL-220 fruit harvested in the field in Charleston, SC.

Description

USVL-220 plants produce elongated ovular-shaped fruits with green dappled rind (rind thickness of 0.7 to 0.8 inch) and red flesh (Fig. 2). In field trials (complete randomized blocks with three entries and three plants with a 3-foot distance between them in each entry/plot) in Charleston, SC (summers of 2008 and 2009), USVL-220 plants produced (on average) 1.7 large mature fruits (36.3 cm long and 18.5 cm wide and weighing 6.08 kg) in mid-to-late season (76 to 78 d post-planting) and 1.1 smaller (26.5 cm long and 13.8 cm width and an average weight of 4.35 kg) fruit per plant that mature at 82 to 84 d post-planting. This yield was similar to that of ‘Charleston Gray’ and ‘Jubilee’ plants grown in the same field in Charleston, SC (2008 and 2009). The ‘Charleston Gray’

Fig. 3. Chloroplast DNA fragments of ‘Charleston Gray’ (368.8 bp) (*Citrullus lanatus* var. *lanatus*), *Citrullus colocynthis* PI 386015 (363.7 bp), and USVL-220 (363.7 bp) amplified by the chloroplast DNA primer pair Cucumis-Cp-4F (5’ CCTTCTCTTCGGGATCG) and Cp-4R (5’ GAGGTTAGAGACCGCTCA) in polymerase chain reaction (PCR) as described by Levi et al. (2009).

Fig. 4. Mitochondrial DNA fragments of ‘Charleston Gray’ (374 bp) (*Citrullus lanatus* var. *lanatus*), *Citrullus colocynthis* PI 386015 (375.8 bp), and USVL-220 (375.8 bp) amplified by the mitochondrial DNA primer pair 355-11F (5’ CTATACTCCATACGGCCTTG) and 355-11R (5’ GAACGAGAGAAGCTATCG AG) in polymerase chain reaction (PCR), as described by Levi et al. (2009).

plants had an average of 1.4 large mature fruits (42.6 cm long and 20.1 cm wide and weighing 7.8 kg) and 1.1 small fruits (29.0 cm long and 17.7 cm wide and an average weight of 4.62 kg), whereas the 'Jubilee' plants produced an average of 1.6 large mature fruits (39.1 cm long and 20.2 cm wide and weighing 7.08 kg) and 1.2 smaller (26.2 cm long and 14.6 cm wide and an average weight of 4.34 kg). Mature fruits of USVL-220 have red flesh color (Fig. 2) with an average soluble solid content of 8.5% to 11.0% [measured in the field for three different fruits in each of three random plots, using an Extech RF15 (0% to 32%) portable refractometer (Extech Instruments Corp., Waltham, MA)]. The fruits of USVL-220 have firm flesh with slightly crispy texture and did not exhibit any hollow heart in the fields in Charleston, SC (2008 to 2009). The fruits contain brown seeds (7.0 mm long and 4 mm width) (Fig. 2). Our greenhouse experiments indicated that USVL-220 is moderately susceptible to southern root-knot nematode (*Meloidogyne incognita*) with an average root gall index of 4.1 (similar to 'Crimson Sweet', which had gall index of 4.3, but better than the *C. colocynthis* PI 386015 which had gall index of 5.0) on a scale of 1.0 to 5.0, in which 1.0 = no galls and 5.0 = greater than 80% of root system galled (as has been described by Thies and Levi, 2003, 2007).

In a previous study (Levi et al., 2006), we released three breeding lines, including USVL-200 (early, globular fruits with dark green rind and yellow-pink flesh), USVL-205 (early, globular fruits with dark green rind and red flesh), and USVL-210 (elongated fruit with light green-gray rind and pink-red flesh), which also contain the chloroplast and mitochondrial genomic background of *C. colocynthis* (Levi and Thomas, 2005). Similar to USVL-210, here USVL-220 also produces elongated fruits (Fig. 2). However, the fruits of USVL-220 are smaller (36 cm long and 18.4 cm wide and weighing 5.80 kg) and slightly more ovular than those of USVL-210 (42 cm long and 18 cm wide and weighing 6.49 kg). The USVL-220 fruits have green dappled rind with red flesh (Fig. 2), whereas the USVL-210 fruits have a light green-gray rind and pink-red flesh color (Levi et al., 2006). The nuclear genome of USVL-210 contains mostly genes of the cultivar Charleston Gray (Levi et al., 2006), whereas USVL-220 also contains genes from 'Sugar Baby' and 'Allsweet' (Fig. 2). As indicated, the possibility of interaction between nuclear and cytoplasmic (mitochondria or chloroplast) genes in controlling flower production may not be excluded and may have an effect on female flowers production in the breeding lines containing cytoplasmic background derived from *C. colocynthis*, including USVL-200 or USVL-205 (one female flower for every five to seven male flowers), and USVL-210 (one female flower for every seven to 10 male flowers) versus USVL-220 (one female flower for every seven to eight male flowers). Further studies using cytoplasmic substitution lines that contain the nuclear genome of these breeding lines (USVL-200,

USVL-205, USVL-210, and USVL-220) and the cytoplasm of *C. colocynthis* versus that of cultivated watermelon (*C. lanatus* var. *lanatus*) are needed to prove this assumption.

Polymerase chain reaction (PCR) analysis using chloroplast and mitochondrial DNA primers was performed, as described for expressed sequence tag primers by Levi et al. (2009), to determine if USVL-220 contains chloroplast and mitochondrial genomes of *C. colocynthis* (PI 386015). In the PCR analysis, we used eight chloroplast DNA primer pairs derived from cucumber (*Cucumis sativus* L.) chloroplast genome (Plader et al., 2007) and 10 mitochondrial DNA primer pairs derived from watermelon (*Citrullus lanatus*) mitochondrial genome (Alverson et al., 2010) (primer pair sequences are not shown). As expected from our primer design-based and sequence data, each of these primer pairs produced chloroplast or mitochondrial DNA fragments in the size range of 300 to 380 bp (data are not shown). However, only one of each of the eight chloroplast primer pairs and one of each of the 10 mitochondrial primer pairs produced polymorphic fragments between *C. colocynthis* (PI 386015) and *C. lanatus* var. *lanatus* ('Charleston Gray') (as shown in Figs. 3 and 4). These primer pairs confirmed that the USVL-220 plants retain chloroplast (Fig. 3) and mitochondrial (Fig. 4) genomes derived from the maternal *C. colocynthis* parent (PI 386015). Restriction fragment length polymorphism and PCR analysis data of plants that were successively used as the maternal parents in early generations (F1, BC1, and BC2) confirmed that they contained increasing amounts of the nuclear genome of watermelon cultivars (*C. lanatus* var. *lanatus*) and chloroplast and mitochondrial genomes derived from *C. colocynthis* (as indicated by Levi and Thomas, 2005).

USVL-220 may be useful for scientists and plant breeders interested in enhancing watermelon cultivars with cytoplasm (chloroplast and mitochondrial genomes) of wild watermelon species. This breeding line may be used for examining the effect of the desert species *C. colocynthis* cytoplasm on photosynthesis and respiration. In breeding programs, USVL-220 should be used as the maternal parent to retain the *C. colocynthis* cytoplasm.

Seed Availability

Small samples of seed of USVL-220 are available for distribution to interested research personnel and plant breeders who should make a written request to Dr. Amnon Levi, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414-5334. Seed of USVL-220 also will 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 acknowledgment of the source be given when this germplasm contributes to research or development of a new breeding line or cultivar.

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