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Novel Watermelon Breeding Lines Containing Chloroplast and Mitochondrial Genomes derived from the Desert Species *Citrullus colocynthis*

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The U.S. Department of Agriculture, Agricultural Research Service, announces the release of three novel watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] breeding lines:USVL-200, USVL-205, and USVL-210. These lines contain the nuclear genome of cultivated watermelon (*C. lanatus* var. *lanatus*) and chloroplast and mitochondrial genomes derived from the desert species *C. colocynthis* (L.) Schrad.

Efforts to develop these breeding lines were initiated in 1999 with our greenhouse observation of plants derived from reciprocal crosses between U.S. plant introductions (PIs) of the wild desert species and watermelon cultivars. We observed that when a watermelon cultivar was the female parent, all F_1 plants produced one female flower for every 5 to 9 male flowers. In contrast, when a wild desert plant (PI 386015 or PI 386016) was the female parent, all F_1 plants (8 to 12 F_1 plants in each cross) produced a higher number of female flowers (one female for every 2 to 4 male flowers). This ratio of one female for every 2 to 4 male flowers was retained in the BC_1 and BC_2 when the F_1 plant was used as the female parent and a watermelon cultivar ('Allsweet', 'Charleston Gray', 'Crimson Sweet', or 'New Hampshire Midget') was the recurrent male parent. However, the ratio of female flowers was reduced to one female flower for every 4 to 7 male flowers in BC_3 through BC_8. These results suggest that, in addition to nuclear genes, the maternal plant cytoplasm [most likely chloroplast or mitochondrial genes, which are known to be maternally inherited in most plant species (Havey et al. 1998)] may affect female flower production in watermelon. Nucleocytoplasmic interaction in sex inheritance has been observed in various plant species (Ehlers et al., 2005; Van der Hulst et al. 2004; Wade and McCauley, 2005).

Chloroplasts and mitochondria are maternally inherited in crosses between cultivated watermelon and the related subspecies *C. lanatus* var. *citroides* (Havey et al., 1998). Our experiments, using DNA markers, confirmed that chloroplast and mitochondria are maternally inherited in crosses between cultivated watermelon and the wild desert species (Levi and Thomas, 2005). In addition, this study confirmed that the chloroplast and mitochondrial genomes of the wild desert species are retained in successive backcrosses, where a watermelon cultivar is the recurrent male parent and the backcross plant (carrying the the wild desert species chloroplast and mitochondrial genomes) is the female parent (Levi and Thomas, 2005; A. Levi, unpublished data).

Origin

USVL-200 was produced by first crossing an F_1 hybrid ['New Hampshire Midget' (*C. lanatus* var. *lanatus*) × Griffin 14113 (*C. lanatus* var. *citroides*)] with the wild desert PI 386015 as the female parent. Then, most of the nuclear genes of the F_1 hybrid plant were replaced with the nuclear genes of cultivated watermelon through a series of successive backcrosses with different watermelon cultivars. The watermelon cultivars that were used as the male (pollinator) parents in the eight successive backcrosses were 1) 'Allsweet', 2) 'Charleston Gray', 3) 'Minilee', 4) 'Allsweet', 5) 'Charleston Gray', 6) 'Black Diamond', 7) 'New Hampshire Midget', and 8) 'Black Diamond'. Then, a BC_8-S_1 was self-pollinated and a plant with yellow flesh fruit was selected in five successive generations to produce USVL-200 seeds (BC_8S_6).

USVL-205 is a sister line of USVL-200. It was developed by self-pollinating a BC_8S_1 plant, and a plant with red flesh fruit was selected in six successive generations to produce USVL-205 seeds (BC_8S_7).

USVL-210 was produced by first crossing 'Charleston Gray' with the wild desert PI 386016 (female parent). Then, most of the nuclear genes of the F_1 hybrid plant were replaced with those of the cultivated watermelon through a series of six successive backcrosses with watermelon cultivars that were used as the male parents. The watermelon cultivars that were used in the six successive backcrosses to replace the the wild desert species nuclear genome were 1) 'Charleston Gray', 2) 'Calhoun Gray', 3) 'Charleston Gray', 4) 'Crimson Sweet', 5) 'New Hampshire Midget', and 6) 'Charleston Gray'. A BC_6S_1 plant was self-pollinated, followed by self-pollination and selection of the plant with best fruit quality in five successive generations to produce USVL-210 seeds (BC_6S_6).

Description and Performance

USVL-200 and USVL-205 produce one female flower for every 5 to 7 male flowers. Each of the plants of these breeding lines produced three fruit in the field in Charleston, S.C., during Summers 2004 and 2005. USVL-200 and USVL-205 produce globular fruit with a thick, dark-green rind. USVL-200 has a yellow-pink flesh with a firm texture devoid of hollow heart, while USVL-205 has the same fruit characteristics except for having a red flesh (Table 1, Fig. 1). The fruit of USVL-200 and USVL-205 have an intermediate solid soluble content (Table 1) and are not as sweet as the cultivars used in their development ('Allsweet', 'Charleston Gray', 'Minilee', 'Black Diamond', and 'New Hampshire Midget'). The fruit of these two lines are ready for harvest in early to midseason, similar to 'New Hampshire Midget'.

USVL-210 is a 'Charleston Gray'-type watermelon. Typical fruit are oblong with light green to gray rind and a pink flesh color (Fig. 1). The flesh has a pleasant flavor and an intermediate-high content of soluble solids (Table 1). USVL-210 plants produce one female flower for every 7 to 10 male flowers, similar to 'Charleston Gray'. An average yield of USVL-210 plants, in the field in Charleston (during Summers 2004 and 2005), was 2.6 to 3 fruit per plant. Overall, fruit of USVL-210 have thick rind, crispy flesh texture, and do

Table 1. Fruit characteristics of the three breeding lines.

Characteristic	USVL-200	USVL-205	USVL-210
Fruit shape	Globular	Globular	Elongated
Fruit size (cm)	25 × 23	25 × 23	42 ×18
Fruit weight (lb)	17.5	17.5	14.3
Rind color	Dark green	Dark green	Light green–gray
Flesh color	Yellow–pink	Pink–red	Pink
Flesh texture	Firm	Firm	Slightly crispy
Soluble solids (%)[z]	6–8.5	6–8.5	8.5–10
Seeds	Large, brown	Large, brown	Small, light brown

[z]Measured by a refractometer.

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Fig. 1. Ripe watermelon fruit of USVL-200 (**top**), USVL-205 (**middle**), and USVL-210 (**bottom**).

not exhibit hollow heart (Table 1). They are ready for harvest in mid to late season, similar to 'Charleston Gray'.

Low genetic diversity exists among watermelon cultivars, and there is a need to broaden their genetic base using related wild species or subspecies that have been collected throughout the world and have wide genetic diversity (Levi et al., 2001). The breeding lines reported here may be useful in introducing the chloroplast and mitochondrial genomes of the wild species into watermelon cultivars. They may be used in studying the effects of foreign cytoplasm (chloroplast and mitochondria of *C. colocynthis*) on photosynthesis, respiration, flower production, fruit quality, and disease or pest resistance in cultivated watermelon. USVL-200 and USVL-205 may be useful in improving watermelon cultivars for production of early globular fruit with a thick dark green rind and a firm flesh, devoid of hollow heart. USVL-210 might be useful in improving watermelon cultivars with respect to production of elongated fruit with a thick light-green rind and a firm pink flesh.

Seed Availability

Small samples of seed of USVL-205 and USVL-210 are available for distribution to interested research personnel who make written request to Amnon Levi, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414-5334. Seeds of USVL-200, USVL-205, and USVL-210 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 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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