

Qualitative Inheritance of Rind Pattern and Flesh Color in Watermelon

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Abstract

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] is a diverse species, with fruits of different sizes, shapes, rind patterns, and flesh colors. This study measured the inheritance of novel rind phenotypes and verified the genetics of white, red, salmon yellow, and canary yellow flesh colors. For each of the 11 crosses, six generations (P_aS_1 , P_bS_1 , F_1 , F_2 , BC_1P_a , and BC_1P_b) were produced to form 11 families. Three new genes were identified and designated as follows: *Scr* for the scarlet red flesh color of Dixielee and Red-N-Sweet, *Yb* for the yellow belly (ground spot) of Black Diamond Yellow Belly, and *ins* for the intermittent stripes of Navajo Sweet. The inheritance of the *C* gene for the canary yellow flesh color was verified as single dominant, and a new inbred type line was developed possessing that gene. Aberrations in the segregation of red, white, and salmon yellow flesh colors were recorded, raising questions on the inheritance of these traits. Finally, the spotted phenotype from Moon and Stars was combined with light green and gray rind patterns for the development of novel cultivars with distinctive rind patterns.

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] has been bred to improve yield, quality, and disease resistance; to diversify fruit and plant type (i.e., seeded versus seedless fruits and large versus dwarf vines); and to adapt useful cultivars to different production areas around the world. Watermelon breeders have contributed to the development of new cultivars and to the understanding of the genetics of useful traits in this crop. In the United States, many cultivars were released in the late 1800s and early 1900s with adaptation to the western or eastern production areas: for example, Angeleno, Chilean, and Kleckley Sweet were popular in California, while Florida Favorite and Georgia Rattlesnake were popular in the southeastern United States (Whitaker and Jagger 1937).

Genetic studies on watermelon were first reported between the late 1930s and early 1940s. The emphasis of these investigations was on major traits, including rind, flesh, and seed coat colors; fruit shape and weight; and sex expression (Poole 1944; Poole and Grimball 1945; Poole et al. 1941; Porter 1933, 1937; Weetman 1937).

The genetics of flesh color in watermelon have been studied, and genes for white, red, orange, salmon yellow, and canary yellow colors have been reported (Guner and Wehner 2003). Three alleles were identified at the *y* locus that produce red, orange, and salmon yellow flesh colors (*Y*, *y*^o, and *y*, respectively). *Y* was dominant to *y*^o and *y*, and *y*^o was dominant to *y* (Henderson et al. 1989, 1998; Poole 1944; Porter 1937). Canary yellow color (*C* gene) was

dominant to pink (*c*) and epistatic to red (*Y*) (Henderson et al. 1998; Poole 1944). Red color was also recorded in individuals homozygous for *C* due to the expression of the *i-C* gene (inhibitor of canary yellow) (Henderson et al. 1998; Rhodes and Dane 1999). A third gene for yellow flesh (*B*) was described in a breeding line as hypostatic to the white flesh gene (*Wf*) (Henderson 1992; Shimotsuma 1963).

Breeding for specific flesh colors is often challenging due to frequent distortions encountered in the inheritance of some of these genes. Abnormal phenotypes are often recovered in populations segregating for red and salmon yellow flesh. Some fruits have differential coloration among portions of the flesh, that is, having a colored center and white margin or white flesh with colored blotches within the carpellar remnants.

The inheritance of the *C* gene has been confirmed in many crosses and breeding populations (T.C. Wehner, unpublished data). However, it has never been verified against the *Wf* gene for white flesh. In addition, the *C* gene was available mainly in the F_1 hybrids Yellow Baby and Yellow Doll, and a homozygous type line was unavailable.

Cultivars with distinctive flesh colors included Dixielee and Red-N-Sweet, released in 1979 and 1987, respectively (Wehner 2002). These cultivars have firm scarlet red flesh of a darker color than popular cultivars such as Charleston Gray or Allsweet. Angeleno Black Seeded was the red (*Y* gene) type line, having a much lighter flesh than these new mutant types. Many breeders have made use of Dixielee

Table 1. Crosses and traits analyzed for qualitative inheritance of phenotypic traits in watermelon fruit during the summer of 2004 in Clinton and Kinston, North Carolina

Cross	Trait of interest	
	Phenotype	Gene
Study of new genes		
Angeleno Black Seeded × Dixielee	Scarlet red flesh color	<i>Scr</i>
Angeleno Black Seeded × Red-N-Sweet	Scarlet red flesh color	<i>Scr</i>
Dixielee × Red-N-Sweet	Allelism of scarlet red flesh color	<i>Scr</i>
Black Diamond Yellow Belly × Black Diamond	Yellow belly (ground spot)	<i>Yb</i>
Navajo Sweet × Crimson Sweet	Intermittent stripes	<i>ins</i>
Verification of known genes		
Cream of Saskatchewan × NC-517	Canary yellow flesh color	<i>C</i>
Golden Honey × Cream of Saskatchewan	Salmon yellow flesh color	<i>y</i>
Charleston Gray × Cream of Saskatchewan	White flesh color	<i>Wf</i>
Moon and Stars × Black Diamond	Spotted dark green rind	<i>Sp</i>
Moon and Stars × King and Queen	Spotted light green rind	<i>Sp</i>
Moon and Stars × Charleston Gray	Spotted gray rind	<i>Sp</i>

or Red-N-Sweet as sources of genes for the scarlet red color for the development of new hybrids (G.W. Elmstrom, personal communication), but the inheritance of this trait has not been studied.

The rind (skin) colors and patterns of watermelon fruit have been among the major objectives of breeding. Watermelon is considered to have a green rind, ranging from light to dark and from solid to striped (Guner and Wehner 2003). During the 1900s, inbred cultivars with unique rind colors were released by watermelon breeders in the United States and Japan, but this germplasm has not been maintained and these cultivars are currently no longer available. Japan 6 had inconspicuous and penciled lines on the rind (*p* allele), China 23 had a medium green-colored network on a striped rind (*P* allele), and Long Iowa Belle and Round Iowa Belle had randomly distributed and irregularly shaped greenish-white spots on a mostly solid dark green rind (*m* gene) (Weetman 1937).

Navajo Sweet has medium green, intermittent stripes on a light green rind, becoming more incomplete toward the stem end of the fruit. Black Diamond Yellow Belly has a solid dark green rind with a dark yellow to orange ground spot unlike the usual creamy white ground spot of Black Diamond. The genetics of these two mutations have not been studied. The intermittent stripes might be interesting to use on new specialty cultivars, such as the mini type (1.5–4.0 kg fruit weight). The yellow belly trait might provide more uniformity of the ground spot color in cultivars having a dark green rind because, typically, the color of the ground spot varies greatly depending on the position of the fruit on the ground. Consumers often interpret this variation as different degrees of maturity of the fruit and do not buy fruits with a lighter belly, thus increasing the amount of fruits discarded by the store, while mutant fruits would have a consistently yellow ground spot, thus increasing the percentage of fruits sold per bin.

Moon and Stars has been a popular novelty cultivar since its release in 1926 (Wehner 2002), having large yellow areas (moons) and small yellow areas (stars) (collectively, yellow

spots) over a dark green rind. Fruits are elongate, with sweet, red flesh, thick rind, and brown seeds with black speckles in the center of the seed. The word “clump” has been used to describe this seed coat color, possibly referring to the concentration of black speckles in the center of the seed and their absence along the margins. After the initial release, many selections have been released that were different from the original type. Major differences are in the shape of the fruit (round or oval versus elongate), color of the seed coat (tan or black or white versus clump), and color of the flesh (yellow versus red). In addition, the moons are absent in some cultivars. The single dominant gene *Sp* has been identified as controlling the presence of yellow spots on leaves and fruits (Rhodes 1986). However, there are no reports regarding the behavior of the spotted type in a cross with inbreds having gray (white-green or yellow-green) fruit rind pattern.

The objectives of these experiments were to study the inheritance of several new traits, including the scarlet red flesh trait from Dixielee and Red-N-Sweet crossed with coral red flesh of Angeleno Black Seeded, yellow belly (yellow ground spot) from Black Diamond Yellow Belly, and intermittent striped rind pattern in Navajo Sweet. We also studied the interaction of canary yellow (NC-517), coral red (Charleston Gray), and salmon yellow (Golden Honey) traits when the type lines were crossed with a white-fleshed line (Cream of Saskatchewan). Finally, we studied the interaction of spotted and gray or solid light green rind traits in segregating families. Our study increases the number of genes known for watermelon and clarifies the genetics of the already known traits.

Materials and Methods

Traits and Crosses

A total of 11 families were developed from 11 single crosses between watermelon inbred cultivars or lines (Table 1). We developed six generations (P_aS_1 , P_bS_1 , F_1 , F_2 , BC_1P_a , and BC_1P_b) for each family in the greenhouses at North Carolina



Figure 1. Intermittent stripes in Navajo Sweet (fruit on the left) and continuous stripes in Crimson Sweet (fruit on the right).

State University in Raleigh, North Carolina. Seeds of the inbred lines used in these experiments were obtained from the gene mutant collection of the Cucurbit Genetics Cooperative (Curators: T. C. Wehner and S. R. King).

Two scarlet red flesh color parents, Dixielee and Red-N-Sweet, were crossed with coral red Angeleno Black Seeded to study the inheritance of red flesh color. Additionally, Dixielee \times Red-N-Sweet cross-progenies (P_aS_1 , P_bS_1 , F_1 , F_2 , BC_1P_a , and BC_1P_b) were examined to test for allelism of scarlet red in these lines.

Black Diamond Yellow Belly has the yellow belly phenotype, and Black Diamond has the regular white ground spot, common to most watermelon cultivars. Thus, Black Diamond Yellow Belly was crossed with Black Diamond to study the inheritance of yellow belly, a dark yellow to orange coloration of the ground spot.

Navajo Sweet has intermittent striped rind (Figure 1), where the stripes are just speckles of dark green pigment on a medium green background color. On some fruits, these speckles become more continuous near the stem end, forming partial stripes extending from the stem end toward the fruit equator. For the study of intermittent stripes, Navajo Sweet was crossed with Crimson Sweet, having continuous stripes extending from the stem end to the blossom end of the fruit.

The *C* gene for canary yellow flesh was studied in the cross Cream of Saskatchewan (white flesh) \times NC-517 (canary yellow flesh). NC-517 was used as an inbred line developed from Yellow Baby and Yellow Doll F_1 hybrids. The two hybrids are the original canary yellow type lines, but we preferred to use an inbred line as the canary yellow parent. Cream of Saskatchewan has white flesh. The white color in this cultivar was less bright than in citron [*C. lanatus* (Thunb.) Matsum. & Nakai var. *citroides*] lines that we have studied (personal observation), making it difficult to classify white and canary yellow individuals when immature. Therefore, at harvest, only data from fully mature fruits were recorded.



Figure 2. Moons and stars induced on watermelon by the *Sp* gene may be recovered on striped fruits, overlapping both dark green stripes and light green background.

Two crosses were made to study the inheritance of the *y* and *Wf* genes for salmon yellow and white flesh color in watermelon. Golden Honey was homozygous recessive for the *y* gene (salmon yellow flesh). The cross Golden Honey \times Cream of Saskatchewan segregated for salmon yellow versus white flesh. Charleston Gray was homozygous recessive for the *wf* gene (nonwhite flesh). The progeny of Charleston Gray \times Cream of Saskatchewan segregated for nonwhite versus white flesh.

The inheritance of spotted rind and leaves (*Sp* gene) in Moon and Stars was verified in a cross with Black Diamond. Moon and Stars had yellow spots (1–5 mm in diameter), called stars, on the dark green rind and on the leaves. Larger spots, called moons, are sometimes present on the fruit of spotted cultivars, but their shape and size vary greatly. Black Diamond had dark green rind and leaves free of spots and was considered homozygous for *sp*. The interaction of spotted rind with light green rind was studied in Moon and Stars \times King and Queen, and the interaction of spotted rind with gray rind was studied in Moon and Stars \times Charleston Gray. The presence of spots on rind and leaves was recorded separately (presence versus absence) because the light green or gray background colors of the fruit made it difficult to identify the presence of spots on the fruit (Figure 2).

Cultural Practices

Seeds of the six generations for each family were sown in 72-cell polyethylene flats in the greenhouses at North Carolina State University. An artificial soilless growing medium was used (Canadian sphagnum peat moss, perlite, vermiculite, processed pine bark). The flats were moistened to capacity after seeding and held in the greenhouse at constant temperature (25°C–30°C) until full emergence. The transplants were moved to an open cold frame at the field site for acclimation 1 week before transplanting. The

seedlings were transplanted by hand at the two-true-leaf stage. Missing or damaged transplants were replaced a week after transplanting.

In the field, raised beds were made up with drip irrigation tubes and covered with black polyethylene mulch. The experiment was conducted using horticultural practices recommended by the North Carolina Extension Service (Sanders 2004).

In order to keep families, generations, and plants separate for data collection, each plant was manually trained each week into a spiral shape by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field set fruit. The vine training allowed easy tracing of the fruit to the plant that produced it, giving high accuracy to the system.

Experiment Design and Data Analysis

Our field test was performed in the summer of 2004 at two North Carolina locations: Horticultural Crops Research Station in Clinton and Cunningham Research Station in Kinston. Even though this was a study of Mendelian traits and replication was not necessary over locations, dividing the families into two sets was a precautionary measure in case of adverse environmental conditions or disease epidemics at one location. All six generations of each family were planted at each location. Transplants were placed in rows in the following order and number: P_aS₁ (10), P_bS₁ (10), BC₁P_a (30), BC₁P_b (30), F₁ (20), and F₂ (100) at Clinton and P_aS₁ (10), P_bS₁ (10), F₁ (20), BC₁P_a (30), BC₁P_b (30), and F₂ (110) at Kinston. At Clinton, each field was 0.4 ha with eight rows 60 m long, and each family occupied four rows. At Kinston, each field was 0.4 ha with six rows 85 m long, and each family occupied three rows. The fields had raised, shaped beds (rows) on 3.1-m centers with single hills 1.2 m apart.

We analyzed the data by family and then pooled data over families for the same gene after testing for homogeneity of variances using the heterogeneity χ^2 test (Ostle and Malone 1988; Steel et al. 1997). We performed segregation analysis and goodness-of-fit tests using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina) and the SASGENE 1.2 program (Liu et al. 1997), based on χ^2 testing of the expected segregation ratios for a single gene. All χ^2 tests were performed with a 95% confidence level.

Names and symbols for new genes proposed herein are in conformance with gene nomenclature rules for the Cucurbitaceae family (Cucurbit Gene List Committee 1982).

Results and Discussion

Scarlet Red Flesh

The heterogeneity χ^2 for the F₂, BC₁P_a, and BC₁P_b data was nonsignificant (0.01, 0.27, and 0.04, respectively). Therefore, we analyzed the data pooled over families (Table 2). In both F₁ generations, all fruits had scarlet red flesh, indicating that scarlet red flesh color was inherited as a dominant trait. Pooled F₂ individuals segregated 169:52 (scarlet red:coral red flesh), which is consistent with a 3:1 ratio ($\chi^2 = 0.25$, P value = .61). Fruits in the BC₁P_a generation (P_a having

coral red flesh) segregated 1:1 (scarlet:coral red flesh), as expected ($\chi^2 = 0.01$, P value = .91). The BC₁P_b generation had two fruits with coral red flesh in one cross, but a misclassification due to differences in maturity of the fruit may have been possible. The pooled χ^2 was 0.04 (P value = .83) confirming the 1:0 expected ratio.

In the test for allelism between the two parents with scarlet red flesh color (Dixielee \times Red-N-Sweet), all fruits of all generations (P_aS₁, P_bS₁, F₁, F₂, BC₁P_a, and BC₁P_b) had scarlet red flesh, thus confirming that both cultivars have the same allele at this locus. Our results confirmed that scarlet red flesh in Dixielee and Red-N-Sweet is controlled by a single dominant gene. We propose naming this new gene *Scarlet red* flesh color, with the symbol *Sr*.

Yellow Belly

In the cross Black Diamond Yellow Belly \times Black Diamond only the color of the ground spot segregated because Black Diamond Yellow Belly is a mutant of Black Diamond differing only for this trait (Table 3). The F₁ generation had all fruits with yellow belly. Therefore, in the next generations, we tested the hypothesis that the yellow color was controlled by a single dominant allele. The F₂ plants segregated 91:32 (yellow:white belly), consistent with this hypothesis ($\chi^2 = 0.07$, P value = .79). For the backcross to the homozygous recessive parent, the segregation was 27:26 (yellow:white belly) and the χ^2 for the 1:1 expected ratio was 0.02 (P value = .89).

Evaluating the color of the ground spot is difficult if the fruits are not fully mature. At full maturity, the wild type had a creamy white-colored belly that was called white, while the mutant type had a dark yellow to orange belly. In addition, the yellow ground spot usually had a more regular shape than the white one.

This test confirmed the hypothesis of a single gene controlling yellow belly in Black Diamond Yellow Belly. We propose naming this new dominant gene *Yellow belly*, with the symbol *Yb*.

Intermittent Stripes

For the intermittent stripe rind pattern, the F₁ generation indicated control by a single recessive gene (Table 4). F₂ plants segregated 154:53 (continuous:intermittent stripes) ($\chi^2 = 0.04$, P value = .84). Plants in the BC₁P_a generation (P_a having intermittent stripes) segregated as expected with a 1:1 ratio and a χ^2 of 0.27 (P value = .60). Our hypothesis testing confirmed that the intermittent stripes in Navajo Sweet were controlled by a single recessive gene. We propose naming this new gene *intermittent stripes*, with the symbol *ins*.

Canary Yellow Flesh

In the F₁ generation, all 34 fruits had canary yellow flesh (Table 5). The F₂ segregated 135:49 (canary yellow:white flesh), showing that the data were consistent with a 3:1 expectation ($\chi^2 = 0.26$, P value = .60). Fruits in the BC₁P_a generation (P_a having white flesh) segregated as expected with a 1:1 ratio ($\chi^2 = 0.27$, P value = .60).

Table 2. Single-locus goodness-of-fit test for scarlet red flesh color in watermelon^a

Generation	Total	Scarlet red ^b	Coral red ^c	Expected ^d	χ^2	df	P value
Angeleno Black Seeded × Dixielee							
P _a S ₁ ^e	20	0	20				
P _b S ₁ ^f	20	20	0				
F ₁	40	40	0				
F ₂	116	89	27	3:1	0.18	1	.66
BC ₁ P _a	44	23	21	1:1	0.09	1	.76
BC ₁ P _b	49	49	0				
Angeleno Black Seeded × Red-N-Sweet							
P _a S ₁ ^e	20	0	20				
P _b S ₁ ^f	20	20	0				
F ₁	33	33	0				
F ₂	105	80	25	3:1	0.08	1	.77
BC ₁ P _a	47	22	25	1:1	0.19	1	.66
BC ₁ P _b	49	47	2	1:0	0.04	1	.83
Pooled							
P _a S ₁ ^e	40	0	40				
P _b S ₁ ^f	40	40	0				
F ₁	73	73	0				
F ₂	221	169	52	3:1	0.25 ^g	1	.61
BC ₁ P _a	91	45	46	1:1	0.01 ^g	1	.91
BC ₁ P _b	98	96	2	1:0	0.04 ^g	1	.83

^a Data are ratings from two crosses of *Citrullus lanatus* var. *lanatus*: Angeleno Black Seeded × Dixielee and Angeleno Black Seeded × Red-N-Sweet; data are presented by family and pooled over families.

^b Scarlet red was the standard red flesh color.

^c Coral red was the mutant red flesh color.

^d Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

^e P_a was the carrier of the recessive gene (coral red).

^f P_b was the carrier of the dominant gene (scarlet red).

^g Heterogeneity $\chi^2_{(0.05; 1)}$: F₂ = 0.01, BC₁P_a = 0.27, BC₁P_b = 0.04.

The experiment confirmed that the *C* gene for canary yellow flesh color was inherited as a single dominant gene (Henderson et al. 1998; Poole 1944). Originally, this gene was identified in Yellow Baby and Yellow Doll, both F₁ hybrids. We propose that the type line NC-517, an inbred developed from Yellow Baby and Yellow Doll and having

the *C* gene, be used in future as a homozygous source for this gene.

Salmon Yellow and Red Flesh

In populations segregating for salmon yellow and red flesh, we recovered intermediate phenotypes. For example, some

Table 3. Single-locus goodness-of-fit test for yellow belly color in watermelon^a

Generation	Total	Yellow ^b	White ^c	Expected ^d	χ^2	df	P value
Black Diamond Yellow Belly × Black Diamond							
P _a S ₁ ^e	17	17	0				
P _b S ₁ ^f	20	0	20				
F ₁	9	9	0				
F ₂	123	91	32	3:1	0.07	1	.79
BC ₁ P _a	22	22	0				
BC ₁ P _b	53	27	26	1:1	0.02	1	.89

^a Data are ratings from one cross of *Citrullus lanatus* var. *lanatus*: Black Diamond Yellow Belly × Black Diamond.

^b Yellow was the mutant belly color.

^c White was the standard belly color.

^d Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

^e P_a was the carrier of the dominant gene (yellow belly).

^f P_b was the carrier of the recessive gene (white belly).

Table 4. Single-locus goodness-of-fit test for intermittent stripes in watermelon^a

Generation	Total	Continuous ^b	Intermittent ^c	Expected ^d	χ^2	df	P value
Navajo Sweet × Crimson Sweet							
P _a S ₁ ^e	20	0	20				
P _b S ₁ ^f	20	20	0				
F ₁	34	34	0				
F ₂	207	154	53	3:1	0.04	1	.84
BC ₁ P _a	60	32	28	1:1	0.27	1	.60
BC ₁ P _b	58	58	0				

^a Data are ratings from one cross of *Citrullus lanatus* var. *lanatus*: Navajo Sweet × Crimson Sweet.

^b Continuous was the standard stripe type.

^c Intermittent was the mutant stripe type.

^d Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

^e P_a was the carrier of the recessive gene (intermittent stripes).

^f P_b was the carrier of the dominant gene (continuous stripes).

fruits had a pigmented center with white margin, patches of color in generally white flesh, colored flesh with white carpel walls, or other intermediate color combinations. In both families, the parental fruits were uniform for the expected flesh color. The F₁ and the F₁-derived generations, instead, had the unexpected colors described above. We did not observe Mendelian segregation patterns for flesh color in the progenies of the crosses Golden Honey × Cream of Saskatchewan and Charleston Gray × Cream of Saskatchewan (Figure 3).

The separate rating of the color of different parts of the flesh could be an alternative approach to gather more information on the genetics of the color of different portions of the fruit. The portions of the fruit to be rated should include (1) the endocarp between the carpel walls and the mesocarp (white rind); (2) the flesh within the carpels, originating from the styler column; and (3) the carpel walls, between (1) and (2).

One possible hypothesis to explain the presence of the abnormal types is that the expression of the pigment is caused by several different genes, one for each portion of the fruit. Thus, the mixed colorations would have been caused by recombination of these genes. Alternatively, tissue-specific expression of one or several of the regulatory genes in the

pigmentation pathway may be the cause of the coloration patterns encountered.

Finally, red and salmon yellow flesh colors in watermelon could be quantitative rather than qualitative traits. In our experiments and in our breeding work, we have not observed a clear quantitative distribution of pigmentation from one parental type to the other, with the abnormal phenotype being intermediate levels. Should this trait be quantitative, the variation would be measurable for the intensity of the flesh color, rather than presence or absence of the pigments in different portions of the fruit.

Spotted Rind and Leaves

The presence of spots on rind and leaves (called stars) in crosses of Moon and Stars with three other cultivars having solid green leaves segregated as expected (Table 6) under the control of the *S_p* gene (Poole 1944; Rhodes 1986). Although linkage analysis confirmed that the *S_p* gene determined the spotted phenotype both of the rind and of the leaves (no significant recombination detected in all three families), the identification of yellow spots on the light green

Table 5. Single-locus goodness-of-fit test for canary yellow flesh color in watermelon^a

Generation	Total	Canary ^b	White ^c	Expected ^d	χ^2	df	P value
Cream of Saskatchewan × NC-517							
P _a S ₁ ^e	20	0	20				
P _b S ₁ ^f	20	20	0				
F ₁	34	34	0				
F ₂	184	135	49	3:1	0.26	1	.60
BC ₁ P _a	56	27	29	1:1	0.27	1	.60
BC ₁ P _b	51	51	0				

^a Data are ratings from one cross of *Citrullus lanatus* var. *lanatus*: Cream of Saskatchewan × NC-517.

^b Canary was the mutant flesh color.

^c White was the standard flesh color.

^d Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

^e P_a was the carrier of the recessive gene (white flesh).

^f P_b was the carrier of the dominant gene (canary yellow flesh).

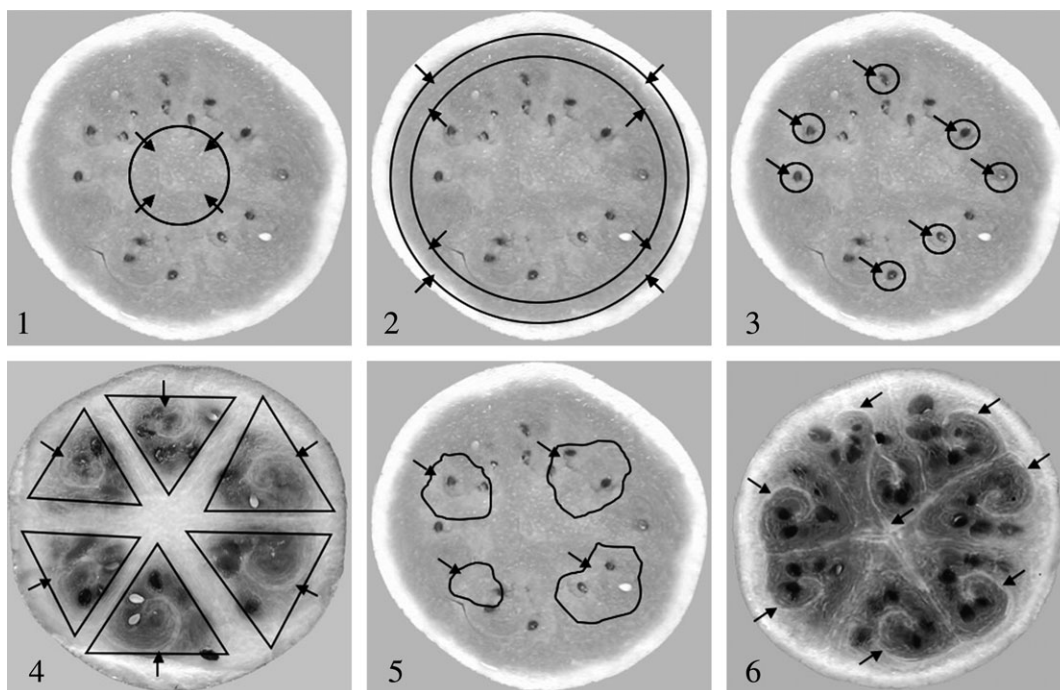


Figure 3. Examples of intermediate flesh colors in the progenies of the crosses Golden Honey \times Cream of Saskatchewan and Charleston Gray \times Cream of Saskatchewan. Arrows indicate areas of differential coloration of the flesh. Different colored areas are (1) center, (2) margin near rind, (3) around seeds, (4) inside the carpels, (5) random blotches, or (6) the carpel septae.

background from King and Queen was difficult and prone to error because the spots blended in with the background color. Therefore, the single-gene hypothesis could not be confirmed using fruit data from the cross Moon and Stars \times King and Queen, and distorted segregation ratios were measured for this family.

The heterogeneity χ^2 for the F_2 and BC_1P_b leaf data was nonsignificant for the null hypothesis (2.39 and 0.16, respectively) (Table 6). In the F_1 generation, all plants in the three crosses had spotted leaves. The pooled F_2 segregated 253:84 spotted:solid green leaves, and the χ^2 was 0.01 (P value = .87), showing that the data were consistent with a 3:1 expectation. The leaves in the BC_1P_b generation (P_b having solid green leaves) segregated 1:1 as expected ($\chi^2 = 0.15$, P value = .70) (Table 6).

Pooled rind data (spotted versus normal) confirmed the expected segregation of the *Sp* gene. The heterogeneity χ^2 for the F_2 and BC_1P_b fruit data were 2.01 and 0.14, respectively (Table 6). In the pooled F_1 generation, all 69 plants had spotted fruits. The pooled F_2 segregated consistently with a 3:1 ratio, counting 181:63 spotted:normal fruit and a χ^2 of 0.09 (P value = .76). The fruits in the BC_1P_b generation (P_b having normal rind) segregated as expected with a χ^2 of 0.14 (P value = .70).

We concluded that the *Spotted* gene from Moon and Stars produced the spotted phenotype in leaves and fruits of cultivars with solid dark green (Black Diamond), solid light green (King and Queen), and gray rind (Charleston Gray) patterns. Nevertheless, the spotted type was inconsistently

visible on the solid light green background due to the lack of contrast between the two colors.

In this study, we observed the presence of F_2 and BC_1 plants with striped rind, thus suggesting that the dark green background of Moon and Stars was generated by dark green stripes on a dark green background (data not shown). On striped fruits, the spots would cover both the dark green stripes and the medium green background (Figure 2).

During our experiments, we did not find a consistent presence of the moons in the parental inbred Moon and Stars. We observed moons ranging in size from a few centimeters in diameter to covering up to one-third of the fruit. Moons were observed in the parental generation of the three crosses only in 21 of 57 P_aS_1 plants tested. On some fruits, we observed that stars close to the border of a moon tended to coalesce with, or be covered by, the moon. A moon may be the result of a random event of coalescence of several stars. Alternatively, the moons could be larger spots caused by differential expression of the *Sp* gene. Moons could also be controlled by a different gene, but this hypothesis would not explain their inconsistent presence in the inbred parent. Further research is needed to clarify the genetics of the moon, and the *Sp* gene should be considered only for the inheritance of the stars on fruits and leaves.

Conclusions

With these genetic studies, we identified three new genes in watermelon. *Scarlet red* flesh (*Scr*) produced more intense red

Table 6. Single-locus goodness-of-fit test for spotted rind and leaves in watermelon^a

Generation	Total	Spotted ^b	Normal ^c	Expected ^d	χ^2	df	P value
Moon and Stars × Black Diamond—leaf and rind rating							
P _a S ₁ ^e	20	20	0				
P _b S ₁ ^f	20	0	20				
F ₁	34	34	0				
F ₂	203	147	56	3:1	0.72	1	.39
BC ₁ P _a	54	54	0				
BC ₁ P _b	56	26	30	1:1	0.29	1	.59
Moon and Stars × Charleston Gray—leaf and rind rating							
P _a S ₁ ^e	17	17	0				
P _b S ₁ ^f	20	2	20				
F ₁	35	35	0				
F ₂	41	34	7	3:1	1.37	1	.24
BC ₁ P _a	52	52	0				
BC ₁ P _b	56	28	28	1:1	0.00	1	1.00
Moon and Stars × King and Queen—leaf rating							
P _a S ₁ ^e	20	20	0				
P _b S ₁ ^f	20	0	20				
F ₁	34	34	0				
F ₂	93	72	21	3:1	0.29	1	.59
BC ₁ P _a	57	57	0				
BC ₁ P _b	59	29	30	1:1	0.02	1	.89
Moon and Stars × King and Queen—rind rating							
P _a S ₁ ^e	20	20	0				
P _b S ₁ ^f	20	0	20				
F ₁	34	34	0				
F ₂	93	55	38	3:1	12.48	1	.00
BC ₁ P _a	57	57	0				
BC ₁ P _b	59	19	40	1:1	7.47	1	.01
Pooled—leaf rating							
P _a S ₁ ^e	57	57	0				
P _b S ₁ ^f	60	0	60				
F ₁	103	103	0				
F ₂	337	253	84	3:1	0.01 ^g	1	.87
BC ₁ P _a	163	163	0				
BC ₁ P _b	171	83	88	1:1	0.15 ^g	1	.70
Pooled—rind rating							
P _a S ₁ ^e	37	37	0				
P _b S ₁ ^f	40	0	40				
F ₁	69	69	0				
F ₂	244	181	63	3:1	0.09 ^b	1	.76
BC ₁ P _a	106	106	0				
BC ₁ P _b	112	54	58	1:1	0.14 ^b	1	.70

^a Data are ratings from three crosses of *Citrullus lanatus* var. *lanatus*: Moon and Stars × Black Diamond, Moon and Stars × King and Queen, and Moon and Stars × Charleston Gray; data are presented by family and pooled over families, where the segregation confirmed the single-gene hypothesis.

^b Spotted was the mutant rind and leaf type.

^c Normal was the standard rind and leaf type.

^d Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

^e P_a was the carrier of the dominant gene (spotted phenotype).

^f P_b was the carrier of the recessive gene (uniform phenotype).

^g Heterogeneity $\chi^2_{(0.05; 1)}$: F₂ = 2.39, BC₁P_b = 0.16.

^h Heterogeneity $\chi^2_{(0.05; 1)}$: F₂ = 2.01, BC₁P_b = 0.14.

color in Dixielee and Red-N-Sweet compared to Angeleno Black Seeded, the type line for red flesh color in watermelon (Porter 1937). We propose to call the original red flesh color coral red to distinguish it from scarlet red. *Scr* was inherited

as a single dominant gene. *Yellow belly* (*Yb*) was classified as a single dominant gene that changed the color of the ground spot in Black Diamond from creamy white to dark yellow. The presence of intermittent versus continuous stripes on

the rind of Navajo Sweet was explained by the action of a single recessive gene that we named *intermittent stripes* (*ins*), with the dominant allele present in Crimson Sweet.

We confirmed the inheritance of the *C* gene for the canary yellow flesh as a single dominant gene. We suggest that the inbred line NC-517, the canary yellow parent in our study, be considered the homozygous type-line for the *C* gene, in addition to the canary yellow F₁ hybrids used previously (Yellow Baby and Yellow Doll).

Dixielee and Red-N-Sweet, Black Diamond Yellow Belly, Navajo Sweet, and NC-517 will be kept in the gene mutant collection as type lines for the *Scr*, *Yb*, *ins*, and *C* genes, respectively, by the watermelon gene curators for the Cucurbit Genetics Cooperative (T. C. Wehner and S. R. King).

Our study highlighted a complex genetic background for the inheritance of red and salmon yellow flesh colors, previously attributed solely to the expression of the *Wf* and *y* genes. Based on our observations, we discarded the hypothesis of a quantitative type of inheritance and suggested that different genes, or tissue-specific expression of the same genes, might be involved in the pigmentation of different portions of the fruit.

A new possibility for the development of watermelon fruit with novel rind type results from our verification of the inheritance of the spotted phenotype (*Sp* gene) when transferred from Moon and Stars to cultivars with gray and light green background. The spotted trait was more evident on the gray background, while it was undetectable on many fruits with light green rind. In addition, the presence of small spots (stars) was consistent on fruits and leaves, while the presence of large yellow blotches (moons) was inconsistent and may be determined by the coalescence of adjacent stars during development. Moons were present only on fruits, even though in a single instance, large, irregular yellow areas were observed on the foliage.

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