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Discovery of Second Gene for Solid Dark Green versus Light Green Rind Pattern in Watermelon

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The watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*) has high variability for fruit size, shape, rind pattern, and flesh color. This study was designed to measure the qualitative inheritance of rind phenotypes (solid dark green vs. light green). For each of the 2 families, "Mountain Hoosier" × "Minilee" and "Early Arizona" × "Minilee," 6 generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) were developed. Each family was tested in summer 2008 in 3 environments in North Carolina. Phenotypic data were analyzed with the χ^2 method to test the segregation of Mendelian genes. Deviations from the expected segregation ratios based on hypothesized single dominant gene for solid dark green versus light green rind pattern were recorded, raising questions on the inheritance of this trait. Inheritance of solid dark green rind versus light (gray) rind showed duplicate dominant epistasis. Duplicate dominant epistasis gives rise to a 15:1 ratio (solid dark green:light rind pattern) in F_2 generation. When both the loci are homozygous recessive, we observe light rind pattern. The *g-1* and *g-2* genes were identified to control light green rind when in homozygous recessive form.

Key words: *Citrullus lanatus, rind pattern,* χ^2*test*

The 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 vs. seedless fruit and large vs. 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, whereas "Florida Favorite" and "Georgia Rattlesnake" were popular in the southeastern United States (Whitaker and Jagger 1937). The first reported genetic studies on watermelon were from the late 1930s and early 1940s and involved the adapted inbred cultivars developed in the previous few decades of watermelon breeding. The emphasis of these investigations was on major traits, such as rind, flesh, and seed-coat colors, fruit shape and weight, and sex expression (Porter 1933, 1937; Weetman 1937; Poole et al. 1941; Poole 1944; Poole and Grimball 1945).

Watermelon breeders are interested in developing elite cultivars using novel phenotypes including different fruit shapes and rind patterns. The rind (skin) colors and patterns of watermelon fruit have been one of the objectives of breeding. Watermelon has a green rind, ranging from light to dark, from solid to striped, and intermittent to spotted (Guner and Wehner 2003), and the inheritance of these rind types has been studied. Researchers have proposed various models of inheritance of rind pattern in watermelon cultivars. In 1937, Weetman proposed that 3 alleles at a single locus determine the inheritance of striped and solid green (dark and light rind). The *D* allele for dark green is dominant to the *d* allele for light green rind, and the d^s allele, which produces stripes, is dominant to *d* and recessive to *D* (Weetman 1937). This allelic series was renamed to *G*, g^s, and *g* by Poole in 1944 and this hypothesis has been reported in all the gene lists for watermelon (Cucurbit Gene List Committee 1979, 1982, 1987; Henderson 1991; Henderson 1992; Rhodes and Zhang 1995; Rhodes and Dane 1999; Guner and Wehner 2003, 2004), using the notation adopted above. Weetman (1937) also hypothesized that 2 loci (S, dominant for striping and D, dominant for dark green rind) could be controlling the background color and foreground stripe pattern. However, Porter (1937) reported that dark green rind was completely dominant to light green in the 2 crosses involving 2 different dark green cultivars ("Angeleno" and "California Klondike"). He reported incomplete dominance of dark green rind in the cross California Klondike × Thurmond Gray, the latter cultivar being described as gray (yellowish green). There are reports of dominance of solid dark green over gray rind

(Wehner 2008). Gusmini and Wehner (2006) also studied the inheritance of spotted (*Sp*), yellow belly (*Yb*), and intermittent rind pattern (*ins*) that can be used to develop specialty cultivars.

To date, there is no strong evidence for either of the 2 hypotheses proposed by Weetman for the inheritance of different shades of solid green rind and striped rind in watermelon. Nevertheless, dark green (*D*, renamed *G*) is completely dominant to light green (*d*, renamed *g*) in crosses with a light green parent. On the other hand, in crosses of dark green cultivars with gray cultivars (light green background), genes for rind color behave as incomplete dominant and produce the medium green type that is also commonly observed in watermelon. Possibly, the multi-allelic series at the *g* locus needs to include an allele for the background of the gray watermelons that is different from the *g* allele for light green rind. The inheritance of gray rind pattern has never been studied directly. Moreover, there is no clear distinction between light green and gray rind pattern. Cultivars used to conduct these studies are no more available to confirm these studies. There is need to verify these studies with current cultivars having solid dark green and light green or gray rind.

In current germplasm pool, "Mountain Hoosier" and "Early Arizona" have solid dark green rinds. These cultivars are hypothesized to have genotype "*GG*" for solid dark green rind, whereas "Minilee" has light green rind pattern (*gg*).

The objectives of these experiments were to study the inheritance of solid dark green versus light green rind pattern from families of Mountain Hoosier × Minilee and Early Arizona × Minilee based on 6 related generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, and BC_1P_b). This study was conducted to confirm already reported alleles, *G* (solid dark green) and *g* (light green).

Materials and Methods

Traits and Crosses

Three families were developed from 2 crosses of watermelon inbred cultivars or lines to study the rind pattern of watermelon. In this way, 2 families were developed; Mountain Hoosier × Minilee and Early Arizona × Minilee. Six generations were developed (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, and BC_1P_b) for each family, which were grown in the greenhouses at Horticultural Field Laboratories, North Carolina State University in Raleigh, North Carolina. Parents were self-pollinated to obtain enough seeds for making future crosses, so they were noted as P_aS_1 and P_bS_1. P_aS_1 and P_bS_2 refers to self-pollinated generation of parent A and parent B, respectively. BC_1P_a and BC_1P_b refers to backcross to parent A and parent B, respectively. Mountain Hoosier × Minilee and Early Arizona × Minilee were studied to determine the inheritance of solid dark green rind from Mountain Hoosier and Early Arizona against light green rind from Minilee (Figure 1).

Cultural Practices

Seeds of the 6 generations for each family were sown in 72-cell polyethylene flats in the greenhouses at North Carolina State University. The artificial soilless growing medium 4P *Fafard* soilless *mix* (Conrad Fafard Incorporated, Agawam, MA) was used. The medium was moistened to capacity after seeding and held in the greenhouse at constant temperature (25–30 °C) until full emergence. The transplants were moved to an open cold frame at the field site for acclimation 2 weeks prior to transplanting. The seedlings were transplanted by hand at the 2-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). The soil types were Orangeburg loamy sand at Clinton and a Norfolk sandy loam at Kinston. 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, resulting in high accuracy.

The field test was run in the summer of 2008 at 2 research stations: Horticultural Crops Research Station in Clinton, North Carolina and Cunningham Research Station in Kinston, North Carolina. In this experiment, we identified locations as Kinston, Clinton (M), and Clinton (P) where M and P stands for 2 site names at Clinton research station. Though this was a study of Mendelian traits, and replication was not necessary over locations, families were divided into 3 sets (one set per location) as a precautionary measure in case of adverse environmental conditions or unpredicted disease epidemics occurs at one location. All 6 generations of each family were planted at each location as one set without replication. Transplants were placed in rows in the following order and number: P_aS_1 (10), P_bS_1 (10), BC_1P_a (30) BC_1P_b (30), F_1 (20), F_2 (100) at Clinton (M) and Clinton (P) locations and P_aS_1 (10), P_bS_1 (10), F_1 (20), BC_1P_a (30), BC_1P_b (30), F_2 (100) at Kinston. At Clinton, each field was 0.4 ha with 8 rows 60 m long and each family occupied 4 rows. At Kinston each field was 0.4 ha with 6 rows 85 m long and each family occupied 3 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 the data over families. We performed segregation analysis and goodness-of-fit tests (Ramsey and Schafer 1997) using the SAS–STAT statistical package (SAS Institute, Cary, NC) 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 ($\alpha = 0.05$). Names and symbols for new genes proposed herein are in conformance with gene nomenclature rules for the *Cucurbitaceae* family (Cucurbit Gene List Committee 1982).

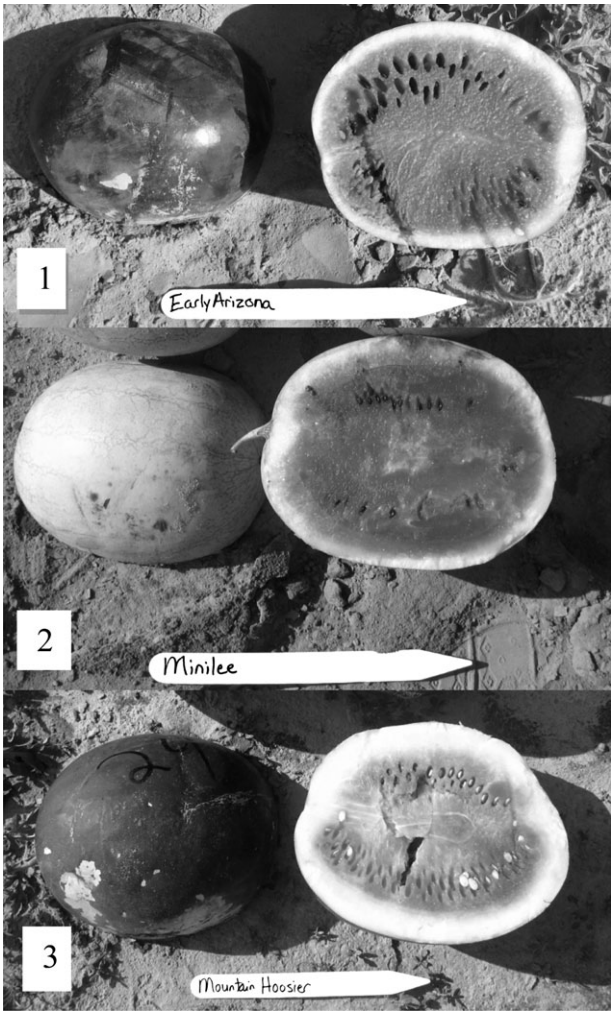

Figure 1. Cultivars used to develop families. 1) "Early Arizona" solid dark green rind; 2) "Minilee" showing light green rind; and 3) "Mountain Hoosier" with solid dark green rind.

Results and Discussion

Solid Dark Green versus Light Green Rind Pattern

The *G* allele for solid dark green rind is dominant to *g* allele which controls light green rind pattern. The F_2 generation should segregate in 3:1 ratio phenotypically and backcross to recessive parent should segregate in 1:1 ratio. In Mountain Hoosier × Minilee and Early Arizona × Minilee families, F_1 indicated control of single dominant gene for solid dark green rind over light green rind pattern as all fruit were solid dark green. F_2 plants segregated 229:20 (χ^2 =38.23, *P* value = 0.00) in Mountain Hoosier × Minilee and 239:18 (χ^2 =44.39, *P* value = 0.0) in Early Arizona × Minilee (Table 1). Plants in the BC_1P_b generation (backcross to Minilee) segregated 59:23 (solid dark green:light green) with χ^2 of 15.80 (*P* value = 0.00) in Mountain Hoosier × Minilee and 62:24 (solid dark green:light green) with 16.79 (*P* value = 0.00) in Early Arizona × Minilee. Both the families showed significant distortion from expected ratio in both F_2 and backcross generations. The hypothesis of single gene controlling solid dark rind against light green rind was disapproved based on this study. Segregation ratios still indicated that solid dark rind is under control of some dominant genes. Different genes might be interacting epistatically to distort the 3:1 segregation ratio. The segregation ratios in the F_2 and backcross generations were found to be closely associated with dominant duplicate epitasis gene action (15:1). Both the families were tested for duplicate dominant epistasis using χ^2 test. If that is true, the F_2 generation should segregate as 15:1 (solid dark green:light green), BC_1P_b should segregate 3:1, and BC_1P_a and F_1 should be all solid dark green rind. All the fruit in the F_1 and BC_1P_a (backcross to parent with solid green rind) were solid dark green in both families, which supported our initial hypothesis (Table 2). F_2 segregated in 229:20 (χ^2 = 1.06, *P* value = 0.30) in Mountain Hoosier × Minilee and 239:18 (χ^2 = 0.27, *P* value = 0.61) in Early Arizona × Minilee and were in conformity with the expected segregation ratio of 15:1. Similar results were also obtained when the data was pooled over families for F_2 (χ^2 = 1.20, *P* value = 0.27). Backcrossing to recessive parent Minilee resulted in expected segregation ratio of 3:1 (solid dark green:light green) with χ^2 of 0.26 (*P* value = 0.61), 0.57 (*P* value = 0.45), and 0.79 (*P* value = 0.37) in Mountain Hoosier × Minilee, Early Arizona × Minilee, and when the data was pooled over families, respectively.

Our hypothesis testing confirmed that solid dark green rind versus light green rind is under control of duplicate dominant epistasis gene action. Holland (2001) illustrated duplicate dominant epistasis that gives rise to 15:1 ratio in F_2 generation. Two loci control production of identical enzymes. If the dominant allele is present at either of the loci then the metabolic pathway functions giving rise to the optimal genotypic value. Only when both the loci are homozygous recessive and the key enzyme is not produced, we observe the alternate genotype. We propose that 2 recessive genes *g-1* and *g-2* are controlling the light green rind. Either of allele of 2 genes can give solid dark green rind when present together or alone in dominant form (*G-1* or *G-2*). When these genes are present in homozygous recessive form, they produce light green rind. *G-1_ g-2g-2_*, *g-1g-1G-2_*, and *G-1-G-1_G-2_* will produce solid dark green rind, where as *g-1g-1g-2g-2* will produce light green rind. We propose naming new recessive genes for light green rind as *g-1* and *g-2*.

Conclusion

Consumers and growers have preference for different rind patterns in watermelon. To meet their demand, watermelon breeders cross cultivars of different rind pattern to develop breeding populations. Solid dark green and light green rinds are very common in watermelons, so it is essential to know how they behave when crossed together. Based on our study, we discarded hypothesis of complete dominance of

Table 1 Goodness-of-fittest for single dominant gene for rind pattern in watermelon[a]

Family	Total	Solid dark rind[b]	Light green rind[c]	Expected ratio[d]	χ^2 test	df	*P* value
Mountain Hoosier × Minilee							
P_aS_1[e]	27	27	0				
P_bS_1[f]	26	0	26				
F_1	58	58	0				
F_2	249	229	20	3:1	38.23	1	0.000
BC_1P_a	84	84	0	1:0			
BC_1P_b	82	59	23	1:1	15.80	1	0.000
Early Arizona × Minilee							
P_aS_1[e]	26	26	0				
P_bS_1[f]	28	0	28				
F_1	56	56	0				
F_2	257	239	18	3:1	44.39	1	0.000
BC_1P_a	83	83	0	1:0			
BC_1P_b	86	62	24	1:1	16.79		0.000

[a] Data are ratings from family Mountain Hoosier × Minilee and Early Arizona × Minilee of *Citrullus lanatus var. lanatus* from 3 locations namely Kinston, Clinton (M), and Clinton (P).

[b] Solid dark was the standard rind pattern.

[c] Light green was the mutant rind pattern.

[d] Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation.

[e] P_a: Parent A was carrier of dominant allele (solid dark green).

[f] P_b: Parent B was carrier of recessive allele (light green).

solid dark green rind over light green rind. This study highlighted that solid dark green rind is inherited in duplicate dominant epistasis fashion with light green rind. The *g-1* and *g-2* genes were named to control light green rind in homozygous recessive form. Presence of any dominant allele in the genotype will produce solid dark green rind.

Table 2 Goodness-of-fit test for duplicate dominant epistasis for rind pattern in watermelon[a]

Family	Total	Solid dark rind[b]	Light green rind[c]	Expected ratio[d]	χ^2 test	df	*P* value
Mountain Hoosier × Minilee							
P_aS_1[e]	27	27	0				
P_bS_1[f]	26	0	26				
F_1	58	58	0				
F_2	249	229	20	15:1	1.06	1	0.30
BC_1P_a	84	84	0	1:0			
BC_1P_b	82	59	23	3:1	0.26	1	0.61
Early Arizona × Minilee							
P_aS_1[e]	26	26	0				
P_bS_1[f]	28	0	28				
F_1	56	56	0				
F_2	257	239	18	15:1	0.27	1	0.61
BC_1P_a	83	83	0	1:0			
BC_1P_b	86	62	24	3:1	0.57		0.45
Pooled over families							
P_aS_1[e]	53	53	0				
P_bS_1[f]	54	0	54				
F_1	114	114	0				
F_2	506	468	38	15:1	1.20	1	0.27
BC_1P_a	167	167	0	1:0			
BC_1P_b	168	121	47	3:1	0.79		0.37

[a] Data are ratings from family Mountain Hoosier × Minilee and Early Arizona × Minilee of *Citrullus lanatus var. lanatus* from 3 locations namely Kinston, Clinton (M), and Clinton (P).

[b] Solid dark was the standard rind pattern.

[c] Light green was the mutant rind pattern.

[d] Expected was the hypothesized segregation ratio for duplicate dominant epistasis inheritance for each segregating generation.

[e] P_a: Parent A was carrier of dominant allele (solid dark green).

[f] P_b: Parent B was carrier of recessive allele (light green).

Pedigrees of Early Arizona and Mountain Hoosier are not known. Seeds of California Klondike are not available that were used in previous studies. In future, it is worthwhile to make crosses between Mountain Hoosier and Early Arizona to check if they are allelic for solid dark rind pattern. There is currently no information if there is any cultivar that has been bred with *G-1* and *G-2* together.

The genotype of light green rind fruited watermelon would be *g-1g-1g-2g-2*. Watermelon breeders should consider this inheritance pattern to develop cultivars with solid dark green or light green rind.

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