

Interaction of Flesh Color Genes in Watermelon

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Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] flesh color is controlled by several genes to produce red, orange, salmon yellow, canary yellow, or white. The objective of these experiments was to study the interaction of three independently reported gene loci, each having two or three alleles: *C* (canary yellow) versus *c* (red), *y* (salmon yellow) versus *Y* (red) versus *y*^o (orange), and *i* (inhibitory to *C*) versus *I* (noninhibitory to *C*). The interaction of *C*, *y*, *y*^o, and *i* is of interest to those developing new cultivars of watermelon and has not been reported previously. Five crosses were used to study gene action: Yellow Baby × Tendersweet Orange Flesh, Yellow Doll × Tendersweet Orange Flesh, Yellow Baby × Golden Honey, Yellow Doll × Golden Honey, and Yellow Baby × Sweet Princess. Based on performance of P_A, P_B, F₁, F₂, BC_{1A}, and BC_{1B}, the parents have the following genotypes: Yellow Baby = *CCYYII*, Yellow Doll = *CCYYII*, Tendersweet Orange Flesh = *ccy^oy^oII*, Golden Honey = *ccyyII*, and Sweet Princess = *ccYYii*. Segregation of flesh color in the progeny of the five families supported the previous report of a multiple allelic series at the *y* locus, where *Y* (red) was dominant to *y*^o (orange) and *y* (salmon yellow). In conclusion, epistasis is involved in the genes for major flesh colors in watermelon, with *ii* inhibitory to *CC* (canary yellow), resulting in red flesh, and *CC* (in the absence of *ii*) epistatic to *YY*, producing canary flesh.

Several genes have been identified that control flesh color in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Henderson 1989, 1991; Robinson et al. 1976). A brilliant yellow flesh color called canary yellow (*C*) is dominant to red flesh (*c*) (Poole 1944). Red flesh (*Y*) is dominant to salmon yellow (a pale yellow; *y*) found in Golden Honey (Poole 1944; Porter 1937). Also, orange flesh (*y*^o) from Tendersweet Orange Flesh is a member of a multiple allelic system at that locus, where *Y* (red flesh) is dominant to both *y*^o (orange flesh) and *y* (salmon yellow flesh), and *y*^o (orange flesh) is dominant to *y* (salmon yellow flesh) (Henderson 1989). In a separate set of studies using genes from cultivated and noncultivated, bitter-fruited forms of *C. lanatus*, Shimotsuma (1963) suggested that two loci with epistatic interaction controlled white, yellow, and red flesh. He showed that *Wf* produced white flesh and was epistatic to the locus he named *b*. The recessive allele *wf* allowed color to develop, producing yellow flesh in combination with *B* and red flesh *b*. Thus Shimotsuma (1963) obtained a ratio of 12 white:3 yellow:1 red in the F₂ generation of a cross between white and red flesh inbred lines. The genotypes of the F₂ must have been as

follows: white = *WfL_B* or *WfL_{bb}*, yellow = *wfwfB*, and red = *wfwfbb*. Both *B* and *C* are dominant to red flesh. It is not known whether *B* for yellow flesh and *C* for canary yellow flesh are the same locus.

The above loci can result in four flesh colors that have commercial utility in the United States watermelon market and are of interest to plant breeders. Those are canary yellow flesh found in Yellow Baby and Yellow Doll, orange flesh found in Tendersweet Orange Flesh, salmon yellow flesh found in Golden Honey, and red flesh found in Sweet Princess. For simplicity, these colors will be referred to hereafter as canary, orange, salmon, and red.

The behavior of combinations of the commercially useful flesh color alleles in watermelon have not been reported previously. The objective of these experiments was to study the inheritance and interaction of *C*, *c*, *Y*, *y*^o, and *y* on flesh color in five watermelon crosses.

Materials and Methods

Controlled pollinations were made in the greenhouse in Raleigh, North Carolina, to generate the following five crosses: (1) Yel-

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Table 1. Segregation and chi-square goodness-of-fit test for watermelon flesh color in the cross Yellow Baby (canary) × Tendersweet Orange Flesh (orange)^a

Generation	Genotype or source	Observed (C+O:red)	Expected (C+O:red)	χ^2	<i>P</i>
Segregation data					
Yellow Baby (P _A)	CCYY	48:0	1:0	0	1
TOF (P _B)	ccy ^o y ^o	21:0	1:0	0	1
F ₁	CcYy ^o	18:0	1:0	0	1
F ₂	Segregating	116:32	13:3	0.801	.371
BC _{1PA}	C.Y.	24:0	1:0	0	1
BC _{1PB}	Segregating	66:24	3:1	0.133	.715
Chi-square heterogeneity test					
F ₂	Sum of 5 chi squares	5 df	13:3	1.747	.833
	Pooled	1 df	13:3	0.801	.371
	Heterogeneity	4 df	13:3	0.946	.918
BC _{1PB}	Sum of 4 chi squares	4 df	3:1	3.357	.500
	Pooled	1 df	3:1	0.133	.715
	Heterogeneity	3 df	3:1	3.224	.358

^a C+O is the combination of canary and orange. F₂ segregated C+O (C.Y., C₂y^oy^o, ccy^oy^o) and red (ccY.), BC_{1B} segregated C+O (CcY^o, Ccy^oy^o, ccy^oy^o) and red (ccY^o). Expected ratios are 13 canary or orange:3 red for F₂, and 3 canary or orange:1 red for BC_{1B}.

low Baby × Tendersweet Orange Flesh, (2) Yellow Doll × Tendersweet Orange Flesh, (3) Yellow Baby × Golden Honey, (4) Yellow Doll × Golden Honey, and (5) Yellow Baby × Sweet Princess. The F₁, F₂, BC_{1A}, and BC_{1B} generations were produced from P_A and P_B, respectively, in each cross. Sufficient numbers of plants could not be handled at one time, so two to six sets were planted using different planting dates or different years, and tested for chi-square heterogeneity before pooling to form the six generations of the five crosses. Data were analyzed for each cross and generation, fitting the observed to expected ratios using a chi-square goodness-of-fit test.

Plants were seeded in a sandy loam soil at the Central Crops Research station near Clayton, North Carolina. Rows were 3.1 m apart, with plants 2.5 m apart within rows. Irrigation was applied to supplement rain-

fall for 25–38 mm total per week. Fertilizer was applied with 90-39-74 kg/ha before planting and 34-0-0 kg/ha at vine tip-over stage.

Fruits were harvested at peak maturity (as indicated by thumping the fruit, the rind color, and ground spot color of the fruit), cut crosswise, and immediately classified for flesh color. One hour later fruits were rechecked for flesh color both before and after cutting a 25 mm slice crosswise off the fruit. Phenotypes such as salmon (and to a lesser extent orange) became noticeably lighter in flesh color after being exposed to the sunlight. Flesh color was based on several fruits for each plant.

In crosses of cultivars having canary versus orange flesh color, or having canary versus salmon flesh color, it was often difficult to separate the different shades of canary, orange, and salmon

flesh color in the segregating progeny. In those cases flesh color was categorized as red versus the other colors (canary, orange, or salmon). Attempts to separate the canary, orange, or salmon flesh colors produced inconsistent results, indicating that the investigator was unable to rate accurately the intermediate types resulting from the interaction of the color genes.

Results and Discussion

Five crosses were studied to determine the effect on flesh color of canary yellow (C) in combination with red (Y), orange (y^o), and salmon (y). The crosses indicated that the alleles and loci did not behave in a simple way, but that there was epistasis. Also it became apparent that in cross 5, there was a third locus involved in flesh color, which appeared to inhibit the expression of canary yellow (C). We named the newly discovered locus *inhibitor of canary yellow*, with the proposed gene symbol *i*. Our evidence is presented in the results for the five crosses as follows.

Cross 1: Yellow Baby × Tendersweet Orange Flesh

Study of the segregation of flesh color in the progeny of cross 1 (Table 1) indicated that the parental genotypes were as follows: Yellow Baby = CCYY and Tendersweet Orange Flesh = ccy^oy^o. Since both parents were homozygous for *I*, *C* should have been expressed fully in all offspring. Consequently *I* was not segregating or observed in the following genotypes. The gene *C* was epistatic to *Y* producing canary flesh. Further, because of difficulty in classifying some segregants as canary versus orange, they were all grouped into one class. Red-fleshed segregants were placed into a second class. The expected F₂ segregation ratio would then be as follows: 13 canary or orange (9 C.Y. + 3 C₂y^oy^o + 1 ccy^oy^o):3 red (3 ccY.). The BC_{1B} (to Tendersweet Orange Flesh) would be expected to segregate 3 canary or orange (1 CcY^o + 1 Ccy^oy^o + 1 ccy^oy^o):1 red (1 ccY^o). The F₁ and BC_{1A} (to Yellow Baby) were all canary as expected. In some plants we observed an intermediate orange-canary flesh color. That may be due to the presence of modifier genes, or to the possibility that canary (C) may not be epistatic to y^o as it is to Y.

The generation chi-square values were a good fit to the proposed ratios (Table 1). The heterogeneity test indicated uniformity among families, confirming the validity of the pooled data and the hypothesis.

Table 2. Segregation and chi-square goodness-of-fit test for watermelon flesh color in the cross Yellow Doll (canary) × Tendersweet Orange Flesh (orange)^a

Generation	Genotype or source	Observed (C+O:red)	Expected (C+O:red)	χ^2	<i>P</i>
Segregation data					
Yellow Doll (P _A)	CCYY	541:0	1:0	0	1
TOF (P _B)	ccy ^o y ^o	21:0	1:0	0	1
F ₁	CcYy ^o	28:0	1:0	0	1
F ₂	Segregating	34:9	13:3	0.134	.714
BC _{1PA}	C.Y.	26:0	1:0	0	1
BC _{1PB}	Segregating	27:10	3:1	0.081	.776
Chi-square heterogeneity test					
F ₂	Sum of 2 chi squares	2 df	13:3	1.707	.426
	Pooled	1 df	13:3	0.134	.714
	Heterogeneity	1 df	13:3	1.573	.210
BC _{1PB}	Sum of 2 chi squares	2 df	3:1	0.176	.916
	Pooled	1 df	3:1	0.081	.776
	Heterogeneity	1 df	3:1	0.095	.773

^a C+O is the combination of canary and orange. F₂ segregated C+O (C.Y., C₂y^oy^o, ccy^oy^o) and red (ccY.), BC_{1B} segregated C+O (CcY^o, Ccy^oy^o, ccy^oy^o) and red (ccY^o). Expected ratios are 13 canary or orange:3 red for F₂, and 3 canary or orange:1 red for BC_{1B}.

Table 3. Segregation and chi-square goodness-of-fit test for watermelon flesh color in the cross Yellow Baby (canary) × Golden Honey (salmon)^a

Generation	Genotype or source	Observed (C+S:red)	Expected (C+S:red)	χ^2	P
Segregation data					
Yellow Baby (P _A)	CCYY	48:0	1:0	0	1
Golden Honey (P _B)	ccyy	26:0	1:0	0	1
F ₁	CcYy	19:0	1:0	0	1
F ₂	Segregating	113:37	13:3	3.447	.063
BC _{1PA}	C ₁ Y ₁	19:0	1:3	0	1
BC _{1PB}	Segregating	55:24	3:1	1.220	.269
Chi-square heterogeneity test					
F ₂	Sum of 5 chi squares	5 df	13:3	6.501	.260
	Pooled	1 df	13:3	3.447	.063
	Heterogeneity	4 df	13:3	3.054	.549
BC _{1PB}	Sum of 4 chi squares	4 df	3:1	4.346	.361
	Pooled	1 df	3:1	1.220	.269
	Heterogeneity	3 df	3:1	3.125	.372

^a C+S is the combination of canary and salmon. F₂ segregated C+S (C₁Y₁, C₁yy, ccyy) and red (ccY₁), BC_{1B} segregated C+S (CcY₁, C₁yy, ccyy) and red (ccY₁). Expected ratios are 13 canary or salmon:3 red for F₂, and 3 canary or salmon:1 red for BC_{1B}.

Cross 2: Yellow Doll × Tendersweet Orange Flesh

Study of the segregation of flesh color in the progeny of cross 2 (Table 2) indicated that the parental genotypes were as follows: Yellow Doll = CCYY and Tendersweet Orange Flesh = ccY^o. As for cross 1, segregants were combined as canary and orange versus red. Once again the expected F₂ segregation ratio would then be as follows: 13 canary or orange (9 C₁Y₁ + 3 C₁y^o + 1 ccY^o):3 red (3 ccY₁). The BC_{1B} (to Tendersweet Orange Flesh) would be expected to segregate 3 canary or orange (1 CcY^o + 1 Ccy^o + 1 ccY^o):1 red (1 ccY^o). The F₁ and BC_{1A} (to Yellow Doll) were all Canary as expected.

The generation chi-square values were a good fit to the proposed ratios (Table 2). The heterogeneity test indicated uniformity among families, confirming the validity of the pooled data and the hypothesis.

Cross 3: Yellow Baby × Golden Honey

Study of the segregation of flesh color in the progeny of cross 3 (Table 3) indicated that the parental genotypes were as follows: Yellow Baby = CCYY and Golden Honey = ccyy. The major difference between crosses 1 and 2 versus crosses 3 and 4 was that yy produced a salmon flesh color, whereas y^o produced an orange flesh color. Similar to crosses 1 and 2, canary and salmon segregants were grouped into one class, while dark red and light red segregants were grouped into a second class. The expected F₂ segregation ratio would be as follows: 13 canary or salmon (9 C₁Y₁ + 3 C₁yy + 1 ccyy):3 red (3 ccY₁). The BC_{1B} (to Golden Honey) would be expected to segregate 3 canary or salmon (1 CcY₁ + 1 Ccy₁ + 1 ccyy):1 red (1 ccY₁). The F₁ and BC_{1A} (to Yellow Baby) were all canary as expected.

The generation chi-square values were a

good fit to the proposed ratios (Table 3). The heterogeneity chi-square test showed uniformity among families, confirming the validity of the pooled data and the hypothesis.

Cross 4: Yellow Doll × Golden Honey

Study of the segregation of flesh color in the progeny of cross 4 (Table 4) indicated that the parental genotypes were as follows: Yellow Doll = CCYY and Golden Honey = ccyy. As in cross 3, canary and salmon segregants were grouped into one class, while dark red and light red segregants were grouped into a second class. The expected F₂ segregation ratio would be as follows: 13 canary or salmon (9 C₁Y₁ + 3 C₁yy + 1 ccyy):3 red (3 ccY₁). The BC_{1B} (to Golden Honey) would be expected to segregate 3 canary or salmon (1 CcY₁ + 1 Ccy₁ + 1 ccyy):1 red (1 ccY₁). The F₁ and BC_{1A} (to Yellow Doll) was all canary as expected.

The generation chi-square values were a good fit to the proposed ratios (Table 4). The heterogeneity chi-square test showed uniformity among families, confirming the validity of the pooled data and the hypothesis.

Cross 5: Yellow Baby × Sweet Princess

Study of the segregation of flesh color in the progeny of cross 5 (Table 5) indicated that there was a third locus acting in the expression of flesh color. The locus was named *inhibitor of canary yellow*, with the proposed gene symbol *i*. In the segregating progeny, it appears that the allele *i* was inhibitory to *C* allowing *Y* to produce red flesh. The allele *I* was noninhibitory, thus allowing *C* to function. The allele *C* was epistatic to *Y*, thus producing canary flesh. Accordingly the parental genotypes must have been as follows: Yellow Baby = CCYYII and Sweet Princess = ccYYii. The expected F₂ segregation would thus be 9 C₁YYL = canary, 3 C₁YYii = red, 3 ccYYL = red, and 1 ccYYii = red to produce a phenotypic ratio of 9 canary:7 red. The BC_{1B} (to Sweet Princess) is expected to produce 3 red (1 CcYYii + 1 ccYYLi + 1 ccYYii):1 canary (1 CcYYII). The F₁ and BC_{1A} (to Yellow Baby) were all canary as expected.

The generation chi-square values showed a good fit to the expected ratios (Table 5). The heterogeneity chi-square test showed that segregation among families was uniform. Therefore reliability can be placed in the pooled segregation data and the proposed hypothesis can be accepted. Interestingly fruits were found

Table 4. Segregation and chi-square goodness-of-fit test for watermelon flesh color in the cross Yellow Doll (canary) × Golden Honey (salmon)^a

Generation	Genotype or source	Observed (C+S:red)	Expected (C+S:red)	χ^2	P
Segregation data					
Yellow Doll (P _A)	CCYY	51:0	1:0	0	1
Golden Honey (P _B)	ccyy	26:0	1:0	0	1
F ₁	CcYy	21:0	1:0	0	1
F ₂	Segregating	53:12	13:3	0	1
BC _{1PA}	C ₁ Y ₁	4:0	1:0	0	1
BC _{1PB}	Segregating	32:10	3:1	0	1
Chi-square heterogeneity test					
F ₂	Sum of 3 chi squares	3 df	13:3	1.118	.773
	Pooled	1 df	13:3	0.004	.950
	Heterogeneity	2 df	13:3	1.114	.573
BC _{1PB}	Sum of 3 chi squares	3 df	3:1	2.586	.460
	Pooled	1 df	3:1	0.032	.858
	Heterogeneity	2 df	3:1	2.554	.279

^a C+S is the combination of canary and salmon. F₂ segregated C+S (C₁Y₁, C₁yy, ccyy) and red (ccY₁), BC_{1B} segregated C+S (CcY₁, C₁yy, ccyy) and red (ccY₁). Expected ratios are 13 canary or salmon:3 red for F₂, and 3 canary or salmon:1 red for BC_{1B}.

Table 5. Segregation and chi-square goodness-of-fit test for watermelon flesh color in the cross Yellow Baby (canary) × Sweet Princess (red)^a

Generation	Genotype or source	Observed (canary:red)	Expected (canary:red)	χ^2	<i>P</i>
Segregation data					
Yellow Baby (P _A)	<i>CCYYII</i>	48:0	1:0	0	1
Sweet Princess (P _B)	<i>ccYYii</i>	0:23	0:1	0	1
F ₁	<i>CcYYIi</i>	21:0	1:0	0	1
F ₂	Segregating	77:52	9:7	0.620	.431
BC _{1PA}	<i>C₋YYL</i>	16:0	1:0	0	1
BC _{1PB}	Segregating	16:64	1:3	1.067	.302
Chi-square heterogeneity test					
F ₂	Sum of 6 chi squares	6 df	9:7	4.087	.665
	Pooled	1 df	9:7	0.620	.431
	Heterogeneity	5 df	9:7	3.467	.628
BC _{1PB}	Sum of 4 chi squares	4 df	3:1	6.511	.164
	Pooled	1 df	3:1	1.067	.302
	Heterogeneity	3 df	3:1	5.444	.142

^a F₂: *C₋YYL* = canary; *ccYYL*, *C₋YYii*, *ccYYii* = red. BC_{1B}: *CcYYii* = canary; *CcYYii*, *ccYYii*, *ccYYii* = red. Expected ratios are 9 canary:7 red for F₂, and 3 red:1 canary for BC_{1B}.

that contained both yellow and red areas (either swirled together or as separate sectors) similar to that found by Navot et al. (1990). Such fruits were classified as red unless the fruit was predominately canary with only a very small, light-pink area. Modifier genes, in addition to the major genes proposed here, may also be present. Also the heterozygote (*Cc*) may not be completely dominant or completely epistatic under all conditions of environment or fruit maturity.

Conclusions

Thus the proposed genotypes of the parents are Yellow Baby = *CCYYII*, Yellow Doll

= *CCYYII*, Sweet Princess = *ccYYii*, Tendersweet Orange Flesh = *ccy^oYII* and Golden Honey = *ccyyII*. The data supported previous findings of a multiple allelic series at the *y* locus, where *Y* (red) is dominant to *y^o* (orange) and *y* (salmon), and *y^o* (orange) is dominant to *y* (salmon). At the other two loci, *ii* is epistatic to *CC* (canary), and in combination with *YY* results in red flesh. Further, *CC* in the absence of *ii* is epistatic to *YY*, thus producing canary flesh.

The homozygous genotypes produced by the three gene loci should have the following eight phenotypes that we have verified: *CCYYII* = canary, *CCYYii* = red,

CCy^oYII = canary-orange, *CCyyII* = canary-salmon, *ccYYII* = red, *ccYYii* = red, *ccy^oYII* = orange, and *ccyyII* = salmon; and the following six phenotypes that are still hypothetical: *CCy^oYii* = orange, *CCy^oyii* = salmon, *ccy^oYii* = orange, and *ccy^oyii* = salmon.

Additional studies are needed to determine whether *CC* is epistatic to *y^o* the way it is to *YY*, to verify the hypothetical phenotypes of the four genotypes listed above that have not yet been verified, and to check for possible allelism of the *B* and *C* loci for canary yellow flesh color.

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