THE GENETICS OF ANTHOCYANIN COLORATION IN EGGPLANT (*SOLANUM MELONGENA* L.)*

E. C. TIGCHELAAR, JULES JANICK, AND H. T. ERICKSON

Department of Horticulture, Purdue University, Lafayette, Indiana

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EARLY genetic studies of anthocyanin coloration in eggplant (HALSTEAD 1918; NOLLA 1932) deal primarily with genes which control the presence or absence of pigmentation in the fruit. TATEBE (1939, 1944), the first to consider the interactions between fruit pigmentation genes, reported six independently inherited factors (*C*, *P*, *D*, *G*, *Gv*, and *Puc*). Three dominant genes (*C*, *P*, and *D*) cooperate in the development of anthocyanin in the fruit. *C* is assumed to control the production of yellow flavone pigments, chromogenic substances for the production of anthocyanin, but genetic evidence for this gene is not given. Genes *P* and *D* are dominant complementary factors which are necessary for the formation of anthocyanin pigments in the fruit. *P* is also necessary for the development of anthocyanin in the corolla and other plant parts. *G* is required for the formation of chlorophyll in the fruit flesh and *Gv* controls green variegation of the fruit. *Puc* (pigment under the calyx) in the presence of *D* and *P* controls the light independent synthesis of anthocyanin in the fruit.

JANICK and TOPOLESKI (1963) report an additional complementary gene *X* closely linked to *D*. It was proposed that gradations in anthocyanin coloration occurred as the number of dominant alleles of *D* and *X* increase from two to four. Thus only rare crossovers between *D* and *X* would produce intensely pigmented recombinants from repulsion phase crosses. Linkage was also indicated between *Puc* and *X* on the basis of an association of plant color intensity with *Puc*. It is the purpose of the present study to examine the proposed linkages of *D*, *X* and *Puc* and to extend information on the genetics of anthocyanin development and distribution in the eggplant.

MATERIALS AND METHODS

The source and description of the varieties used in the present study is presented in Table 1.

The linkage of *D* and *X* was examined by F_3 progeny tests involving the anthocyanin complementing varieties Kantoao (*d X*) and White Beauty (*D x*) (JANICK and TOPOLESKI, 1963). These varieties, whose fruit lack anthocyanin, produce an F_1 with lightly pigmented fruit. In the backcross to Kantoao ($d\ X/D\ x \times dX$), progeny testing using hypocotyl coloration as a seedling marker permitted identification of genotypes for *X* (only the heterozygote *Xx* segregates for hypocotyl color). Genotypes for *D* were determined directly, (segregates with anthocyanin pigmented fruit are *Dd*, non-pigmented segregates are *dd*). Tests of linkage were thus based on genotype frequencies rather than on phenotype frequencies. In a similar manner, progeny tests

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TABLE 1

Source and description of eggplant varieties

Variety or accession	PI accession number	Source*	Origin	Anthocyanin pigmentation†			Remarks
				Corolla	Hypocotyl	Fruit	
A1	213026	(1)	India	+	—	—	Variegated fruit
A12	164286	(1)	India	+	—	—	.....
A29	175915	(1)	Turkey	+	+	—	White flesh
A43	198330	(1)	India	—	—	—	White flesh
A52	213194	(1)	Greece	+	+	—	Striped fruit
A63	249569	(1)	Thailand	—	—	—	Variegated fruit
A64	249568	(1)	Thailand	+(dilute)	+	—	Flower tinge
Aonasu	.....	(2)	Japan	—	—	—	Small seed
Kantoao	.....	(2)	Japan	+	+	—	.....
Long White	.....	(3)	U.S.A.	+	—	—	.....
Osaka Maru	.....	(2)	Japan	+ DP	+ DP	+ DP	White flesh
Puerto Rican Beauty	.....	(4)	Puerto Rico	+ LV	+ LV	+ DV	.....
Rosita	.....	(4)	Puerto Rico	+ LV	+ LV	+ LV	White flesh
Sinkuro	.....	(2)	Japan	+ DP	+ DP	+ DP	No pigment under calyx
White Beauty	.....	(3)	U.S.A.	+	—	—	White flesh

* (1) W. R. LANGFORD, Coordinator, Southern Regional Plant Introduction Station, Experiment, Ga.
(2) S. NISHI, Dept. of Vegetable Crops, Hort. Research Station. Hiratsuka, Kanagawa, Japan.
(3) Gleckers Seedsman, Metamora, Ohio.
(4) H. AZZAM, University of Puerto Rico, Rio Piedras, Puerto Rico.

† + = anthocyanin pigmented, — = non-pigmented, D = dark, L = light, P = purple, V = vinaceous.

were used to determine genotypes for *X* in colored segregates of crosses involving Sinkuro (*D X puc*) and Long White (*D x Puc*) to test the proposed linkage of *X* and *Puc*.

New complementary genes were identified by tests of allelism in which accessions with non-anthocyanin pigmented fruit were crossed with Kantoao and White Beauty. Accessions which complemented with both testers or which failed to complement with either were considered as new genotypes and were subsequently intercrossed. All resulting F_1 hybrids were selfed and F_2 progenies scored for pigmentation in the corolla, hypocotyl, and fruit.

The inheritance of anthocyanin quantity, quality, and distribution was studied in crosses in which both parents formed anthocyanin in fruit and other plant parts. Four contrasting color phenotypes were selected: Sinkuro, Puerto Rican Beauty, Rosita, and Osaka Maru. Segregating crosses were scored visually for color intensity using a numerical color rating scale from 1 to 5 (1 being the color rating of the light colored parent and 5 of the dark parent). F_3 progeny tests were employed as a further aid in determining the inheritance of color intensity.

In the crosses involving Sinkuro, Rosita, and Puerto Rican Beauty the anthocyanins were examined qualitatively. Fruit peel tissue was dried in a forced air oven at 40°C for 36–48 hours, ground in a Wiley mill, and subsequently extracted overnight with a small portion (3–5 ml) of 1% acidified methanol. A single solvent system (n-butanol : acetic acid : water; 120:30:50 v/v) which gave clear separation of the component pigments was used in all chromatographic screening studies. Descending paper chromatography using known anthocyanins and several solvent systems was employed for identification of the major anthocyanin of each genotype.

RESULTS

Tests of linkage: Close linkage between *D* and *X* was proposed to explain the lack of intensely anthocyanin pigmented recombinants in the progeny of crosses of Kantoao (*d X*) and White Beauty (*D x*) (JANICK and TOPOLESKI 1963). It was assumed that the major variations in color intensity resulted from dosage effects of the complementary genes *D* and *X*. The quantitative effects of complementary genes on color intensity are, however, difficult to assess and furthermore, genetic ratios of colored to non-colored do not clearly identify linkage. Backcrosses to either parent give ratios of 1 colored: 1 non-colored with or without linkage. Expected F_2 ratios of 9 colored: 7 non-colored with independence and 1 colored : 1 non-colored with complete linkage are difficult to distinguish statistically.

The proposed linkage of *D* and *X* can be tested, however, by progeny tests of segregates from backcrosses to Kantoao to determining genotype frequencies. Two genotypes are expected with complete linkage while four genotypes are expected with independence. Four genotypes in equal frequencies were identified which precludes close linkage and indicates independence of *D* and *X* (Table 2).

To test the independence of *X* and *Puc*, an analysis was made using F_3 families segregating for both *X* and *Puc* from a cross of Sinkuro (*X puc*) and Long White (*x Puc*). Since *Puc* is expressed only when fruit pigment is formed, only colored plants (*X* –) were progeny tested to determine the genotypes for *X*. Results in Table 2 indicate independence of *X* and *Puc*.

The linkage model of *D*, *X*, and *Puc* proposed by JANICK and TOPOLESKI (1963) accounted for the failure to recover "darkly pigmented recombinants" in the progeny of crosses of White Beauty × Kantoao and explained the association of color intensity with *Puc*. This model fails, however, to conform to critical

TABLE 2

Association of D *with* X *and* X *with* Puc

Cross	Progeny genotypes			
Dd Xx × *dd XX*	*Dd XX*	*Dd Xx*	*dd XX*	*dd Xx*
Observed	12	13	10	13
Expected with independence	12	12	12	12
Xx Puc puc self	*XX Puc-*	*Xx Puc-*	*XX puc puc*	*Xx puc puc*
Observed	54	114	21	39
Expected with independence	57	114	19	38

genetic analysis indicating that other genetic factors which modify anthocyanin intensity must be involved. This has led to the present search for other genes controlling anthocyanin coloration.

In this paper the genes involved in anthocyanin coloration are classified into two functionally distinct groups: 1. Basic color genes which are required to be in the dominant condition for the development of anthocyanin in the fruit. 2. Modifier genes which are hypostatic to the basic color genes and which quantitatively or qualitatively alter anthocyanin color.

Basic color genes: New basic color genes were identified by tests of allelism between accessions with non-anthocyanin pigmented fruit and Kantoao and White Beauty. Of eight accessions tested (Table 3), two show a unique behavior for fruit color complementation. A43 complements with both testers while A63 fails to complement with either. Both accessions complement with White Beauty for hypocotyl coloration. The remaining accessions complement with one tester

TABLE 3

F_1 complementation between accessions with non-anthocyanin pigmented fruit and tester varieties Kantoao and White Beauty

Accession or variety	Anthocyanin pigmentation		F_1 complementation*			
			Kantoao (*dd XX*)		White Beauty (*DD xx*)	
	Hypocotyl	Fruit	Hypocotyl	Fruit	Hypocotyl	Fruit
A1	—	—	(+)	+	—	—
A12	—	—	(+)	+	—	—
A29	+	—	(+)	—	(+)	+
A43	—	—	(+)	+	+	+
A52	+	—	(+)	—	(+)	+
A63	—	—	(+)	—	+	—
A64	+	—	(+)	—	(+)	+
Aonasu	—	—	(+)	+	—	—

* Kantoao = + hypocotyl, — fruit; White Beauty = — hypocotyl, — fruit.
(+) = No test of complementation since one parent pigmented.
\+ = Complementation (F_1 anthocyanin pigmented).
— = Non-complementation (F_1 non-pigmented).

only, indicating allelism (for basic color genes) with the tester with which there is no complementation. Aonasu, although similar to White Beauty in terms of complementation, is phenotypically different in that anthocyanin is absent from all plant parts including the corolla. According to TATEBE (1944) the genotype of Aonasu is *DD pp*.

The F_1 and F_2 complementation behavior of the three selected accessions (Aonasu, A43, and A63) and the tester parents is given in Table 4. Aonasu and White Beauty must have recessive basic color genes in common since they fail to complement for hypocotyl or fruit coloration and ratios in complementing crosses[1] indicate that each carries a single recessive basic color gene. Since the recessive allele carried by Aonasu was originally symbolized *p* (TATEBE 1939), we have adopted this symbolism to replace *x*. Three alleles are proposed at this locus (*P*, *p*, and p^w in order of dominance) where *p* prevents anthocyanin development in the hypocotyl and fruit and p^w blocks anthocyanin synthesis in all plant parts including the corolla. The genotypes of White Beauty and Aonasu are now symbolized as *DD pp* and *DD* p^wp^w, respectively.

A43 complements for fruit color with all accessions except A63 and complements for hypocotyl and corolla color in all testable cases. Observed F_2 ratios for corolla, hypocotyl, and fruit coloration (Table 4) in crosses with Kantoao, White Beauty, and Aonasu show a good fit to the expectation assuming that A43 carries a third basic color gene in the homozygous recessive condition which prevents pigment development in all plant parts. This gene has been symbolized *Y*. The proposed genotype of A43 is thus *DD PP yy*; all other genotypes are *YY*.

A63 fails to complement for fruit anthocyanin coloration in all F_1 hybrids except in crosses with Aonasu. However, colored recombinants are observed in anomalous ratios in the F_2's. Complementation for corolla and hypocotyl coloration occurs in all testable cases and normal F_2 ratios are observed.

The absence of anthocyanin colored fruit in the F_2 of A63 × Kantoao indicates allelism for the recessive allele of the basic color gene *D*. The gene carried by A63 is, however, functionally distinct from the *d* allele of Kantoao because in A63 anthocyanin develops neither in the corolla, hypocotyl, nor fruit. The allele carried by A63 has been symbolized d^w so that three alleles (*D*, *d*, and d^w in order of dominance) occur at this locus. The proposed genotypes of Kantoao and A63 are *dd PP YY* and d^wd^w *PP YY*, respectively.

Fruit color ratios in the F_2 of complementing crosses involving A63 deviate from the expected 9:7 ratio, indicating the existence of other factors which specifically inhibit fruit pigmentation. The ratio in the F_2 of A43 × A63 (Table 4) fits a ratio of 9 colored: 55 non-colored and can be explained by assuming that A63 carries a dominant inhibitor of fruit pigmentation in addition to the basic color gene d^w. This inhibitor gene has tentatively been symbolized *R*. Results from other complementing crosses (A63 × White Beauty; A63 × Aonasu), how-

[1] F_2 are interpreted as follows: 9:7 indicates normal 2 factor complementation; 3:1 indicates single gene segregations where one parent forms pigment; 0:1 indicates allelism for basic color genes in non-complementing F_1's; other ratios indicate additional factors to be involved.

TABLE 4

F_1 complementation and F_2 ratios for corolla, hypocotyl, and fruit coloration (+ = anthocyanin pigmented; — = non-pigmented) in complementing crosses

Cross	Plant part	F_1* complementation	F_2 Observed ratio +	—	F_2 Expected ratio‡	χ^2	P†
Aonasu × Kantoao	Corolla	(+)	156	63	3:1	1.65	.20
	Hypocotyl	(+)	156	63	3:1	1.65	.20
	Fruit	+	122	97	9:7	0.03	.85
Aonasu × White Beauty	Corolla	(+)	40	20	3:1	2.22	.15
	Hypocotyl	—	0	60	0:1	. . .	. .
	Fruit	—	0	60	0:1	. . .	. .
Aonasu × A43	Corolla	+	28	21	9:7	.02	.85
	Hypocotyl	+	28	21	9:7	.02	.85
	Fruit	+	28	21	9:7	.02	.85
Aonasu × A63	Corolla	+	44	33	9:7	.03	.80
	Hypocotyl	+	44	33	9:7	.03	.80
	Fruit	+	28	49	9:7§	12.31	<.01
Kantoao × A43	Corolla	(+)	74	26	3:1	0.05	.85
	Hypocotyl	(+)	74	26	3:1	0.05	.85
	Fruit	+	55	45	9:7	0.06	.80
Kantoao × A63	Corolla	(+)	73	26	3:1	0.08	.75
	Hypocotyl	(+)	73	26	3:1	0.08	.75
	Fruit	—	0	99	0:1	. . .	. .
White Beauty × A43	Corolla	(+)	89	19	3:1	4.00	.04
	Hypocotyl	+	113	81	9:7	0.31	.60
	Fruit	+	53	55	9:7	2.27	.15
White Beauty × A63	Corolla	(+)	145	48	3:1	0.02	.85
	Hypocotyl	+	638	519	9:7	0.57	.90
	Fruit	—	46	147	9:7§	77.6	<.01
A43 × A63	Corolla	+	66	55	9:7	0.02	.85
	Hypocotyl	+	66	55	9:7	0.02	.85
	Fruit	—	16	102	9:7§	85.00	<.01
					9:55	0.02	.85

* (+) = no test of complementation since one parent pigmented.
\+ = complementation.
— = no complementation.
† Probability of a larger Chi-square value.
§ = Observed ratio significantly different from the expected.
‡ Refer Table 11 for genotypes.

ever, do not conform to those expected with a single dominant inhibitor gene.

Crosses involving A64 (Table 5) segregated for a lighter corolla color phenotype from that in other complementing crosses. The corolla color phenotype of A64 is designated "tinge". Ratios for corolla color in crosses with the tester parents

TABLE 5

Association of flower tinge with fruit coloration in crosses of A64 × Kantoao and A64 × White Beauty

Variety or cross	Fruit anthocyanin pigmented		Fruit non-pigmented		Expected ratio*	χ^2	P†
	Flower normal	Flower tinge	Flower normal	Flower tinge			
A64	. .	. .	. .	24	. .	. . .	. . .
Kantoao (K)	. .	. .	25	. .	. .	. . .	. . .
(A64 × K)F_1	. .	. .	22	. .	. .	. . .	. . .
(A64 × K) × K	. .	. .	117	0	1:0	. . .	. . .
(A64 × K) × A64	. .	. .	91	81	1:1	0.58	.45
(A64 × K)F_2	. .	. .	138	46	3:1	0.00	1.00
White Beauty (WB)	. .	. .	25	. .	. .	. . .	. . .
(A64 × WB)F_1	25	. .	. .	. .	. .	. . .	. . .
(A64 × WB) × WB	97	0	73	0	1:0:1:0	3.38	.08
(A64 × WB)F_2	90	0	35	44	27:9:21:7	(a)‡	(b)‡

* = Expected assuming independence of a single factor for flower and fruit coloration.
† = Probability of a larger Chi-square value.
‡ = (a) indicates a very large number: (b) a very small probability.

Kantoao and White Beauty fit the expected for single gene control of the "tinge" phenotype. Furthermore, the relationship of fruit color and corolla color in the F_2 of A64 × White Beauty indicates complete linkage between *D* or *P* and the gene controlling the tinge phenotype. Since A64 is recessive for an allele of *D*, an additional allele at this locus (or very close coupling phase linkage) is indicated. This gene is assumed to be an allele of *D* (symbolized d^t) so that four alleles (*D*, *d*, d^t, and d^w in order of dominance) occur at the *D* locus. The proposed genotype of A64 is $d^t d^t$ *PP YY*.

Modifier genes: These are considered in three functionally distinct groups: 1. Qualitative genes which induce changes in color through alterations in the chemical structure of the anthocyanins. 2. Distributor genes which control the formation of anthocyanin in specific parts of the plant. 3. Diluter genes which intensify or dilute anthocyanin color.

Four contrasting color phenotypes which differed in color quality, quantity, and distribution were used: Sinkuro and Osaka Maru produce intense purple pigmentation in all plant parts; Rosita produces a light vinaceous coloration in the hypocotyl, plant, and fruit; and Puerto Rican Beauty, similar to Rosita with respect to hypocotyl and plant color, produces intense vinaceous fruit color (Table 1).

Qualitative genes: Chromatographic analyses of crosses and backcrosses of Rosita × Sinkuro and Puerto Rican Beauty × Sinkuro (Table 6) indicate that there are two major anthocyanins present in these varieties. The major anthocyanin formed by Rosita and Puerto Rican Beauty is chromatographically identical with delphinidin-3-rhamnoglucoside while the major anthocyanin of the variety Sinkuro is the acylated anthocyanin "nasunin" (SAKAMURA, WATANABE

TABLE 6

Segregation for Ac *in crosses and backcrosses of Rosita × Sinkuro and Puerto Rican Beauty × Sinkuro*

Variety or cross	Major pigment		Expected ratio*	χ^2	P†
	Nasunin	Delphinidin-3-rhamnoglucoside			
Rosita (R)	..	9	..	...	..
Sinkuro (S)	9	..	..	...	..
(R × S)F_1	10	..	..	...	..
(R × S) × R	71	83	1:1	0.94	.35
(R × S)F_2	105	44	3:1	1.63	.20
Puerto Rican Beauty (PRB)	..	99	..	...	..
(PRB × S)F_1	8	..	..	...	..
(PRB × S) × PRB	68	76	1:1	0.44	.50
(PRB × S)F_2	100	39	3:1	0.69	.45

* Following genotypes assumed: R = *acac*; S = *AcAc*; PRB = *acac*.
† P = Probability of a larger Chi-square value.

and OBATA 1963). Co-chromatography with known anthocyanins[2] has been used to confirm these identifications. A single qualitative gene *Ac* (acylated anthocyanin) is proposed which determines the nature of the major anthocyanin of the fruit, converting dephinidin-3-rhamnoglucoside to nasunin. These structural alterations are reflected in a change in color from vinaceous (*ac ac*) to purple (*Ac–*).

Distributor genes: In this discussion, the term distributor gene refers to those factors which control anthocyanin formation in specific plant parts. Alleles of *D*, *P*, and *Y* differentially influence anthocyanin development in specific plant parts but were already discussed as "basic color genes." Gene *Puc* is considered a distributor gene because it controls the formation of anthocyanin under the calyx (in a light independent path of synthesis). Anthocyanin striping of the anthers (Table 7) is also controlled by a single gene which, in crosses of Rosita × Sinkuro and Puerto Rican Beauty × Sinkuro, shows complete coupling phase linkage with *Puc*. In crosses of Osaka Maru × Sinkuro, however, segregation for *Puc* without segregation for anther striping was observed. A single gene *Sa* is proposed which controls the formation of anthocyanin in the anthers. *Sa* may be closely linked to *Puc* or there may exist several allelic forms of *Puc* (see Table 13).

Diluter genes: Color intensity was extremely difficult to evaluate objectively. In crosses of Rosita × Sinkuro, the F_1 was intermediate in color intensity and a wide array of color combinations and intensities was evident in the F_2. In crosses of Puerto Rican Beauty × Sinkuro, a similar range of segregation occurred for

[2] Nasunin obtained through the generosity of DR. S. SAKAMURA, Department of Agr. Chem. Hokkaido University, Sapporo, Japan. Delphinidin-3-rhamnoglucoside reported to be the major anthocyanin of the eggplant variety Black Beauty (ABE and GOTOH 1957).

TABLE 7

Segregation for Puc *(anthocyanin pigment under the calyx) and* Sa *(anthocyanin striping of the anthers) in crosses of Rosita × Sinkuro, Puerto Rican Beauty × Sinkuro, and Osaka Maru × Sinkuro*

Variety or cross	Pigment under calyx		No pigment under calyx		Expected* ratio	χ^2†	P
	Anther stripe	No anther stripe	Anther stripe	No anther stripe			
Rosita (R)	32	..	..	..	.....	...	...
Sinkuro (S)	..	..	..	32	.....	...	...
(R × S)F_1	9	..	..	..	.....	...	...
(R × S) × R	154	0	0	0	1:0:0:0	...	...
(R × S) × S	84	0	0	72	1:1:1:1	(a)	(b)
(R × S)F_2	119	0	0	38	9:3:3:1	(a)	(b)
Puerto Rican Beauty (PRB)	32	..	..	..	.....	...	...
(PRB × S)F_1	32	..	..	..	.....	...	...
(PRB × S) × PRB	144	0	0	0	1:0:0:0	...	...
(PRB × S) × S	73	0	0	78	1:1:1:1	(a)	(b)
(PRB × S)F_2	115	0	0	42	9:3:3:1	(a)	(b)
Osaka Maru (OM)	14	..	..	..	.....	...	...
(OM × S)F_1	12	..	..	..	.....	...	...
(OM × S) × S	0	32	0	24	0:1:0:1	1.14	.30

* Following genotypes assumed: R = *PucPuc SaSa*; S = *pucpuc sasa*; PRB = *PucPuc SaSa*; OM = *PucPuc sasa.*

† (a) = indicates a very large number; (b) = a very small probability.

hypocotyl and plant color, however, there was little or no variation in fruit color.

Because of the difficulty in evaluating color intensity, F_3 progeny tests of hypocotyl color were used to determine the number of major genes involved. An estimate of two major genes was obtained from the percent of F_3 progenies which bred true for one or the other of the two parental phenotypes (Table 8). These genes (symbolized Dil_1 and Dil_2) appear to be incompletely dominant and additive in their effects on color intensity. Sinkuro is assumed to carry the dominant alleles (Dil_1, Dil_2) and Rosita and Puerto Rican Beauty recessive alleles (dil_1, dil_2).

In the F_1 of Rosita × Puerto Rican Beauty fruit color was intermediate between the two parental types and the color of vegetative plant parts was similar to that of the parents. The similarity in genetic behavior of Rosita and Puerto Rican Beauty in crosses with Sinkuro, and failure of these varieties to complement for plant color intensity indicates functionally distinct alleles at one (or both) diluter loci. Alleles carried by Rosita (symbolized dil^{pf}) repress both plant and fruit coloration, whereas alleles carried by Puerto Rican Beauty (symbolized dil^{p}) repress plant color only. The proposed genotypes of Sinkuro, Puerto Rican Beauty, and Rosita are Dil_1Dil_1 Dil_2Dil_2, $dil_1^{p}dil_1^{p}$ $dil_2^{p}dil_2^{p}$ and $dil_1^{pf}dil_1^{pf}$ $dil_2^{pf}dil_2^{pf}$, respectively.

Associations: In crosses of Sinkuro × Long White, JANICK and TOPOLESKI

TABLE 8

Percent of F_3 progenies which breed true for parental color phenotypes and intermediate phenotypes in crosses of Rosita × Sinkuro and Puerto Rican Beauty × Sinkuro

Cross		Percent homozygous F_3 progenies				Number tested	χ^2	P
		Percent segregating	Light pigmented	Inter-mediate	Dark pig-mented			
(Rosita × Sinkuro) × Sinkuro	Obs.	76.3	0	0	23.7	80	0.10	.75
	Exp.*	75.0	0	0	25.0			
(Rosita × Sinkuro)F_2	Obs.	75.6	7.7	12.8	5.1	78	0.82	.65
	Exp.*	75.0	6.25	12.5	6.25			
(Puerto Rican Beauty × Sinkuro) × Sinkuro	Obs.	51.4	0	0	48.6	35	32.2	.01
	Exp.*	75.0	0	0	25.0			
(Puerto Rican Beauty × Sinkuro) F_2	Obs.	70.6	5.9	13.2	10.3	68	0.92	.80
	Exp.*	75.0	6.25	12.5	6.25			

* Expected assuming two major diluter genes and parental genotypes as follows: Rosita—*dil_1dil_1 dil_2dil_2*; Puerto Rican Beauty—*dil_1dil_1 dil_2dil_2*; Sinkuro—*Dil_1Dil_1, Dil_2Dil_2*.

(1963) proposed linkage between *X* and *Puc* because of an association between *Puc* and color intensity. The association of *Puc*, as well as of *Ac*, with plant color intensity in crosses of Rosita × Sinkuro and Puerto Rican Beauty × Sinkuro is presented in Table 9. The dominant allele at *Puc* is associated with reduction in plant color intensity (.6–1.4 units) whereas the dominant allele at *Ac* is associated with increased plant coloration (.6–.9 units).

TABLE 9

Association of Ac *and* Puc *with mean visual evaluation of plant color intensity (1 = light, 5 = dark)*

Cross†	*Puc-*		*puc puc*		Effect of	
	Ac-	*ac ac*	*Ac-*	*ac ac*	*Puc*	*Ac*
Rosita (R)	. .	1.0	. .	. .	. .	. .
Sinkuro (S)	. .	. .	5.0	. .	. .	. .
(R × S) × R	2.4	1.7	. .	. .	. .	+.7*
(R × S) × S	3.9	. .	4.6	. .	—.7*	. .
(R × S)F_2	3.1	2.0	3.6	2.8	—.6*	+.9*
Puerto Rican Beauty (PRB)	1.0	. .	. .	. .	. .	. .
PRB × S	. .	. .	3.0	. .	. .	. .
(PRB × S) × PRB	2.2	1.7	. .	. .	. .	+.6*
(PRB × S) × S	4.0	. .	4.5	. .	—.6*	. .
(PRB × S)F_2	2.7	1.7	3.8	3.3	—1.4*	+.7*

* = significant at the 5% level
† = 148 plants examined for each cross

TABLE 10

Association of Ac *with* Dil_1 *and* Puc *with* Dil_2 *as determined by* F_3 *progeny analysis*

Cross	F_3 hypocotyl color segregation					
	All light		Intermediate or segregating		All dark	
	Ac-	*ac ac*	*Ac-*	*ac ac*	*Ac-*	*ac ac*
(Rosita × Sinkuro)F_2	2	4	49	19	4	0
(Puerto Rico Beauty × Sinkuro)F_2	1	3	45	12	5	1
(Puerto Rican Beauty × Sinkuro) × Puerto Rican Beauty	7	16	21	24	0	0
	Puc-	*puc puc*	*Puc-*	*puc-puc*	*Puc-*	*puc puc*
(Rosita × Sinkuro)F_2	6	0	54	13	0	4
(Puerto Rican Beauty × Sinkuro)F_2	4	0	48	9	0	6
(Rosita × Sinkuro) × Sinkuro	0	0	49	12	0	19
(Puerto Rican Beauty × Sinkuro) × Sinkuro	0	0	14	4	0	17

These associations of *Ac* and *Puc* with color intensity suggest a relationship of these genes with Dil_1 and Dil_2. This may involve linkage or pleiotropic effects of *Ac* and *Puc* on color intensity. A test of linkage or pleiotropy is possible by F_3 progeny analysis for hypocotyl coloration to determine genotypes for Dil_1 and Dil_2. With complete linkage or pleiotropic effects all homozygous light colored F_3 progenies should also be homozygous recessive for *Ac* and all homozygous dark progenies should be recessive for *Puc*.

The relationship of the diluters with *Ac* and *Puc* is given in Table 10. While an association of *Ac* and one of the diluters (arbitrarily designated Dil_1) is indicated, complete linkage is precluded. The relationship of *Puc* with Dil_2 indicates complete linkage. The significance of these linkages is considered in the discussion.

DISCUSSION

Proposed genotypes of material used in this study are summarized in Table 11. An inheritance chart showing interactions between genes controlling anthocyanin coloration in eggplant is presented in Figure 1. Genes are listed in a horizontal line with resulting color phenotype at the right. The three basic color genes (epistatic genes) are placed at the left and modifier genes (hypostatic genes) to their right. The genotype of each homozygous color phenotype is determined by tracing the lines from left to right until all genes are accounted for.

Three basic color genes (*D*, *P*, and *Y*) have been identified by tests of allelism. At two of these loci (*D* and *P*) series of alleles control the distribution of anthocyanin in different plant parts (Table 12). The *D* gene exhibits four allelic forms (*D*, *d*, d^t, d^w in order of dominance). The *P* gene exhibits three allelic forms (*P*, *p.* and p^w in order of dominance). At the *Y* locus only two alleles (*Y* and y) have been observed. Tests of complementation and F_2 progeny analyses indicate that the *D*, *P*, and *Y* loci are functionally distinct and genetically independent.

TABLE 11

Proposed genotypes of varieties used in genetic analysis

Variety	Anthocyanin pigmentation genes									G†	Gv†
	D	*P*	*Y*	*Ac*	*Dil*$_1$*	*Dil*$_2$*	*Puc*	*Sa**	*R*		
A43	*DD*	*PP*	*yy*	*ac ac*			*Puc Puc*		*rr*	*gg*	*gv gv*
A63	$d^w d^w$	*PP*	*YY*	*Ac Ac*			*Puc Puc*		*RR*	*gg*	*Gv Gv*
A64	$d^t d^t$	*PP*	*YY*	*Ac Ac*			*Puc Puc*		*rr*	*GG*	*gv gv*
Aonasu	*DD*	$p^w p^w$	*YY*	*Ac Ac*	*Dil*$_1$ *Dil*$_1$	*Dil*$_2$ *Dil*$_2$	*puc puc*		*rr*	*GG*	*gv gv*
Kantoao	*dd*	*PP*	*YY*	*Ac Ac*			*puc puc*		*rr*	*GG*	*gv gv*
Osaka Maru	*DD*	*PP*	*YY*	*Ac Ac*	*Dil*$_1$ *Dil*$_1$	*Dil*$_2$ *Dil*$_2$	*Puc Puc*	*sa sa*	*rr*	*gg*	*gv gv*
Puerto Rican Beauty	*DD*	*PP*	*YY*	*ac ac*	*dil*$_1^p$ *dil*$_1^p$	*dil*$_2^p$ *dil*$_2^p$	*Puc Puc*	*Sa Sa*	*rr*	*GG*	*gv gv*
Rosita	*DD*	*PP*	*YY*	*ac ac*	*dil*$_1^{pf}$ *dil*$_1^{pf}$	*dil*$_2^{pf}$ *dil*$_2^{pf}$	*Puc Puc*	*Sa Sa*	*rr*	*gg*	*gv gv*
Sinkuro	*DD*	*PP*	*YY*	*Ac Ac*	*Dil*$_1$ *Dil*$_1$	*Dil*$_2$ *Dil*$_2$	*puc puc*	*sa sa*	*rr*	*GG*	*gv gv*
White Beauty	*DD*	*pp*	*YY*	*ac ac*	*dil*$_1^{pf}$ *dil*$_1^{pf}$	*dil*$_2^{pf}$ *dil*$_2^{pf}$	*puc puc*		*rr*	*gg*	*gv gv*

* Genotypes of green-fruited varieties for *Dil*$_1$, *Dil*$_2$ and *Sa* are unknown. Italicized genes give probable genotypes.
† G—fruit have green flesh; gg fruits have white flesh.
Gv—fruits are variegated, gv gv fruits are non-variegated.

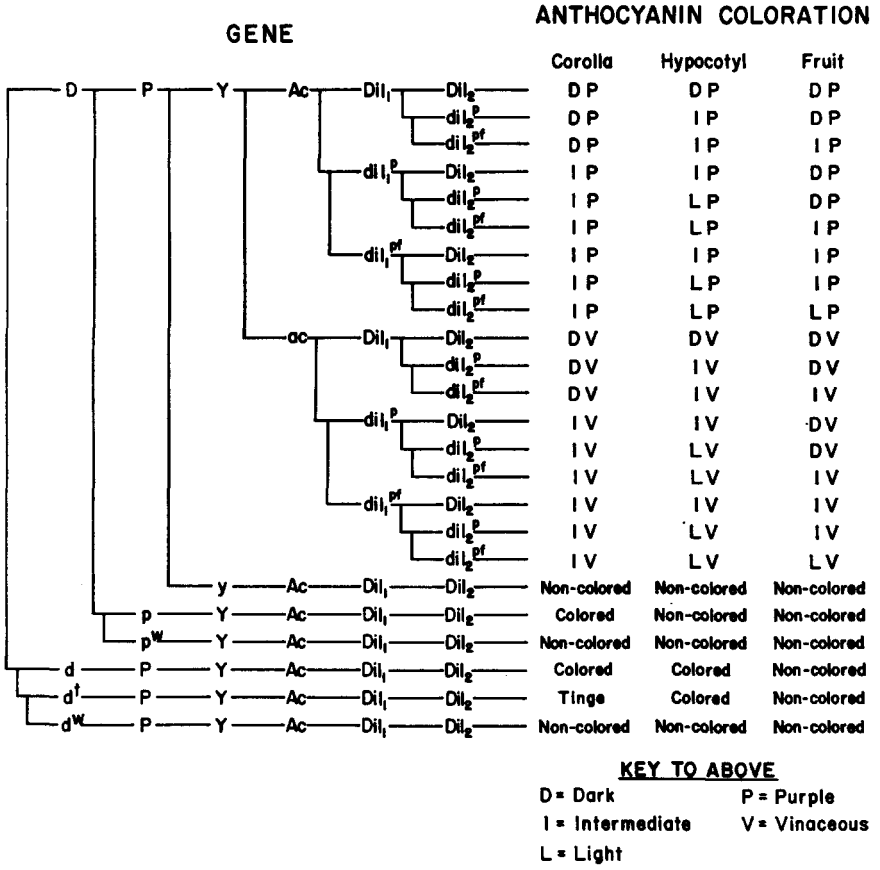

FIGURE 1.—Inheritance chart illustrating interactions among genes for anthocyanin coloration.

TABLE 12

Phenotypic expression of alleles at D, P, *and* Y

Gene Symbol	Anthocyanin distribution		
	Corolla	Hypocotyl	Fruit
D	+	+	+
d	+	+	—
d^t	+(tinge)	+	—
d^w	—	—	—
P	+	+	+
p	+	—	—
p^w	—	—	—
Y	+	+	+
y	—	—	—

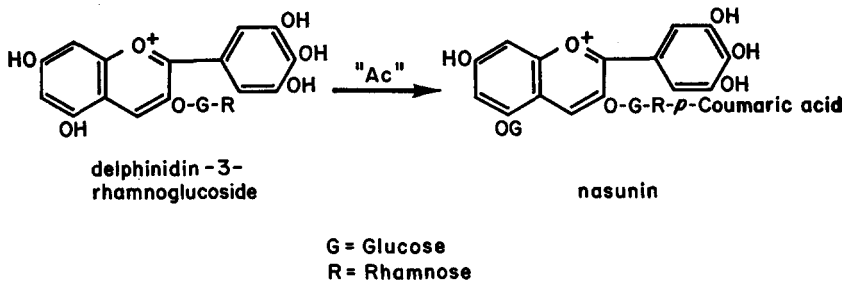

FIGURE 2.—Action of the *Ac* gene. Delphinidin-3-rhamnoglucoside gives a vinaceous color, nasunin gives a purple color.

The major variations in anthocyanin intensity, distribution, and quality are the result of five genes: *Ac*, Dil_1, Dil_2, *Puc*, and *Sa*.

The gene *Ac* determines the nature of the major anthocyanin in the fruit through the conversion of delphinidin-3-rhamnoglucoside to nasunin (Figure 2). Both acylation and glycosylation are involved and these structural alterations are reflected in a change in color from vinaceous (*ac ac*) to purple (*Ac-*).

An estimate of two major genes controlling color intensity has been obtained from F_3 progeny tests in crosses of three contrasting color varieties. These genes (symbolized Dil_1 and Dil_2) are incompletely dominant and additive in their effects on color intensity. Furthermore, three alleles must occur at one or both of these loci to account for the variation in color intensity in different plant parts. Alleles carried by Rosita (symbolized dil^{pf}) repress both plant and fruit color while alleles carried by Puerto Rican Beauty (symbolized dil^{p}) repress plant color only.

A single gene *Puc* (TATEBE 1944; JANICK and TOPOLESKI 1963) controls the light independent synthesis of anthocyanin in the fruit. A number of other effects are associated with this locus. *Sa* (controlling anthocyanin striping of the anthers) shows complete coupling phase linkage with *Puc* in most varieties while Dil_2 appears to be completely linked in repulsion. Results may be interpreted to indicate a complex of functional genetic subunits at *Puc* or a series of alleles at this locus (Table 13). In order to distinguish between the given alternatives, large populations are required to detect recombinant types. No recombination has been observed in segregating lines to date.

Eggplant varieties showing recombinant types suggestive of a complex locus at *Puc* are, however, found among certain Japanese varieties. Assuming $Puc/Sa/dil_2^{pf}$ to represent the complex genotype (order not designated) in the variety Rosita and $puc/sa/Dil_2$ in the variety Sinkuro, then recombination between *Puc* and *Sa* would account for the observed genotype ($Puc/sa/Dil_2$) found in the variety Osaka Maru (Table 13).

The genetic control of anthocyanin coloration in the eggplant shows a striking similarity to that observed in other species intensively examined. In rice (NAGAO, TAKAHASHI, and MIYAMOTO 1956) five loci, two with series of alleles, govern the

TABLE 13

Suggested gene designations for a complex locus or an allelic series at Puc

Anthocyanin phenotype	Linkage	Allelic series	Representative variety
Pigment under calyx, anther stripe, light plant, dark fruit	*Puc/Sa/dil*$_2^p$	*Puc*^{sa}1	Puerto Rican Beauty
Pigment under calyx, anther stripe, light plant, light fruit	*Puc/Sa/dil*$_2^{pf}$	*Puc*^{sa}2	Rosita
Pigment under calyx, no anther stripe, dark plant, dark fruit	*Puc/sa/Dil*$_2$	*Puc*	Osaka Maru
No pigment under calyx, no anther stripe, dark plant, dark fruit	*puc/sa/Dil*$_2$	*puc*	Sinkuro

production and distribution of anthocyanin. The two loci exhibiting multiple allelic series are complementary for anthocyanin synthesis and thus show a behavior similar to the basic color genes *D* and *P* in eggplant.

In the potato (Dodds and Long, 1957) three closely linked factors *B*, *F*, and *I* influence anthocyanin pattern. I is required for pigmentation in the tubers (a light independent synthesis), *F* governs flecking of the flowers, and *B*, with five alleles, controls the distribution of anthocyanin in different anatomical parts. The *B*, *F*, *I* linkage group in potato thus exhibits a dual complexity involving a group of pseudo-alleles, one of which exhibits a true allelic series. This situation is much like that proposed at the *Puc* locus where three closely linked factors (*Puc/Sa/Dil*$_2$) are postulated, one of which exhibits three alleles (i.e. *Dil*$_2$, *dil*$_2^p$, and *dil*$_2^{pf}$).

Genetics studies of anthocyanin coloration in higher plants have revealed several cases of alleles and "pseudoalleles" governing anthocyanin development and distribution (Alston 1959). Complex loci of this type have been reported in cotton (Stephens 1951), maize (Stadler 1951; Laughnan 1955; Emmerling 1958), potato (Dodds and Long 1956) as well as in several other species (Lawrence and Sturgess 1957; Davis, Taylor and Ash 1958). The preponderance of such loci raises several questions with regard to gene action in anthocyanin synthesis.

In the eggplant, the basic color genes *D*, *P*, and *Y* appear to influence a step directly in the path to anthocyanin synthesis. All are necessary in the dominant condition to produce color in the fruit and the various recessive alleles lead to inhibition of anthocyanin synthesis in different plant parts. However, the absence of anthocyanin in the corolla (genotypes $d^w d^w$, $p^w p^w$, or yy) is always associated with inhibition of anthocyanin formation in all other plant parts, while the absence of anthocyanin in the hypocotyl (genotype *pp*) is always associated with absence of anthocyanin in the fruit although pigment may form in the corolla.

No exceptions to these generalizations have been found. A hierarchy thus exists with respect to corolla, hypocotyl, and fruit coloration producing only four possible phenotypes, respectively, (+ + +, + + −, + − − and − − −). Apparently fruit pigmentation is physiologically most complex for it is most readily inhibited. Corolla coloration, on the other hand, is least readily inhibited.

It is possible that each locus (*D*, *P*, and *Y*) either consists of two or more functional sites (cistrons) which regulate distribution, or that the alleles at each locus exert their effect similarily, differing only in efficiency. Since only four possible phenotypes are known, if discrete sites exist, they do not show recombination or mutate independently. The most plausible interpretation, therefore, implies functionally distinct alleles which differ in efficiency so that in certain tissues the enzyme produced by a particular allele can compete for substrates, while in other tissues, it fails to compete and inhibition of pigment synthesis results. The efficiency hypothesis is supported by the fact that Kantoao (*dd*) can develop fruit pigmentation under low temperatures (JANICK and TOPOLESKI 1963).

The many effects associated with the *Puc* locus (See Table 13) are more difficult to interpret. Results are interpreted to indicate three closely linked genes (*Puc/Sa/Dil*$_2$) one of which (*Dil*$_2$) exhibits a true allelic series. Present information does not, however, exclude the possibility of an allelic series in which alleles exert pleiotropic effects on anthocyanin development in different plant parts. A more complete understanding of the "mode of action" of the *Puc* gene and/or recovery of reciprocal recombinant types will be required to definitely establish the existence of a complex locus at *Puc*.

Other reported cases of multiple factor linkage groups and allelic series controlling anthocyanin distribution appear to be associated with loci similar in action to *Puc* (i.e., involved in the light independent synthesis of anthocyanin). The *I* locus of the *BFI* linkage group in potato (DODDS and LONG 1956) controls the light independent synthesis of anthocyanin in the tubers. Similarly, the *B* locus in maize (COE 1966) controls the dark synthesis of anthocyanin in the seed. This association of multiple factor linkage groups and allelic series with genes controlling the light independent synthesis of anthocyanin may be strictly fortuitous, however, it may also reflect a peculiarity of the genes responsible for this light independent synthesis. Studies of the mode of action of the *Puc* gene, and others like it, may further clarify the genetic nature of such loci.

Present evidence indicates that the genetic control of anthocyanin development in eggplant is not simple. Genetic analyses are complicated by genes exhibiting epistatic effects (*D*, *P*, and *Y*), allelic series (*D*, *P*, *Dil*$_1$, and *Dil*$_2$), and close linkages or pleiotropic effects (*Puc/Sa/Dil*$_2$).

SUMMARY

Nine genes that determine anthocyanin development and distribution in eggplant have been identified. Three independent complementary factors *D*, *P*, and

Y cooperate to affect anthocyanin development in the corolla, hypocotyl, and fruit. Of four alleles identified at *D* (in order of dominance), *d* inhibits anthocyanin coloration in the fruit only, d^t inhibits fruit coloration and dilutes flower color, and d^w inhibits anthocyanin coloration in all plant parts. Of three alleles at *P* (in order of dominance), *p* inhibits anthocyanin coloration in the hypocotyl and fruit, p^w inhibits anthocyanin coloration development in all plant parts. Of two alleles at *Y*, *y* inhibits anthocyanin development in all plant parts. Dominant *Ac* converts the anthocyanin delphinidin-3-rhamnoglucoside to nasunin and is accompanied by a change in fruit color from vinaceous to purple. *Puc* (pigment under the calyx) permits the dark synthesis of anthocyanin in the fruit and *Sa* the formation of an anthocyanin stripe on the anthers. *Puc* and *Sa* appear tightly linked. At least two genes, Dil_1 and Dil_2 affect color intensity and three alleles are indicated: dil^p represses plant color only and dil^{pf} represses both plant and fruit color. A dominant gene *R* inhibits fruit pigmentation in certain genetic backgrounds. Partial linkage of Dil_1 with *Ac* and complete linkage of Dil_2 with *Puc* is indicated.

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