

tant was different from the mutant reported by Bergh and Lippert (1964). The mutant observed in this study was fertile and had a broader, deep green, and very rough leaf surface. It was also dwarfed reaching only a height of 6 cm. The light green-green mottle (*mot-1g*) had leaves curling downward and was fertile. The golden yellow-green mottle (*mot-gy*) mutant was characterized by long petioles with longitudinal green stripes. The stems and fruits were yellow and longitudinal green stripes were also present. The dwarf virescent (*dvw*) mutant was characterized by uniform golden yellow young leaves which turned green as the leaves matured. A dwarf yellow (*dwy*) mutant, 6 cm tall, had similar leaf color as dwarf virescent (*dvw*). Similarly, a dwarf green (*dug*) mutant with the same size as the dwarf yellow mutant was observed. However, this mutant was characterized by yellow young leaves that changed to a dark green color when fully expanded. The mature leaves also had rugose midribs and bigger veins. Leaf variegation is a common mutation which can be either a nuclear or cytoplasmic mutation. EMS may have a high specificity for mitochondrial and plastid genomes (Miller et al. 1984). It is known that many plastome mutations interfere with the development of the photosynthetic apparatus (Redei et al. 1984) and can cause male and female sterility.

Interestingly, some mutants were observed only after specific treatments. For example, the twisted long leaf mutation was only observed with the 1% EMS at 10°C for 3 h of treatment. The shoestring-leaf mutants were only observed from the 1.5% EMS at 20°C for 9 h of treatment. Campanulate fruits were observed in several treatments with 1.5% EMS and were most abundant in the treatment at 20°C for 9 h. This may indicate that a specific mutation, at least in *Capsicum*, can only be induced by a certain dose of the mutagen used. Some of the mutants described above appeared later in the M<sub>3</sub> generation. This delayed expression of mutation can be explained by an unstable change in the G-C nucleotide pair due to methylation or ethylation (Bird and Neuffer 1987).

Our results also suggest that *C. annuum* seeds are not particularly sensitive to EMS. The highest dosage (1.5% EMS, 20°C, 9 h) used in this experiment did not reduce the germination percentage to <50%. Hence, it may still be possible to increase the concentration and/or duration of treatment to induce more mutations. However, further increase in the solution tem-

perature may affect the germinability and viability of the seeds. In addition, it will cause serious damage to the developing plant as a result of other cells, other than the germ cells, being affected by the chemical treatment (Neuffer and Chang 1989). The mutants with abnormal and distorted growth induced and described in this study may have been due to the mutations in these cells.

Several unique and interesting mutants were induced in this study. There were some mutants that were completely sterile and cannot be used for further studies. The fertile mutants generated in this study will be valuable for linkage and mapping studies of *Capsicum*. All of the fertile mutants reported herein are preserved in the Capsicum Genetics Cooperative at the Department of Agronomy and Horticulture, New Mexico State University, Las Cruces.

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The Journal of Heredity 1996:87(3)

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## The Inheritance of Flower Color in *Petunia hybrida* Vilm

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The inheritance of specific flower colors in *Petunia hybrida* Vilm can be explained through the combined inheritance of anthocyanin pigmentation and pH. The inheritance of anthocyanin pigmentation is controlled by multiple independent genes (*Hf* and *Mf*) that follow simple Mendelian genetics. The inheritance of pH is more complex, being controlled by two independent codominant genes (*Ph1* and *Ph2*). Linkage of the various pH and anthocyanin genes prevents the expression of all of the potential gene combinations.

In most species, the inheritance of flower color is not clearly correlated with pigment composition. In rose (*Rosa* L.), the heritable interaction among the different anthocyanins was determined; however, flower color was not always correlated with pigment composition (Marshal et al. 1983). Cultivars that contained cyanidin had flowers that were red through laven-

**Table 1. Anthocyanin composition of red parent (P1), violet parent (P2), F<sub>1</sub> hybrid between P1 and P2, and the F<sub>2</sub> segregants**

Plant	Anthocyanin								
	de-gl	pe-gl	cy-gl	cy-ru	mv-ca	mv-co	pt-ca	pn-ca	pn-co
P1 hfhfmmf	—	8.0 <sup>a</sup> 0.7 <sup>a</sup>	57.8 1.5	34.2 1.1	—	—	—	—	—
P2 HfHfMfMf	—	—	—	—	55.0 2.9	22.3 2.9	22.7 0.5	—	—
F <sub>1</sub> HfHfMfMf	—	—	—	—	30.4 2.4	64.8 2.7	—	4.8 1.3	—
F <sub>2</sub> Hf-Mf	—	—	—	—	46.4 6.4	51.0 6.1	0.7 1.7	1.9 1.5	—
hfhfMf-	—	—	—	—	—	—	—	16.6 9.5	83.4 9.5
Hf-mfmmf	72.0 8.2	5.7 2.4	14.3 3.4	8.0 3.4	—	—	—	—	—
hfhfmmf	—	3.2 1.6	47.8 8.7	48.4 8.8	—	—	—	—	—

<sup>a</sup> Percentage of total.

<sup>b</sup> Standard deviation.

Abbreviations: de-gl: delphinidin-3-glucoside; pe-gl: pelargonidin-3-glucoside; cy-gl: cyanidin-3-glucoside; cy-ru: cyanidin-3-rutinoside; mv-ca: malvidin-3-caffeoylrutinoside; mv-co: malvidin-3-coumarylrutinoside; pt-ca: petunidin-3-caffeoylrutinoside; pn-ca: peonidin-3-caffeoylrutinoside; pn-co: peonidin-3-coumarylrutinoside.

der. Similarly, those cultivars that contained peonidin had flowers that were red through purple.

In tulip (*Tulipa* L.), cultivars were found that had the same anthocyanin composition but different flower colors (Nieuwhof et al. 1989). Hybrids were not always intermediate in pigment composition between the parents (van Raamsdonk 1993). *T. kaufmanniana* Regel × *T. fosteriana* Hoog hybrids had the same flower color as the *T. kaufmanniana* parent, but a different pigment composition than either parent.

Flower color in *Petunia hybrida* Vilm has been studied for a long time (Hooker 1837). One of the first major genetic studies identified nine genes that were involved in the inheritance of flower color (Paris and Haney 1958). However, in the final paragraph of their report it was concluded: "There was no clear cut and definite expression that could be linked with any one gene."

As more detailed data became available on the anthocyanin composition of the various color classes in *Petunia hybrida* (Griesbach et al. 1991; Muszynski 1964; Wiering and deVlaming 1984), emphasis shifted from the inheritance of flower color to the inheritance of specific anthocyanins (Wiering 1974; Wiering and deVlaming 1977). Even though the complete biosynthetic pathway has now been determined (Wiering and deVlaming 1984), the

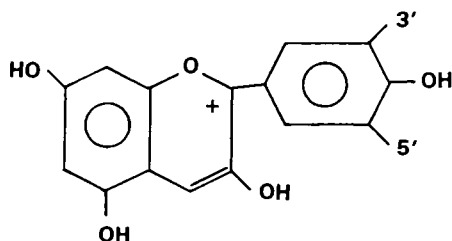
inheritance of specific flower colors is still not well understood. Two plants with the same genotype for flavonoid pigments might not have the same flower color.

There are two reasons for this poor correlation between genotype and phenotype. First, a specific genotype will not produce exactly the same array of anthocyanins in every cultivar (Wiering and deVlaming 1977). Second, flower color is a very complex characteristic that involves the chemical interaction of two different types of flavonoids—the anthocyanins and copigments (Kondo et al. 1992). Within the cell, multiple anthocyanin molecules complex with multiple copigment molecules. In this complex, the aromatic rings of the various anthocyanins and copigments stack on top of one another. Differences in color are the result of changes in the physical interaction or hydrogen bonding between the stacked rings. Changes in the pH of the vacuole and the presence of metal ions can influence the hydrogen bonding and the degree of compression of the stacked rings. The degree of compression affects the perceived color.

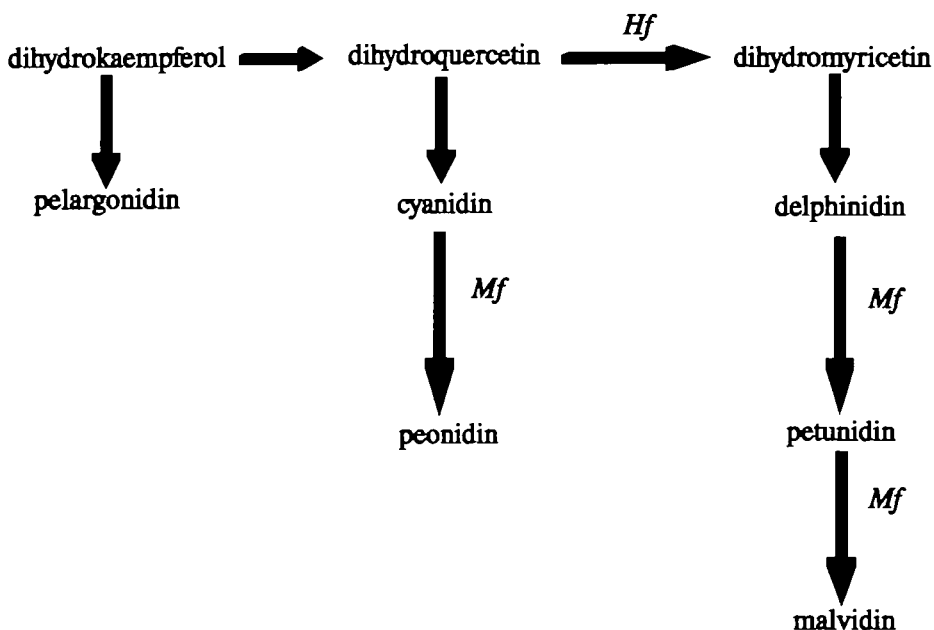
This article describes the inheritance of specific flower colors in *Petunia hybrida* and their correlation with pigment composition and pH.

## Materials and Methods

Inbred lines of a red-flowered and violet-flowered *Petunia hybrida* were developed



**Figure 1.** Structural backbone of the anthocyanin molecule.



**Figure 2.** Anthocyanin biosynthetic pathway. One *Mf* allele can function at multiple steps in the pathway.

from the commercially available Magic Series (Pan American Plant, West Chicago, Illinois). A population of F<sub>1</sub> and F<sub>2</sub> hybrids were produced from these lines. The Royal Horticultural Society's (RHS) Colour Chart was used to denote the color of the flowers. The pH and anthocyanin pigments of each flower were determined.

The anthocyanins were analyzed by high-pressure liquid chromatography (HPLC) as previously described (Griesbach et al. 1991). The HPLC profiles of individual plants were obtained from single flowers that were replicated three times. Individuals were then grouped into classes based on their anthocyanin composition. The specific anthocyanins within each of the classes were reported as a mean percentage of the total.

The pH was determined microspectrophotometrically as previously described (Stewart et al. 1975). An individual plant's pH was reported as the mean of five measurements.

## Results

### Inheritance of Flower Color

The red-flowered parent (RHS 45A) contained cyanidin (92%) and pelargonidin (8%), while the violet-flowered (RHS 89C) parent contained malvidin (77.3%) and petunidin (22.7%) (Table 1). Both parents were inbred and were homozygous for flower color genes. Since the red parent did not contain either delphinidin, petunidin, or malvidin, it was recessive for hydroxylation at the 5' position (Figures 1 and 2). This gene is denoted as *Hf*. The red parent was also recessive for methylation, since it did not contain peonidin. This gene is denoted as *Mf*. The genotype of the red parent must have been *hfhfmmf*. The violet parent, since it contained malvidin and petunidin, was dominant for both 5'-hydroxylation and methylation. The genotype of the violet parent must have been *HFHMMf*. As expected of a heterozygote (*HfhMfmf*), the F<sub>1</sub> hybrid contained both 5'-hydroxylated and methylated anthocyanins. The F<sub>1</sub> hybrid contained malvidin (95%) and peonidin (4.8%).

The F<sub>2</sub> population segregated as expected for two independent genes (9 *HfMf*:3 *Hfmmf*:3 *hfhMfmf*:1 *hfhmmf*) with a chi-square value of 0.125 (acceptance at the 99% probability level). Twenty-five plants contained the 5'-hydroxylated and methylated anthocyanins malvidin and petunidin (Table 1). The genotype of these plants was *HfMf*. Nine plants contained the methylated but not the 5'-hydroxylat-

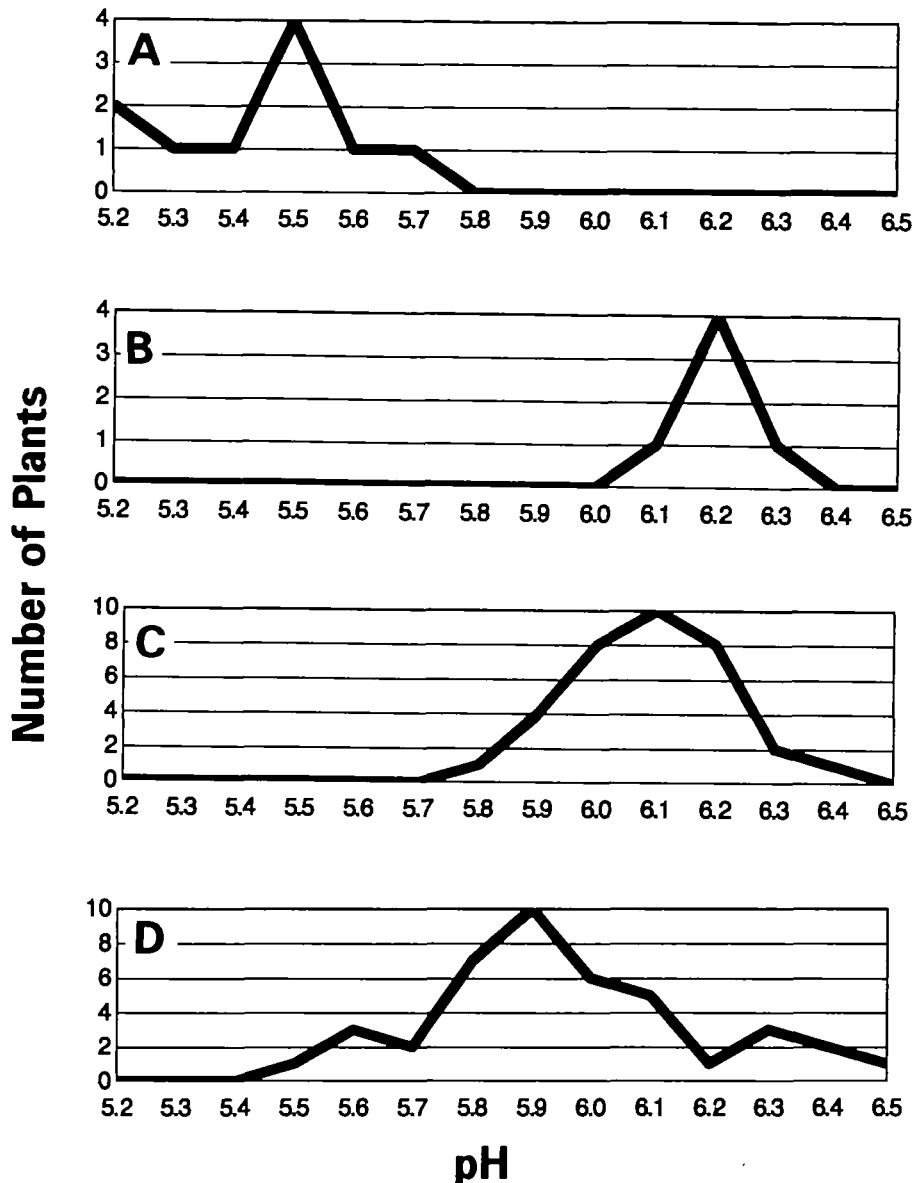


Figure 3. The vacuolar pH of individual plants of the (A) red parent, (B) violet parent, (C) F<sub>1</sub> hybrid, and (D) F<sub>2</sub> hybrid.

ed anthocyanin peonidin. The genotype of these plants was *hfhMf*. Eight plants contained the 5'-hydroxylated but not the methylated anthocyanin delphinidin. The genotype of these plants was *Hfmmf*. Three plants contained cyanidin and pelargonidin which are not 5'-hydroxylated or methylated. The genotype of these plants was *hfhmmf*.

### Inheritance of pH

The red parent (*hfhmmf*) had a pH of 5.5 with a standard deviation of 0.2 (Figure 3A). The violet parent (*HFHMMf*) had a pH of 6.2 with a standard deviation of 0.1 (Figure 3B). The F<sub>1</sub> hybrid population had a mean pH of 6.1, which was slightly more acidic than the violet parent (Figure 3C).

The F<sub>2</sub> population segregated (Figure 3D). The predominant class of individuals within the F<sub>2</sub> population had a pH of 5.9. A few individuals (9 out of 45) had a more acidic pH (mean of 5.6) that was similar to that of the red parent. A few individuals (7 out of 45) had a more alkaline pH (mean of 6.3) that was similar to that of the violet parent.

The color of the F<sub>1</sub> hybrid was not violet (RHS 89C) as expected from its genotype and anthocyanin composition; it was purple (RHS 80A). Similarly, all of the F<sub>2</sub> seedlings that contained cyanidin were not as red (RHS 45A) as expected, but salmon (RHS 52). All of the F<sub>2</sub> seedlings that contained peonidin were pink (RHS 66A). All of the F<sub>2</sub> seedlings that contained delphin-

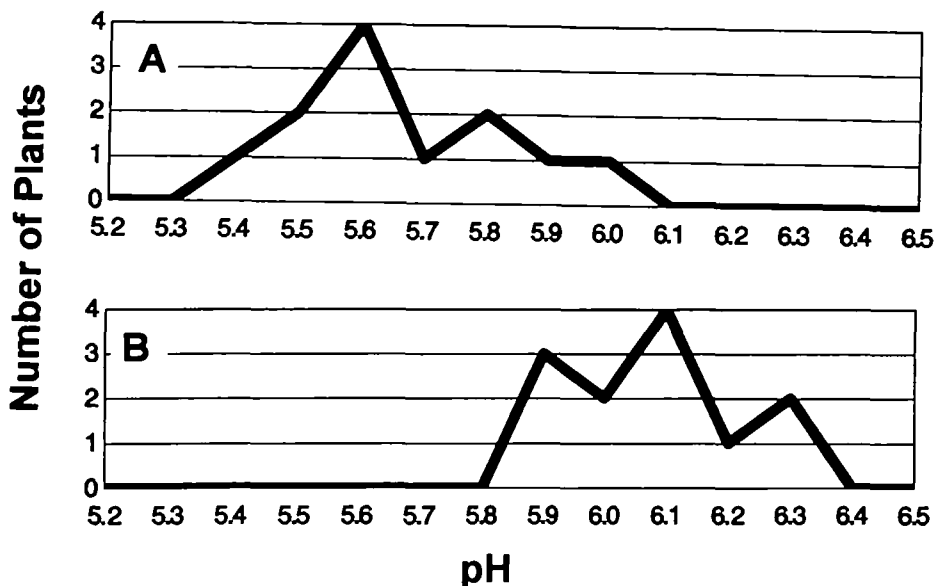


Figure 4. The vacuolar pH of individual plants containing (A) cyanidin and (B) delphinidin.

idin were lavender (RHS 87C). Not all of the  $F_2$  seedlings that contained malvidin were purple; 3 were violet (RHS 89C) and 22 were various shades of purple (RHS 78A–87A).

## Discussion

There are four different flavonoid hydroxylation genes in *Petunia* (*Ht1*, *Ht2*, *Hf1*, and *Hf2*) (Stotz et al. 1985). The expression of the *Ht2* gene, unlike the other hydroxylation genes, is limited to the tube of the flower. The *Ht* genes control the hydroxylation at the 3' position, while the *Hf* genes control the hydroxylation at the 5' position (Figures 1 and 2). The *Hf2* gene is not completely dominant like the *Hf1* gene.

The data suggest that the red-flowered parent was homozygous dominant for the *Ht1* gene and recessive for the *Hf* genes, since it did not accumulate delphinidin derivatives. The violet-flowered parent accumulated delphinidin, therefore it must have been homozygous dominant for one of the *Hf* genes. Because the violet parent did not contain cyanidin derivatives, it must have been dominant for the *Hf1* gene, since the *Hf2* gene is incompletely dominant.

There are four different anthocyanin-O-methyltransferase genes in *Petunia* (*Mt1*, *Mt2*, *Mf1*, and *Mf2*) (Jonsson et al. 1984). Each gene controls a distinct and independent enzyme. Both the *Mt* and *Mf* enzymes are capable of methylating the 3' and 5' positions (Figures 1 and 2). If one or more of the *Mt* genes is dominant and all four of

the *Mf* genes are recessive, then 3'-methylated anthocyanins (peonidin or petunidin) accumulate as the major product. If at least one of the *Mf* genes is dominant, then 3',5'-methylated anthocyanin (malvidin) accumulates as the major product. The *Mf1* enzyme has a greater substrate affinity ( $K_m = 8 \mu\text{M}$ ) than the *Mf2* enzyme ( $K_m = 21 \mu\text{M}$ ), thereby producing a higher ratio of malvidin to petunidin.

The data suggest that the red parent was recessive for all of the methylation genes, while the violet parent was homozygous dominant for only one of the methylation genes. The violet parent must have been dominant for one of the *Mf* genes since it accumulated 3',5'-methylated anthocyanin malvidin. The very high ratio of malvidin to petunidin in the violet parent (3:1), the  $F_1$  hybrid (20:1), and the  $F_2$  segregants (37:1) (Table 1) suggests that the violet parent was dominant for the *Mf1* gene and not the *Mf2* gene. This was confirmed in testcrosses with plants of known genetic background.

The  $F_1$  hybrid had the same anthocyanin composition as the violet parent but was not the same color. The difference in color could be attributed to its more acidic pH. Very slight changes in pH are known to effect flower color (Stewart et al. 1975). In *Fuchsia* L., a difference of 0.2 units resulted in a noticeable difference in color.

Vacuoles are more acidic than the cytoplasm. The acidic pH of the vacuole is maintained through the activity of a pyrophosphatase enzyme. A vacuolar  $\text{H}^+$  translocating pyrophosphatase concentrates  $\text{H}^+$  ions from the cytoplasm into the vac-

uole (Rea and Poole 1993). The resulting pH difference is used to energize the transport of other metabolites into the vacuole including organic acids. The vacuolar pH of leaves and petals are under different regulation. The violet and red parent had the same leaf vacuolar pH, but different petal vacuolar pHs (data not shown).

There are six different genes in *Petunia* that determine the vacuolar pH of petals (*Ph1*, *Ph2*, *Ph3*, *Ph4*, *Ph5*, and *Ph6*) (Chuck et al. 1993; deVlaming et al. 1983). The dominant allele of each *Ph* gene reduces the pH of the vacuole. It is not known how the *Ph* genes regulate pH. In addition to determining pH, the *Ph3*, *Ph5*, and *Ph6* genes are also involved in seed development. The dominant alleles of these genes interfere with normal seed development and lead to reduced fertility. The *Ph3* and *Ph4* genes are involved in the regulation of anthocyanin biosynthesis. The dominant alleles of these genes inhibit malvidin synthesis.

There was considerable variation in the pH of individual plants with the same flavonoid genotype (Figure 3). This variation could be due to the differences in the pH of individual cells. Significant cell-to-cell variation within a petal was found for vacuolar pH, even though there was little variation in the mean pH between different petals from the same plant (Kurkdjian and Guern 1989). The cell-to-cell variation was reported to be the result of small differences in the concentration of organic acids. A small two-fold increase in the concentration of malate led to a 2 unit decrease in pH (Kurkdjian and Guern 1989).

In this study, the inheritance of vacuolar pH can be most simply explained through the action of two codominant genes with five different phenotypic classes. All four recessive alleles would result in the least acidic pH (6.3). The addition of each dominant allele would incrementally reduce the pH. The lowest pH (5.5) would be the result of all four dominant alleles. Because of the influence of the cellular environment as previously described, it would be difficult to distinguish the pH of the different intermediate phenotypic classes.

The  $F_2$  population segregated as expected for two independent codominant genes (1 *PhPhPhPh*:10 intermediate classes:1 *phphphph*) with a chi-square value of 0.096 (acceptance at the 95% probability level). Because both parents had normal fertility, the two pH genes must have been *Ph1* and *Ph2*. The red parent had the genotype *Ph1Ph1Ph2Ph2*, while the violet parent had the genotype *ph1ph1ph2ph2*.

Even though all of the F<sub>2</sub> seedlings that had the genotype *Hf-Mf* contained the same anthocyanin composition, they did not have the same color. The F<sub>2</sub> seedlings that were red (RHS 78A) had more acidic pHs than those seedlings that were blue (RHS 89C). The full range of pHs (5.5–6.3) found in the entire F<sub>2</sub> population was not found within this *Hf-Mf* subpopulation (Figure 4). None of the *Hf-Mf* seedlings expressed the more acidic pHs. The most acidic pHs were only found in the *hfhfMf* and *hfhfmmf* subpopulations. This is expected since the *Hf1* and *Ph1* genes are closely linked on chromosome 1 (Wiering and deVlaming 1984). Our data suggests that the dominant *Hf1* allele is linked to the recessive *ph1* allele. Flowers that contain cyanidin/peonidin should not possess a recessive *ph1* allele.

Several conclusions can be drawn from this study.

1. The inheritance of specific flower colors can be explained through the combined inheritance of anthocyanin pigmentation and vacuolar pH.

2. The inheritance of anthocyanin pigmentation was controlled by multiple independent genes (*Hf* and *Mf*) that followed simple Mendelian genetics.

3. The inheritance of vacuolar pH was more complex, being controlled by two independent codominant genes (*Ph1* and *Ph2*) and being influenced by the cellular environment.

4. Linkage of the various pH and anthocyanin genes prevented the expression of all of the potential gene combinations. It was not possible to obtain seedlings expressing cyanidin/peonidin at the least acidic pHs or delphinidin/malvidin at the more acidic pHs.

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The Journal of Heredity 1996.87(3)

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## Yellow-Tip: A Cytoplasmically Inherited Trait in Melon (*Cucumis melo* L.)

D. T. Ray and J. D. McCreight

A new chlorophyll-deficient mutant is the first cytoplasmically inherited trait de-scribed in melon. This mutant is charac-terized by yellow apices, with the leaves and stems progressively turning green in color as the branches mature. A protocol is proposed for naming and symbolizing cytoplasmic traits in melon. This mutation is named *yellow-tip* and is symbolized *cyt-Yt*. As a chlorophyll-deficient mutation, it is potentially useful in genetic, physiological, and biochemical studies.

In melon (*Cucumis melo* L.), 111 mutant phenotypes have been described, but only 62 are maintained by the collection cura-tors (Pitrat 1994). Of these mutants, nine are chlorophyll-deficient mutants, six of which are maintained (Pitrat 1994). Chlorophyll-deficient mutants are potentially useful in genetic, physiological, and bio-chemical studies. These mutants have dis-crete phenotypes that are easily identified and readily manipulated. Eight of the nine chlorophyll-deficient mutants are condi-tioned by recessive alleles at different loci (Cox and Harding 1986; Dyutin 1979; Hoff-man and Nugent 1973; Nugent and Hoff-man 1974; Pitrat et al. 1986, 1991; Whitaker 1952; Zink 1977). The ninth mutant, *Pale (Pa)* green foliage, is conditioned by a nu-clear semidominant allele that is lethal (white) when homozygous, and viable, but yellow in color, when heterozygous (McCreight and Bohn 1979).

Dominant mutants are especially useful in pollination studies, and now are often used to test pollen distribution of geneti-cally engineered plants (Umbeck et al. 1991). In melon, dominant mutations have been suggested for use in the screening of hybrid seedlings (Foster 1968; Lee and Janick 1978).

A new chlorophyll-deficient phenotype was found in the breeding lines of Dr. R. E. Foster (University of Arizona) and given to D. T. Ray for genetic analysis. The mu-tant line was slow-growing, with the coty-ledons and growing tips (leaves, stem, and tendrils) yellow in color, but later turning green (Figures 1 and 2). We report inheritance studies on this chlorophyll-deficient phenotype.

## Materials and Methods

Reciprocal crosses were made between the chlorophyll-deficient line and Top Mark, (*C. melo* subsp. *melo* Cantalupensis Group; Kirkbride 1993) the current stan-dard cultivar for western U.S. shipping-type cantaloupes. It is characterized by dark-green foliage and heavily netted fruit with orange-colored flesh. Crosses were performed in a greenhouse in Salinas, Cal-ifornia, to produce F<sub>1</sub> (reciprocal), F<sub>2</sub>, and BC<sub>1</sub> families. Evaluations were done in a greenhouse at the University of Arizona. Plants were grown in a medium of equal parts by volume of peat, sand, and ver-miculite in 21.6 cm high, 21.6 cm top di-iameter, and 17.8 cm bottom diameter con-tainers (7.6 L volume). Greenhouse tem-perature throughout the experiment (June to August 1993) ranged between