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ORANGE FLOWER COLOR INHERITANCE

IN *PELARGONIUM* X *HORTORUM*

By

Shifeng Pan

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ABSTRACT

ORANGE FLOWER COLOR INHERITANCE IN *PELARGONIUM* X *HORTORUM*

By

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Five genes are hypothesized to control the orange flower color Inheritance in *Pelargonium* x *hortorum* . They are *P*, *Or*, *Sa*, *W* and *I*. The *P*, *Sa*, and *W* are found to have the same function as reported in previous research. The dominant *P* or *Sa* gives red, *pp* gives pink, and *sasa* gives deep salmon. The *W* gives colored plant, and *ww* gives white. The homozygous recessive *W* is epistatic to all other genes. The *Or*,*I* and *sa* are new in this research. *Or* gives crimson color; while the recessive *oror* with the dominant *P*, *Sa*, *W* and recessive modifier *ii* gives orange flower color. A recessive allele to *sa*, sa^1, gives light salmon in the homozygous recessive condition. The *I* dilutes and *ii* intensifies the flower color in the presence of dominant *Sa* and *W*.

The anthocyanin analysis results showed that the genes controlling the flower color were also controlling the specific anthocyanin pigment synthesis. The orange flowered phenotype contained only pelargonidin.

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INTRODUCTION

Pelargonium x *hortorum* L. H. Bailey (*P* x *h*), commonly called geraniums, has been in cultivation for about 300 years, and has become one of the most important plants for the pot and bedding plant industries. Floriculture statistics showed that seed produced pelargonium sales in 1986 were $45.6 million, and $58.9 million in 1987 (1).

In recent years progress has been made concerning genetic control of geranium flower color. Vegetative produced *P* x *h* plants with attractive orange flowers are in the trade, but the genetic inheritance of the color is not clear. The research on the inheritance of orange color was initiated as a first step to the possible development of a seed produced orange flowered *P* x *h* cultivar for the bedding plant industry. By determining the inheritance relationship between orange flower color and the other flower anthocyanin pigments in *P* x *h*, it could also lead to the development of other colors.

The objectives of this research were 1) to study the inheritance of orange flower color in diploid *P* x *h* lines, and 2) to determine the major anthocyanin pigments related to the orange flower color.

LITERATURE REVIEW

Pelargonium, containing about 280 species and a large number of hybrids and cultivars, is native to South Africa (11,15). It is believed that *Pelargonium*, probably *Pelargonium zonale*, was introduced into Europe by the Dutch governor of the Cape Colony in 1609 (7). Between 1800 and 1830, two groups of hybrids began to be distinguishable: 1) *Pelargonium* x *hortorum*, the zonale geranium, and 2) *Pelargonium* x *domesticum*, Martha Washington geranium. The third group, the ivy leaved *Pelargonium* was not introduced into Europe until after 1850 (11).

Pelargonium x *hortorum* (*P* x *h*) cultivars continue to be rapidly developed especially after many new flower and foliage colors and forms appeared in the late 1800's. There are many flower color available in *P* x *h*, including orange, purple, magenta, varied shades of red, salmon, pink, white and many bicolors.

All of these colors in *P* x *h* are the result of water soluble pigments - anthocyanins which absorb light (10). There are three known factors that change the color of anthocyanins; namely, pH, metal chelation, and co-pigment mentation, which can operate in plant tissues (9, 13). Furthermore, the colored pigments are synthesized by a variety of chemical reactions controlled by enzymes, which are proteins determined by genes(13). Usually, several different anthocyanins exist within the same flower. The different anthocyanins may be synthesized from each other by reactions controlled by enzymes. Altering the enzyme, which is controlled by genes, might change the chemical reaction and produce a different pigment. This is the foundation of developing new flower colors and of studying flower color inheritance.

Early P. x h. research reported that any colored flower was dominant to white, and that darker colors were dominant to light colors (6). Craig (9) studied seven distinct flower color phenotypes in inbred lines of P x h and determined that three independent genes P, Sa, and V controlled these colors. Dominant V gives solid flower color, while homozygous recessive gives variegation in the red phenotype. When all three genes were dominant, the phenotype was red. The reaction of these loci varied in different genetic backgrounds. Pink flowered phenotypes are always variegated, regardless of the conditions of the V locus. Recessive Sa and V interact to give picotee, a flower having one basic color with a margin of another color. When these three loci interact, they produce the genotypes and phenotypes listed in table 1.

Table 1. Flower color inheritance
In P x h.(Craig, 1963)

GENOTYPE	PHENOTYPE
P_Sa_V_	red solid color
P_Sa_vv	red variegated color
P_sasaV_	salmon solid color
P_sasavv	picotee
ppSa_V_	medium pink variegated
ppSa_vv	bright pink variegated
ppsasaV_	light pink variegated
ppsasavv	Picotee

Nugent and Snyder (17) studied a character in geranium flowers called 'Center Color', which seems to be the same as the variegated character identified by Craig (9). They reported that this phenotype was conditioned by one gene C with the dominant condition producing a solid colored flower, whereas, the homozygous recessive resulted in a white area in the center of the flower.

Badr and Horn (3) confirmed Craig's loci of P and Sa. They also observed a locus C which acted as a diluter within flower groups, and a gene W which most likely was a major gene that acted with only incomplete dominance upon anthocyanin hydrolysis. Williams (26) found a locus A, and when dominant the gene conditions purple flower color in the presence of ppSaSaVV.

Wernett (25) studied the inheritance of an orange flower color mutant derived by x - ray treatment. She proposed 5 loci to condition orange flower color in diploid P x h, in which homozygous dominant O gives the greatest amount of orange color. When heterozygous, an intermediate level of orange color is present, and when homozygous recessive, a small amount of orange color is present. Two loci E and H interact to modify the effect of locus of O. However, either diluter or intensifier effect of E and H is dependent on the dosage of orange color present. Homozygous recessive m gave a red-orange color. When M is dominant, orange color is observed. She found the V locus had the same function as described by Craig (9).

Six basic anthocyanins are found in plants and all six have been reported in P x h (10). They are pelargonidin, peonidin, malvidin, cyanidin, petunidin and delphinidin. Robinson and Robinson (20) investigated P x h for anthocyanin content in flower petals. They found the anthocyanin in blue-red colored cultivars to be malvidin, and salmon-pink to be pure pelargonidin. They also found the red flowered cultivar 'Henry Jacoby' to contain mostly pelargonidin with some malvidin 3,5-dimonoside. Scott-Moncriff (23) studied salmon flowered progenies derived from the self - pollinated rose - pink flowered

flowered 'Constane'. She found malvidin 3,5-dimonoside, small amount of pelargonidin, and some flavone and pyrogallol tannin.

A paper chromatographic method was first used for anthocyanin pigment analysis by Bate-Smith in 1948 (14). Since then the method has become the most valuable application in the identification of anthocyanins and in the study of biochemical genetics. Anthocyanins were the first group of plant substances in which the relationship between the single gene and simple biochemical differences was demonstrated (14).

Admedullah et al (2) found pelargonidin in the P x h cultivars 'Red Irene', 'Madame Salleron' and Pelargonium cucullatum.

Williams (26) in her anthocyanin studies of orange flower color in P x h suggested the presence of pelargonidin as the only anthocyanin. The source of the orange color in her study was not mentioned. She also found that purple flower petals lacked pelargonidin, but appeared to contain peonidin, malvidin and either delphinidin or petunidin.

Buswell (4) studied flower color inheritance on tetraploid P x h. He proposed that flower color seemed to be related to a gene dosage effect with pink - flowered plants containing no dominant alleles, red-flowered plants containing a single dominant allele and orange - red flowered plants containing two or more dominant alleles.

After TLC studies, he found that pink flowered phenotypes contained only peonidin and cyanidin, while both red and red-orange flowered phenotypes contained pelargonidin, peonidin and cyanidin. Increasing amounts of pelargonidin was present with increasing numbers of dominant alleles.

With spectrophotometry and TLC, Wernett (25) found all orange and orange related flower color phenotypes to contain only pelargonidin 3,5-diglucoside, while red-orange and red-orange related phenotypes to contain pelargonidin 3-monoglucoside.

MATERIALS AND METHODS

The orange flower color used in this research originally was obtained by Dr. Lowell C. Ewart from a private grower. The cultivar, Maxim Kovalevski, was tall growing with very big orange flowers. Later the cultivar was crossed with the very short growing, cherry red colored cultivar 'Robin Hood' to reduce the plant height for possible pack production. After selfing the F1 hybrids, orange flowered plants were selected, and selfed for three or four generations (Figure 1). The selected orange flowered plants were checked for the homozygosity of the orange color in the beginning of this research. The results are presented in Table 2. Other homozygous plants for flower color which were crossed with the selected orange flowered plants in this study are listed in Table 3.

To study the orange flower color inheritance of the diploid P x h, the following crosses were made:

Orange x Crimson

Crimson x Orange

Deep salmon x Orange

Rose/white x Orange

Light salmon x Orange

Light pink x Orange

White x Orange

Orange x White

Picotee x Orange

Figure 1. Derived Single Flowered Orange Background Lines From Maxim Kovalevski Crossed With Robin Hood

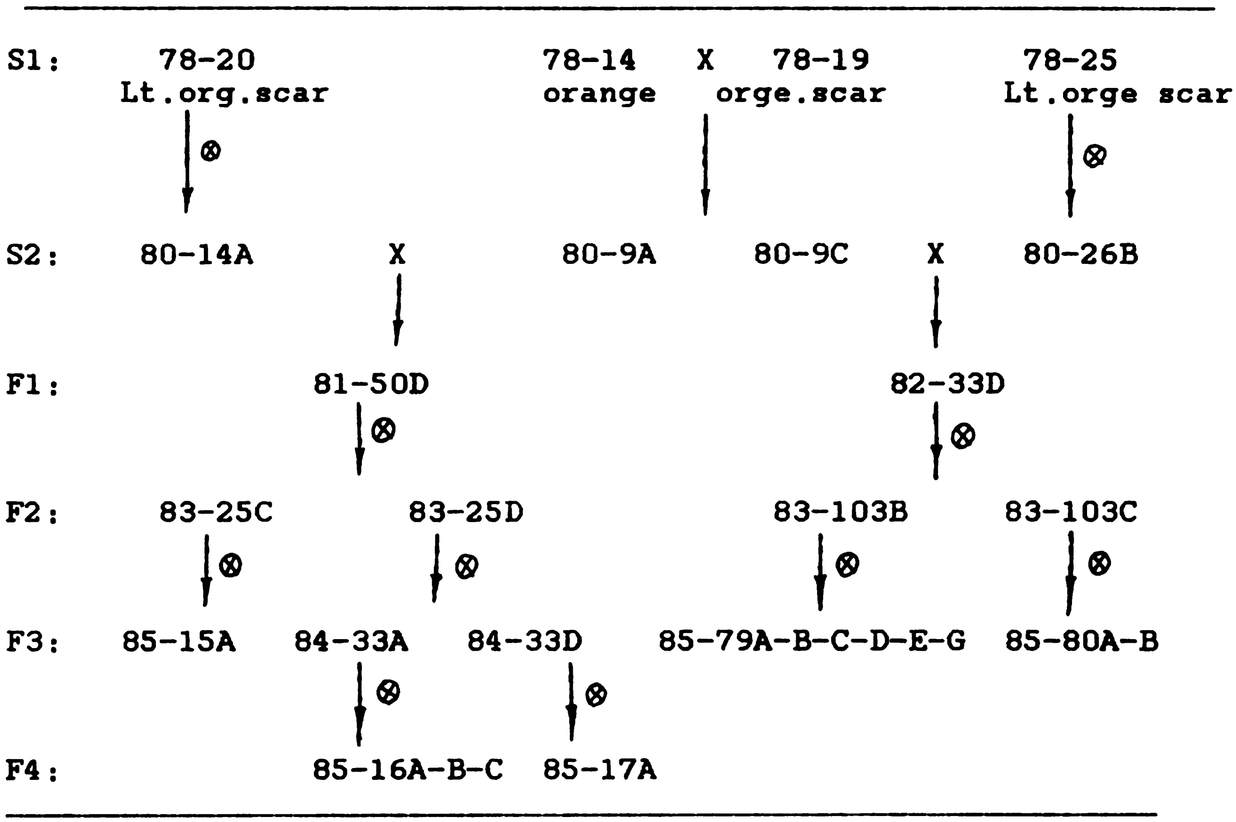


Table 2. Population Ratios of the Orange Flower Colored Parents Following Selfing

PEDIGREE	PHENOTYPE	R.H.S.COLOR CHART NUMB.	GENERATION	SEGREGATION ORANGE	DEEP SALM.
84-33D	ORANGE	33B	F3	14	4
85-15A	ORANGE	33B	F3	44	0
85-16A	ORANGE	33B	F4	27	0
85-79B	ORANGE	33B	F3	14	0
85-79D	ORANGE	33B	F3	13	0
85-79E	ORANGE	33B	F3	11	0

Table 3. Other Flower Colored Parents Used In the Orange Flower Color Inheritance

PEDIGREE	PHENOTYPE	R.H.S.COLOR CHART NUMB.	GENERATION	HOMOZYGOSITY OF FLOWER COLOR
81-10C	LIGHT SALM.	48C	F5	HOMOZYGOUS
82-27A	DEEP SALM.	39B	F5	HOMOZYGOUS
82-60C	ROSE/WHITE	57B	F5	HOMOZYGOUS
83-54B	CRIMSON	47B	F5	HOMOZYGOUS
84-10A	WHITE	-a	F5	HOMOZYGOUS
80-38A	PICOTEE	-a	F5	HOMOZYGOUS

-a. No R.H.S. Color Chart number available.

POLLINATION AND SEED HANDLING

Standard pollination techniques were used to produce the necessary seeds. For cross pollination, emasculation was done when the anthers were immature. Both small soft camel hair brushes and forceps were used in removing the anthers or applying pollen on the stigma.

Self and cross pollinations were performed using fresh pollen released from the anthers and applied to the stigma of the parent. The stigma was most acceptable when the five branches of the stigma were starting to open.

Usually about one month after pollination, the resulting seed was harvested, and put in a 4 °C seed storage room and dried for two to three weeks before any seed treatment.

P x h seed was treated using concentrated Sulfuric acid (H2SO4) to affect proper germination as described by Ewart (12). Seed was put in a 100 ml glass beaker, and concentrated H2SO4 added until the seed was covered. The treatment was timed after the acid was added and was carried out at room temperature (about 21°C). The average time for treatment was seven to eight minutes. A glass rod was used to stir the seed several times during the treatment. The acid and seed were poured into a wire hand strainer at the end of the treatment, and washed with water to stop the acid action. The treated seed was dried and put into a seed bag.

PLANT CULTURE AND FLOWER COLOR CLASSIFICATION

All H_2SO_4 treated seeds were sown in a peat-lite planting mix and covered with about 1/8 inch layer of fine perlite. The sowing mix was drenched with Banrot to prevent damping off problems. After sowing and watering, the seed trays were placed in clear plastic bags (for uniform moisture and temperature) and placed in a production room at 21 to 23 C. Cool white fluorescent light was provided for quick germination. The plastic bags were removed when the seeds germinated.

The seedlings were transplanted to 24 or 32 cell flats 2 to 3 weeks after sowing. After transplanting the progeny seedlings to the flats, Banrot and Subdue were used to drench the growing medium for disease control. To keep the plants compact and to hasten flowering, Cycocel (1500ppm) was sprayed on the plants two and three weeks after transplanting. Standard greenhouse practices were followed for disease and insect control.

The parental plants and all selected F1 progenies were grown in 6 inch pots containing a peat—lite growing medium under normal greenhouse conditions.

All plants were grown in a screened greenhouse to avoid uncontrolled pollinations.

P x h is very responsive to cumulative light energy for early flowering (5). Therefore 24 hours supplemental light was supplied using high - pressure sodium lights.

Temperature was maintained at 24°C days and 16 °C nights.

Flower color of the plants was classified using the Royal Horticultural Society Color Chart (RHS) (21) at a constant light condition and using the first open floret.

ANTHOCYANIN ANALYSIS --- PAPER CHROMATOGRAPHY

One to two day old flower petals were taken from the parental and F1 plants for drying in a forced draft oven at 40°C for one week and stored in glass bottles in desiccators until needed for analysis.

Half gram samples of dried flower petals were extracted with 50 ml of 1% HCL in methanol. If dry flower petal samples were not available, ten grams of fresh flower petals were extracted with 50ml of 1% HCL in methanol. The extract was filtered and concentrated in a rotary evaporator at 40 °C or in a boiling water bath for about 30 minutes. The pigment concentrate was rinsed from the flask by using a 0.05 M citrate phosphate buffer of pH 5.5. To adjust the pH of the concentrate to 2.8, a 2N NaOH solution was used. The concentrate was centrifuged at 7500 rpm for 10 minutes, and the resulting supernatant was stored in the dark at 2 ° C.

Whatman number 1 chromatography paper was used for analysis. Three solvent systems as described by Harborne (14) were used. These solvents were BAW (n-butanol-acetic acid-water 4:1:5), 1% HCL (water-12 N hydrochloric acid 97:3), and BuHCL (n - butanol - 2N hydrochloric acid 1:1). When using the BuHCL solvent system, the paper and the chromatographic tank were equilibrated with the bottom layer of separated n-butanol - 2N HCL (1:1) for at least 24 hours before the tests started. Special attention was also paid to the BAW solvent system. The solvent was prepared 3 days before use since the length of time this solvent storage affected the Rf values (14). The pigment concentrate was applied to sheets of papers in 2.5 centimeter streaks, using drumond 10 ul pipettes. Each sample was run two times on a different sheet of chromatographic paper. The Rf values were taken by averaging the two replications. Pigments were identified by observation their color in visible light, under ultraviolet light, and by observing their Rf (relative positions) on the chromatographic paper as described by Harborn (14).

The Rf values were calculated as,

$$Rf = \frac{\text{distance of the spot from the origin (cm)}}{\text{distance of the solvent from the origin (cm)}}$$

ANTHOCYANIN ANALYSIS -- THIN-LAYER CHROMATOGRAPHY

Thin - layer chromatography was also done for the parents and some of the F1 hybrids. Precoated silica gel thin-layer plates were marked 2 cm from the bottom, 15 cm from the origin (bottom mark). The extract was run in the chromatography tank until the solvent front moved about

15 cm, and then the plates were air dried in a fume hood. Pigments were identified according to their color in both visible and ultraviolet light, and by observing their Rf values (16,18, 19 and 24).

STATISTICAL ANALYSIS

Chi - Square analysis was used to test the fit of segregation ratios to Mendelian ratios. The non - significant null hypothesis was accepted when the probability was equal or greater than 0.05.

RESULTS AND DISCUSSION

ORANGE FLOWER COLOR INHERITANCE

The phenotypes of the parents and their F1 hybrids are listed in table 4. Genetic studies were not possible for the crosses of red with orange and light pink with orange due to a seed setting problem.

Craig(9) showed that reciprocal crosses in his flower color inheritance study did not produce different F1 hybrids. This study suggested the same conclusion from the results obtained from reciprocal crosses of crimson with orange, white with orange and white with rose/white. All these crosses and their F1 phenotypes are listed in table 5.

By checking the Royal Horticulture Society Color Chart Cross Inference Table (22), it was found that the crimson, deep salmon and rose/white flower colors used in this research were very close or the same respectively to the colors of red, salmon and medium pink which were studied by Craig (9). Therefore, Craig's genetic models for those colors were considered when hypothesizing new genetic models in this research. The gene $\underline{V}$, which is designed to control flower variegation by Craig (9), was not included in this research.

Table 4. Phenotypes and the Related R.H.S. numbers For The Parents and Their Cross For Orange Flower Color Inheritance In P x h

CROSSES				CROSS (F1)	
PARENT 1	RHS #	PARENT 2	RHS #	PHENOTYPE	RHS #
Orange	33B	Crimson	46B	Crimson	47B
		Red	43A	Red	42A
		Deep Salm.	39B	Red Org.	40A
		Lt. Salm.	48C	Red Org.	40B
		Rose/wh.	57B	Red Org.	40A
		Lt. Pink	49B	Red Org.	40B
		Picotee	-z	Peach	52C
		White	-z	Peach	52C

z no R.H.S. # available.

Table 5. Phenotypes of Reciprocal Crosses For Orange Flower Color Inheritance In P x h

FEMALE	CROSS R.H.S.#	MALE	R.H.S.#	CROSS(F1) PHENOTYPE	R.H.S.#
83-54B Crimson	46B	85-79E z Orange	33B	86-1 Crimson	47B
85-79D z Orange	33B	83-54B Crimson	46B	86-2 Crimson	47B
84-10A White	-y	85-79B z Orange	33B	86-15 Peach	52C
85-79D z Orange	33B	84-10A White	-y	86-16 Peach	52C
84-10A White	-y	82-60C Rose/Wh	57B	86-29 Lt.Rose/Wh	69C
82-60C Rose/Wh	57B	84-10A White	-y	86-28 Lt.Rose/Wh	69C

z sister plants.
y no R.H.S.# available.

RESULTS OF THE CROSS INVOLVING CRIMSON AND ORANGE

The pedigree information for the cross of crimson and orange is listed in Table 6.

Three plants were selected from the cross of crimson x orange, and 2 plants for the reciprocal cross of orange x crimson to produce the F2 families. The colors of the F1 plants differed only very slightly from the crimson parent going to a color chart rating of 47B which is slightly lighter in color.

The F2 segregating results from both cross combinations are listed in tables 7 and 8. A monogenic difference between these two parents is hypothesized. The crimson color used in this research (RHS # 47B) is very close to Craig's red color (HCC # 819)(9). The red genotype in Craig's study was reported as PPSaSaVV. Possibly a new gene Or is operating for the color difference in this cross. Dominant Or gene is proposed to govern the crimson flower color, and the homozygous recessive or gene will give the orange flower color. The crimson color is completely dominant to the orange. The F2 segregation fits a 3 to 1 ratio, and the population was homogenous for both F2 populations. The hypothesis was also verified by the backcross results of Table 40 in the appendix. The small color difference of the F1 phenotype to the crimson parent was probably due to the function of a modifier gene designated as i in this research. The proposed genetic model for the cross crimson and orange is listed in Table 9.

Table 6. Pedigree Information of the Cross Crimson and Orange For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

PEDIGREE				NUMBER	
CROSSES	P1	P2	F1 Phenotype and RHS #	F1 Plants	F2 Families
86 - 1	83-54B	85-79E [z]	Crimson(47B)	27	3
86 - 2	85-79D [z]	83-54B	Crimson(47B)	12	2

z sister plants.

Table 7. CHi - Square test on F2 Progenies of the Hybrids From the Cross 86-1 (Crimson and Orange) For Orange Flower Color Inheritance Of <u>P</u>. x <u>h</u>.

FAMILY	OBSERVED CRIMSON	ORANGE	TOTAL	D.F.	X^2 [z] (3:1)	P
86-1-1	10	4	14	1	.095	.75
86-1-6	12	8	20	1	2.400	.15
86-1-4	15	6	21	1	.138	.70
Total [y]	37	18	55	3	2.633	.45
Expect	41.25	13.75	55	1	1.752	.20

z all the chi-square values are non-significant at the .05 level.

y the population homogeneity P=.70

Table 8. Chi - Square test On F2 Progenies of the Hybrids Resulting From the Cross (86-2) of Orange x Crimson For the Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILY	OBSERVATION CRIMSON	ORANGE	TOTAL.	D.F.	X^2 (3:1) [z]	P
86-2-8	17	6	23	1	.014	.90
86-2-9	12	5	17	1	.176	.70
Total [y]	29	11	40	2	.190	.90
Expect	30	10	40	1	.133	.75

z all the chi-square values are non-significant at .05 level.
y the popurlation homogeneity P=.95.

Table 9. Proposed Genotypes For The Cross Involving Crimson and Orange For Orange Flower Color Inheritance In *P* x *h*.

	Crimson		Orange
Parents	OrOrPPSaSaii		ororPPSaSaii
F1		Crimson [z] Ororii	
F2		Crimson [z] 3 Or-ii	
		Orange [z] 1 ororii	

z PPSaSa is omitted.

RESULTS OF THE CROSSES INVOLVING DEEP SALMON AND ORANGE

The pedigree information for deep salmon and orange is listed in Table 10.

In the cross combination of 86-8 and 86-24, twenty nine and forty six F1 plants respectively were obtained, which were all red orange(40A). The F2 families generated from these two crosses gave the same segregation phenotypes, red orange, orange and deep salmon (Tables 11 and 12). The chi - square and family homogeneity tests for the 9:3:4 segregation ratio suggest that 2 genes are operating in the color inheritance of deep salmon with orange. The deep salmon and the orange parents were all homozygous for flower color (table 2 and 3), and the genotype for the (deep) salmon flower color was proposed as PPsasaVV by Craig (9). The salmon flower color he used had a 618/620 rating from the Horticultural Color Chart of 1938 (9). The deep salmon flower color used in this research had a 39B rating from the Royal Horticultural Society Color Chart (21). These two salmon colors matched each other (22). Previously, in the cross of crimson x orange, it was hypothersized that orange flower phenotype has a genotype of ororPPSaSaii. The genotype for the (deep) salmon flower color as reported by Craig is PPsasaVV (9). Therefore, the genotypes for the deep salmon flower in this research can be postulated as ororPPsasaII, and the orange flower colored parents (85-79D and 85-15A) are ororPPSaSaii, where the dominant I has a

Table 10. Parental information of the Cross Deep Salmon and Orange For the Orange Flower Color Inheritance In P x h.

CROSSES	PEDIGREE P1	P2	F1 PHENOTYPE AND RHS #	NUMBER F1	F2
86-8	82-27A	85-79C	RED ORANGE (40A)	29	3
86-23	84-33D	82-27A	RED ORANGE (40A)	25	2
			DP. SALMON (39B)	25	5
86-24	85-15A	82-27A	RED ORANGE (40A)	46	6

Table 11. Chi - Square test On F2 Progenies of the Hybrids Resulting From the Cross 86-8 (Deep Salmon x Orange) For the Orange Flower Color Inheritance In Pelargonium x hortorum.

FAMILY	OBSERVED RED ORG.	ORG.	DP.SALM	TOTAL	D.F.	X^2 (9:3:4)[z]	P.
86-8-5	6	3	3	12	2	.33	.80
86-8-23	15	4	6	25	2	.17	.93
86-8-32	19	7	10	36	2	.20	.90
Total[y]	40	14	19	73	6	.70	.95
Expect	41.06	13.69	18.25	73	2	.07	.97

z all the chi-square values are non-significant at 0.05.
y the population homogeneity P=.96.

diluting effect and the recessive ii has an intensifying effect in the presence of the dominant Sa red gene. This cross showed a two gene epistatic inheritance. When the recessive ii gene appears with oror, an orange color is present; while the Ii with oror shows a red orange color. These all require the presence of the dominant Sa_ gene. A deep salmon flower is produced when the recessive Sa appears with either the dominant or recessive of the I gene. The hypothesized genotypes for this cross are listed in Table 13. The backcross results as shown in Table 41 and 42 in the Appendix support this two gene interaction inheritance pattern.

Table 12. Chi - Square test On F2 Progenies of the Hybrids Resulting From the Cross 86-24 of Orange x Deep Salmon For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	OBSERVED RED ORANGE	ORANGE	DP.SALM.	TOTAL	D.F.	X^z (9:3:4)^z	PROB.
86-24-3	28	18	16	56	2	4.85	.10
86-24-9	12	2	8	22	2	2.24	.30
86-24-14	50	20	29	99	2	1.42	.50
86-24-21	5	4	4	13	2	1.90	.40
86-24-48	23	10	17	50	2	2.60	.25
86-24-57	24	9	8	46	2	.35	.80
Total ^{y}	142	63	87	292	12	13.36	.30
Expect	164.25	54.75	73	292	2	5.75	.06

z all chi-square values are non-significant at 0.05 level.
y the population homogeneity P=.75.

Table 13. Proposed Genotypes of The Cross Deep Salmon and Orange For The Orange Color Inheritance In <u>P</u> x <u>h</u>.

	Deep Salmon	Orange	
parents	<u>PPororsasaII</u>	<u>PPororSaSaii</u>	
F1		Red Orange ^{z} <u>ororSasaIi</u>	
F2		Red Orange ^{z} <u>ororSaSaII</u>	9
		Orange ^{z} <u>ororSaSaii</u>	3
		Deep Salmon ^{z} <u>ororsasaI_</u> <u>ororsasaii</u>	4

z the homozygous <u>PP</u> is omitted.

The cross 86-23 (Table 10) produced 50 F1 plants which segregated 25 red orange (RHS # 40A) and 25 deep salmon (RHS # 39B). This segregation suggests that the 84-33D orange flowered parent had one heterozygous locus sa which was proposed to produce the deep salmon color when the homozygous recessive condition (Table 2). When the orange flower line 84-33D was selfed, the segregation was very close to a 3 orange to 1 deep salmon ratio.

The F2 segregation results in table 14 and 15 supported this hypothesis. The genotype for 84-33D orange flower colored parent was postulated as PPororSasaii, and the deep salmon parent was PPororsasaII. The red orange F1 plant, with a possible genotype of PPororSasaIi, generated red orange, orange and deep salmon phenotypes in F2 (Table 14). The deep salmon F1 plant PPororsasaIi generated only deep salmon F2 progenies (Table 15). The hypothesized genotypes for the cross of orange (84-33D) and deep salmon (82-27A) are given in Table 16.

The chi - square and family homogeneity tests (Tables 11, 12, 14 and 15) support these hypotheses. The low number of red-orange plants produced in the family 86-23-17 may possibly be due to the chance alone because of the small population size.

Table 14. Chi - Square test On the F2 Progenies of the Orange Red Hybrid Plants Resulting From the Cross 86-23 of Orange x Deep Salmon For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	OBSERVED			TOTAL	D.F	X^2	P
	RED ORANGE	ORANGE	DEEP SALM.			$(9:3:4)^z$	
86-23-9	108	32	44	184	2	1.04	.70
86-23-17	32	13	23	68	2	5.34	.07
Total [y]	140	45	60	252	4	6.38	.20
Expect	141.75	47.25	63	252	2	2.54	.45

z all the chi-square values are non-significant at .05 level.
y the population homogeneity P=.20.

Table 15. F2 Results of the Deep Salmon Hybrid Plants Resulting From the Cross 86-23 of Orange x Deep Salmon For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	OBSERVED [z] DP.SALM.	Total
86-23 -3	7	7
86-23-4	24	24
86-23-13	12	12
86-23-16	17	17
86-23-18	23	23
Total	83	83
Expect	83	83

z non-segregating F2 families.

Table 16. Hypothesized Genotypes For the Cross 86-23 of Orange x Deep Salmon For the Orange Flower Color Inheritance In P x h.

Parents	Orange PPororSasaii	Deep Salmon PPororsasaII
F1	Red Orange [z] ororSasaIi	
	Deep Salmon [z] ororsasaIi	
F2 From Red Orange(ororSasaIi) hybrid		
	Red Orange [z] ororSa_I_	9
	Orange [z] ororSa_ii	3
	Deep Salmon [z] ororsasaI_ ororsasaii	4
F2 from Deep Salmon(ororsasaIi) Hybrid		
	Deep Salmon [z] ororsasaI_ ororsasaii	All

z Homozygous PP is omitted.

RESULTS OF THE CROSS INVOLVING LIGHT SALMON AND ORANGE

The pedigree information is listed in Table 17.

Two plants were selected from the F1 of cross 86-11 and 10 from 87-157 to check F2 segregation ratios (Table 18 and 19). Due to a greenhouse freeze in January of 1987 before this group and some other backcross plants flowered, only a few plants survived within the family to record the flower color data. Because of the small population size, the data from all those 87-157 families were pooled.

The F2 gave similar results as for the cross deep salmon and orange. The red orange flower color (RHS # 40B) is slightly lighter than the the red orange (RHS #40A) in the cross of orange and deep salmon. The non-significant results for the chi-square test, the population homogeneity test and the backcross results (Tables 43 and 44 in the Appendix) suggest that a recessive allele to _Sa_, _sa sa_ interacts with _PPororII_. The genotype for the light salmon parent is hypothesized as _PPororsa^{l} sa^{l} II_ and for orange _PPororSaSaii_. The red orange hybrid is produced when the heterozygous _Ii_ interacts with the homozygous recessive _or_ orange color gene in the presence of _Sasa^{l} PP_. The hypothesized inheritance patterns for this cross and their progenies are listed in Table 20.

Table 17. Pedigree and F2 Family numbers of the Cross Light Salmon and Orange For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>. (for the cross of 86-11 and 87-157).

CROSS	PEDIGREE P1	P2	F1 PHENOTYPE AND RHS #	NUMBER F1	F2 FAMILIES
86-11	81-10C [z] (LT.SALM)	85-79D [y] (ORANGE)	RED ORANGE (40B)	6	2
87-157	85-5B [z] (LT.SALM)	85-79B [y] (ORANGE)	RED ORANGE (40B)	53	10

z sister plants.
y sister plants.

Table 18. Chi - square test On F2 Progenies of the Hybrids Resulting From the Cross 86-11 of Orange x Light Salmon For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	OBSERVED RED ORG.	ORG	LT. SALM	TOTAL	D.F.	X^2 (9:3:4) [z]	P
86-11-3	16	8	6	30	2	1.34	.50
86-11-4	14	3	5	22	2	.56	.75
Total [y]	30	11	11	52	4	1.90	.75
Expect	29.25	9.75	13	52	2	.49	.80

z all the chi-square values are non-significant at .05 level.
y the population homogeneity P=.45.

Table 19. Chi - Square test On Pooled F2 Progenies of the Hybrids Resulting From the Cross of Orange x Light Salmon For the Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

Families	Observed Red Orange	orange	Light Salm.	Total	D.F.	X^2 (9:3:4)[z]	P
87-157-1							
87-157-2							
87-157-4							
87-157-6							
87-157-8							
87-157-9							
87-157-11							
87-157-12							
87-157-13							
87-157-14							
Total	40	14	14	68	2	0.73	.70
Expect	38.25	12.75	17	68			

z the chi-square value is non-significant at .05 level.

Table 20. Hypothesized Genotypes For The Cross of Light Salmon x Orange and Their Progenies In the Orange Flower Color Inheritance In *Pelargonium* x *hortorum* of the Crosses 86-7 and 87-157.

	Light Salmon	Orange	
Parents	PPororsa^{l}sa^{l}II	PPororSaSaii	
F1		Red Orange [z] ororSasa^{l}Ii	
F2		Orange [z] ororSaSaii	3
		Red Orange [z] ororSasa^{l}I_	9
		Light Salmon [z] ororsa^{l}sa^{l}I_ ororsa^{l}sa^{l}ii	4

z PP is omitted.

RESULTS OF THE CROSS INVOLVING ORANGE AND WHITE

Twenty-one and 22 hybrids were produced from the orange x white and reciprocal cross respectively (Table 21). All of these plants had a peach flower color (RHS# 52C).

Within the 2 groups, 10 F2 populations were generated. In the F2 (Tables 23 and 24) progenies, there was a close fit to a 1:2:1 segregation ratio for orange, peach and white. Chi - square results, the population homogeneity test data (Tables 22 and 23) and the backcross results (Tables 47, 48 and 49) suggest that an incomplete dominant orange over white inheritance pattern is operating in this cross. The *W* gene is proposed to control this monohybrid inheritance in the presence of the homozygous recessive *or*. The homozygous recessive *w* gives white color, the homozygous dominant *W* gives orange color, and the heterozygous *Ww* shows peach flower color (Table 25), all in the presence of *oror*.

Table 21. Parental information of the Cross of Orange and White For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u> (For the Crosses 86-15 and 86-16).

CROSS	PEDIGREE P1	P2	F1 PHENOTYPE AND RHS #	NUMBER F1	F2 FAMILY
86-15	85-79B[z]	84-10A	PEACH (52C)	21	3
86-16	84-10A	85-79D[z]	PEACH (52C)	22	7

z sister plants.

Table 22. Chi - Square Test Results On F2 Progenies of the Hybrids Resulting From the Cross 86-15 of Orange x White For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILIES	OBSERVED ORANGE	PEACH	WHITE	TOT.	D.F.	X^2 (1:2:1)[z]	P
86-15-1	6	20	6	32	2	2.00	.40
86-15-2	9	6	4	19	2	5.21	.07
86-15-15	7	10	5	22	2	.59	.75
Total [y]	22	36	15	73	6	7.80	.25
Expect	18.75	37.5	18.75	73	2	1.74	.45

z all the X^2 values are non-significant at .05 level.
y the population homogeneity P=.20.

Table 23. Chi - square Test Results On F2 Progenies of the Hybrids Resulting From the Cross 86-16 of White x Orange For the Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILIES	OBSERVED			Total	D.F.	X^2	P
	ORANGE	PEACH	WHITE			(1:2:1)[z]	
86-16-1	10	18	6	34	2	1.06	.60
86-16-2	2	11	6	19	2	2.16	.30
86-16-13	1	3	1	5	2	.20	.90
86-16-15	9	14	8	31	2	.35	.85
86-16-16	9	37	15	61	2	3.95	.18
86-16-7	2	2	4	8	2	3.00	.22
86-16-12	10	15	5	30	2	1.67	.50
Total [y]	43	100	45	188	14	12.39	.60
Expect	47	94	47	188	2	.81	.70

z all the X^2 values are non-significant at the .05 level.
y the population homogeneity P=.50.

Table 24. Hypothesized Genotypes For The Crosses 86-15 and 86-16 of White and Orange and Their Progenies In the Orange Flower Color Inheritance In P x h.

Parents	orange ororWW		White ororww
F1		Peach [z] Ww	
F2		Orange [z] WW	1
		Peach [z] Ww	2
		White [z] ww	1

z the homozygous recessive oror is omitted.

RESULTS OF THE CROSS INVOLVING ORANGE AND ROSE/WHITE

Ten red-orange flowered plants (RHS # 40A) were produced from the cross of orange x rose/white (Table 25).

Seven red-orange F1 plants were selfed to generate F2 families. The segregation data for the F2 progenies are shown in Table 26. The data showed a monogenic difference between the orange and the rose/white flower parents. The rose/white (RHS# 57B) is the same color as the medium pink (HCC# 627/1) which was studied by Craig (9). Craig reported the genotype for medium pink as ppSaSaVV. This genotype was confirmed later by Badr and Horn (3). With the genotype for the orange flower parent being PPSaSaWWii in the presence of oror as proposed in this study, the F2 data suggests that the P gene, which has been reported to control red and pink (rose/white)(9), interacts with the homozygous recessive Or orange color gene to give orange, red orange and rose/white flowers. Thus PPoror gives orange, pporor gives rose/white, and Pporor gives a red orange flower color. The data did not show any function of the modifier gene I proposed in previous crosses, which means that the gene P is probably epistatic to gene I. The hypothesis of the monogenic inheritance between orange and rose/white is accepted by chi - square and family homogeneity tests (Table 26), and by the backcross results (Table 50 in the Appendix).

The proposed genotypes for the cross of orange with rose/white and their progenies are presented in Table 27.

Table 25. Pedigree information of the Cross of Orange and Rose/White (86-20) For the Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

CROSS	PEDIGREE P1	PEDIGREE P2	F1 PHENOTYPE AND RHS #	NUMBER F1 PLANTS	NUMBER F2 FAMILIES
86-20	85-16A (ORANGE)	82-60C (ROSE/WHITE)	RED ORANGE (40A)	10	7

Table 26. Chi - square test On F2 Progenies of the Hybrids Resulting From the Cross 86-20 of Orange x Rose/white For the Orange Flower Color Inheritance In *P* x *h.*

FAMILIES	OBSERVED ORANGE	OBSERVED RED ORANGE	OBSERVED ROSE/WH.	TOTAL	D.F.	X^2 (1:2:1)^z	P
86-20-1	4	5	5	14	2	1.29	.60
86-20-3	33	59	19	111	2	3.97	.15
86-20-4	5	5	9	19	2	5.95	.05
86-20-6	2	5	4	11	2	.82	.70
86-20-7	7	9	5	21	2	.81	.70
86-20-8	2	3	1	6	2	.33	.85
86-20-9	30	43	19	92	2	3.02	.20
Total ^{y}	83	129	62	274	14	16.19	.13
Expect	68.5	137	68.5	274	2	4.15	.13

z all the X^2 values are non-significant at the .05 level.
y the population homogeneity P=.40.

Table 27. Hypothesized Genotypes For The Cross 86-20 of Orange x Rose/white and Their Progenies In the Orange Flower Color Inheritance In P x h.

	Orange	Rose/White	
Parents	PPororSaSaWWii	ppororSaSaWWII	
F1		Red Orange [z] Pporor	
F2		Orange [z] PPoror	1
		Red Orange [z] Pporor	2
		Rose/White [z] pporor	1

z SaSaWWIi is omitted.

RESULTS OF THE CROSS INVOLVING PICOTEE X ORANGE

From the cross of picotee x orange, four peach hybrids were selected to produce F2 families (Table 29).

The F2 result is similar to the cross of white x orange in that a diluting factor in the picotee parent diluted the orange color to the peach color. There were six distinctive flower color phenotypes in the F2 segregation. They were peach, orange, rose/white, deep salmon, light pink and picotee. All the colors have been described above except the light pink. The F2 segregation data (Table 51 in the Appendix) suggests a trihybrid inheritance, but no suitable genetic model could be derived to explain the segregation ratio. This may be due to the possibility that the picotee parent was not really homozygous and can appear as two different genotypes (9). Further research will be necessary to determining the inheritance for the cross of picotee and orange by first determine the actual genotype of possible picotee parent plants through testing them with other known colored plants and then crossing them with the orange genotype for analysis.

Table 28. Pedigree information For The Cross of Picotee and Orange For the Orange Flower Color Inheritance In P x h.

PEDIGREE				NUMBER	
CROSS	P1	P2	F1 PHENOTYPE AND RHS #	F1 PLANTS	F2 FAMILIES
86-27	80-38 (PICOTEE)	85-15A (ORANGE)	PEACH (52C)	40	4

SUMMARY OF THE ORANGE FLOWER COLOR INHERITANCE

Five genes have been hypothesized to control the orange flower color inheritance in *Pelargonium* x *hortorum*. They are *P*, *Or*, *Sa*, *W* and *I*. The *P* and *Sa* genes have been previously reported by Craig (9); The *P* and *Sa* give red, *pp* gives pink, *sasa* gives deep salmon. The *W* gene in this research has the same function as that reported by Badr and Horn (3). *W_* gives colored phenotype and *ww* gives white. The recessive *w* is epistatic to all other genes. An allele to *sa*, sa^1 gives light salmon in the homozygous recessive condition. *Or_* gives crimson, *oror* gives orange. The *I* gene dilutes the flower color and it's homozygous recessive *ii* intensifies the flower color in the presence of the dominant *Sa* and *W* genes. Both complete and incomplete dominant inheritance patterns have been found for the inheritance of orange flower color in this research.

The *Or* gene is different from the *O* gene hypothesized to control the X - ray mutation originated orange flower color in *P* x *h* studied by Wernett (25). The homozygous recessive *or* gene combining with dominant *P*, *Sa*, *W*, and recessive intensify gene *ii* gives the orange flower color in this research. The difference in the results between Wernett's and the present research may be due to the different sources of the orange material used. Wernett used the orange, picotee and blush sister plants as parents, which came from the same X - ray originated plant.

Besides Or, the gene I and the allele sa^{1} are new in this study. Considering all 5 genes, the genotypes for the parents used in this research are shown in Table 29.

The line chart form (19) is used to represent all the genotypes and the related phenotypes (Figure 2) which were studied in this research. The genes are listed in a horizontal line with the dominant gene on top of the line, and the incomplete dominant and the recessive gene below the line. The epistatic genes are represented by parallel lines down from the hypostatic genes to the right a short distance below the one from the epistatic genes. To determine the genotype of any given phenotype start at the selected color and follow the line to the left and upward until all genes are accounted for.

Table 29. Proposed Genotypes Of The Parents Used In This Research For the Orange Flower Color Inheritance In *P* x *h*

Parents	Phenotype	Genotype
83-54B	Crimson	OrOrPPSaSaWWii
82-27A	Deep Salm.	ororPPsasaWWII
85-79B	Orange	ororPPSaSaWWii
85-79D	Orange	ororPPSaSaWWii
84-33D	Orange	ororPPSasaWWii
81-10C	Light Salmon	ororPP$sa^{l}sa^{l}$WWII
82-60C	Rose/white	ororppSaSaWWII
84-10A	White	ororPPSaSawwII

Figure 2. Line Chart Presentation For The Interactions Of Or,Sa(Sa),P,W and I, and The Related Phenotypes For the Inheritance Of Orange flower color in *Pelargonium* x *hortorum*.

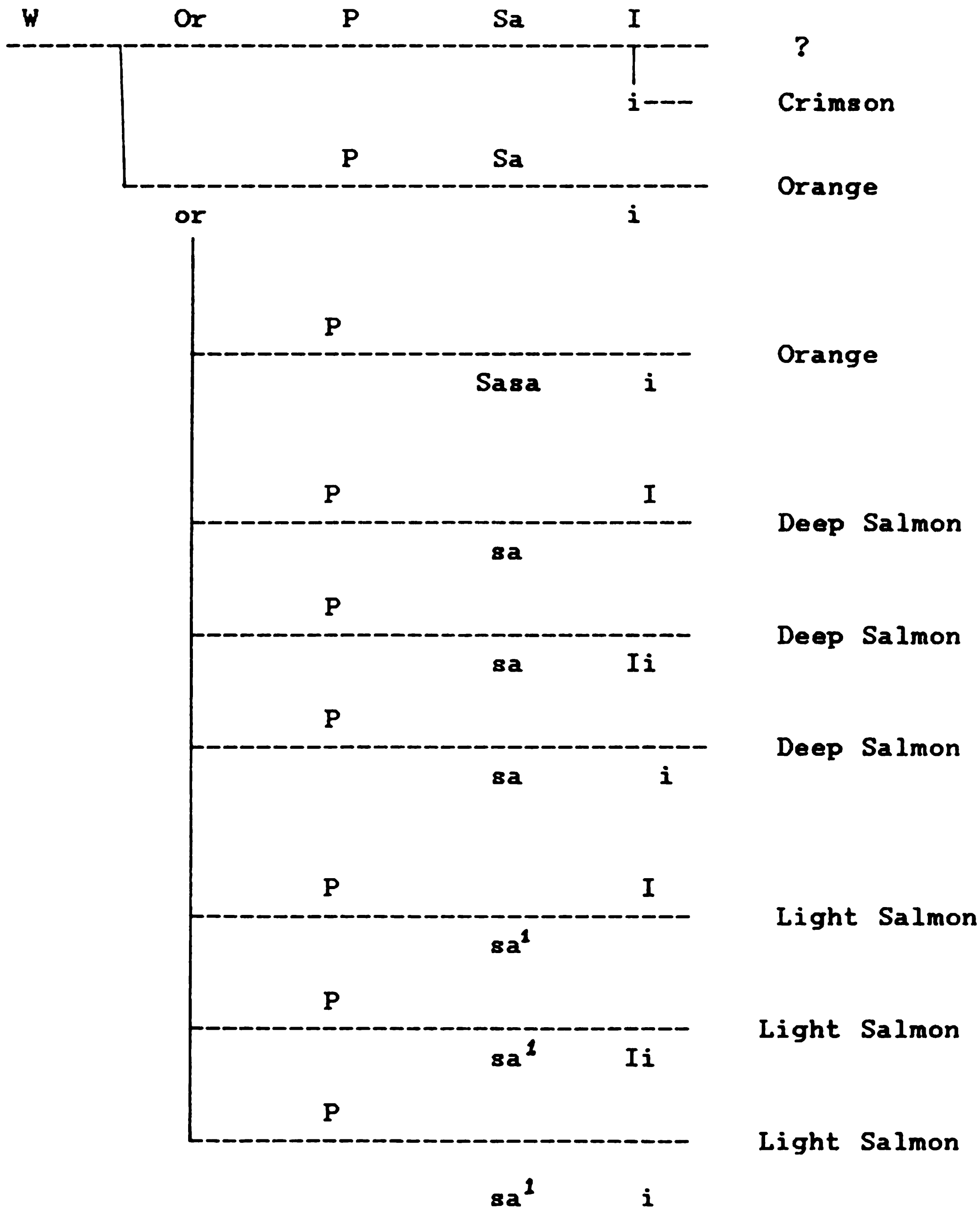
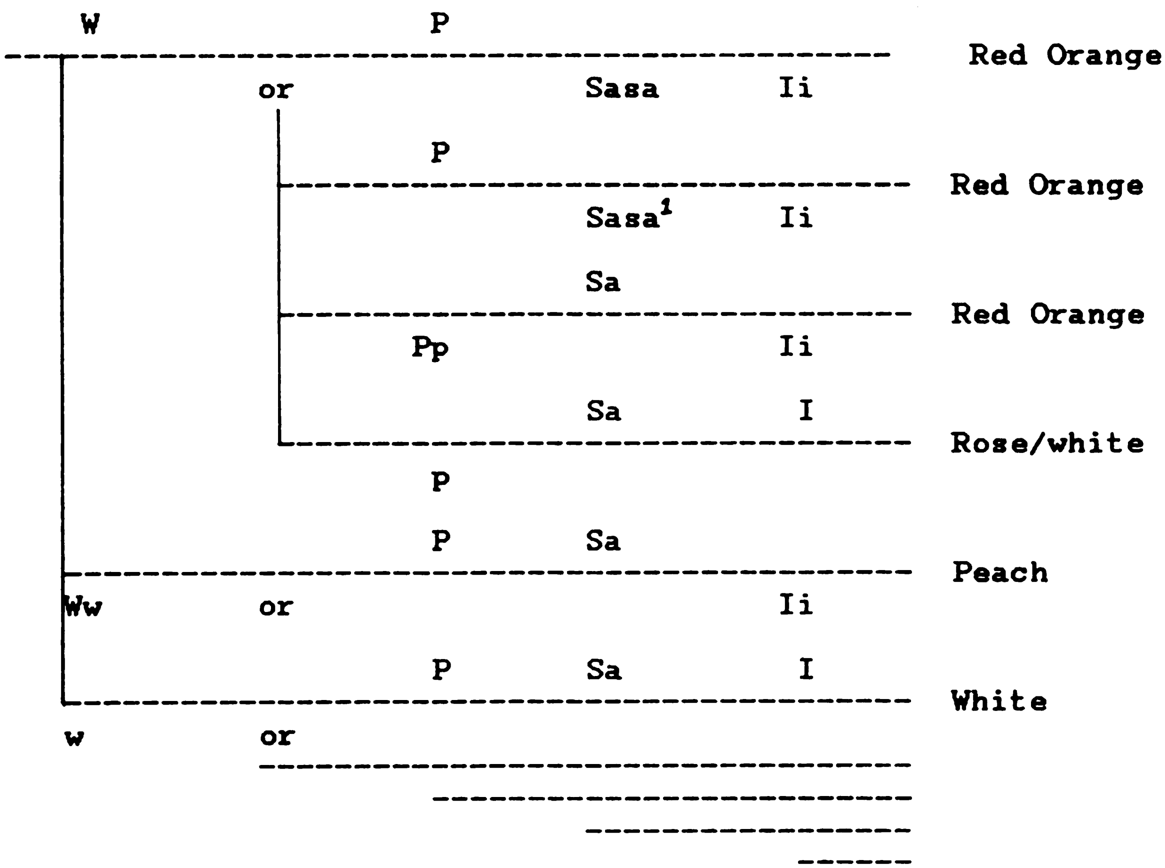


Figure 2. (cont'd)



PIGMENT ANALYSIS

Paper chromatography (P. C.) and thin - layer chromatography (T.L.C.) methods were conducted to analyze the anthocyanin pigments of the parents and their F1 hybrids. In the P.C. method the three solvents BAW, BuHCL and 1% HCL were tried. The 1% HCL solvent system gave very poor separation results and was discarded. To confirm the results of the P.C. method, the T.L.C. procedure was used with the BAW solvent system. In each cross combination, the Rf values, band numbers, visible band colors and band colors under ultra violet light for both the parents and their F1 hybrids were investigated, and the anthocyanin pigments were identified.

<u>Pigment Analysis For The Cross Of Crimson and Orange</u>

In the cross of Crimson x orange (Table 30), two bands were found in the crimson parent (83-54B). They were orange and purple respectively under visible light. Under U.V. light they were fluorescent yellow and dull purple colors. After comparing these band colors and the tested Rf values with published standards (13, 15, 17 and 23), they were identified as petunidin. The orange parent (85-79E) showed only one band, which gave orange color under visible light, and fluorescent yellow color under U.V. light. It was identified as pelargonidin. The F1 hybrid showed both bands which had been appeared in the crimson parent.

Table 30. Pigment Analysis of the Cross Involving Crimson x Orange For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
85-79E	P	orange	33B	ororii	1	or.	f.y.	.33	.10	.63	Pg.
83-54B	P	Crimson	46B	OrOrii	1	or.	f.y.	.26	.12	.63	Pg.
					2	Pu.	d.p.	.21	.08	.58	Pt.
86-1	F1	Crimson	47B	Ororii	1	or.	f.y.	.32	.10	.63	Pg.
					2	Pu.	d.p.	.19	.06	.58	Pt.

1 or. - orange; Pu. - Purple.

2 f.y. - fluorescent yellow; d.p. - dull purple.

3 Pg. - Pelargonidin; Pt. - Petunidin.

This result further confirmed the hypothesis that the crimson color is completely dominant to the orange flower color. The _Or_ gene, supposed to control the crimson color, is hypothesized also to control the pelargonidin and petunidin biosynthesis. When it is homozygous recessive, _oror_, only pelargonidin is produced. This hypothesis is further supported by the pigment analysis result in the reciprocal cross orange x crimson (Table 31).

Pigment Analysis For The Cross Of Deep Salmon and Orange

Two bands were found in the deep salmon (82-27A) parent. They were identified as pelargonidin and delphinidin. The orange parent 85-79D, 84-33D and 85-15A all showed only one band, identified as pelargonidin (Tables 32, 33 and 34). F1 hybrids (86-8) of deep salmon (82-27A) x orange (85-79B) showed three bands (Table 32). Two of them were identified as pelargonidin, and one band was delphinidin. The reason for the two pelargonidin bands appearing is not quite clear. This may possibly be caused by the modifier gene _I_ which was postulated in the genetic studies of the cross involving deep salmon and orange. The genes _Or_, _Sa_ and _I_ which control flower colors in this cross are proposed also to control the pigment synthesis. Thus _ororSaSaii_ controls the pelargonidin production, and _ororsasaII_ controls the pelargonidin and delphinidin production. The hybrid _ororSasaIi_ produces both pelargonidin and delphinidin.

Table 31. Pigment Analysis of the Cross Involving Orange x Crimson

For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
85-79B	P	Orange	33B	ororii	1	or.	f.y.	.328	.10	.633	Pg.
83-54B	P	Crimson	46B	OrOrii	1	or.	f.y.	.26	.12	.63	Pg.
					2	Pu.	d.p.	.21	.08	.58	Pt.
86-2	F1	Crimson	47B	Ororii	1	or.	f.y.	.28	.12	.63	Pg.
					2	Pu.	d.p.	.17	.08	.58	Pt.

1 or-orange; Pu.- Purple.

2 f.y. - fluorescent yellow; d.p. - dull purple.

3 Pg. - Pelargonidin; Pt - petunidin.

Table 32. Pigment Analysis of the Cross Involving Deep Salmon x Orange

For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype*	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
82-27A	P	deep salmon	39B	sasaII	1	or.	f.y.	.25	.11	.57	Pg.
					2	Pu.	d.p.	.17	.07	.37	Dp.
85-79D	P	Orange	33B	SaSaii	1	or.	f.y.	.33	.10	.63	Pg.
86-8	F1	Red orange	40A	SasaIi	1	or.	f.y.	.28	.11	.63	Pg.
					2	r.o.	d.r.	.24	.09	-	Pg.
					3	Pu.	d.p.	.18	.07	.38	Dp.

* homozygous recessive oror is omitted.

1 or. - orange; Pu. - Purple; r.o. - red orange.

2 f.y. - fluorescent yellow; d.p. - dull purple;

3 Pg. - Pelargonidin; Dp. - Delphinidin.

Table 33. Pigment Analysis of the Cross Involving Orange x Deep Salmon

For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype*	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
84-33D	P	orange	33B	Sasaii	1	or.	f.y.	.34	.10	.63	Pg.
82-27A	P	Deep Salmon	39B	sasaII	1	or.	f.y.	.25	.11	.57	Pg.
					2	Pu.	d.p.	.17	.07	.37	Dp.
86-23	F1	Red orange	40A	SasaIi	1	or.	f.y.	.28	.12	-	Pg.
					2	Pu.	d.p.	.23	.09	-	Dp.
	F1	Deep Salmon	39B	sasaIi	1	or.	f.y.	.28	.14	-	Pg.
					2	Pu.	d.p.	.23	.09	-	Dp.

* the homozygous recessive oror is omitted.
\- not available.
1 or. - orange; Pu. - Purple
2 f.y. - fluorescent yellow; d.p. - dull purple.
3 Pg. - Pelaegonidin; Dp. - Delphinidin.

Table 34. Pigment Analysis of the Cross InvolvingO Orange x Deep Salmon

For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype*	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
85-15A	P	orange	33B	SaSaii	1	or.	f.y.	.39	.09	.62	Pg.
82-27A	P	deep salmon	39B	sasaII	1 2	or. Pu.	f.y. d.p.	.25 .17	.11 .07	.57 .37	Pg. Dp.
86-24	F1	red orange	40A	SasaIi	1 2	or. Pu.	f.y. d.p.	.22 .15	.12 .09	- -	Pg. Dp.

* the homozygous recessive oror is omitted.
\- the data is not available.
1 or. - orange; Pu. - Purple.
2 f.y. - fluorescent yellow; d.p. - deep purple.
3 Pg. - Pelargonidin; Dp. - Delphinidin.

In the cross 86-23 (orange 84-33D x deep salmon 82-27A), two different colored plants appeared, red orange and deep salmon. Both hybrids showed the same pigments of pelargonidin and delphinidin.

As shown in Table 33, the 84-33D orange parent showed only one pelargonidin band, and the deep salmon parent (82-27A) showed 2 bands which were identified as pelargonidin and delphinidin. Similarly as that in cross 86-8, the genes of _Or_, _Sa_ and _I_, which are hypothesized to control the flower color inheritance in this cross, are also proposed to control the pigment production. Thus, _ororsasaII_ controls pelargonidin and delphinidin production; while _ororSasaii_ controls only pelargonidin production. The hybrids _ororSasaIi_ and _ororsasaIi_ produce both pelargonidin and delphinidin.

For the cross of 86-24 (Table 34), the orange parent (85-15A) which is hypothesized as _ororSaSaii_, produces only pelargonidin, while The deep salmon parent (82-27A) _OrOrsasaII_ produces pelargonidin and delphinidin. Their F1 hybrids _ororSasaIi_ Contains both pelargonidin and delphinidin.

Pigment Analysis For The Cross Of Light Salmon and Orange

The results for the cross involving light salmon and orange are shown in Table 35. Only one band was found for each parent. In the light salmon parent (84-10A), the band was magenta and dull magenta color under visible and

Table 35. Pigment Analysis of the Cross Involving Light Salmon and Orange For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype*	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
81-10C	P	light salmon	49B	sa^1sa^1II	1	mg.	d.m.	.21	.08	.55	Cy.
85-79D	P	orange	33B	SaSaii	1	or.	f.y.	.33	.10	.63	Pg.
86-11	F1	red orange	40B	$Sasa^1Ii$	1 2	or. mg.	f.y. d.m.	.29 .20	.11 .07	.62 .55	Pg. Cy.

* the homozygous recessive oror is omitted.

1 mg. - magenta; or. - orange.

2 d.m. - dull magenta; f.y. - fluorescent yellow.

3 Cy. - Cyanidin; Pg. - Pelargonidin.

U.V. light respectively. It was identified as cyanidin. The band in the orange parent (85-79D) was identified as pelargonidin, which is proposed to be controlled by ororSaSaii. The genotype $ororsa^{i}sa^{i}II$, which is hypothesized to control the light salmon flower color, allowing cyanidin production. Their hybrid contains both pelargonidin and cyanidin produced by the genotype $ororSasa^{i}Ii$. The results in this cross show that the sa^{i}, an allele to sa, does not produce pelargonidin and delphinidin but cyanidin. Thus the resulting flower color is light salmon.

Pigment Analysis For The Cross Of White And Orange

In cross 86-15, no band appeared for the white parent (84-10A), thus no pigment is being produced (Table 36). One band was found for the orange parent (85-79B). This band was identified as pelargonidin. A second band appeared besides the pelargonidin band in their F1. This second band was identified as petunidin. Assuming homozygous recessive w controls white flower color and non - pigment production; WW colored flower phenotype governs the pelargonidin production. Their hybrid Ww gives not only pelargonidin but also the pigment petunidin. This new pigment is obviously related to the recessive suppresser of the w gene, but not the modifier(s), such as I, since the recessive w is epistatic to any other genes in function as proposed in the flower color segregation study of white and

Table 36. Pigment Analysis of the Cross Involving White x Orange

For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
84-10A	P	white	no	ororww	no	no	no	-	-	-	no
85-79B	P	orange	33B	ororWW	1	or.	f.y.	.37	.09	.62	Pg.
					1	or.	f.y.	.25	.11	.63	Pg.
86-15	F1	peach	52C	ororWw	2	Pu.	d.p.	.21	.07	.57	Pt.

\- no observable values.

1 no - no visiable band; or. - orange; Pu. - Purple.

2 no - no visiable band; f.y. - fluorescent yellow; d.p. - dull purple.

3 Pg. - Pelargonidin; Pt. - Petunidin.

Table37. Pigment Analysis of the Cross Involving Orange x White For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
85-79D	P	orange	33B	ororWW	1	or.	f.y.	.33	.10	.63	Pg.
84-10A	P	white	no	ororww	no	no	no	-	-	-	no
86-16	F1	peach	52C	ororWw	1	or.	f.y.	.25	.11	.63	Pg.
					2	Pu.	d.p.	.21	.07	.57	Pt.

\- no visible values.

1 no - no visiable band and color; or. - orange; Pu. - Purple.

2 no - no visiable band and color; f.y. - fluorescent yellow; d.p. - dull purple.

3 no - no pigment identified; Pg. - Pelargonidin; Pt. - Petunidin.

orange. This hypothesis is further verified by the result of the pigment analysis for the reciprocal cross orange x white (Table 37).

Pigment Analysis For The Cross Of Orange x Rose/White

In Table 38, the orange parent (86-16) contained only pelargonidin. The rose/white parent (82-60C) showed only one band identified as cyanidin. Their red orange hybrid gave both pelargonidin and cyanidin. The ppororSaSaII genotype which controls rose/white flower color would also control the cyanidin pigment production and the PPororSaSaii genotypes which controls orange flower color would control pelargonidin production. Their hybrid PpororSaSaIi synthesized both cyanidin and pelargonidin. This result matches the flower color segregation results showed in table 26.

Table 38. Pigment Analysis of the Cross Involving Orange and Rose/white For the Orange Flower Color Inheritance In P x h

Plant number	Generation	Phenotype	RHS number	Genotype*	Bands	Visb. band color[1]	U.V. band color[2]	P.C.Rf BAW	P.C.Rf BUHCL	TLC Rf BAW	Pigment Identified[3]
85-16A	P	orange	33B	PPii	1	or.	f.y.	.34	.07	.60	Pg.
82-60C	P	Rose/white	57B	ppII	1	mg.	d.m.	.18	.08	.53	Cy.
86-20	F1	red orange	40A	PpIi	1	or.	f.y.	.28	.11	-	Pg.
					2	mg.	d.m.	.23	.07	-	Cy.

* the homozygous recessive oror is omitted.

1 or. - orange; mg. - magenta.

2 f.y. - fluorescent yellow; d.m. - dull magenta.

3 Pg. - Pelargonidin; Cy. - Cyanidin.

\- data is not available.

SUMMARY OF PIGMENT ANALYSIS

The identified pigments for each parents used in this research are shown in table 39.

The pigment analysis results show that OrOrSaSaii synthesizes pelargonidin and petunidin, and ororsasaII synthesizes pelargonidin and delphinidin in the presence of PPWW. The homozygous recessive or gene controls only pelargonidin synthesis in the presence of PP, SaSa, WW and recessive gene ii. In the presence of oror, PP gene together with recessive $sa^1 sa^1$ or Sa together with homozygous pp control cyanidin production; while the genotype PPSasa or PpSaSa produces both pelargonidin and cyanidin. The w in the homozygous recessive condition governs no pigment production, proposed as epistatic to all other genes. Figure 3 shows all the pigment analysis results. These results fit what would be expected from the flower color inheritance results.

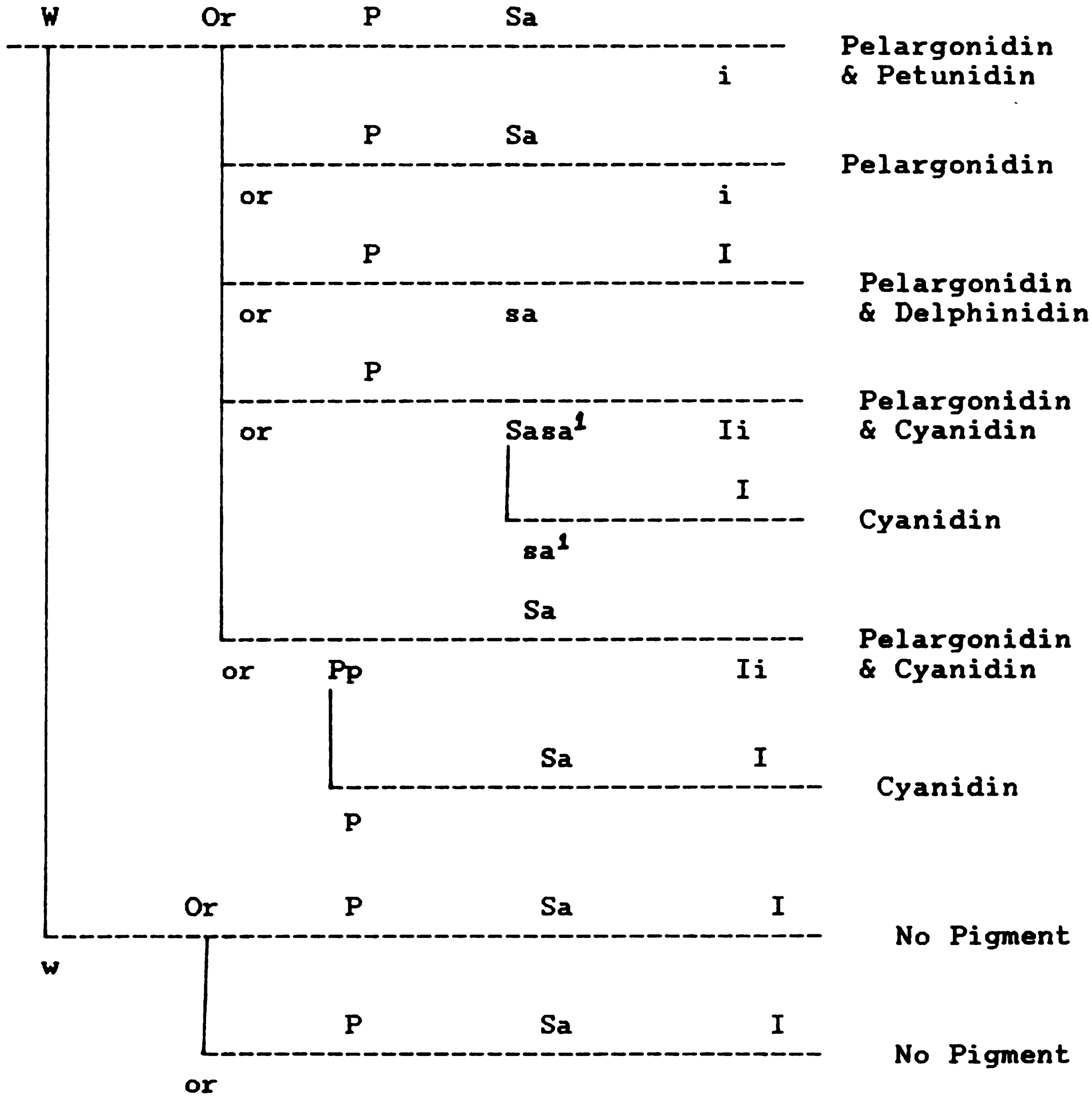
The result that the orange flower color contains only pelargonidin agrees with previous researches (25, 26). The genetic segregation results which differ from the Wernett's study (25) may possibly be explained as: 1) the lack of a wide range of crosses between orange and other flower colors in that research; 2) the different sources of the orange flower color; 3) the same problem that a flower color is controlled by both dominant and recessive gene(s) from different research has been found in some other ornamental plant species (19).

It has been shown from this research that the orange flower color is controlled by multi-loci. It seems that the only way to produce a seed produced hybrid orange flower colored P x h is by crossing orange inbreds together. This is true at least for the materials used in this research.

Table 39. Pigments Identified For All Parents Used For The Orange Flower Color Inheritance In *P* x *h*.

Phenotype	Genotype	Pigments Identified
Crimson	PPOrOrSaSaWWii	Pelargonidin and Petunidin
Orange	PPororSasaWWii	Pelargonidin
Deep Salmon	PPororsasaWWII	Pelargonidin and Delphinidin
Light Salm.	PPororsa^{l}sa^{l}WWII	Cyanidin
White	PPororSaSawwII	None
Rose/white	ppororSaSaWWII	Cyanidin

Figure 3. Line Chart Presentation Of The Genotypes And The Synthesized Pigments For The Orange Flower Color Inheritance In P x h.



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APPENDIX

Table 40. Backcross Result Of (Crimson x Orange) x Orange For The Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILY	OBSERVED CRIMSON	OBSERVED ORANGE	Total	D.F.	X^2 (1:1)	P
87-163	9	7	16	1	.250	.55
EXPECT	8	8	16			

Table 41. Backcross Result Of (Orange x Deep Salmon) x Orange For The Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILY	CROSS	OBSERVED RED ORANGE	OBSERVED ORANGE	Total	D.F.	X^2 (1:1)	P
87-251	86-24-3 X 85-15A	9	7	16	1	.25	.60
87-220	86-24-57 X 85-15A	4	3	7	1	.14	.70
	Total [z]	13	10	23	2	.39	.80
	Expect	11.5	11.5	23	1	.39	.53

z the population homogeneity P=1.0.

Table 42. Backcross result Of (Orange x Deep Salmon) x Deep Salmon For The Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	CROSS	OBSERVED RED ORANGE	DP. SALM.	Total	D.F.	X^2 (1:1)	P
87-216	86-24-3 X 82-27A	3	4	7	1	.14	.70
87-217	86-24-14 X 82-27A	14	14	28	1	.00	1.00
87-218	86-24-23 X 82-27A	3	2	5	1	.20	.60
87-219	86-24-57 X 82-27A	8	10	18	1	.22	.58
	Total [z]	28	30	58	4	.56	.80
	Expect	29	29	58	1	.07	.80

z the population homogeneity P=.94.

Table 43. Backcross Result Of (Light Salmon X Orange) x Light Salmon For The Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	CROSS	OBSERVED RED ORANGE	OBSERVED LT. SALM.	Total.	D.F.	X^2 (1:1)	P
87-192	86-11-3 X 81-10C	5	3	8	1	.50	.50
87-194	86-11-4 X 81-10C	7	6	13	1	.08	.80
87-196	86-11-5 X 81-10C	5	8	13	1	.69	.40
	Total [z]	17	17	34	3	1.27	.75
	Expect	17	17	34	1	0.00	1.00

z the population homogeneity P=.55.

Table 44. Backcross result Of (Light Salmon x Orange) x Orange For The Orange Flower COlor Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	CROSS	OBSERVED ORANGE	OBSERVED RED ORANGE	Total	D.F.	X^2 (1:1)	P
87-195	86-11-5 X 85-79D	8	9	17	1	.06	.80
	Expect	8.5	8.5	17			

Table 45. Backcross Result Of (Light Salmon X Orange) x Light Salmon For The Orange Flower Color Inheritance In Pelargonium x hortorum.

FAMILIES	OBSERVED RED ORANGE	LIGHT SALMON	Tot.	D.F.	X^2 (1:1)	P
87-251						
87-252						
87-255						
87-258						
87-261						
87-262						
87-266						
87-270						
87-271						
Total	63	57	120	1	.30	.50
Expect	60	60	120	1		

Table 46. Backcross Result of (Light Salmon x Orange) x Orange For The Orange Flower COlor Inheritance In Pelargonium x hortorum.

FAMILIES	OBSERVED RED ORANGE	ORANGE	Total	D.F.	X^2 (1:1)	P
87-246						
87-249						
87-256						
87-264						
87-269						
87-276						
Total	19	21	40	1	.10	.75
Expect	20	20	40	1		

Table 47. Backcross Result of (Orange x White) x Orange For the Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	CROSS	OBSERVED ORANGE	OBSERVED PEACH	Total	D.F.	X^2 (1:1)	P
87-37	85-79B						
	X 85-15-1	7	6	13	1	.08	.75
	EXPECT	6.5	6.5	13			

Table 48. Backcross Result of (Orange x White) x White For The Orange Flower Color Inheritance In <u>Pelargonium</u> x <u>hortorum</u>.

FAMILY	CROSS	OBSERVED PEACH	OBSERVED WHITE	Total	D.F.	X^2 (1:1)	P
87-38	86-15-2						
	X 84-10A	13	11	24	1	.17	.65
	EXPECT	12	12	24			

Table 49. Backcross Result of (White x orange) x White For The Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILIES	CROSSES	OBSERVED PEACH	OBSERVED WHITE	Total	D.F.	X^2 (1:1)	P
87-46	86-16-7 X 84-10A	5	8	13	1	.69	.45
87-48	86-16-12 X 84-10A	3	2	5	1	.20	.60
87-202	86-16-16 X 84-10A	7	6	13	1	.08	.75
	Total [z]	15	16	31	3	.89	.80
	EXPECT	15.5	15.5	31	1	.03	.85

z the population homogeneity P=.40.

Table 50. Backcross Result of (Orange x Rose/White) x Rose/White For The Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILY	CROSS	OBSERVED RED ORANGE	OBSERVED ROSE/WH.	Total	D.F.	X^2 (1:1)	P
	86-20-7 X 82-60C	8	7	15	1	.067	.97
	EXPECT	7.5	7.5	15			

Table 51. F2 Segregation Progenies of the Peach Hybrids Resulting From the Cross of Picotee x Orange For the Orange Flower Color Inheritance In *Pelargonium* x *hortorum*.

FAMILY	OBSERVED						
	PEAC.	ORANGE	ROSE/ WHITE	DEEP SALM.	LT. PK.	PICT.	TOTAL
86-27-8	43	15	15	17	3	6	99
86-27-12	37	12	13	15	5	6	88
86-27-36	33	11	13	18	5	3	83
86-27-43	40	14	12	10	7	5	88
Total	153	52	53	60	20	20	358