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Cytogenetic Evaluation and Foliage Color Inheritance Within Interspecific Begonia Inbreds

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Yue Sun

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Lowell C. Ewart

Major professor

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CYTOGENETIC EVALUATION AND FOLIAGE COLOR INHERITANCE WITHIN INTERSPECIFIC *BEGONIA* INBREDS

BY

Yue Sun

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ABSTRACT

CYTOGENETIC EVALUATION AND FOLIAGE COLOR INHERITANCE WITHIN INTERSPECIFIC BEGONIA INBREDS

By

Yue Sun

The cytogenetic investigation of the germplasm used in this research showed the plant material to be tetraploid with about 64 somatic chromosomes. A dominant gene, *Ru* (R), is hypothesized to control the red underfoliage color inheritance in the tetraploid fibrous-rooted *Begonia x semperflorens-cultorum* Hort. germplasm of this study. This dominant gene also affects the intensity of the foliage color with RRRR and RRRr giving dark red color on the underside of the leaves. The combination of RRrr and Rrrr gives intermediate red coloration, and homozygous recessive rrrr gives all-green foliage. Age of plants, daylength, and temperature also affect underfoliage color. Test crosses and analyses of F_2 generations were used to determine inheritance information, and hybrids also were evaluated for potential commercial value. After nine generations, three triplex plants were selected as a base to develop a dominant homozygous inbred line through selfing and sib mating.

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INTRODUCTION

An interspecific *Begonia* cross between *B. Schmidtiana* and *B.* x *semperflorens-cultorum* produced a population that segregated several plants having deep green color in the top tissue of the leaves and red color in the underside tissue of the foliage, making an attractive color contrast. The plants also had large, drooping stems ideal for hanging basket use. However, red underfoliage does not breed true by seed. Inheritance of this type of begonia foliage coloration has never been reported. The primary objective of this research was to investigate red underfoliage color inheritance patterns, and introduce a true inbred line that could be used to produce a cultivar with new leaf color and possible value for hanging basket production.

Preliminary test crosses and selfs of red-underfoliaged plants indicated a dominant inheritance pattern and possibly a tetraploid chromosome number. To analyze the problem in more detail, increased numbers of selfs, test crosses, and sib matings were performed along with cytological analysis and seed germination studies.

The genus *Begonia* is a large group with perhaps 2,500 species (Thompson, 1981). Before the genus name was established, species of *Begonia* were discovered under other names. Earlier history records the names *Totoncaxoxo coyollin* (Mexican) and *Qiu-hai-tang* (Chinese). The name *Begonia* was founded by Charles Plumier, a Franciscan Monk

and botanist, in 1690. Plumier discovered six species on the Antilles Islands and named the genus in honor of Michael Begon, Governor of Santo Domingo (Thompson, 1981).

Begonia is native to tropical and subtropical areas, with the greatest number having been discovered in the Americas. The plant stems are succulent or woody. Some stems grow in an erect or semi-erect fashion, while others creep or climb. Leaves are alternate and asymmetrical with many shapes. Leaf sizes range from less than 2 to more than 45 centimeters in diameter. The leaf surfaces vary from glabrous to densely hairy and felted. Flowers are characteristically monoecious. Most often the staminate flowers have either two or four petals and the pistillate flowers have two to five petals. Usually the ovaries have two or three locules with axil placentae. The flower color ranges from white to red. Purple and blue are the only two colors not found. Blooming times vary greatly, most being seasonal (Thompson, 1981).

Until 1800, only about five species were cultivated, but after that time the number increased rapidly. After 1850, there were four major developments in *Begonia* cultivation. First, in 1856, *B. rex* was introduced into England and evolved into the Rex Cultorum Group. Second, in the 1860s, six tuberous species that led to the development of Tuberhybrida group were discovered. Sometime between 1814 and 1821, *B. cucullata* var. *Hookeri* (BCH), formerly called *B. semperflorens* (Bailey, 1978), accidentally was introduced into the Berlin Botanical Gardens by Ferkinand Sello. It was found growing in soil brought in from Brazil with other collected plants. This germplasm was not recognized until it was crossed with a newly discovered species, *B. Schmidtiana,* which was introduced by Haage and Schmidt in 1878. This cross was the third major

development in *Begonia* cultivation and was the beginning of the intensive hybridization that resulted in today's very important *Begonia* group, *Begonia* x *semperflorens-cultorum* Hort (BSC). The fourth major development occurred in 1880, when the winter-flowering bulbous *B. socotrana* was found, from which the Hiemalis and Cheimantha groups originated (Thompson, 1981).

Nearly all BSC come from South America, and all the plants are bushy with glossy or hairy leaves. The BSC group grown commercially today as fibrous-rooted begonias has similar characteristics. The plants have glossy, smooth, and sometimes hairy foliage. Leaf colors are green, bronzy-red, dark mahogany, and variegated. *Begonia* x *semperflorens-cultorum* Hort usually blooms throughout the year. Flowers are single, semidouble, or double. The colors of the flowers range from white to deep wine red, and some varieties are bi-colored. F_1 hybrids of BSC were introduced commercially early in the twentieth century. The cultivar Primadonna, introduced in 1909, is listed for being the first F_1 hybrid ever introduced commercially (Ewart, 1995). Today in the United States, the named cultivars number more than 200 (Thompson, 1981).

Fibrous-rooted begonias have been among the top five crops in the bedding plant mix since 1984. Their popularity is due to their versatility and beauty in beds, hanging baskets, window boxes or pots (Ewart, 1995). In 1994 begonias were third in the bedding plant production crop mix, accounting for an average 7.3% of the total bedding plants produced (Behe and Walker, 1994). The bedding plant industry significantly increased sales by 9% from 1993 to 1994, reaching $1.28 billion (Agricultural Statistical Board, 1995).

Zeilinga (1962) studied the inheritance of dwarfness in BSC and found it to be recessive. Holley (1945) studied several different characters in BSC, including doubleness, flower color, plant habit, and foliage color. He suggested that a dominant gene S controlled flower singleness but he could not explain adequately the inheritance of double and semidouble flowers. He also could not explain fully the inheritance of flower and foliage color, and he felt that two or three factors determine flower color, white being double recessive and red being homozygous dominant. In studying foliage color, he found that dark foliage (bronze leaf) was dominant to green foliage. A dark red-leaved cultivar, Carmen, was crossed with several green-leaved cultivars, and all the hybrids had dark foliage. The F_2 progenies, however, segregated into approximately 50% dark foliage, 25% intermediate-red foliage, and 25% green foliage. He gave no further explanation as to the possible reason for this segregation. With regard to plant habit, Holley thought that a spreading, branched habit was recessive to upright growth, the relationship being monogenic.

Matsuura and Okuno (1943) described several advantages for *Begonia* as a subject for cytogenetic studies: existence of a number of species and cultivars, abundance of seeds per capsule and rather rapid growth from seed to flower, monoecious condition that facilitates making crosses, and striking diversity in nuclear organization, even within a single species. In spite of these advantages, only a few cytological studies have been conducted on *Begonia*. Pastrana (1932) described a sex chromosome in *B. Schmidtiana* and observed 13 chromosomes in the sporophytic tissue. Matsuura and Okuno (1943) determined the chromosome numbers for 20 species and stated that chromosomal numbers

in *Begonia* showed wide variation. They also suggested 6, 7, or 13 as the possible basic number, 13 being of secondary origin. They noted two plants morphologically different from *B. Schmidtiana*, and both plants had a somatic chromosome number of 32. In pollen mother cells (PMCs) the chromosomal configuration was $15_{II} + 2_{I}$. The researchers studied chromosomal numbers of seven different BSC plant groups, but did not mention any cultivar names. Finally, they counted 66 chromosomes in a BSC cultivar that had normal meiotic configuration (Table 1).

Zeilinga (1962) described the development of another group from BSC called *B. x semperflorens-cultorum* var. *gracilis*. It was the result of a hybrid between BSC x *B. Schmidtiana* back-crossed with BSC. He studied the historical aspect of cultivar development of BSC, as well as the chromosomal numbers of the different cultivars. He then made crosses between different ploidy levels and reported the cultivars of the BSC group to be diploid. On the other hand, the BSC var. *gracilis* group was triploid or tetraploid as a result from back-crossing with diploid BSC. Zeilinga (1962)found that chromosomal numbers for BSC var. gracilis were 2n = 4x = 68; for *Luminosas compacta* cv. Scarlet, 2n = 4x = 66. He also found that the dense cytoplasm and the very small chromosomes made counting chromosomes in *Begonia* very difficult. He observed that cell divisions were scarce in the small root tips, with extremely low numbers of metaphase plates available for study.

Doorenbos and Legro (1968) conducted a cytological analysis of a winter-flowering *Begonia* called 'Konkurrent' type. They felt that this type was derived somehow from an interspecific cross between *B. socotrana* (2n = 28) and *B. dregei* (2n = 26). They made

Table 1. Chromosomal numbers in *Begonia* from previous investigations.

Cultivar names	Species (Group)	Chromosomal numbers reported by Matsuura	Zeilinga	Doorenbos
	dregei	13(n)		26
	socrotrana	28		28
	Schmidtiana[z]	32	32	
No. I[y]	*semperflorens*[x]	60		
No. III[y]	*semperflorens*[x]	60		
No. V[y]	*semperflorens*[x]	33		
No. VI[y]	*semperflorens*[x]	36		
No. VII[y]	*semperflorens*[x]	33		
HORT rose	*versaliensis*	33		
Ball's Red	*semperflorens*[x]		34	
Christmas Cheer	*semperflorens*[x]		34	
Rosea	*semperflorens*[x]		32	
Vernon	*semperflorens*[x]		34	
Luminosa	*gracilis*		68	
Luminosa compacta	*gracilis*		66	
Indian Maid	*gracilis*		66	
Organdy	*gracilis*		66	
Rosa Wunder	*gracilis*		66	

[z]Pastrana (1932) gave 2n = 13 for *B.Schmidtiana*.

[y]Matsuura and Okuno (1943) studied chromosomal numbers of seven different *B.* x *semperflorens-cultorum* plant groups, plants No. II and No. IV were lost during research.

[x]*B.* x *semperflorens-cultorum* Hort.

a cross between *B. socotrana* and *B. dregei* and used colchicine to obtain tetraploid seedlings. The tetraploid plants (2n = 4x = 54) were fertile and produced triploid plants when pollinated by *B. socotrana*. The triploid plants had the same appearance as the 'Konkurrent' type. They also found that counting *Begonia* chromosomes was very difficult, and that intraplant chromosomal numbers varied frequently in this interspecific hybrid group. Some of the chromosomal determinations from these studies are shown in Table 1.

The previous cytogenetic studies from Zeilinga (1962) and Doorenbos (1968) suggest that many tetraploid cultivars have been derived from diploid hybridization involving similar genomes with high fertility. In most cases, diploids were used in back-crosses with tetraploids to produce new triploid cultivars. Such a developmental strategy suggests the possibility of autotetraploid development, because the genomes of these diploids are very close.

Burnham (1962) demonstrated two types of segregation in autopolyploids: chromosomal segregation and maximum equational segregation. With chromosomal segregation, genes closely linked with the centromeres. An autotetraploid with a triplex AAAa genotype form only AA and Aa gametes, which occur in a ratio of 1:1. When crossovers occur between the gene locus and the centromere, the two pairs of resulting sister chromatids pass to the same pole in anaphase I, and aa gametes can be expected. This behavior has been termed maximum equational segregation (Figure 1). This segregation type requires quadrivalent formation and random separation of the chromatids at anaphase II. Table 2 shows the different segregation ratios between these two

segregation types.

Table 2. Expected frequency of gametic types from chromosomal and maximum equational segregation in tetrasomics or tetraploids heterozygous at a single locus.

Genotype	Chromosomal segregation	aa (%)	Maximum equational segregation	aa (%)
AAAa	AA+Aa	0	13AA+10Aa+aa	4.2
AAaa	AA+4Aa+aa	16.7	2AA+5Aa+2aa	22.2
Aaaa	Aa+aa	50.0	AA+10Aa+13aa	54.2

Muller (1923) developed a formula for determining the number of individuals required at various probabilities to avoid missing a particular genotype:

$n = -\log_e F\,(P-1/2)$

n = number of offspring to be raised

F = a chance of failure

P = reciprocal of the chance that any one individual will be of the desired type

According to this formula, with a 99% chance of success and 1% chance of failure, F = 0.01. If an autotetraploid plant with a triplex (RRRr) genotype is selfed in an attempt to find one possible homozygous-dominant individual, 16 plants would need to be raised for chromosomal segregation: P = 4, F = 0.01, n = 16 (Table 3). Based on maximum equational segregation, thirteen plants would be needed. If, however, a duplex (Rrrr)

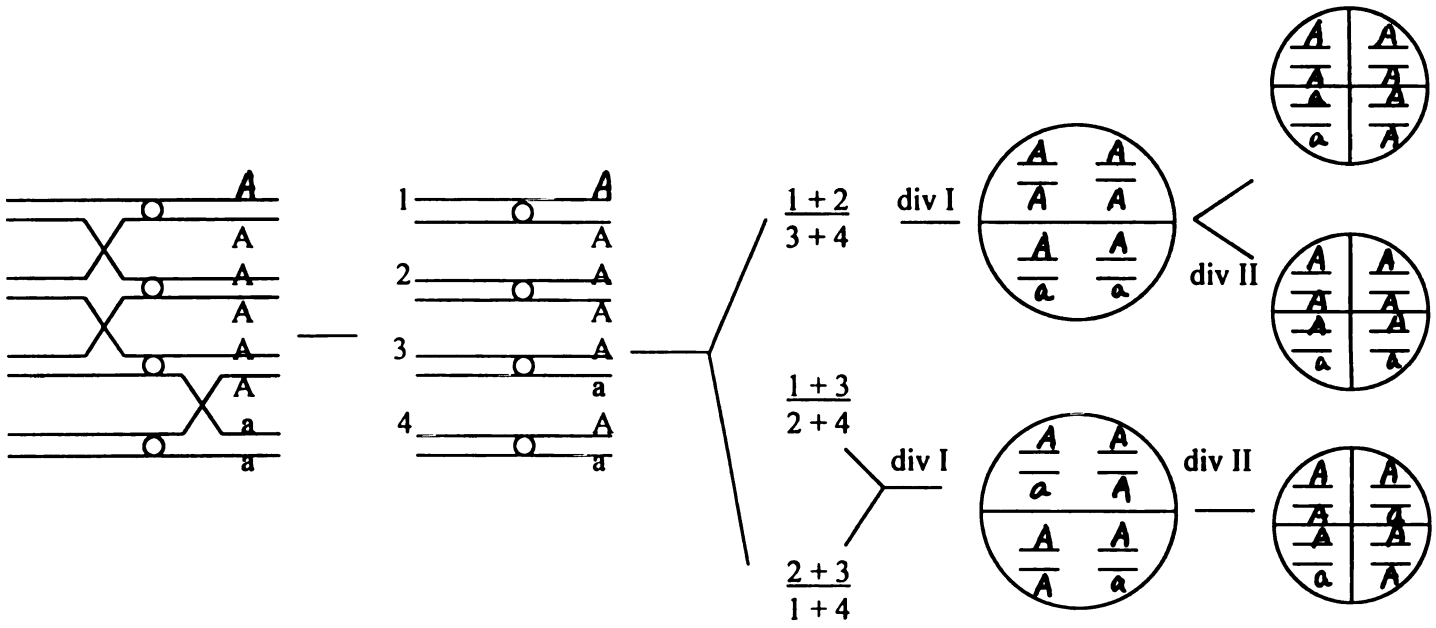

Figure 1. Scheme for determining the theoretical maximum equational segregation for a triplex. Two alleles at a single locus, designated A a, are shown with crossovers between the locus and the centromeres, numbered 1 to 4. By assuming equal frequencies of alternate and two adjacent segregations of the centromeres, 1 + 4/2 + 3, 1 + 3/2 + 4, 1 + 2/3 + 4, respectively, the gametic ratio may be determined as 13AA : 10Aa : aa (modified from Figure 51; Burnham, 1962).

Table 3. Expected ratios of different genotypes from selfed plants for the number of individuals required to possibly find one homozygous-dominant individual (red-underfoliaged plant) according to Muller's formula (Muller, 1923) and from the different type of segregation by Burnham (1962) for under-foliage color inheritance within interspecific *Begonia* inbreds.

Genotype	Type of Segregation	RRRR	RRRr	RRrr	Rrrr	rrrr	P[z]	F[y]	n[x]
RRRr	Chromosome[w]	1	2	1			4	0.01	16
	Max. eq.[v]	169	260	126	20	1	3.4	0.01	13
RRrr	Chromosome[w]	1	8	18	8	1	35	0.015	144
	Max. eq.[v]	4	20	33	20	4	19.25	0.01	86
Rrrr	Chromosome[w]			1	2	1			
	Max. eq.[v]	1	20	126	260	169	407	0.01	1870

[z]Reciprocal of the chance that any one individual shall be of such type.
[y]A chance of failure.
[x]Number of offspring to be raised.
[w]Chromosomal segregation.
[v]Maximum equational segregation.

individual is selfed, 144 plants would be needed at F = 0.015 to find one homozygous dominant plant for chromosome segregation , but only 86 plants would be needed at F=0.01 for maximum equational segregation. Thus, if a triplex dominant plant could be identified, theoretically the possibility of finding a homozygous-dominant individual would be greater.

In this study, the desired number of plants for both selfs and test crosses was 144, which used three flats of 48 cells. This number for each population was workable for the space and growing containers available and the number of populations to be analyzed. This number also would yield a good possibility of finding a homozygous-dominant plant from a self of any triplex plant. The number of plants did vary because of seed availability and plant mortality.

The germplasm for red underfoliage color used in this research was developed by the ornamental breeding research program at Michigan State University. The germplasm, designated SCV, was generated from the interspecific cross between *B. Schmidtiana* and a BSC plant selected from a cross of the BSC cvs.Charm and Vodka. *B. Schmidtiana* has pink flowers and dark green leaves with red underfoliage, and the plants are upright in habit with rather hairy stems. The BSC cv. Charm has variegated leaves, long stems, light red flowers, and is vegetatively propagated. The hybrid BSC cv. Vodka has dark bronze-colored leaves with bright red flowers, a compact upright habit, and is seed-propagated. A green-foliaged inbred plant, now designated as CV, selected from a cross of 'Charm' and 'Vodka' in the F_2 generation, was used to make the cross with *B. Schmidtiana*. The CV inbred germplasm selected for this foliage inheritance study has bright red flowers, an

upright habit, and it breeds true for all-green foliage. It was used in test crosses with SCV inbred selections to get underfoliage color segregation information. Such crosses also could be considered F_1 hybrid crosses, because both parents are inbreds. Several such populations were planted in the summer of 1995 for potential commercial application.

The SCV red-underfoliaged inbred plants used in this study have red flowers, long drooping stems, and are seed-propagated. After several generations of selfing (F_6 to F_9), three inbred individuals that did not segregate all-green-foliaged plants after selfing were found. An all-green-foliaged inbred that was developed from the hybrid cultivar Pink Avalanche was used in a special crossing sequence to help determine polyploidy of the germplasm for red underfoliage.

Germplasm development and relationship of the darkest red-underfoliaged inbred individuals in this study are shown in Figure 2.

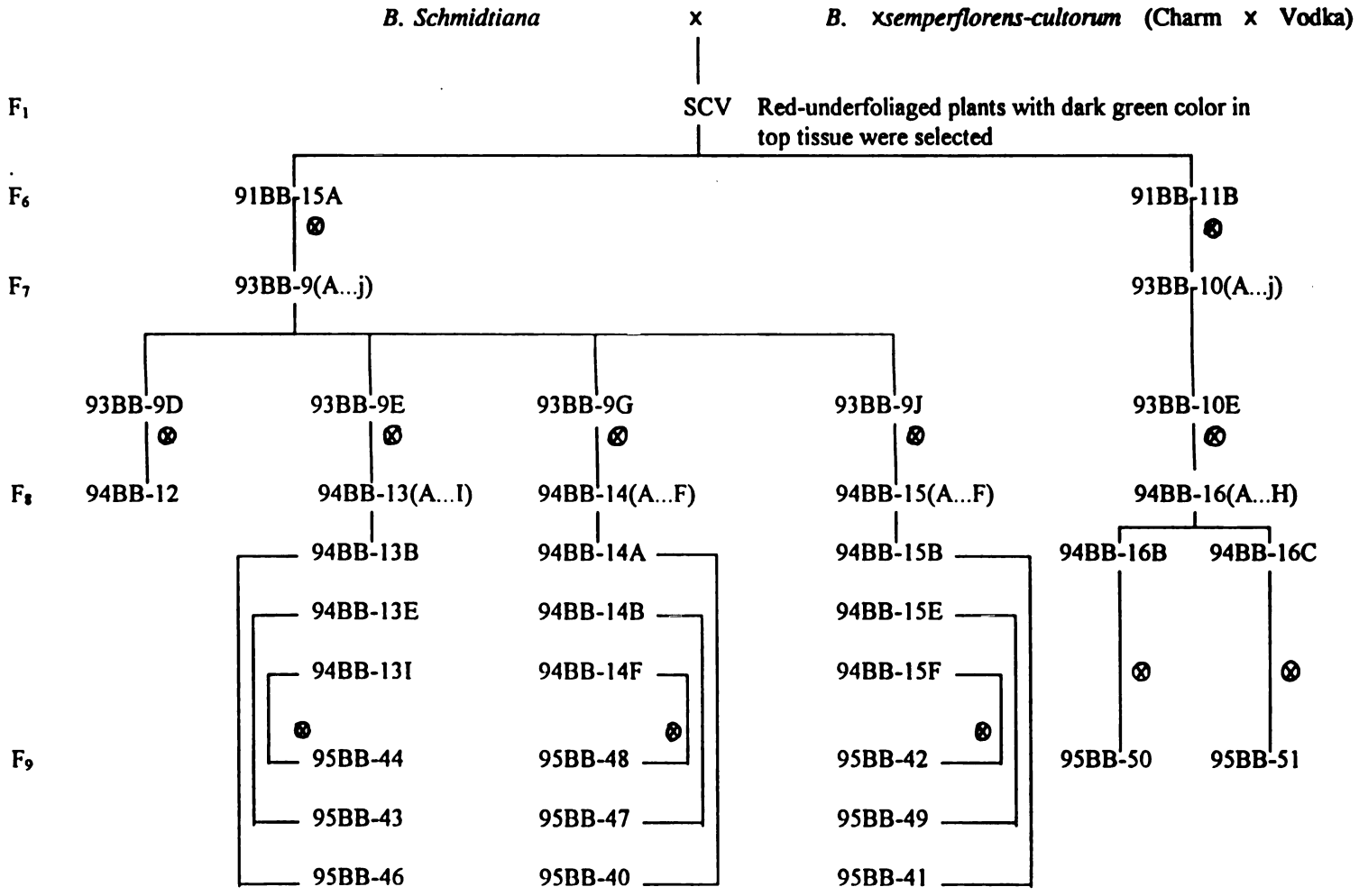

Figure 2. Germplasm development and relationship of inbred individuals with the darkest red-underfoliage in the foliage color inheritance study from the interspecific *Begonia* cross *B. Schmidtiana* x (*B.* x *semperflorens-cultorum* cvs. Charm x Vodka)SCV.

MATERIALS AND METHODS

Plant Materials

The development of the germplasm involved in this research is described in the introduction. Seeds from two F_6 inbred plants provided by Dr. Ewart were used to start this research.

Seed Handling

All seeds were sown in 20-row, plastic germination trays using a peat-lite planting mix. The seeds were covered with a very light dusting of fine vermiculite. After sowing and watering, clean clear plastic covers were placed on top of the seed trays for moisture control, and the trays then were placed in a germination room at 80°F. Radicle emergence took place five to seven days after sowing. After 10 days, the plastic covers were removed. After three weeks, seed flats were moved to a cooler greenhouse with 65°F and 55°F day/night (D/N) temperatures respectively. Cool-white fluorescent or high-pressure sodium (HPS) lamps were provided to encourage rapid growth. The seedlings were fertilized with 0.13% KNO_3 every 10 days.

Approximately seven weeks after sowing, the seedlings were transplanted to 48-cell flats and 0.2% KNO_3 was applied every two weeks. Standard greenhouse practices were followed for disease and insect control.

Hybridization Procedure

Selected plants from the transplant flats were planted directly into five-inch standard plastic pots. As plants came into flower, the required pollinations were made. To avoid unwanted fertilization, female flowers just at the verge of opening were used. Tweezers were used to handle the male flowers, using direct flower-to-flower pollen transfer. All greenhouse crosses and selfs were performed in a screened house. Four to six weeks were required for the seeds to mature. After cleaning, the seeds were placed in a 5°C, 35% relative humidity (RH) seed storage room for at least two weeks before sowing.

Color Classification

Underfoliage coloration was categorized as dark red, intermediate red, and green for inbred and test-cross populations.

Foliage color segregation data were collected about 25 days after transplanting and every seven to nine days for another two months to note any changes over time. At each generation of red-underfoliaged inbreds, five to 10 of the darkest red-underfoliaged plants were selected and transplanted into five-inch standard plastic pots.

Statistical Analysis

Chi-square and goodness-of-fit analyses were used to test the segregating generations to Mendelian ratios. The non significant null hypothesis was accepted when

the probability was equal to or greater than 0.05.

Analysis of variance (ANOVA) was used to analyze seed germination and vigor rates. If F tests showed a significant difference, the least significant difference (LSD) was used for comparing the results in the seed germination and vigor experiment.

Pollinations Performed

The types of pollinations performed and the reason for each self, sib, and test cross are given in Table 4.

Each new selfed population was obtained by selfing the darkest red-underfoliaged individuals selected from the previous inbred populations. Each test-cross population was obtained by crossing the darkest red-underfoliaged selections from inbred populations with the homozygous all-green-foliaged CV inbred. To increase inbred vigor and possibly help hedge against missing any homozygous red-underfoliaged plants caused by inbreeding depression resulting from repeated selfing, sib-mating was performed among some of the dark red-underfoliaged individuals.

Seed Germination Test and Seedling Vigor Rates

Seed germination tests were performed on various populations. The purpose was to check germination in the various populations representing different genotypes on the assumption that any unusual results might help explain unexpected ratios, should they occur in the material. These tests were handled by counting out 50 seeds of each line to be tested and spreading the seeds on filter paper in 100-x-15 mm glass petri dishes in

Table 4. Pollinations performed for the underfoliage color inheritance study within interspecific *Begonia* inbreds.

Parental Types	Objective
Red-underfoliaged inbreds selfed: 91BB-11B, 91BB-15A 93BB-9-D,E,G,J 93BB-10-B,E 94BB-13-A,B,E,I 94BB-14-A,B,F 94BB-15-B,E,F 94BB-16-B,C	To continue the lines, to get segregation counts, to obtain a homozygous red-underfoliaged individual.
Test-cross pollination of individuals selected for red-underfoliaged plants with homozygous all-green-foliaged inbred.	To obtain segregation ratio counts, to determine if any inbred plant homozygous for red underfoliage was possible to obtain.
Sib pollination within individual lines and between sister lines of red-underfoliaged inbreds with the darkest red color.	To increase vigor, to check segregation ratios, to make further selections for possible homozygous red-underfoliaged individuals.

which enough distilled water had been added to moisten the paper thoroughly. The petri dish covers were sealed with Parafilm®, and the dishes were placed in a tissue-culture room where the temperature averaged 25°C with a light intensity of 12 to 14 $uE.M^{-2}.S^{-1}$. Germination counts were made one week from the time of sowing. The counts are shown in Table 17.

Seedling vigor rating was on a scale of 1 to 5 (poor to excellent), which was determined on seedling vigor qualities, qualities of seedling tissue, and development of seedling structure.

Pollen-Fertility Test

Empty or poorly stained pollen grains are an indication of poor fertility.

A pollen-fertility test was conducted on a plant selected from an apparently sterile hybrid population. The stain used was cotton blue (Darlington and LaCour, 1976). Prepared by adding equal parts of water, 85% liquified lactic acid, 88% liquified phenol (liquified carbolic acid), and U. S. P. glycerine, in that order, and mixing well before each addition (Ewart, 1963).

The anthers were removed from flower buds, placed in a drop of cotton blue on a slide, cut in half, and mixed with the stain, and the excess debris was removed. A cover slip then was placed over the drop of stain containing the pollen. The slide then was observed under an Olympus binocular microscope at x200 magnification.

Cytological Techniques

In order to get chromosome number information, root tips and pollen mother cells were used. The stain used was aceto-carmine (Smith, 1947). It was prepared by boiling an excess of carmine in 45% acetic acid for five minutes, which then was cooled and filtered.

Three pretreatment methods were attempted on root tips: 1) cold treatment, keeping root tips at 0°C overnight (Singh, 1993; Whallon, 1993 a), 2) placing the root tips in paradichlorobenzene (Meyer, 1945; Palmer and Heer, 1973) for four hours at room temperature, 3) placing the root tips in 0.1% colchicine for three to four hours at room temperature (Singh, 1993; Whallon, 1993 a). These pretreatments were tried to prevent spindle fiber formation and keep the chromosomes from clumping. Methods 2 and 3 gave the best results, but none of them were outstanding.

The root tips usually were harvested between 8:00 and 10:00 a.m. during the summer and a little later during the fall and winter. The root tips were fixed for 24 hours in Farmer's Solution (Carnoy's Solution I), which consisted of one part glacial acetic acid and three parts absolute or 95% ethanol (Singh, 1993; Whallon, 1993 a). The root tips were maintained in 70% ethanol until they could be analyzed.

The root tips were placed in a drop of aceto-carmine on a slide, which then was placed on a dissecting microscope at x10 to remove the root caps. Next the tip portion was macerated and smeared using a dissecting needle and a spear-point needle. After the cover slip was in place, further smearing was obtained by pressure on the cover slip applied with the slide between folds of a paper towel. At this point two small drops of

stain were added to the edge of the cover slip, and the whole preparation was heated gently.

Besides being used to determine chromosomal number, pollen mother cells were examined to try to determine chromosomal meiotic configurations. Aceto-carmine again was used. Small male flower buds approximately 5 to 7 mm long usually were picked between 8:00 and 10:00 a.m. The flower buds were fixed 18 to 24 hours in a propionic acid-alcohol solution that consisted of one part propionic acid, three parts absolute ethanol and one gram ferric chloride ($FeCl_3$) per 100 ml of fixative (Swaminathan et al., 1954) without any pretreatment. The male flower buds then were washed with two changes of 70% ethanol and stored in 70% ethanol until used. The anthers were removed from flower buds and placed in a drop of aceto-carmine on a slide and cut in half using a dissecting needle and spear-point needle. The macerated anthers were pressed to force the pollen mother cells out into the stain. The excess debris then was removed and the material covered with a cover slip. The procedures above were performed under a dissecting microscope. Further spreading of the cells was obtained by applying pressure on the cover slip with the slide between folds of a paper towel. Two small drops of stain then were added to the edge of the cover slip and whole preparation was heated gently.

Observations for root tips and pollen mother cells were made using an Olympus binocular microscope at x200 to 1,000. Photomicrographs for root tips were made at x1,000.

Because the chromosomes were extremely small, a Zeiss Laser Scanning Confocal

Microscope (LSM) was used to take photomicrographs from x4,000 to x10,000.

The LSM improves contrast by removing of out-of-focus light in the final image, resulting in increased resolution. With LSM, the magnification can vary from x100 to a high of x16,000 with combinations of objectives and zoom values, which is more than ten times the magnification of a conventional light microscope (Whallon, 1993 b).

RESULTS

Cytogenetic Evaluation

The overall raw data from root-tip chromosomal counts of the germplasm examined in this study ranged from 32 to 72 (Figures 3-9, Table 5), which indicated that ploidy levels ranged from diploid to tetraploid. The clearest observed somatic chromosomal numbers and the suggested ploidy level are shown in Table 6.

The interspecific inbred SCV is polyploid (tetraploid) with 60 to 64 chromosomes (Figure 3). A BSC inbred derived from the cultivar Pink Avalanche was diploid with 32 chromosomes (Figure 4). When this inbred was crossed with the interspecific inbred SCV, the resulting hybrid had 48 chromosomes in somatic cells (Figure 5), and the plants were sterile. The cotton blue test revealed empty pollen grains. Self and test-cross pollinations were unsuccessful. These results indicate that this hybrid was a triploid, reinforcing previous indications that SCV is tetraploid. *Begonia* x *semperflorens-cultorum* cv. Charm had 32 to 34 chromosomes (Figure 6) and was considered diploid, whereas BSC cv. Vodka had 40 to 42 chromosomes (Figure 7). The hybrid of these two cultivars (CV) was polyploid with 68 to 72 chromosomes and self-fertile (Figure 8). *Begonia Schmidtiana* had 62 to 64 chromosomes (Figure 9).

Pollen mother cells also were used to determine chromosomal numbers and meiotic configurations, but proved to be even more unsatisfactory than root tips. It was

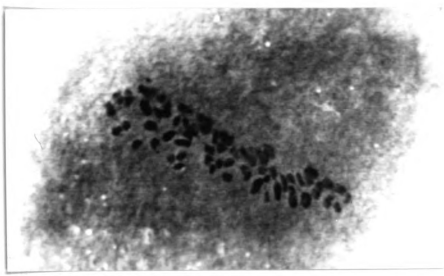

Figure 3. *Begonia* root tip cell from inbred SCV (*B. Schmidtiana* x CV) (2n = 4x = 62-64; x3,128).

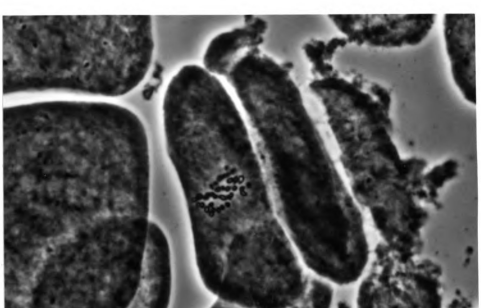

Figure 4. Root tip cell from *B.* x *semperflorens-cultorum* inbred 'Pink Avalanche' (2n = 2x = 32; x1,100).

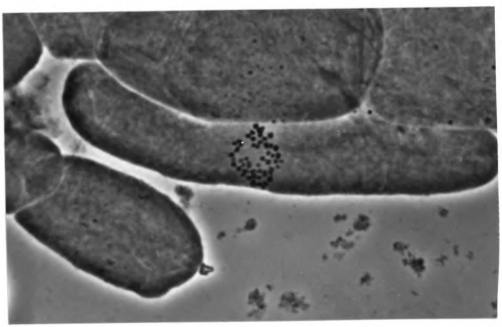

Figure 5. *Begonia* root tip cell from SCV x *B.* x *semperflorens-cultorum* inbred 'Pink Avalanche' (2n = 3x = 48; x1,100).

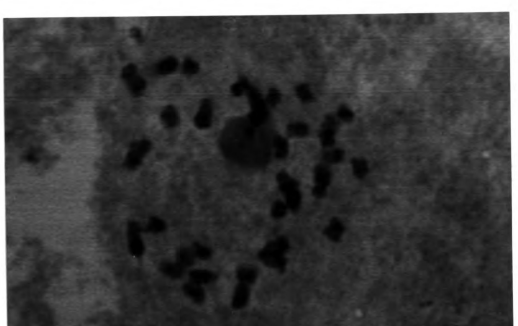

Figure 6. Root tip cell from *Begonia* x *semperflorens-cultorum* cv. Charm (2n = 2x = 32-34; x5,244).

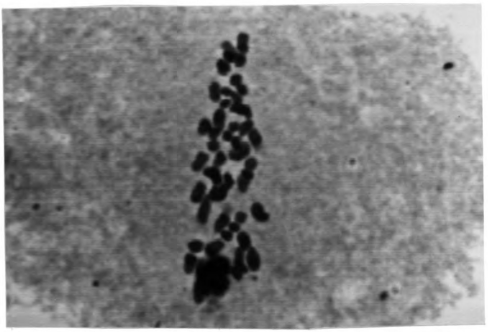

Figure 7. Root tip cell from *Begonia* x *semperflorens-cultorum* cv. Vodka (2n = 2x = 40-42; x3,600).

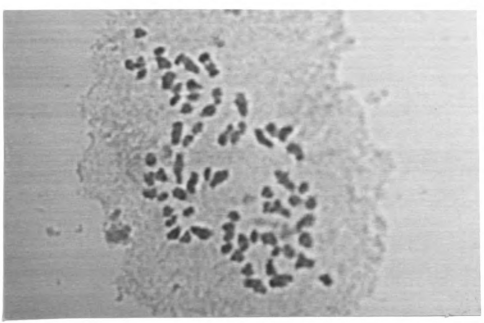

Figure 8. Root tip cell from *Begonia* x *semperflorens-cultorum* inbred developed from 'Charm' x 'Vodka' (CV) (2n = 4x = 68-72; x3,848).

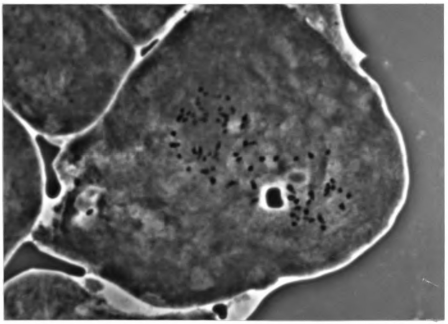

Figure 9. Root tip cell from *Begonia Schmidtiana* (2n = 4x = 62-64; x1,100).

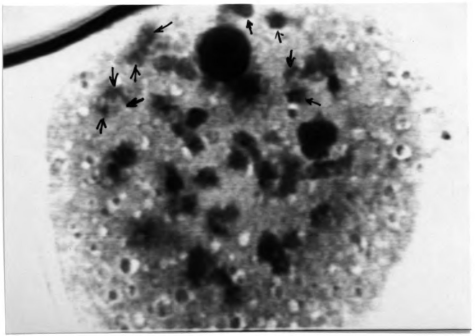

Figure 10. *Begonia* pollen mother cell from inbred SCV (*B. Schmidtiana* x CV) (2n = 4x = 62-64; x5,167; ↑ indicates univalent).

Table 5. The overall raw data from *Begonia* root tip chromosomal counts of the germplasm examined in the underfoliage color inheritance study.

Species (cultivar)	Countable cells observed	Chromosomal number per cell		
		Maximum	Minimum	Mean
B. Schmidtiana	3	68	57	64.3
Charm[z]	4	34	32	32
Vodka[z]	6	50	40	44.4
CV[y]	11	72	58	64.7
SCV[y]	17	68	57	65.2
Avalanche[w]	14	34	26	31.4
SCV x Avalanche	5	49	42	46.8

[z]*Begonia* x *semperflorens-cultorum* cvs.
[y]The inbred developed from *B.* x *semperflorens-cultorum* cvs. Charm x Vodka.
[x]The inbred developed from *B. Schmidtiana* x CV.
[w]The inbred developed from *B.* x *semperflorens-cultorum* cv. Pink Avalanche.

Table 6. Somatic chromosomal numbers of the germplasm examined for underfoliage color inheritance within interspecific *Begonia* inbreds.

Species (cultivar)	Chromosomal Numbers	Ploidy level
Begonia Schmidtiana	62-64	4x
*B*egonia cultivar[z] Charm	32-34	2x
*B*egonia cultivar[z] Vodka	40-42	3x
CV[y]	68-72	4x
SCV[x]	64	4x
Begonia cultivar[z] Pink Avalanche	32	2x
SCV x *B.*cultivar[z] Pink Avalanche	48	3x

[z]*Begonia* x *semperflorens-cultorum.*

[y]The inbred developed from *Begonia* x *semperflorens-cultorum* cv. Charm crossed with *Begonia* x *semperflorens-cultorum* cv. Vodka.

[x]Inbred developed from *B. Schmidtiana* crossed with an F_2 plant from a *B.* x *semperflorens-cultorum* cross between cultivar Charm and Vodka.

extremely difficult to spread chromosomes. Figure 10 shows meiotic chromosomes of SCV. About 30 bivalent and four to six univalent chromosomes were found at diakinesis.

Red Underfoliage Color Inheritance Patterns and Selection of Homozygous Red-Underfoliaged Plants

Crossing *B. Schmidtiana* (red underfoliage color) with the all-green-foliaged inbred CV (*B.* x *semperflorens-cultorum* cv. Charm x *B.* x *semperflorens-cultorum* cv. Vodka) produced 46 plants, all of which had red under-foliage.

A dominant gene, now designated (Sun and Ewart) '*Ru*' (R) with its recessive allele '*ru*' (r), is hypothesized to control the red underfoliage color inheritance in the germplasm used in this research.

Table 7 shows the segregation ratios and statistical results from the F_2 population of three red-underfoliaged plants and one all-green-foliaged plant. These three red-underfoliaged plant selections segregated out red-, intermediate red-, and all-green-foliaged plants. The all-green-foliaged plant, when selfed, produced all-green-foliaged plants. These results showed that the all-green foliage color is recessive to the red underfoliage character.

Tables 8 through 16 present the Chi-square determinations of the various population categories studied. We hoped that some homozygous dominant red-underfoliaged plants would be found during this research.

Based on chromosomal segregation, any selfed triplex, duplex, and simplex plants should segregate 0%, 2.8% and 25% recessive genotype individuals, respectively, and

Table 7. Segregation ratios and statistical analyses of F_2 populations from SCV x CV for underfoliage color inheritance within interspecific *Begonia* inbreds.

Pedigree	Segregation ratio[z] Red[x]	In. red[w]	Green[v]	Green (%)	Possible genotype	Chromosomal segregation Ratio R:r	Recessive (%)[u]	Max. eqa. segregation[y] Ratio R:r	Recessive (%)[u]
95BB-18	68	44	32	22.2	RRRr	All R	0.0**	575:1	0.174**
					RRrr	35:1	2.8**	77:4	4.94**
					Rrrr	**3:1**	**25.0^{NS}**	**407:169**	**29.34^{NS}**
95BB-20	25	65	54	37.5	RRRr	All R	0.0**	575:1	0.174**
					RRrr	35:1	2.8**	77:4	4.94**
					Rrrr	3:1	25.0**	**407:169**	**29.34^{NS}**
95BB-21	39	48	56	39.2	RRRr	All R	0.0**	575:1	0.174**
					RRrr	35:1	2.8**	77:4	4.94**
					Rrrr	3:1	25.0**	407:169	29.34*
95BB-19	0	0	48	100	RRRr	All R	0.0**	575:1	0.174**
					RRrr	35:1	2.8**	77:4	4.94**
					Rrrr	3:1	25.0**	407:169	29.34**
					rrrr	**All a**	**100.0^{NS}**	**All a**	**100.0^{NS}**

^{NS},*,**Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.

[z]Actual segregation data for one population.

[x]Red-underfoliaged plants.

[y]Maximum equational segregation.

[w]Intermediate red-underfoliaged plants.

[v]All-green-foliaged plants.

[u]Percentage of recessive genotype.

0.174%, 4.94%, and 29.34% if based on the maximum equational segregation system. Therefore, in this research the statistical analysis was conducted by combining the dark and intermediate red- underfoliaged plants to represent the dominant genotype. The percentage of all-green-foliaged plants whose genotype was recessive in various pollination populations was calculated and analyzed.

Table 8 shows observed segregation ratios obtained from three F_7 populations of dark red underfoliage of SCV germplasm. The percentage of all-green-foliaged plants of 93BB-9 is 10.5, not significantly different from the expected duplex, based on maximum equational segregation, but significantly different based on chromosomal segregation. In 93BB-10, the percentage of all-green-foliaged plants is 7.7, which was not significantly different from the expected level for a duplex segregation ratio, either for the chromosomal or maximum equational segregation systems. The results of the statistical analysis suggest that these two populations were derived from a duplex plant with RRrr genotype.

The all-green-foliage segregation percentage is 18.1 for the population of 93BB-5, a result that does not fit any expected genotype. However, the closest fit was for Rrrr (simplex) based on chromosomal segregation. This was significant at 0.05. Possibly some of the all-green-foliaged plants were classified incorrectly, since coloration changed over time.

Ten of the darkest red-underfoliaged plants from the three populations (93BB-5, -9 and -10) were selected and selfed to produce seed for the next generation. These plants also were test-crossed with a homozygous all-green-foliaged inbred to check for selections homozygous for the red underfoliage character. The test crosses also can be considered

Table 8. Segregation ratios and statistical analyses of selfed inbred populations of SCV obtained by selecting plants with the darkest red underfoliage for the underfoliage color inheritance study within interspecific *Begonia* germplasm.

		Segregation ratio[z]				Possible	Chromosomal segregation		Max.eqa. segregation[y]	
Pedigree	Gen[x]	Red[w]	In. red[v]	Green[u]	Green (%)	genotype	Ratio R:r	Recessive (%)[t]	Ratio R:r	Recessive (%)[t]
93BB-9	F_7	26	76	12	10.5	RRRr	All R	0.0**	575:1	0.174**
						Rrrr	**35:1**	**2.8****	**77:4**	**4.94^{NS}**
						Rrrr	3:1	25.0**	407:169	29.34**
93BB-10	F_7	24	108	11	7.7	RRRr	All R	0.0**	575:1	0.174**
						Rrrr	**35:1**	**2.8^{NS}**	**77:4**	**4.94^{NS}**
						Rrrr	3:1	25.0**	407:169	29.34**
93BB-5	F_7	23	117	31	18.1	RRRr	All R	0.0**	575:1	0.174**
						Rrrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0*	407:169	29.34**

$^{NS, *, **}$Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation ratio in one population.
[y]Maximum equational segregation system.
[x]Generations.
[w]Dark red-underfoliaged plants.
[v]Intermediate red-underfoliaged plants.
[u]All-greenfoliaged plants.
[t]Percentage of recessive genotype.

hybrids because the two parents were inbreds and were, therefore, selfed for F_2 segregation ratio data (Table 7).

Seed was obtained successfully from six individuals of 93BB-5: 93BB-5A, 5B, 5C, 5D, 5E, and 5H, which were test-crossed with the all-green-foliaged inbred and yielded six populations (Table 9). The results of these six populations were combined according to underfoliage color classification to represent the segregation ratio of 93BB-5. The all-green-foliaged segregation percentage was 49.4, not significantly different from the expected simplex segregation ratio, based on chromosomal segregation. The results indicate that 95BB-5 was simplex with a genotype of Rrrr, which correlates with the information for 93BB-5 in Table 8. Since the data indicated all the selections from 93BB-5 were simplex, and it was not possible to select any homozygous-dominant individuals, the inbred line was abandoned.

Tables 10 and 11 show the results from the eight successful test-cross populations from 93BB-9 and nine test-cross populations from 93BB-10, respectively. Both results show that the all-green-foliaged segregation percentage is higher than expected (16.7 to 22.2% for a duplex genotype), probably because some simplex plants were selected from 93BB-9 and 93BB-10. When these possible simplex selections were test-crossed with an all-green-foliaged inbred, they produced more all-green-foliaged plants than would be produced from duplex or triplex plants. Since the segregation numbers of these populations were combined for analysis, the all-green-foliaged percentage would be higher than expected. These results also show the difficulty in selecting for the various dominant genotypes based on the red color intensity.

Table 9. Segregation ratios and statistical analyses of test-cross populations obtained by crossing selections with the darkest red underfoliage from SCV inbred 93BB-5 with the homozygous green-foliaged CV inbred for underfoliage color inheritance within interspecific *Begonia* inbreds.

	Segregation ratio[z]				Possible	Chromosomal segregation		Max. eqa. segregation[y]	
Pedigree	Red[x]	In. red[w]	Green[v]	Green (%)	genotype	Ratio R:r	Recessive (%)[u]	Ratio R:r	Recessive (%)[u]
94BB-28 (5A)[t]	9	73	62						
94BB-29 (5B)[t]	22	54	68						
94BB-30 (5C)[t]	4	106	34						
94BB-31 (5D)[t]	5	48	91						
94BB-32 (5E)[t]	6	37	101						
94BB-35 (5H)[t]	0	24	120						
Total	46	318	356	49.4	RRRr	All R	0.0**	23:1	4.2**
					Rrrr	5:1	16.7**	7:2	22.2**
					Rrrr	**1:1**	**50.0^{NS}**	11:13	54.2**

^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[x]Dark red-underfoliaged plants.
[y]Maximum equational segregation.
[w]Intermediate red-underfoliaged plants.
[v]All-green-foliaged plants.
[u]Percentage of recessive genotype.
[t]Six individual plants selected from 93BB-5.

Table 10. Segregation ratios and statistical analyses of test-cross populations obtained by crossing selectins with the darkest red underfoliage from SCV inbred 93BB-9 with the homozygous green-foliaged CV inbred for underfoliage color inheritance within interspecific *Begonia* inbreds.

	Segregation ratio[z]				Possible	Chromosomal segregation		Max. eqa. segregation[y]	
Pedigree	Red[x]	In. red[w]	Green[v]	Green (%)	genotype	Ratio R:r	Recessive (%)[u]	Ratio R:r	Recessive (%)[u]
94BB-36 (9A)[t]	16	46	82						
94BB-37 (9B)[t]	20	37	87						
94BB-38 (9C)[t]	60	69	15						
94BB-41 (9F)[t]	2	30	64						
94BB-42 (9G)[t]	72	71	1						
94BB-43 (9H)[t]	25	42	77						
94BB-44 (9I)[t]	28	79	37						
94BB-45 (9J)[t]	58	77	9						
Total	281	451	372	33.7	RRRr	All R	0.0**	23:1	4.2**
					Rrrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**

^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[x]Dark red-underfoliaged plants.
[y]Maximum equational segregation.
[w]Intermediate red-underfoliaged plants.
[v]All-green-foliaged plants.
[u]Percentage of recessive genotype.
[t]Six individual plants selected from 93BB-9.

Table 11. Segregation ratios and statistical analyses of test-cross populations obtained by crossing selections with the darkest red underfoliage from SCV inbred 93BB-10 with the homozygous green-foliaged CV inbred for underfoliage color inheritance within interspecific *Begonia* inbreds.

	Segregation ratio[z]				Possible	Chromosomal segregation		Max. eqa. segregation[y]	
Pedigree	Red[x]	In. red[w]	Green[v]	Green (%)	genotype	Ratio R:r	Recessive (%)[u]	Ratio R:r	Recessive (%)[u]
94BB-46 (10A)[t]	30	91	48						
94BB-47 (10B)[t]	21	83	51						
94BB-48 (10C)[t]	24	59	74						
94BB-49 (10D)[t]	34	90	20						
94BB-50 (10E)[t]	56	81	7						
94BB-51 (10F)[t]	13	66	65						
94BB-52 (10G)[t]	20	80	44						
94BB-53 (10H)[t]	21	84	38						
94BB-55 (10J)[t]	13	56	75						
Total	232	690	422	31.7	RRRr	All R	0.0**	23:1	4.2**
					Rrrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**

^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[x]Dark red-underfoliaged plants.
[y]Maximum equational segregation.
[w]Intermediate red-underfoliaged plants.
[v]All-green-foliaged plants.
[u]Percentage of recessive genotype.
[t]Six individual plants selected from 93BB-10.

Two populations from these test crosses, 94BB-42, 94BB-45 from 93BB-9, and one population 94BB-50 from 93BB-10, show the highest percentage of red-underfoliaged plants. The statistical results of the all-green-foliaged segregation percentage of these three test-cross populations are shown in Table 12. The 94BB-46 and -48 populations were used for comparison. The results indicate that two of the three selections, 94BB-45 and 94BB-50 (6.2% and 4.9% all-green-foliage, respectively) fit a triplex inheritance pattern based on the maximum equational segregation. The 94BB-42 population had only one all-green-foliaged plant, and this segregation pattern does not fit any logical segregation system. It is possible that this all-green-foliaged plant is a mixed seedling. If it is, therefore, ignored, the segregation ratio of 94BB-42 would fit the expected triplex inheritance pattern based on the chromosomal segregation. Consequently, the three individuals, 93BB-9G, 93BB-9J, and 93BB-10E, that corresponded to the three test crosses with the lowest number of all-green-foliaged plants were selfed to produce seed for the next inbred generation, F_8.

Table 13 shows the segregation ratios for the inbred populations 94BB-13, -14, -15 and -16, which were derived from the selfs of 93BB-9E, -9G, -9J, and 93BB-10E. The seeds from the test cross of 93BB-9E had poor germination, so no test-cross data were possible. However, 93BB-9E was included here because of the intense red color of the underfoliage. The all-green-foliaged segregation percentages of 94BB-14 and 94BB-15 were 6.25 and 8.3, respectively. The data show no significant differences from expectations for duplex segregation based on the maximum equational segregation. The statistical result indicates that the 94BB-14 and 94BB-15 populations were from duplex plants (93BB-9G, 93BB-9J), which is contradictory to their test-cross segregation results

Table 12. Segregation ratios and statistical analyses of test-cross populations obtained by crossing selections with the darkest red underfoliage from SCV inbred 93BB-9 and 93BB-10 with the homozygous green-foliaged CV inbred for underfoliage color inheritance within interspecific *Begonia* inbreds.

Pedigree	Segregation ratio[z] Red[x]	In. red[w]	Green[v]	Green (%)	Possible genotype	Chromosomal segregation Ratio R:r	Recessive (%)[u]	Max. eqa. segregation[y] Ratio R:r	Recessive (%)[u]
94BB-42	72	71	1	0.69	RRRr	All R	0.0**	23:1	4.2**
9G[t] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
94BB-45	58	77	9	6.2	**RRRr**	All R	0.0**	**23:1**	**4.2^{NS}**
9J[t] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
94BB-50	56	71	7	4.9	**RRRr**	All R	0.0**	**23:1**	**4.2^{NS}**
10E[s] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
94BB-46	30	91	48	28.4	RRRr	All R	0.0**	23:1	4.2**
10A[s] x CV					RRrr	5:1	16.7**	7:2	22.2*
					Rrrr	1:1	50.0**	11:13	54.2**

Table 12 (cont'd).

Pedigree	Segregation ratio[z] Red[x]	In. red[w]	Green[v]	Green (%)	Possible genotype	Chromosomal segregation Ratio R:r	Recessive (%)[u]	Max. eqa. segregation[y] Ratio R:r	Recessive (%)[u]
94BB-48 10C[s] x CV	24	59	74	47.1	RRRr	All R	0.0[**]	23:1	4.2[**]
					RRrr	5:1	16.7[**]	7:2	22.2[**]
					Rrrr	1:1	50.0[NS]	11:13	54.2[**]

[NS,*,**]Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[x]Dark red-underfoliaged plants.
[y]Maximum equational segregation.
[w]Intermediate red-underfoliaged plants.
[v]All-green-foliaged plants.
[u]Percentage of recessive genotype.
[t]Individual plants selected from F_7 SCV inbred 93BB-9.
[s]Individual plants selected from F_7 SCV inbred 93BB-10.

Table 13. Segregation ratios and statistical analyses of F_8 SCV inbred populations obtained by selfing inbreds with the darkest red underfoliage from SCV F_7 inbreds for foliage color inheritance within interspecific *Begonia* inbreds.

		Segregation ratio[z]				Possible	Chromosomal segregation		Max.equa. segregation[y]	
Pedigree	Gen[x]	Red[w]	In. red[v]	Green[u]	Green (%)	genotype	Ratio R:r	Recessive (%)[t]	Ratio R:r	Recessive (%)[t]
94BB-13	F_8	62	73	8	5.6	RRRr	All R	0.0**	575:1	0.174**
93BB-9E[s]						**RRrr**	35:1	2.8*	**77:4**	**4.94**^{NS}
						Rrrr	3:1	25.0**	407:169	29.34**
94BB-14	F_8	83	52	9	6.25	RRRr	All R	0.0**	575:1	0.174**
93BB-9G[s]						**RRrr**	35:1	2.8*	**77:4**	**4.94**^{NS}
						Rrrr	3:1	25.0**	407:169	29.34**
94BB-15	F_8	52	80	12	8.3	RRRr	All R	0.0**	575:1	0.174**
93BB-9J[s]						**RRrr**	35:1	2.8**	**77:4**	**4.94**^{NS}
						Rrrr	3:1	25.0**	407:169	29.34**
94BB-16	F_8	49	79	16	11.1	RRRr	All R	0.0**	575:1	0.174**
93BB-10E[s]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.34**

$^{NS, *, **}$Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation ratio in one population.
[y]Maximum equational segregation system.
[x]Generations.
[w]Dark red-underfoliaged plants.
[v]Intermediate red-underfoliaged plants.
[u]All-greenfoliaged plants.
[t]Percentage of recessive genotype.
[s]Four individual plants selected from F_7 SCV inbred 93BB-9 and 10.

(Table 12). The inbred population 94BB-16 produced an even higher percentage of all-green-foliaged plants than did 94BB-14 and 94BB-15. Also the all-green-foliaged percentage shows that it does not fit any logical genotypic segregation. Some of the darkest red-underfoliaged individuals, however, were selected from this inbred population to be test-crossed and selfed to produce the next generation. The inbred population 94BB-13 had the lowest percentage of all-green-foliaged plants (5.6%), but the statistical data suggest it may be a duplex. Either there were errors in identifying the red-underfoliaged plants, or other factors influence the intensity of this characteristic. Daylength, temperature, and age of plants influenced the intensity of the red under-foliage characteristic. These factors are discussed later.

Six to ten of the darkest red-underfoliaged individuals were selected from 94BB-13, 94BB-14, 94BB-15 and 94BB-16 (F_8 populations), and each selection was selfed and test-crossed with the homozygous all-green-foliaged inbreds. The test-cross results are shown in Table 14, and the selfed population results are shown in Table 15.

Table 14 shows the segregation ratios of the six test-cross populations derived from 94BB-13, 94BB-14, 94BB-15 and 94BB-16. Two of them, 95BB-61 and 95BB-63, did not segregate all-green-foliaged plants and were not significantly different from the expected triplex segregation ratio. These two populations, however, were not large. Two other test-cross populations 95BB-60 and 95BB-69 segregated only a few all-green-foliaged plants and were not significant for maximum equational triplex segregation. The statistical results indicate that these four selected plants, 94BB-13E, 94BB-14A, 94BB-15F, and 94BB-16C, may be triplex.

Table 14. Segregation ratios and statistical analyses of test-cross populations obtained by crossing six selections with the darkest red underfoliage from SCV F_8 inbred 94BB-13, 94BB-14, 94BB-15, and 94BB-16 with the homozygous green-foliaged CV inbred for underfoliage color inheritance within interspecific *Begonia* inbreds.

	Segregation ratio[z]				Possible	Chromosomal segregation		Max. eqa. segregation[y]	
Pedigree	Red[x]	In. red[w]	Green[v]	Green (%)	genotype	Ratio R:r	Recessive (%)[u]	Ratio R:r	Recessive (%)[u]
95BB-63	39	9	0	0	**RRRr**	**All R**	**0.0^{NS}**	23:1	4.2**
15F[t] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
95BB-61	30	17	0	0	**RRRr**	**All R**	**0.0^{NS}**	23:1	4.2**
13E[s] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
95BB-60	44	47	3	3.2	**RRRr**	All R	0.0**	**23:1**	**4.2^{NS}**
14A[r] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
95BB-69	38	8	2	4.2	**RRRr**	All R	0.0**	**23:1**	**4.2^{NS}**
16C[q] x CV					RRrr	5:1	16.7**	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**
95BB-65	49	34	12	12.6	RRRr	All R	0.0**	23:1	4.2**
13B[s] x CV					**RRrr**	**5:1**	**16.7^{NS}**	7:2	22.2*
					Rrrr	1:1	50.0**	11:13	54.2**

Table 14 (cont'd)

Pedigree	Segregation ratio[z] Red[x]	In. red[w]	Green[v]	Green (%)	Possible genotype	Chromosomal segregation Ratio R:r	Recessive (%)[u]	Max. eqa. segregation[y] Ratio R:r	Recessive (%)[u]
95BB-68	31	12	5	10.4	RRRr	All R	0.0**	23:1	4.2**
16B[q] x CV					**RRrr**	**5:1**	**16.7**[NS]	7:2	22.2**
					Rrrr	1:1	50.0**	11:13	54.2**

[NS,*,**]Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[x]Dark red-underfoliaged plants.
[y]Maximum equational segregation.
[w]Intermediate red-underfoliaged plants.
[v]All-green-foliaged plants.
[u]Percentage of recessive genotype.
[t]Individual from 94BB-15.
[s]Individual from 94BB-13.
[r]Individual from 94BB-14.
[q]Individual from 94BB-16.

In Table 15 the first three selfed lines correspond to the first three test-cross populations in Table 14. Two F_8 inbred plants, 94BB-15F and 94BB-13E, did not segregate any all-green-foliaged plants in their selfed populations, 95BB-42 and 95BB-43. The all-green-foliaged segregating percentage fit the expected triplex segregation ratio, based on chromosomal segregation. The selfed 95BB-40 population from 94BB-14A produced only one all-green-foliaged plant, and the segregation pattern does not fit any of the segregation systems. It is possible that this all-green-foliaged plant is a mixed seedling from other population. Therefore, without counting this all-green foliage, the segregation ratio of 95BB-40 was not significantly different from that expected for triplex segregation, based on chromosomal segregation. The statistical results indicate that these three F_8 inbred plants, 94BB-15F, 94BB-13E, and possibly 94BB-14A, are triplex with RRRr genotype. The population from the self of 94BB-16C, because of poor seed germination, did not have enough plants to determine a segregation ratio properly.

The two test-cross populations in Table 14, 95BB-65 and 95BB-68, which were derived from 94BB-13B and 94BB-16B, showed a higher percentage of all-green foliage than the other four test-cross populations. The statistical results reveal no significant difference from the expected duplex segregation ratio, based on the chromosome segregation system.

Table 15 also shows the selfed segregation ratios for the two F_8 individuals, 94BB-13B and 94BB-16B, which were involved in sib matings. The all-green-foliaged segregation percentages were 3.75 and 2.1 and were not significantly different from the expected duplex segregation for maximum equational segregation for 94BB-13B or from

Table 15. Segregation ratios and statistical analyses of F_9 SCV inbred populations obtained by selfing inbreds with the darkest red under-foliage from SCV F_8 inbreds for foliage color inheritance within interspecific *Begonia* inbreds.

		Segregation ratio[z]				Possible	Chromosomal segregation		Max.eqa. segregation[y]	
Pedigree	Gen[x]	Red[w]	In. red[v]	Green[u]	Green (%)	genotype	Ratio R:r	Recessive (%)[t]	Ratio R:r	Recessive (%)[t]
95BB-42	F_9	26	22	0	0	**RRRr**	**All R**	**0.0^{NS}**	575:1	0.174**
15F[s]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.34**
95BB-43	F_9	31	16	0	0	**RRRr**	**All R**	**0.0^{NS}**	575:1	0.174**
13E[r]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.03**
95BB-40	F_9	63	32	1	1.0	RRRr	All R	0.0**	575:1	0.174*
14A[q]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.34**
95BB-48	F_9	38	9	0	0	**RRRr**	**All R**	**0.0^{NS}**	575:1	0.174**
14F[q]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.34**
95BB-49	F_9	52	23	0	0	**RRRr**	**All R**	**0.0^{NS}**	575:1	0.174**
15E[s]						RRrr	35:1	2.8**	77:4	4.94**
						Rrrr	3:1	25.0**	407:169	29.34**

Table 15 (cont'd)

Pedigree	Gen[x]	Segregation ratio[z] Red[w]	In. red[v]	Green[u]	Green (%)	Possible genotype	Chromosomal segregation Ratio R:r	Recessive (%)[t]	Max.eqa. segregation[y] Ratio R:r	Recessive (%)[t]
95BB-46	F_9	32	45	3	3.75	RRRr	All R	0.0**	575:1	0.174**
13B[r]						**RRrr**	35:1	2.8**	**77:4**	**4.94** NS
						Rrrr	3:1	25.0**	407:169	29.34**
95BB-50	F_9	19	27	1	2.1	RRRr	All R	0.0**	575:1	0.174**
16B[p]						**RRrr**	**35:1**	**2.8**NS	**77:4**	**4.94**NS
						Rrrr	3:1	25.0**	407:169	29.34**

NS, *, **Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation ratio in one population.
[y]Maximum equational segregation system.
[x]Generations.
[w]Dark red-underfoliaged plants.
[v]Intermediate red-underfoliaged plants.
[u]All-greenfoliaged plants.
[t]Percentage of recessive genotype.
[s]Individuals from F_8 SCV inbreds population 94BB-15.
[r]Individuals from F_8 SCV inbreds population 94BB-13.
[q]Individuals from F_8 SCV inbreds population 94BB-14.
[p]Individuals from F_8 SCV inbreds population 94BB-16.

Table 16. Segregation ratios and statistical analyses of sib-cross populations obtained by crossing two selections with the darkest red underfoliage from SCV F_9 inbreds for underfoliage color inheritance within interspecific *Begonia* inbreds.

	Segregation ratio[z]				Pos. parental[y]	Chromosomal segregation		Max. eqa. segregation[x]	
Pedigree	Red[w]	In. red[v]	Green[u]	Green (%)	genotype	Ratio R:r	Recessive (%)[t]	Ratio R:r	Recessive (%)[t]
95BB-84 (14F x 13B)[s]	19	29	0	0	**RRRr X Rrrr**	**All R**	**0^{NS}**	214:2	0.9**
					RRrr X RRrr	35:1	2.8	77:4**	4.94**
95BB-85 (16B x 15E)[r]	15	33	0	0	**RRrr X RRRr**	**All R**	**0^{NS}**	214:2	0.9**
					RRrr X RRrr	35:1	2.8	77:4**	4.94**

$^{NS, *, **}$Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.
[z]Actual segregation data for one population.
[y]Possible parental genotype.
[x]Maximum equational segregation system.
[w]Dark red under-foliage plants.
[v]Intermediate red under-foliage plants.
[u]All-green foliage plants.
[t]Percentage of recessive genotype.
[s]Two individuals selected from F_8 inbred 94BB-13 and 14.
[r]Two individuals selected from F_8 inbred 94BB-15 and 16.

either chromosomal and maximum equational segregation for 94BB-16B. These results correlate with the results of test-cross segregations in Table 14, which indicates 94BB-13B and 94BB-16B are duplex.

Table 16 shows the segregation ratios for two sib-mated populations. The 95BB-84 was derived by sibbing 94BB-14F x 94BB-13B. The 95BB-85 population was derived from the sib cross of 94-16B and 94BB-15E. No all-green-foliaged plants segregated out of these sib matings. The results also indicate that the two F_8 inbred plants, 94BB-14F and 94BB-15E, are triplex. Otherwise, a few all-green-foliaged plants should have segregated out of the sib-mated populations. The population sizes, however, were rather small.

Starting with F_6 germplasm, and after three additional generations of selection, five individuals, 94BB-13E, 94BB-14A, 94BB14F, 94BB-15E, and 94BB-15F were selected and are possibly triplex. Two of them, 94BB-14F and 94BB-15E, need further verification by test-crossing with an all-green-foliaged inbred.

The Possible Effect of Age of Plants, Daylength, and Temperature on Red Underfoliage Coloration

During this research, we found that the age of the plants, daylength, and temperature possibly affected under-foliage coloration. Figures 11 through 22 show the variation we encountered.

Figures 11 through 13 show the underfoliage color changes over time along with age of plants for three test-cross populations, 94BB-42, 94BB-45, and 94BB-50, from selected inbreds of F_8 SCV in 1994. After 72 days from the time the seed was sown, the

plants were either all green or intermediate red. As the plants aged, the number with all-green foliage decreased, and the dark red underfoliage started to appear.

Figures 14 through 16 give the result of three test-cross populations, 95BB-61, 95BB-63, and 95BB-69, from selected inbreds of F_9 SCV in 1995. In these populations the number of intermediate red- and all-green-foliaged plants also decreased, and the number of dark red-underfoliaged plants increased as the plants aged.

The difference between the two years' results is that the dark red-underfoliaged plants in 1995 appeared earlier than in 1994. The difference may be due to daylength and temperature influences for the time of year. In 1995 the populations were grown from late June through late October. The first count was on September 6. Most of this experiment covered a considerable period with long days and warm temperatures. In 1994, the populations were grown from early August through early November, the first underfoliage color count occurring on October 21. During this period, the daylength was getting shorter and the greenhouse temperatures were lower. Longer daylength and warmer temperatures may, therefore, increase photosynthetic activity, which could influence the earlier pigment formation in the leaves. The same difference also was found in the selfed populations in 1994 and 1995.

Figures 17 through 19 show the 1995 results over time for the F_9 populations 95BB-40, 95BB-48, and 95BB-49 from the self-pollinations of three selected inbreds of F_8 SCV for the underfoliage color changes. These populations were grown during the hot summer of 1995. At day 71 there were mostly dark red- and intermediate red-underfoliaged plants. Over time, the number of dark red-underfoliaged plants increased,

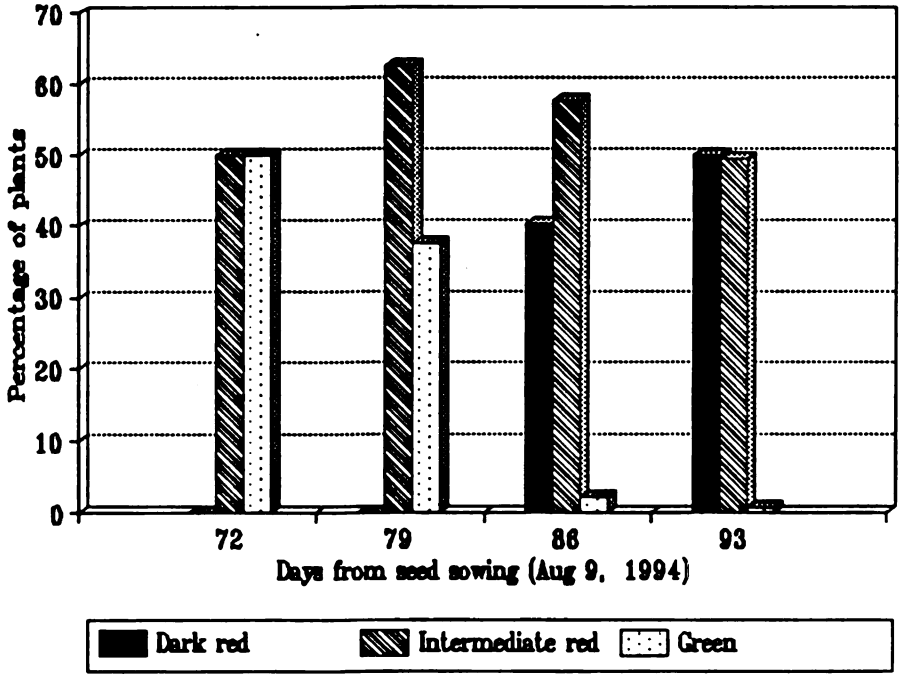

Figure 11. Variation in *Begonia* underfoliage color determination over time for the test-cross population 94BB-42 (a possible triplex SCV plant x CV).

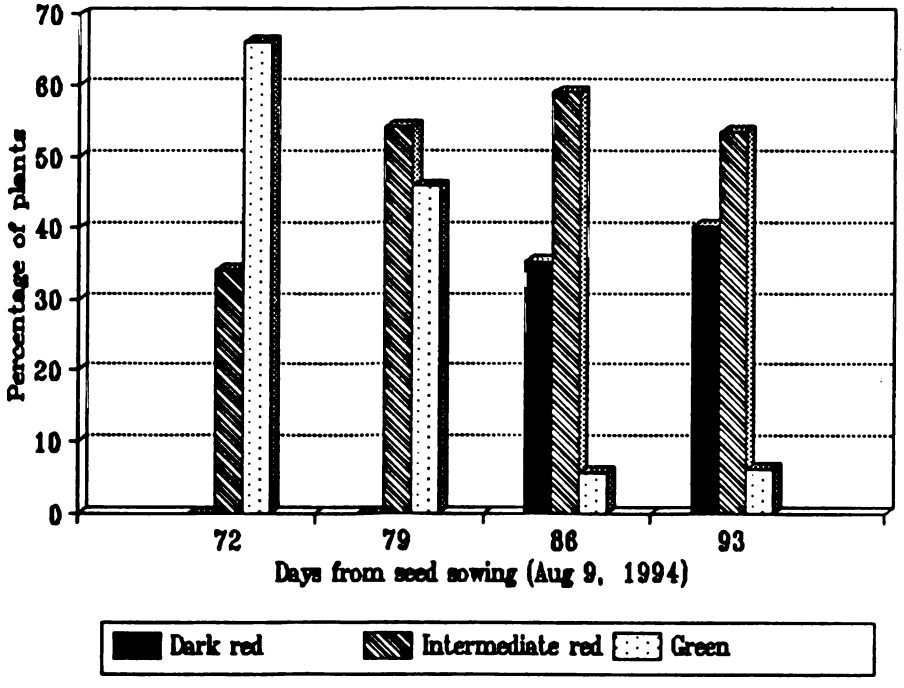

Figure 12. Variation in *Begonia* underfoliage color determination over time for the test-cross population 94BB-45 (a possible triplex SCV plant x CV).

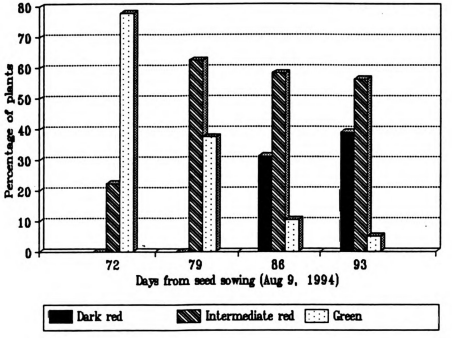

Figure 13. Variation in *Begonia* underfoliage color determination over time for the test-cross population 94BB-50 (a possible triplex SCV plant x CV).

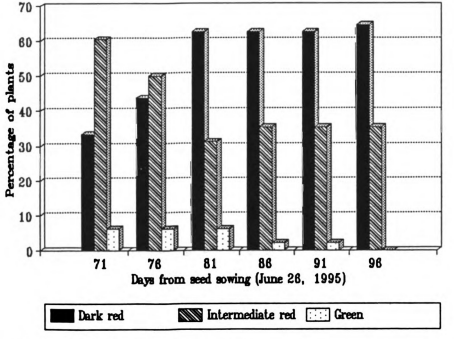

Figure 14. Variation in *Begonia* underfoliage color determination over time for the test-cross population 95BB-61 (a possible triplex SCV plant x CV).

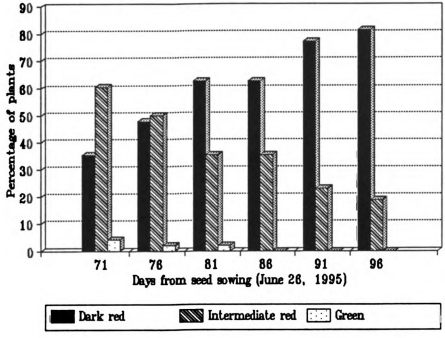

Figure 15. Variation in *Begonia* underfoliage color determination over time for the test-cross population 95BB-63 (a possible triplex SCV plant x CV).

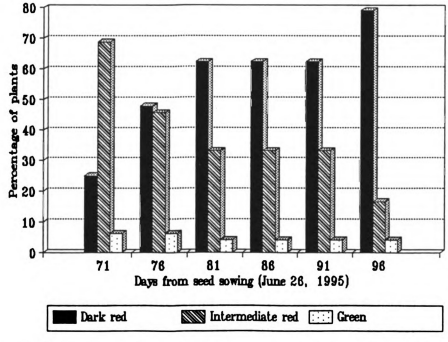

Figure 16. Variation in *Begonia* underfoliage color determination over time for the test-cross population 95BB-69 (a possible triplex SCV plant x CV).

and the number of intermediate red- and all-green foliaged plants decreased. The 95BB-48 and 95BB-49 populations at the end showed only intermediate red- and dark red-underfoliaged plants (Figures 18 and 19). These three populations are possibly from triplex plants.

Figures 20 through 22 show three F_8 SCV populations, 94BB-13, 94BB-14, and 94BB-15, in 1994 for underfoliage color change over time. The parent plants that produced these populations were possibly duplex. These populations were grown during the winter and did not show red underfoliage color until 98 days from sowing. At 114 days from sowing there were still some all-green-foliaged plants in the populations. The results obtained from these populations covered a period from November 15 to the middle of March under short days and cooler temperatures. Compared to the possible triplex 95BB-40, 95BB-48, and 95BB-49 population results, the underfoliaged color changed much more slowly. Red-underfoliaged plants did not appear until 91 days from sowing, generally 20 days later than when grown at warm temperatures. Two all-green-foliaged plants from the F_8 inbred population 94BB-13 were selected and potted. Within two weeks, both of these plants had a foliage change to intermediate red. This phenomenon might help to explain the contradictory results between Tables 12 and 13. It is possible that if the three populations represented in these tables had been saved longer, the number of all-green-foliaged plants would have decreased.

These results strongly suggest that the determination for pigmentation of the leaves for the red-underfoliage character should be done during long and warm days in late spring and summer.

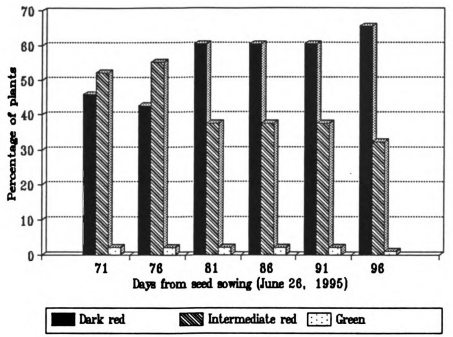

Figure 17. Variation in *Begonia* underfoliage color determination over time for the F_9 SCV inbred population 95BB-40 (F_9 from a possible triplex plant).

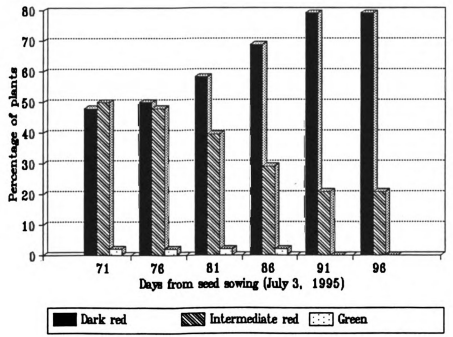

Figure 18. Variation in *Begonia* underfoliage color determination over time for the F_9 SCV inbred population 95BB-48 (F_9 from a possible triplex plant).

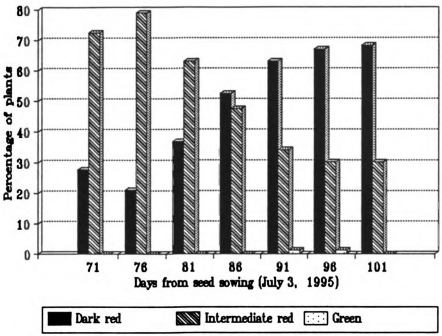

Figure 19. Variation in *Begonia* underfoliage color determination over time for the F_9 SCV inbred population 95BB-49 (F_9 from a possible triplex plant).

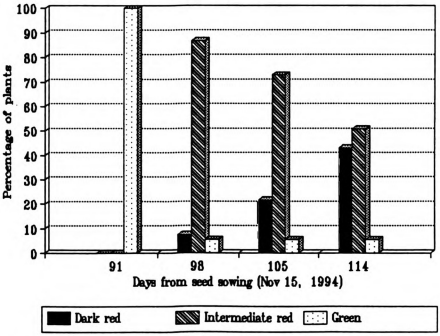

Figure 20. Variation in *Begonia* underfoliage color determination over time for the F_8 SCV inbred population 94BB-13 (F_8 from a possible duplex plant).

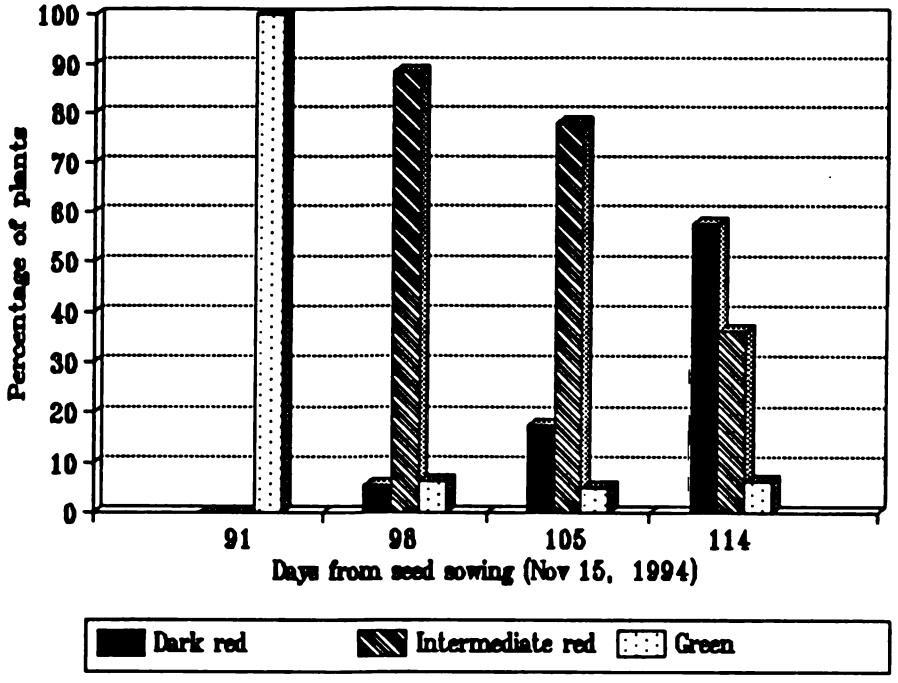

Figure 21. Variation in *Begonia* underfoliage color determination over time for the F_8 SCV inbred population 94BB-14 (F_8 from a possible duplex plant).

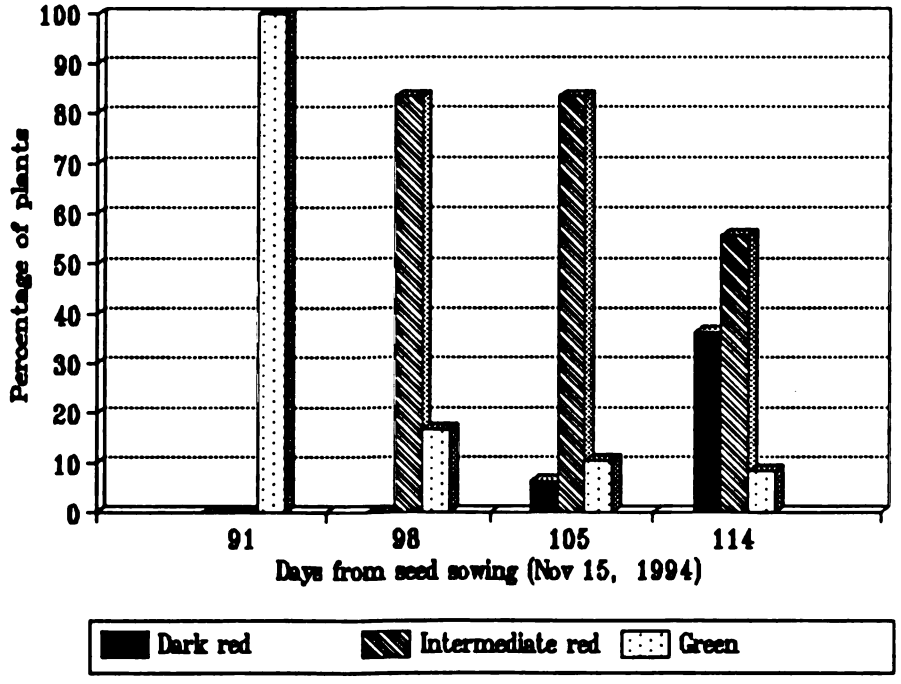

Figure 22. Variation in *Begonia* underfoliage color determination over time for the F_8 SCV inbred population 94BB-15 (F_8 from a possible duplex plant).

Germination Percentage and Vigor Ratings

To obtain germination percentages and vigor information for self-pollinated lines, sib-mated lines, and test-cross lines, germination tests were performed. Seedling vigor was rated from 1 to 5, 5 being the strongest and 1 being the weakest. The results are shown in Table 17.

The two test-cross lines had nearly 100% germination, and the seedlings were the most vigorous of all the groups tested. The LSD test indicated test-cross lines having significant differences from the selfed and sib-mated lines. The germination rates of the self-pollinated inbred lines varied from 46.7% to 92.0% and had fair vigor. The sib-mated lines had relatively uniform germination rates and better seedling vigor than the self-pollinated inbred lines. The LSD test showed significant differences among selfed and sib-mated lines for seedling vigor.

The red-under-foliaged inbred germplasm was maintained by selfing over several generations. Loss of vigor is so prevalent that it possibly could negatively affect the selection for a red-underfoliaged homozygous quadriplex individual. Future inbred line maintenance probably would benefit from sibbing the darkest red-underfoliaged inbred plants.

Table 17. Seed germination tests giving percentage of germination and vigor from various types of pollinations for underfoliage color inheritance within interspecific *Begonia* inbreds.

Pedigree	Generation	Pollination type	Germination (%)				Vigor			
			I[z]	II[z]	III[z]	Mean	I[z]	II[z]	III[z]	Mean
95BB-40	F_9	Self	98	78	48	74.7b[y]	3	3	2	2.7b[x]
95BB-42	F_9	Self	100	92	84	92.0d	3	3	3	3.0b
95BB-45	F_9	Self	50	38	52	46.7a	1	2	2	1.7a
95BB-46	F_9	Self	40	78	98	72.0b	1	2	2	1.7a
95BB-60	F_1	Test cross	98	98	98	98.0d	5	5	5	5.0d
95BB-61	F_1	Test cross	100	98	98	98.7d	5	5	5	5.0d
95BB-80	S_1	Sib mating	94	84	74	84.0c	5	4	4	4.3c
95BB-81	S_1	Sib mating	74	86	84	81.3bc	4	4	4	4.0c

[z]Replications.
[y]$LSD_{0.05} = 9.11$.
[x]$LSD_{0.05} = 0.51$.

DISCUSSION

The major germplasm components studied in this research were tetraploid as determined by cytogenetic evaluation and population segregation analysis from selfs and test-crosses. A diploid inbred from the BSC cv. Pink Avalanche also was used to help verify the ploidy level giving a triploid hybrid when crossed with the SCV inbred.

The complicated red-underfoliage segregation ratios and meiotic configuration indicated an autotetraploid inheritance pattern. The segregation ratios also indicated a dominant gene, designated 'Ru', which controls red underfoliage color in the SCV interspecific inbred. In this research, the polyploid plants had smaller leaves and flowers when compared to the diploid inbred from 'Pink Avalanche', which is not the case in many species.

Counting chromosomes was very difficult, mainly because they were very small and numerous. Usually a polyploid with a higher chromosome number will have a greater cell volume than the diploid counterpart (Burnham, 1962). In *Begonia*, however, the cell size and volume of a tetraploid look the same as that of the related diploid species. With more chromosomes in the same-sized cell, they were more crowded and overlapped, which made chromosomal determination tenuous. Only a few countable cells were found for *B. Schmidtiana* and BSC cvs. Charm and Vodka. The root tips were very small and grew

very slowly, with few proper cell divisions. Counting the chromosome number for SCV, 'Pink Avalanche', and the hybrid between SCV and 'Pink Avalanche' was easier since the root tips of these plants were larger and grew faster.

The pretreatment procedures using 0.1% colchicine, paradichrobenzene, and cold temperature to prevent spindle fiber formation did not make chromosomal counting easier. The chromosomes were stained better if the root tips remained in aceto-carmine for one hour or overnight, which also helped soften the root tips for easier spreading. The polyploid SCV and CV germplasm in this study was developed from material that was reported earlier to be diploid (*B. Schmidtiana*) or determined to be diploid (*B.*x *semperflorens-cultorum* cvs. Charm [2n = 32-34] and Vodka [2n = 40-42]). These two diploids of differing chromosomal number cross to produce a fertile, tetraploid inbred (CV) with 2n = 4x = 68 to 72 chromosomes, which in turn could cross with *B. Schmidtiana,* with a chromosomal number of 64, to produce the SCV inbred with a chromosome number of 64 (2n = 4x = 64). Matsuura and Okuno (1943) observed two morphologically different plants from *B. Schmidtiana*, and in both plants the somatic chromosomal number was 32. In this research, however, the *B. Schmidtiana* germplasm with red underfoliage color had 64 chromosomes. It is possible that the *B. Schmidtiana* germplasm used in this research increased in ploidy level since the work of Matsuura and Okuno in 1943 or that the germplasm evolved from completely different sources. Before the Matsuura and Okuno, Pastrana (1932) observed 13 chromosomes in the sporophytic tissue of *B. Schmidtiana*. It is possible that there is a wide range of chromosomal diversity in this species, as well as various morphological characteristics and ploidy levels.

Matsuura and Okuno (1943) suggested basic numbers for *Begonia* to be 6, 7, or 13, but in looking at the information in Table 1 and the general chromosomal numbers found in this study (Tables 5 and 6), a basic number of 8 also is a possibility.

The tetraploid *B.* x *semperflorens-cultorum* inbred CV with 68 to 72 somatic chromosomes, as mentioned previously, may have developed from nonreduced gametes when the cross was made. Zeilinga (1962) reported the formation of a tetraploid group called *B. semperflorens-cultorum* var. *gracilis* with 68 chromosomes, which was developed from the hybridization between two diploids, *B. semperflorens-cultorum* cv. Vernon with 2n = 34 and *B. versaliensis* Hort rose with 2n = 33. Doorenbos and Legro (1968) also felt that a winter-flowering *Begonia,* 'Konkurrent', was somehow derived from an interspecific cross between two diploid species, *B. socotrana* (2n = 28) and *B. dregei* (2n = 26), which have great morphological differences. They found that tetraploid plants (2n = 4x = 54) from this cross, when backcrossed with diploid *B. socotrana,* produced plants having the same appearance as the 'Konkurrent' group.

The previous and present hybridization studies in *Begonia* indicate that tetraploid cultivars or species can arise easily through hybridization between diploid species, even though these species or cultivars have different chromosomal numbers and morphological differences. Matsuura and Okuno (1943) felt that this tendency brought about a large range of chromosomal diversity in *Begonia*.

Meiotic chromosomal configurations were found only in the SCV material. The results came late in the study, even though much time was devoted to this area. A propionic acid-alcohol solution containing ferric chloride ($FeCl_3$) was much better as a

fixative for meiotic study than Farmer's solution. It may be that iron plays an important role in improvement of chromosomal staining ability. The male flower bud size is also very critical for meiotic analysis. Male flower buds 5 to 7 mm long gave the best results. The bivalent chromosomes appeared to be only slightly larger black spots in comparison to univalents. In some cases, quadrivalent association was found at diakinesis. Chromosomal pairing indicated *B. Schmidtiana* and CV are closely related. This would be expected, since *B. Schmidtiana* is considered one of the ancestors of *B.* x *semperflorens-cultorum*.

A major dominant gene is characterized to affect red underfoliage color in the interspecific *Begonia* inbreds in this study. The cytogenetic results, the intermediate red underfoliage color, and the complicated inheritance pattern show that the SCV interspecific inbred probably is autotetraploid. Variation in the red underfoliage color intensity indicates possible additive or dosage effects for this characteristic. Individual plants with the darkest red underfoliage were selected from each generation to self for the next generation. The selected SCV inbred plants were always darker than the test-cross population plants.

Holley (1945), in his research on foliage color inheritance in *B.* x *semperflorens-cultorum*, found that the F_2 progenies segregated approximately 50% bronze, 25% intermediate bronze, and 25% green instead of the expected 3:1 ratio. The intermediate bronze-foliaged plants could indicate that the germplasm was tetraploid. According to the chromosomal segregation system, a plant with a simplex genotype (Rrrr) should segregate out 50% Rrrr (intermediate bronze-foliaged plants), 25% RRrr (bronze foliage plants) and

25% rrrr (all-green-foliaged plants). However, Holley reported 50% bronze-foliaged and 25% intermediate bronze-foliaged plants, which does not fit a simplex segregation ratio. Possibly some intermediate bronze foliage plants had a color change to bronze over time, similar to what was observed in this study.

Many attempts were made to select homozygous plants for red underfoliage from large heterozygous populations. The results show that it is a difficult task. A major problem is that it is impossible to distinguish homozygous red-underfoliaged plants from heterozygous triplex and duplex plants. Selections were made visually for the perceived darkest red underfoliage. The segregation results for red underfoliage, however, indicate duplex, triplex, and any possible quadriplex plants had nearly the same red color intensity. Test-crossing with all-green-foliaged plants proved to be the most reasonable way to distinguish the number of dominant genes involved. A quadriplex inbred would, of course, be expected to give 100% red-underfoliaged plants in a test-cross progeny. Triplex individuals give much the same results, especially if chromosomal segregation is operating. The duplex and simplex levels, however, show segregation for foliage color, and these can be identified. It is, therefore, necessary to select as many individuals as can be handled for test-crossing until a homozygous, dominant, quadriplex plant can be identified positively. With constant selfing, any minor genes that might affect underfoliage color could segregate and influence the segregation ratio of underfoliage color, making determinations more complicated. Underfoliage color change over time is a possible response to plant age, daylength, and temperature which could indicate that minor genes also exist. The selection of dark red-underfoliaged plants, therefore, must be done under

proper conditions to minimize environmental influence and any minor gene effects.

Competition among plants possibly could influence the availability of homozygous, red-underfoliaged plants if such plants were less vigorous. Weaker plants covered by stronger and taller plants possibly would have less red underfoliaged color intensity and a greater chance of elimination on final selection. In future selections for homozygous-dominant plants, some weak red-underfoliaged inbred plants should be selected purposely.

Maximum equational segregation as stated by Burnham (1962) was used to explain some of the underfoliage color segregation ratios in this study. The statistical results show that some populations had no significantly different segregation ratios, based on maximum equational segregation, but did have significant differences based on chromosomal segregation. It is possible that crossovers between the red underfoliage gene locus and centromere occurred occasionally. Such a crossover has been reported in autotetraploid *Datura stramonium* (Burnham, 1962). However, it has never been reported in *Begonia*. In this study, the statistical results were based on the all-green-foliaged segregating percentage in each self and test-cross population with 144 plants (three 48-cell flats). Some populations had only one or two flats because of a shortage of seedlings after germination. A small variation in all-green plant numbers can change the results dramatically. Burnham (1962) reported maximum equational segregation in *Datura stramonium*: several thousand plants were planted, reinforcing the knowledge that the determination of an autotetraploid inheritance pattern should be based on as large a population size as possible. In this study over three years, several thousand plants were grown, but within several different selections. This procedure was deemed necessary in order to better understand whatever

inheritance patterns might be involved, in selecting a true-breeding line.

Germination tests showed that sib-mated lines had higher germination rates than self-pollinated lines. These populations also had greater vigor and reached transplanting stage much sooner. Since several of the inbred lines are at the F_9 generation, sib mating probably should be used to maintain the germplasm and develop useful breeding lines.

This new red underfoliage characteristic has potential for commercial application. Even though a true homozygous red-underfoliaged plant has not been identified, several plants, which are possibly triplex, could be used for hybrid seed production. Segregation results indicate that triplex plants crossed with all-green-foliaged plants produce almost 100% red-underfoliaged hybrid plants. Even if a few all-green-foliaged plants appeared, they could be discarded.

Experimental hybrids have produced vigorous plants that have long, drooping stems and red underfoliage, which should be suitable for hanging basket use. Also, these red-underfoliaged hybrids exhibited exceptional heat tolerance under full sunlight. In the summer of 1995, some of these hybrids were planted in the MSU Demonstration Gardens for hanging basket and landscape application and in both instances, performance was excellent.

Summary

The germplasm involved in this research included *Begonia Schmidtiana*, BSC cvs. Charm and Vodka, an inbred developed from BSC 'Charm' x 'Vodka' (CV), and *Begonia Schmidtiana* x (BSC cvs. Charm x Vodka) (SCV). The interspecific inbred SCV showed the red underfoliage color, and the hybrid between SCV x CV showed potential for hanging basket production.

Cytogenetic results indicated that *Begonia Schmidtiana* is tetraploid with about 64 chromosomes and BSC 'Charm' and 'Vodka' are diploid with 2n = 32 to 34 and 40 to 42, respectively. The interspecific inbred SCV exhibited about 64 chromosomes in root-tip tissue, and the meiotic analysis of SCV indicated it was autotetraploid, suggesting that the germplasm is closely related.

A major dominant gene, *Ru,* is hypothesized to control red underfoliage color inheritance in SCV, and dosage effect is involved with the expression of color intensity. All-green foliage is recessive. Daylength, temperature, and plant age affected red underfoliage color intensity and the time it took for expression.

Several inbred individuals that are probably at least triplex from SCV were selected to continue the possible development of a homozygous, dominant, inbred line.

LIST OF REFERENCES

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Agricultural Statistical Board. 1995. Floriculture Crops 1994 Summary. Washington D.C., USDA, NASS, April 1995, Sp Cr 6-1 (95) P:2.

Bailey, L. H. and E. Z. Bailey. 1978. Hortus Third. Macmillan Publishing Co., Inc. New York.

Behe, B. K. and C. M. Walker. 1994. 1994 PPGA Season Sales Summary. Professional Plant Growers Association and Greenhouse Manager. Lansing-Fort Worth.

Burnham, Charles R. 1962. Discussions in cytogenetics, p 168-250. Minneapolis, Burgess Publishing Company.

Darlington, C. D. and L. F. LaCour. 1976. The handling of chromosomes. 6th ed. Wiley, New York.

Doorenbos, J. and R. A. H. Legro. 1968. Breeding gloire de lorraine *Begonias*. Mededelingen Landbouwhogeschool Wageningen. Netherlands. 68(19):1-14.

Ewart, L. C. 1963. The inheritance of flower size in grandiflora and multiflora petunias. Ph.D. Diss., The Pennsylvania State University.

Ewart, L. C. 1995. Bedding plant production manual. 2nd ed. Michigan State University.

Holley, W. D. 1945. Inheritance in *Begonia Semperflorens*. Proc. Amer. Soc. Hort. Sci. 46:389-396.

Matsuura, H. and S. Okuno. 1943. Cytogenetical studies on *Begonia*. Cytologia 13:1-18.

Meyer, J. R. 1945. Prefixing with paradichlorobenzene to facilitate chromosome study. Stain Tech. 20:121-125.

Palmer, R. G. and H. Heer. 1973. A root tip squash technique for soybean chromosomes. Crop Sci. 13:389-391.

Pastrana, M. D. 1932. Sporogenesis and sex determination in *Begonia Schmidtiana*. Amer. J. Bot. Vol 19(4):365-384.

Singh, R. J. 1993. Plant cytogenetics. CRC Press, Boca Raton.

Smith, L. 1947. The aceto-carmine smear technique. Stain Tech. 22:17-31.

Swaminathan, M. S., M. L. Magoon, and K. L. Mehra. 1954. A simple propiono-carmine PMC smear method for plants with small chromosomes. India J. Genet. Plant Breeding. 14:87-88.

Thompson, M. L. and D. J. Thompson. 1981. *Begonias*. The complete reference guide. Times Books, New York.

Whallon, Joanne. 1993 a. Laboratory notes of techniques for cytogenetics. Michigan State University, Department of Crop and Soil Sciences, East Lansing.

Whallon, Joanne. 1993 b. Laser scanning confocal microscope (lab notes). Michigan State University, Department of Crop and Soil Sciences, East Lansing.

Zeilinga, A. E. 1962. Cytological investigation of hybrid varieties of *Begonia Semperflorens*. Euphytica 11:126-136.