

# Genetics of Flower Color in Periwinkle *Catharanthus roseus* (L) G. Don

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**Abstract:** Genetics of flower Color in winka *Catharanthus roseus* (L) G.Don were investigate by inheritance two types (strains) of plants with different flowers color were used in this study, violet (V) and White (W) color as parents, to determine the number of genes involved. This study was conducted at the Department of Chemistry & Biology at the Faculty of Education, University of Kassala, kassala State, Sudan, during: the autumn, seasons for two years 2010- 2012. First the two parents were covered to ensure self pollination. Reciprocal cross has been carried out between the two inbred parents. The study showed that a single pair of genes is probably involved in flower colour and that gene for violet color is incompletely dominant over that for white color. The reciprocal crosses gave the same results indicating no role of cytoplasmic genes in the inheritance of these colors.

**Keywords:** *Catharanthus roseus* (L) G. Don, Inheritance, Self Pollination, Incompletely Dominant, Reciprocal Crosses, Cytoplasmic Genes

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## 1. Introduction

*Catharanthus roseus* (synonymous with *Vinca rosea* Linn.) is a perennial herbaceous subshrub plant, this plant belongs to the Magnoliophyta division, under the class of Manoliopsida, in the order of Gentianales, family Apocynaceae [1], Apocynaceae family represents a large family which includes about 1500 species, found mainly in tropical regions many of them are large trees found in rainforest, some are smaller shrubs, the sap of the most parts of the plant is milky latex which is important and useful for medicinal uses, and the members of the family usually have simple leaves, calyx with five parts, flowers in clusters, large five petals and five stamens [2].

Simmond (1960), found that the flowers of periwinkle, *Lochnera* (*Vinca*) *rosea* (L.) contained a pigment of which the aglycone corresponded with none of the common types recognized in other plants [3]. Harbone, and Sherratt (1960) They showed that the anthocyanidin wastrimethyl deiphinidin and corresponded in its properties with hirsutidin,

a pigment which has other wise been recognised only in the Primulaceal [4].

Flory (1944), working in U.S.A, recognised three phenotypes which, from his descriptions and from his mention of forms in the "light pink range", Pink(V), Red eye(E) and White (W) [5]. The characters of the various color forms are summarised by Wanscher (1953), who described the flower color phenotypes of this plant [6].

The bloom of a natural wild plant is pale pink with a purple eye in the centre, but horticulturist has developed varieties of more than one hundred with color ranging from white to pink to purple Aslam et al (2010) [7]. In periwinkle flower colors, the most commonly observed are pink, white corolla and red eye, and white, Flory (1944) attributed these three corolla colors to the epistatic interaction of two genes, R and W, with the R-W-genotype being pink, R-ww being red-eyed, and rrW and rrww being white flowered. Simmonds (1960) implicated two additional genes, A and B in the determination of flower colour, with A being complementary to R, also A and R are necessary for the production of colored flower, without both which flowers

would be white, A-rr----, aaR-----, and aarr----, colored (AR) genotypes are modified by W, B, with W flower being fully colored, and B being aco pigmentation gene which blues the pink pigment (A-R-W-) background bening violet color A-R-W-B-genotype, and A-R-W-bb being pink corolla and purple violet eye, ww plants having pigment confined to the eye of the flower, A-R-wwB- benig white corolla and purple eye, A-R-wwbb being white corolla and reddish purple eye. Milo et al (1985) [8] added another new flower color, pale pink corolla and red eye, with R-wwI genotype. They implicated this type of colour to one additional gene I, which like gene W is also epistatic to the gene R, R allele produces three anthocyanidins A1, B1, and C1 (located in the center of the corolla) and is epistatic to the W and I alleles, which function only in its presence. The W allele produces pigments A2, B2, and C2. The I allele also produces the same pigments as the W allele, but in smaller quantities and mainly in the center of the corolla. The r, w, and i alleles do not produce any pigment, so that R-W-I-, R-W-ii- benig pink, he also discovered and identified another flower colour, pale pink center R-wwI-are reported to be governed by the epistatic interaction between four genes A, R, W, and I. And R-wwii being White corolla and red eye, rrW-I-, rrwwI-, rrW-ii, and rrrwwii being white.

In spite importance of periwinkle as an ornamental and medicinal plant, there is very little work done on the genetics or breeding of this plant Boke (1949) [9]. In genetics of periwinkle flower color, Simmonds (1960) carried out experiment which consists of five phenotypes of flower color in periwinkle V, P, E, F, and W, they are all diploid, and with eight of chromosomes and cytological, in preliminary simmonds selfed these plants, and isolated vegetative from local gardens in several hundred progeny and they raised. All the parents proved to be true breeding at the St Augustine Nursery of the Trinidad Department of Agriculture, it is nursery experience that V, P, E, F and W stocks breed true year after year from open-pollinated seed makes clear that the periwinkle is fairly highly inbred, he crossed nineteen out of the twenty involving five phenotypes (V, P, E, F, W) were made and F1 and F2, he found the result of F1 generation in all of crossed were produced violet flower color phenotype, and when he selfed F1 generation to produce F2 generation he got many types of flower colors Simmond (1960).

## 2. Materials and Methods

The accession LV was found to be true breeding for light violet corolla and purple eye. It was crossed with a white flowered variety, Nirmal. In an earlier study the flower colour genotype of Nirmal (W) was found to be rrWW [11]. Parent plants were raised in a glasshouse from seeds obtained by artificial self-pollination pollen is powdery and the flowers are easily selfed by a means of a blunt needle inserted into the top of the tube [9]. Reciprocal crosses were made as described earlier by Kulkarni, and Baskaran et al (1999) [10]. All of the F1 plants were selfed and also backcrossed to both parents. Altogether 195,103 and 93

plants of F2, and two backcrosses, F1xV and F1x W respectively, were scored for corolla color, along with 10 plants each of the parental and F1 generations. Chi-square were used for testing the goodness- of-fit of observed and expected frequencies of different phenotypic classes in the F2 and backcross generations by Kulkarni, Sreevalli et al (2001) [11].

The source of seeds for the two parental strains of periwinkle (*Catharanthus roseus*) plants with two different flower colour were used in this study violet (V) and White (W) color Figure 1, and Figure 2 were obtained from lants growing naturally in the University lawns (University of Kassala). in Sudan, Kassala state, Department of Chemistry and Biology at the Faculty of Education.



Figure 1. V(Violet) violet corolla and purple violet eye.



Figure 2. W(White) white lobe colour and greenish white eye.

In this paper, the two stocks of parental strains were raised in pot. Two to three seeds was put in each pot and later thinned to one seedling per pot.

Pots of each strain was kept separately in the green house covered with nets to provide partial shade and later to exclude visiting pollinating insects (butterflies). Plants of each strain were grown in isolation for one generation to induce self pollination and selfed progeny bred true flower color for each of the two strains, and were therefore considered homozygous lines.

### 2.1. Production of F1 Seeds

To produce F1 seeds, we need about 100 plants from each parental line were raised in pots and polythene bags of 20 cm diameter filled with soil to a depth of 15 to 20 cm. Hand emasculation of flowers destined to be females was done before the staminal tube opens, the flower is covered by cloth.

Pollen from male flowers from the other line was collected carefully and put on the stigma of emasculated flower bud. The latter was covered and tied with a colored string for distinction. Reciprocal crosses were made between the two parental lines. Seeds resulting from successful hybridization in each reciprocal cross (about 25 each) harvested separately and grown in isolation to produce F1 plants.

### 2.2. Production of F2 and Backcross Plants

All F1 plants from the two reciprocal crosses produced one type of flower color (violet) and therefore were treated as one type of F1 plants.

Some flowers from each F1 plant were allowed to self pollinate by covering the flower bud by soda straw and some other flowers were backcrossed to one of the two parental lines following the procedure of the production F1 plants.

Seeds produced from selfed flowers (F2 seeds) are raised to produce F2 plants. Seeds harvested from emasculated flowers comprised the backcross seeds and were raised separately to produce the backcross plants to each of the two parental lines.

### 2.3. Statistical Analysis

The Chi-square test ( $\chi^2$ ) was used for testing the goodness of fit of observed and expected of different phenotypic classes in the F2 plants and plants of the two backcrosses reference as described by Simmond (1960).

## 3. Results

### 3.1. The F1 Plants

All F1 plants from the cross violet x white and the reciprocal cross white x violet had violet flowers, however the colour was slightly light violet indicating that the violet colour trait is dominant over the white colour. However, the fact that the violet colour is light violet and not as dark as the violet parent indicates that other modifiers for colour intensity, or partial dominance was existed. The results of the two reciprocal crosses were the same therefore, excluding the role of the cytoplasmic mode of inheritance.

### 3.2. The F2 Progeny

A total of 195 F2 plants were scored for flower colour. 60 plants had violet flower color like the parental stock, 82 plants had light violet color like the F1 plants and 53 plants had white flowers. When fitted to a 1: 2: 1 ratio, the  $\chi^2$  score was not significantly larger than the table value at 0.01 which indicates that a 1: 2: 1 genetic ratio fits significantly (Table 1).

**Table 1.** Phenotypes, of observed and expected frequencies of plants with different corolla colours in F2 generation of the cross violet corolla (V) × white corolla (W) in periwinkle.

Phenotype	Violet flower (V)	Light violet flower	White flower (W)	Total
Observed (O)	60	82	53	195
Expected (E)	48.7	97.5	48.5	195
$\frac{(O-E)^2}{E}$	2.62	2.46	0.42	$\chi^2 = 5.5$



**Figure 3.** slightly light violet flower color in (F1) plants plate.

### 3.3. The Backcross Progeny

Table 2 shows the results of the cross between F1 plants and the violet flower colour parent.

A total of 103 backcross progeny was classified in two flower colour phenotypes. 55 plants had violet flower colour as the parent, and 48

Plants had light violet flower colour like the F1 plants this fits significantly with a 1 :1 ratio.

**Table 2.** Phenotypes, and observed and expected frequencies of plants with different flower colors in the backcross generation of the cross F1 × (V) violet corolla in periwinkle.

Phenotype	Light violet flower	Violet flower (V)	Total
Observed (O)	48	55	103
Expected (E)	51.5	51.5	103
$\frac{(O-E)^2}{E}$	0.237	0.237	$\chi^2 = 0.47$



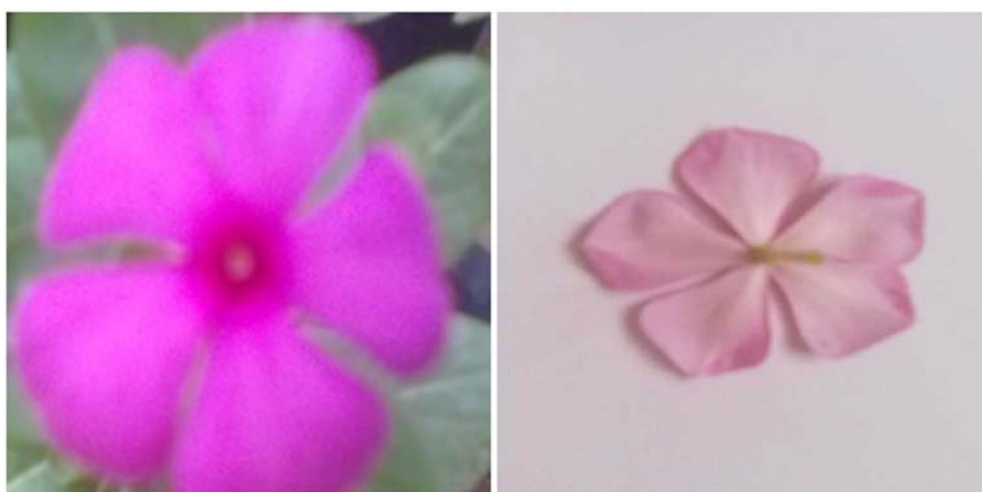
The results of the backcross between F1 and the white colour parent are presented in (Table 3). The backcross progeny consist of 93 plants comprising two groups of flower colour: 43 with light violet colour, similar to F1, and 50 with white flower colour. When fitting the observed value to the 1 :1 genetic ratio, the calculated  $\chi^2$  value is less than the table value at 0.01 level of probability which indicates a good fit to a 1 :1 ratio.

**Table 3.** Phenotypes, and observed and expected frequencies of plants with different flower colours in the backcross generation of the cross  $F1 \times (W)$  white corolla in periwinkle.

Phenotype	Light Violet flower	White flower (W)	Total
Observed (O)	43	50	93
Expected (E)	46	46	93
$\frac{(O-E)^2}{E}$	0.21	0.35	$\chi^2 = 0.56$



**Figure 4.** Face and back of violet flower colour parental (V).



**Figure 5.** Face and back of light violet flower color in (F1) plants.

## 4. Discussion

This study show that the F1 plants produced from crossing of violet flower colored plants with white flowered plants were all with light violet flowers Figure 3. This indicates that the F1 plants had intermediate flower color between the two parents which is probably due to incomplete dominance or there may be some minor modifier gene. Simmonds (1960) produced violet F1 plants from a similar cross between white and violet plants and suggested that four loci are involved in flower colour determination.

Kulkarni et al., (1999) suggested that white flower colour genotype to be  $rrWW$  and violet flower as  $RRWW$ . In

another study Kulkarni et al., (1999) suggested the genotype of the white flowered plant as  $AArrWWbb$  and that of the violet colour as  $AARRWWBB$ . In this study the F1 plants were heterozygous for the R locus. Further detailed genetic study involving other flower colors and also the color of the eye in the base of corolla is suggested. Biochemical studies of colour pigments are also suggested.

The F2 plant segregations were in line with the above results. The white colour character reappeared indicating that it is recessive.

As shown in Table 1, the calculated value of  $\chi^2$  is 5.5 which is less than the table value at 0.01 level of probability and this indicates that we could not reject the null hypothesis

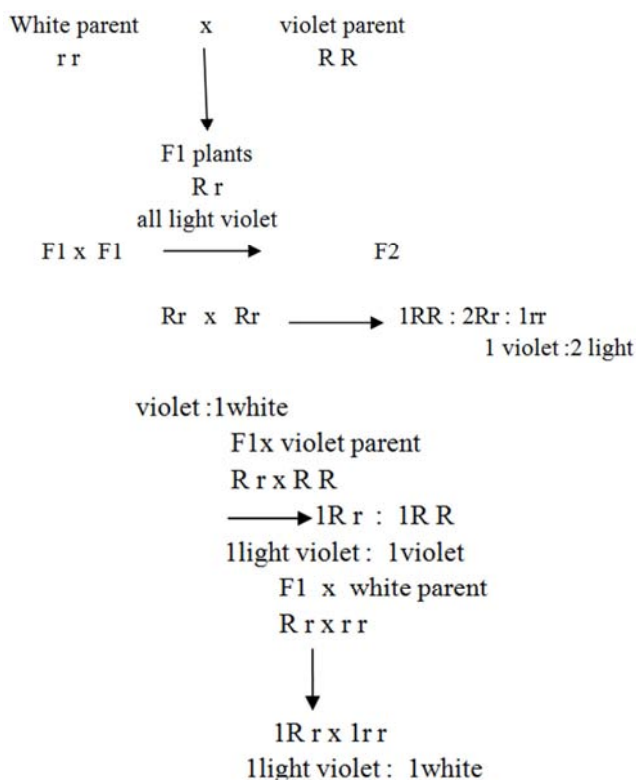
that the expected ratio is different from 1:2:1 this results indicate that this is a monohybrid ratio with only one locus involved. Incomplete dominance or lack of dominance of the violet color allele or its recessive counterpart is a reasonable explanation.

This result indicating that the violet colour trait is dominant over the white color. However, the fact that the violet color is light violet and not as dark as the violet parent indicates that other modifiers for colour intensity, or partial dominance was existed (Figure 4 & Figure 5).

This designated of inheritance has not been reported earlier before in inheritance studies in periwinkle by Simmonds (1960).

The backcross to the recessive parent i.e F1 x white gave a ratio of 1light violet: 1white which is a typical ratio for attest cross of a monohybrid ratio which also verifies our earlier results that the light violet colour F1 plants were heterozygous for this trait.

The result of backcrossing the F1 plants to the violet flower parent revealed a ratio of 1violet: 1light violet which again verifies that the F1 plants were heterozygous for the light violet flower colour. Based on these results the following genetic diagram for the parents, F1, F2 and backcross genotypes could be illustrated as following:



In this study the colour of the eye at the base of the corolla was not considered which could produced more variations in flower color.

Simmonds (1960) when considering the intensity of colour of the eye concluded that two complementary dominant genes control the distribution of color of the eye.

No consideration, in this study was given to the

complicated mode of reproduction of periwinkle reported in the literature e.g. self sterility [12], pollen less anthers [13].

## 5. Conclusion and Recommendations

The study indicated that the cross pollination (hybrids) between violet flower (V), and white flower (W) produced the F1 generation with light violet colour.

The second generation (F2) produced three flower colours, violet corolla (V), light violet corolla and white corolla (W) with a ratio 1:2: 1 respectively.

The heredity of flower colour, between the two strains, violet flower colour (V), and white colour (W) in periwinkle (*Catharanthus roseus*) has been found to be governed by only one locus (monogenic).

The study indicated that the violet corolla (V) has incomplete dominance or some gene modifier was existed over the white flower (W) colour ratio 1: 2:1.

The backcross of (F1) to the violet parent produced only violet and light violet colours in a ratio of 1: 1 indicating the incomplete dominance.

The test cross to the recessive white parent produced only two colours, light violet and white colours in a ratio of 1: 1 which was in line with the monogenic control and the incomplete dominance.

In this study the inheritance of only two flower colour has been investigated. Other colours and shades should be studied.

The study concentrated on the colour of the flower lobe and ignoring the variation in colour of the spot (eye) at the base of the flower which should be considered for the modification.

Further detailed study of the biology of flowering and mode of pollination of this plant species should be studied.

The literature of this plant and its medicinal importance is very extensive, and genetic improvement of its medicinal properties is important. For this reason, study of flower colour genes could be used as genetic markers for useful strains.

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