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Genetics of flower colour in pink flowered “Rosea” and white flowers “Alba” in periwinkle *Catharanthus roseus* (L) G. Don

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Abstract

Genetics of flower Colour in winka *Catharanthus roseus* (L) G. Don were in vestigate by inheritance two types (strains) of plants with different flowers colour were used in this study, pink corolla, and strong violet-purple eye color, and strong pink stem, and dark green leaf lamina (P), and White corolla, and yellow and greenish eye, and strong pink stem, and yellow and green leaf lamina (W) as parents, to determine the number of genes involved. This study was conducted at Horticulture Administration, Ministry of Agriculture, Kassala State, Sudan during for three years the period: Jan 2016 to Oct. 2020.

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 pink corolla, and strong violet-purple eye color, and strong pink stem, and dark green leaf lamina (P) is incompletely dominant over that for White corolla, and yellow and greenish eye, and strong pink stem, and yellow and green leaf lamina (W). The reciprocal crosses gave the same results indicating no role of cytoplasmic genes in the inheritance of these colors.

Keywords: Apocynaceae; Magnoliphyta; Anthocyanid; Cancer; Phenotypes; Iheritance; Self-Pollination

1. Introduction

Catharanthus roseus (L.) G. Don (family Apocynaceae) is an ornamental plant with medicinal values, these plant (synonymous with *Vinca rosea* Linn.) is a perennial herbaceous subshrub plant, this plant belongs to the Magnoliphyta division, under the class of Manoliopsida, in the order of Gentianales, family Apocynaceae [1], Apocynaceae family represents a large family which in cludes about 1500 species, found mainly in tropical regions many of them are large trees found in rainforest, some are smaller shurbs, 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]. *Catharanthus roseus* (L.) is an important medicinal plant of the family Apocynaceae which contains a virtual cornucopia of useful alkaloids, used in diabetes, blood pressure, asthma, constipation, and cancer and menstrual problem. There are about two common cultivars of *C. roseus* which is named on the basis of their flower colour that is the pink flowered “Rosea” and the white flowers “Alba”. *Catharanthus roseus* which is pridely known as the Madagascar periwinkle is found to be a species of *Catharanthus* native and also endemic to Madagascar Monika (2013) [3].

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Simmond (1960), found that the flowers of periwinkle, *Lochnera* (*Vinca*) *rosea* (L.) contained a pigment of which the aglycone corresponded with one of the common types recognized in other plants [4]. Harbone, and Sherratt (1960). They showed that the anthocyanidin was trimethyldephinidin and corresponded in its properties with hirsutidin, a pigment which has otherwise been recognised only in the Primulaceae (Harborne 1958). 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) [6]. The characters of the various color forms are summarised by Wanscher (1953), who described the flower color phenotypes of this plant [7]. The bloom of a natural wild plant is pale pink with a purple eye in the center, but horticulture is that developed varieties of more than one hundred with color ranging from white to pink to purple Alam et al (2010) [8]. 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 a co-pigmentation gene which blues the pink pigment (A-R-W-) background being violet color A-RW-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 being white corolla and purple eye, AR-wwbb being white corolla and reddish purple eye. Milo et al (1985) [9] 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- being 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 rrwwii being white. In spite of 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) [10]. 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). Also in genetics of flower color in winka *Catharanthus roseus* (L) G. Don Abdelmageed and Abdelrahman (2017) [11] who made experimental studies by inheritance two types (strains) of plants with different flowers color was 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. Introductorily the two parents were covered to ensure self-pollination. then reciprocal crosses 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.

2. Material and methods

The accession LP was found to be true breeding for Light pink corolla, pale red eye flower, pale pink stem, and pale green leaf lamina (Li.P). 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 [12].

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) [13]. All of the F1 plants were selfed. Altogether 195, 103 and 93 plants of F2. Chi-square were used for testing the goodness-of-fit of observed and expected frequencies of different phenotypic classes in the F2 by Kulkarni, Sreevalli et al., (2001) [12]. The source of seeds for the two parental strains of periwinkle (*Catharanthus roseus*) plants with two different flower colour were used in this study pink corolla, and strong violet-purple eye color, and strong pink stem, and dark green leaf lamina (P), and White corolla, and yellow and greenish eye, and strong pink stem, and yellow and green leaf lamina (W), Figure 1 and Figure 2 were obtained from

plants growing naturally in kassala, later they were grown in the green house at Horticulture Administration, Ministry of Agriculture, Kassala State, Sudan, by using cloth of bags. The seeds were obtained from crossing the two parents.



Figure 1 The Parent with Pink corolla and strong violet -purple eye color and strong pink stem, and dark green leaf lamina (P)



Figure 2 The Parent with White corolla and yellow and greenish eye color ,and green stem, and yellow and green leaf lamina (W)

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 greenhouse 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 one-hundred 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. When all the parental lines being in full flowering stages in about 90 days after planting Figure. 3.



Figure 3 Pink corolla and White corolla parents in flowering stage.

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. The hand emasculation, and reciprocal crosses were made between the two parental lines, were done according to the method of Abdelmageed and Abdelrahman (2017) [13] (Figure 4 & Figure 5). Seeds resulting from successful hybridization in each reciprocal cross (about 25 each) harvested separately and grown in isolation to produce F1 plants.



Figure 4 Cross- pollination in white parent fixed with a piece of soda straw tied with red string.



Figure 5 Reciprocal crosses in pink parent fixed with a piece of soda straw.

2.2. Production of F2

All F1 plants from the two reciprocal crosses produced one type of flower color F1 generation with Light pink corolla, pale red eye flower, pale pink stem, and pale green leaf lamina (Li.P), 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. Seeds produced from selfed flowers (F2 seeds) are raised to produce F2 plants.

2.3. Statistical Analysis

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

3. Results

3.1. The F1 Plants

All F1 plants from the cross pink corolla and strong violet -purple eye color, strong pink stem, and dark green leaf lamina (P) x White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (W) and the reciprocal cross white corolla and yellow and greenish eye color x pink corolla and strong violet eye color (P) had Light pink corolla, pale red eye flower, pale pink stem, and pale green leaf lamina (Li.P), however the colour was slightly light pink indicating that the pink corolla and strong violet -purple eye color, strong pink stem, and dark green leaf lamina (P) trait is dominant over the White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (W). However, the fact that the pink colour is light pink and not as dark as the pink 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 485 F2 plants were scored for flower colour. 125 plants had pink corolla, strong violet-purple eye color, strong pink stem, and dark green leaf lamina (F2 P) like the parental stock, 227 plants had Light pink corolla, pale red eye flowers, and pale pink stem, and pale green leaf lamina (F2 Li.P) like the F1 plants and 133 plants had White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (F2 W).

When fitted to a 1:2:1 ratio, the χ^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), and also the flower and eye, stem, and leaf lamina color in two parents, the F1, and F2 plants in periwinkle. (Table 2)

Table 1 Phenotypes, of observed and expected frequencies of plants with different corolla colours in F2 generation of the cross pink corolla (P) x white corolla (W) in periwinkle.

Phenotype	Pink flower and strong violet eye (P)	Light pink flower and pale violet eye (Li.P)	White flower and yellow and greenish (W)	Total
Observed(O)	125	227	133	485
Expected (E)	121.3	242.5	121.3	485
$\frac{(O - E)^2}{E}$	0.11	1.00	1.13	$\chi^2 = 2.241$

Table 2 Morphological features in two parents, the F1, and F2 plants in periwinkle.

Genotype	Leaf	Stem	Flower colour
Pink parent (P.P)	dark green lamina	Pigmented	Pink corolla and strong violet and purple eye
White parent (W.P)	yellow green lamina	Light green	White corolla and yellow greenish eye
F1: light pink (F1.L.P)	dark, and yellow green lamina	Pigment Light green	Light pink corolla and pale violet eye (ring in the orifice region)
F2: Pink (F2.P)	dark green lamina	Pigmented	Pink corolla and strong violet and purple eye
F2: light pink (F2.L.P)	Dark, and yellow green lamina	Pigment Light green	Light pink corolla and pink (pale violet) eye (ring in the orifice region).
F2: white (F2.W)	yellow green lamina	Light green	White corolla and yellow and greenish eye



Figure 6 F1 Light pink corolla, pale red eye flower, pale pink stem, and pale green leaf lamina (Li.P).

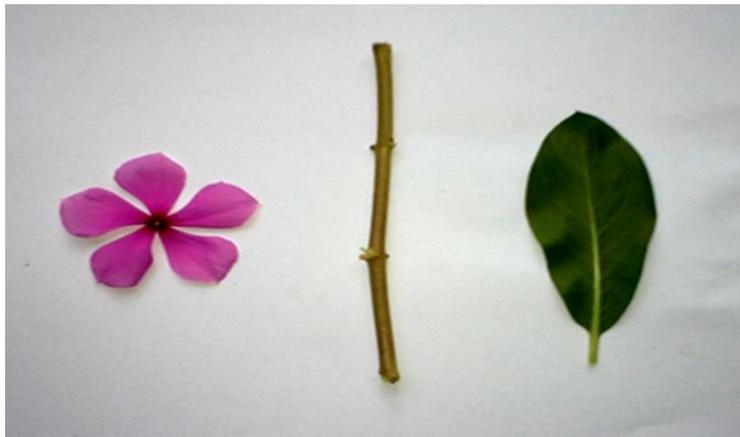


Figure 7 F2 Pink corolla and strong violet-purple eye, strong pink stem, and dark green leaf lamina (F2 P).



Figure 8 F2 White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (F2 W).



Figure 9 F2 Light pink corolla, pale red eye flowers, and pale pink stem, and pale green leaf lamina (F2 Li.P)

4. Discussion

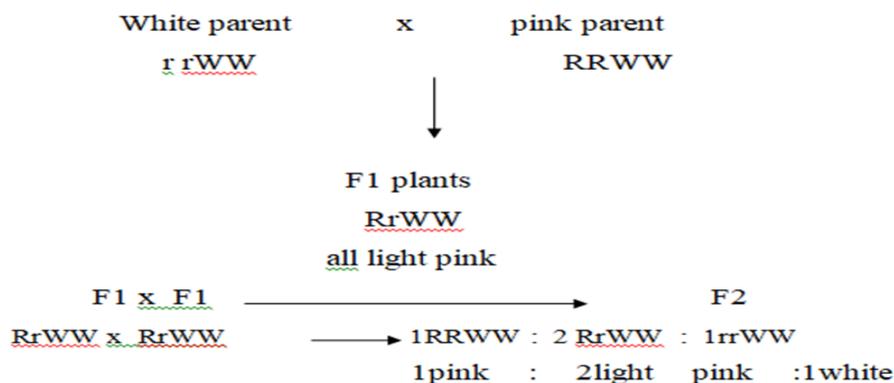
This study shows that the F1 plants produced from crossing of pink flower coloured and red eye plants, with white flowered and yellow (greenish) plants, were all with light pink flower coloured and pale red eye. Figure 6. This indicates that the F1 plants had intermediate flower colour between the two 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 pink and white plants and suggested that four loci are involved in flower colour determination. Flory *et al.*, (1944) suggested that white flower colour genotype to be $rrww$, and Pink flower as $R-W$ - that is means that the crossing between them will produce F1 progeny with $RrWw$. In another study Simmonds *et al.*, (1960) suggested the genotype of the white flowered plant as $A-rr----$, $aaR-----$, and $aarr----$, and Pink flower as $A-R-W-bb$, that is means the crossing between them, will produce F1 progeny with $AaRrWwbb$. Also In another study Milo *et al.*, (1985) suggested the genotype of the white flowered plant $asrrW-I-$, $rrwwI-$, $rrW-ii$, and $rrwwii$, and Pink flower as $R-W-I-$, $R-W-ii-$, thus that is means the crossing between them, will produced F1 progeny with $RrWwIi$. Also Kulkarni *et al.*, (1999) suggested that white flower colour genotype to be $rrWW$ and pink flower as $RRWW$, with the $R-W$ -genotype being pink, and with $R-ww$ being red-eyed, and with rrW , and $rrww$ being white flowered. In another study Kulkarni *et al.*, (1999) suggested the genotype of the white flowered plant as $AArrWWbb$ and that of the pink colour as $AaRrWwBb$, $ARWb$, $AaRWBb$ or $ARrWBb$ AB or RB , $EeR-WwO$, $E-RrW-Oo$, $E-RrW-oo$. In this study the F1 plants were heterozygous for the R locus, and W .

The plants had is relatively dark pink flower color than the parental pink flower, similar to parental white flower. However, the fact that the pink colour is mor darks pink, and also the pink colour is lightpink corolla, pale violet-purple eye color, pale pink stem and not as the pink parent, indicats that other modifiers for colour intensity, or partial dominance was existed. The result of F2 plant segregations were could be classified into three Phenotype of the flower colour. F2 strong pink corolla, strong violet-purple eye color, strong pink stem, and dark green leaf lamina (F2.P), F2 Light pink corolla, pale red eye flowers, and pale pink stem, and pale green leaf lamina (F2.L.P), and White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (F2.W) Figure 7, Figure 8, and Figure 9. As shown in Table 4.1 the calculated value of χ^2 is 2.24 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 pink colour allele or its recessive counterpart is a reasonable explanation. The F2 plant segregations were in line with the above results. The white colour character reappeared indicating that it is recessive, and the pink flower colour from dark pink and light pink like F1 generation, and not similar to the colour of the pink parental indicate that there were not similar to the pink parental flowered, it indicate that the genotype of them ($ARWB$) Kulkarni *et al.*, (1999), and $ARWb$ by Simmonds *et al.*, (1960), and absent of the flower with white corolla with the red eye in F2 generation it indicate that there were no genotype carried $R-ww$ Kulkarni *et al.*, (1999).

The observed frequencies of these three categories of flower colors fit a ratio of 1: 2: 1, and from dark pink and the light pink plants, however, showed some differences among themselves in the intensities of their flower and their eyes, and stem, and leaf lamina colour, this results indicate that this is a monohybrid ratio with only one locus involved. Incomplete dominance or lack of dominance of the pink colour allele or its recessive counterpart is a reasonable

explanation. these results in were in agreement with previous studies in Abdalmageed and Abdelrahman (2017), they were indicated that the 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, and also this result were agreement with Gupta Sarika & Pandey *et al.*,(2007) [14], they were indicate that the this result in this study are expected to be a result of coordinated expression of multigenes. However, the genetic backgrounds of the parents may be polymorphic for only one to several genes determining the traits [14] and also this result were agreement with (kulkarni *et al.*, 2005) [15] who reported that this result the inheritance pattern of the corolla petal colour, in *Cathranthus roseus*, known to be multigenic.

Based on these results the followinggeneticdiagramforthe parents,F1, F2 genotypes could be illustrated as following:



In this study the colour of the eye at the base of the corolla was considered which could produce more variations in flower colour. And also there were no white corolla and red eye because there is no individual in F1, and F2 generation carried (R-ww) genotypes, this result were agreement with (Kulkarni & Baskaran *et al.*, 1999). Simmonds (1960) when considering the intensity of colour of the eye concluded that two complementary dominant genes control the distribution of colour of the eye.

5. Conclusion

The study indicated that the cross pollination (hybrids) between Pink corolla and strong violet-purple eye, strong pink stem, and dark green leaf lamina (P), White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (W), produced the F1 generation with Light pink corolla, pale red eye flowers, and pale pink stem, and pale green leaf lamina.

The second generation (F2) produced three flower colours, F2 Pink corolla and strong violet-purple eye, strong pink stem, and dark green leaf lamina (F2. P), Light pink corolla, pale red eye flowers, and pale pink stem, and pale green leaf lamina (F2 L.P), and White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (F2.W) with a ratio 1:2:1.

The heredity of flower colour, between the two strains, Pink corolla and strong violet-purple eye, strong pink stem, and dark green leaf lamina (P), and White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (W) in periwinkle (*Catharanthus roseus*) has been found to be governed by only one locusto several genes determining the traits (multigenic).

The study indicated that Pink corolla and strong violet-purple eye, strong pink stem, and dark green leaf lamina (P), has incomplete dominance or some gene modifier was existed over the and White corolla, yellow and greenish eye color, green stem, and yellow and green leaf lamina (W) ratio 1:2:1.

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

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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