

Genetics of corolla colour in periwinkle: relationship between genes determining violet, orange-red and magenta corolla

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Abstract

In periwinkle (*Catharanthus roseus*), pink, white and red-eyed (white corolla with red centre) are three common corolla colours, which are due to epistatic interaction between two genes *R* and *W*. Violet corolla, which is not found commonly in natural populations, is reported to be due to another gene *B* which blues the pigment in pink genotypes (*R- W-*). Recently, another gene *O* and its allele *O^m* have been reported to determine two uncommon corolla colours, orange-red and magenta corolla, respectively. Since, the relationship between genes determining violet, orange-red and magenta corolla was not known, a strain possessing violet corolla and white eye (VI) was crossed with strains possessing orange-red corolla and white eye (OR) and magenta corolla and white eye (MJ-1) to study: (i) the relationship between genes involved in the production of violet, orange-red and magenta corolla, (ii) to study the possibility of producing novel corolla colours and (iii) to determine the validity of the gene interaction models proposed earlier. The F_1 plants of both crosses, VI x OR and VI x MJ-1, had violet corolla. The F_2 generation of the cross VI x OR segregated into plants with (i) violet corolla, (ii) pink corolla, (iii) orange-red corolla, and (iv) white corolla in the ratio of 45:12:3:4, while the progeny of the backcross F_1 x OR segregated into three types of plants, (i) violet corolla, (ii) pink corolla, and (iii) orange-red corolla in the ratio of 2:1:1. The F_2 generation of the cross VI x MJ-1 segregated into five kinds of plants viz., (i) violet corolla, (ii) pink corolla, (iii) magenta corolla, (iv) rose corolla, and (v) white corolla in the ratio of 144:48:12:36:16, while the progeny of the backcross, F_1 x MJ-1 segregated into four types of plants viz., (i) violet corolla, (ii) magenta corolla, (iii) rose corolla and (iv) pink corolla in the ratio of 1:1:1:1. The results suggested that genes involved *B*, *R*, *W*, *O/O^m* and *J* were inherited independently and that the gene *B* blues the corolla pigment in *B-RRwwO-* genotypes but not in *B-RRwwO^m-jj* and *B-RRwwO^m-JJ* genotypes. No new corolla colours were observed in the studied crosses due to the interaction between genes governing violet, orange-red and magenta corolla. The observed segregation for different corolla colours in the studied crosses was same as that expected from independent segregation and known interactions between the genes involved, validating the earlier proposed models.

Key words: *Catharanthus roseus*, ornamental plant, medicinal plant, corolla colour, inheritance

Introduction

Periwinkle [*Catharanthus roseus* (L.) G. Don] is commercially grown for the extraction of highly valued anti-cancer and anti-hypertension alkaloids, which are present in its leaves and roots, respectively. It is also widely grown as a flowerbed plant in gardens because of its coloured flowers and hardy nature. The most commonly observed corolla colours in periwinkle are pink, red-eyed (white corolla with red eye), and white. Apart from these common corolla colours, uncommon corolla colours viz., violet, orange-red, scarlet-red, magenta and rose corolla colours have also been reported. These corolla colours pink, red-eyed, pale pink, white, violet, orange-red, scarlet-red, magenta and rose have been found to be determined by interactions between alleles at seven loci, *A*, *R*, *W*, *B*, *I*, *O/O^m*, and *J* (Flory, 1944; Simmonds, 1960; Milo *et al.*, 1985; Kulkarni *et al.*, 1999; Sreevalli *et al.*, 2002; Kulkarni *et al.*, 2005). Genes *A* and *R* were reported to be basic complementary genes without both of which flowers would have white corolla (Simmonds, 1960). The genotypes *A-R-W-* produce pink corolla. The *R* allele produces anthocyanin pigments in the center of the corolla and is epistatic to the *W* and *I* alleles, *i.e.* they function only in the presence of *R* allele. In the presence of the *W* allele, the pigment in the eye region spreads

into the corolla limb. The *I* allele also produces anthocyanin pigments but in smaller quantities, and produces pale pink corolla with red eye in the absence of the *W* allele (Milo *et al.*, 1985). The gene *B* is a copigmentation gene which blues the pigment in *R-W-* genotypes resulting in violet corolla with purple eye and, probably, also in *R-ww* genotypes resulting in white corolla with purple eye (Simmonds, 1960). A newly identified gene *O* was found to be responsible for the production of orange-red corolla even in the absence of *R* allele, the basic gene required for the production of coloured corolla. The gene *O*, however, did not express in the presence of the *W* allele. Another gene *O^m*, allelic to the gene *O*, was found to be responsible for the production of magenta corolla. An inhibitory gene *J*, which by itself did not produce any corolla colour, partially inhibited gene *O^m* to produce rose corolla. The allele *O^m*, like the *O* allele, does not express in the presence of the *W* allele, but unlike the *O* allele produces magenta corolla only in the presence of the *R* allele. Heterozygotes *O/O^m* were found to produce scarlet-red corolla (Kulkarni *et al.*, 2005). Another gene *E* was found to determine the presence or absence of pigmentation in the eye region *i.e.*, in the center of the corolla (Sreevalli *et al.*, 2002).

Periwinkle plants with violet corolla colour are not common

in naturally growing populations of periwinkle. Although the genetics of violet corolla colour has been reported earlier (Simmonds, 1960), its relationship with the recently reported genes determining novel corolla colours *viz.*, orange-red and magenta (Kulkarni *et al.*, 2005), is not known. A knowledge of genes and gene interactions involved in the production of different corolla colours in ornamental plants is essential for understanding the biochemical basis of different corolla colours as well as for producing novel corolla colours either by conventional or molecular breeding. (Meyer *et al.*, 1987; Forkmann, 1991; Davies *et al.*, 2003; Zufall and Rausher, 2003). We recently obtained a strain, designated as VI, possessing violet corolla and white eye from a local dealer in horticultural plants and crossed it with strains possessing orange-red corolla and white eye (OR) and magenta corolla and white eye (MJ-1) to study (i) the relationship between genes involved in the production of violet, orange-red and magenta corolla, (ii) to study the possibility of producing novel corolla colours and (iii) to determine the validity of the gene interaction models proposed earlier.

Materials and methods

Three strains, with orange-red, magenta and violet corolla colours, were procured from a local dealer in horticultural plants and were designated as OR, MJ-1 and VI, respectively. All the three strains had white eye and were found to be true-breeding for their respective corolla colours and white eye. The strain VI was crossed reciprocally with strains OR and MJ-1 to determine the relationship between genes determining violet, orange-red and magenta corolla colours. Parental plants were raised in a glass house from seeds obtained by artificial self-pollination. Reciprocal crosses were also made as described earlier (Kulkarni *et al.*, 2001). The F_1 plants were selfed and also backcrossed to both the parents. Seedlings of parental, F_1 , F_2 and backcross generations were raised from their respective seeds, in perforated plastic trays filled with sterilized soil. Three months old seedlings of all six generations were transplanted into perforated polythene bags containing a mixture of soil, sand and farm yard manure in the ratio of 1:1:1 and placed on the benches in the glass house. The plants were scored for corolla colour after they flowered. Chi-square and G tests were used for testing the goodness of fit of observed and expected frequencies of different phenotypic classes in the F_2 and backcross generations (Sokal and Rohlf, 1981).

Results and discussion

Cross: VI (violet corolla and white eye) x OR (orange-red corolla and white eye): Since both parental strains had white eye, all plants of F_1 , F_2 and backcross generations also had white eye. Therefore, only corolla colours of plants of these generations are mentioned in the following. Also since both parental strains had coloured corolla they are expected to be homozygous for both *A* and *R* alleles and therefore, the genotype at *A* locus of different phenotypes observed in the F_2 and backcross generations is not mentioned in the following.

The flowers of F_1 plants had violet corolla resembling the parent VI. There were no differences in the corolla colour of F_1 plants of reciprocal crosses. The progeny of the F_2 generation could be classified into four classes based on their corolla colour, namely, (i) violet corolla, (ii) pink corolla, (iii) orange-red corolla, and (iv)

white corolla. The observed ratio of these four corolla colours fitted a ratio of 45:12:3:4 both by Chi-square and G tests (Table 1).

The progeny of the backcross, F_1 x OR, produced three types of plants namely those with (i) violet corolla, (ii) pink corolla, and (iii) orange-red corolla and their observed frequencies fitted a ratio of 2:1:1 both by Chi-square and G tests (Table 1). The progeny of the backcross, F_1 x VI, produced all plants with violet corolla.

From an earlier study, it was known that the genotype of the parental strain OR with orange-red corolla and white eye is *eeRRwwOO* and that the *O* allele produces orange-red corolla even in the absence of *R* allele but does not express in the presence of *W* allele (Sreevalli *et al.*, 2002). According to Simmonds (1960), violet corolla is due to the interaction between *R*, *W*, and *B* genes *i.e.*, the gene *B* blues the pink corolla colour in plants with *R-W*- genotypes. Therefore, the genotype of the strain VI with violet corolla and white eye should be *eeRRBBWW*. Thus, the genotypes of parental strains OR and VI with respect to *B*, *R*, *O*, *W* and *E* would be *eebbRRwwOO* and *eeBBRRWWoo*, respectively. With these genotypes of the parental strains OR and VI and above mentioned interactions between these genes and their independent segregation, the expected frequencies of different genotypes and their phenotypes in F_2 and backcross generations are given in Table 1. The observed frequencies of plants with different corolla colours in both F_2 and backcross generations fitted the expected frequencies (Table 1). It was not known if the *B* gene would blue the orange-red corolla of *B-RRwwO*- genotypes. If the gene *B* did not blue the pigment to produce violet corolla colour in *B-RRwwO*- genotypes, then the expected ratio of different corolla colours in the F_2 generation would be 36 violet: 12 pink: 12 orange-red: 4 white. This ratio, however, did not fit the observed ratio of plants with these corolla colours ($\chi^2_{(d.f.3)}=13.573$, $P < 0.01$, $G_{(d.f.3)}=17.962$, $P < 0.01$). If the gene *B* did produce violet corolla in *B-RRwwO*- genotypes, the expected ratio of corolla colours in the F_2 generation would be 45 violet: 12 Pink: 3 orange-red: 4 white. The observed ratio of corolla colours in the F_2 generation, as already mentioned above, fitted this expected ratio (Table 1).

Cross: VI (violet corolla and white eye) x MJ-1 (magenta corolla and white eye): Since the parental strains had white eye, all plants of F_1 , F_2 and backcross generations also had white eye. Therefore, only corolla colours of plants of these generations are mentioned in the following. Also since both parental strains had coloured corolla they are expected to be homozygous for both *A* and *R* alleles and therefore, the genotype at *A* locus of different phenotypes in the F_2 and backcross generations is not mentioned in the following.

The F_1 plants of this cross also had violet corolla. There were no differences in the corolla colour of F_1 plants of reciprocal crosses. In the F_2 generation, plants could be classified into five classes, namely, (i) violet corolla, (ii) pink corolla, (iii) magenta corolla, (iv) rose corolla, and (v) white corolla. The observed frequencies of these five kinds of plants fitted a ratio of 144:48:12:36:16 (Table 2).

The progeny of the backcross, F_1 x VI had violet corolla while the progeny of the backcross F_1 x MJ-1 segregated into four types of plants *viz.*, those with (i) violet corolla, (ii) magenta corolla, (iii) rose corolla and (iv) pink corolla in the ratio of 1:1:1:1 (Table 2).

Table 1. Phenotypes, genotypes, expected proportions, and observed and expected frequencies of plants with different corolla colours in the parental, F_1 , F_2 and backcross generations of the cross VI (violet corolla and white eye) x OR (orange-red corolla and white eye) in periwinkle, according to the proposed model

Generation	Phenotype	Genotype	Expected proportion	Observed frequency	Expected frequency
OR	Orange-red corolla and white eye	<i>eebbRRwwOO</i>	1	All	All
VI	Violet corolla and white eye	<i>eeBBRRWwoo</i>	1	All	All
F_1	Violet corolla and white eye	<i>eeBbRRWwOo</i>	1	All	All
F_2	Violet corolla and white eye	<i>eeB-RRW-O-</i>	27/64		
		<i>eeB-RRW-oo</i>	9/64		
		<i>eeB-RRwwO-</i>	9/64		
		Total	45/64	70	71.7
	Pink corolla and white eye	<i>eebbRRW-O-</i>	9/64		
		<i>eebbRRW-oo</i>	3/64		
	Total	12/64	19	19.1	
	Orange-red corolla and white eye	<i>eebbRRwwO-</i>	3/64		
		Total	3/64	5	4.8
	White corolla	<i>eeB-RRwwoo</i>	3/64		
<i>eebbRRwwoo</i>		1/64			
Total		4/64	8	6.4	
Total			102	102	

$\Sigma\chi^2_{(d.f.3)}$ for the expected ratio of 45:12:3:4 = 0.448 (P = 0.95 – 0.90), $G_{(d.f.3)} = 0.422$ (P = 0.95 – 0.90)

Backcross

F_1 x OR	Violet corolla and white eye	<i>eeBbRRWwO-</i>	1/4		
		<i>eeBbRRwwO-</i>	1/4		
		Total	2/4	6	6.5
Pink corolla and white eye	<i>eebbRRWwO-</i>	1/4			
	Total	1/4	3	3.25	
Orange-red corolla and white eye	<i>eebbRRwwO-</i>	1/4			
	Total	1/4	4	3.25	
Total			1	13	13

$\Sigma\chi^2_{(d.f.2)}$ for the expected ratio of 2:1:1 = 0.230 (P = 0.90 – 0.80), $G_{(d.f.2)} = 0.216$ (P=0.90-0.80)

From an earlier study, it was known O^m is allelic to O and does not express in the presence of W allele. Further, it was also known that unlike its allele O , it produces magenta corolla only in the presence of R allele, and that an independent gene J , which does not produce any corolla colour on its own, partially inhibits the gene O^m to produce rose corolla (Kulkarni *et al.*, 2005). Therefore, the presence of plants with rose corolla in the F_2 and backcross (F_1 x MJ-1) generations suggested the presence of gene J in the parent VI. Thus, the genotypes of parental strains VI and MJ-1 would be *eeBBRRWwooJJ* and *eebbRRwwO^mO^mjj*, respectively. With the already known interactions between genes R , W , B , O^m and J mentioned above and their independent segregation, expected frequencies of different genotypes and their phenotypes in F_2 and backcross generations are given in Table 2. The observed frequencies of plants with different corolla colours in both F_2 and backcross generations fitted the expected frequencies (Table 2). The allele B , however, did not appear to blue the magenta corolla colour in *B-RRwwO^m-jj* genotypes. If it were to produce violet corolla colour in genotypes *B-RRwwO^m-jj*, the expected ratio of different corolla colours in F_2 would have been 153 violet: 48 pink: 3 magenta: 36 rose: 16 white, which however did not fit the observed ratio ($\chi^2_{(d.f.=4)}=80.756$, $P\leq 0.001$; $G_{(d.f.=4)}=39.732$ $P\leq 0.001$).

The results of the present study revealed that genes B , R , W , O / O^m and J involved in the production of violet, pink, orange-red, magenta, rose and white corolla were inherited independently

and that only corolla colours, expected on the basis of the gene interaction models proposed earlier (Simmonds 1960, Kulkarni *et al.*, 2005) for the production of these corolla colours were observed in the crosses VI (violet corolla) x OR (orange-red corolla), and VI (violet corolla) x MJ-1 (magenta corolla), validating the proposed genetic models. It was also evident that the gene B blue the corolla pigment in *B-RRwwO-* genotypes but not in *B-RRwwO^m-jj* and *B-RRwwO^m-JJ* genotypes and that no new corolla colours were produced due to the interactions between B , R , W , O and O^m genes.

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Table 2. Phenotypes, genotypes, expected proportions, and observed and expected frequencies of plants with different corolla colours in the parental, F₁, F₂ and backcross generations of the cross VI (violet corolla and white eye) x MJ-1 (magenta corolla and white eye) in periwinkle, according to the proposed model

Generation	Phenotype	Genotype	Expected proportion	Observed frequency	Expected frequency
VI	Violet corolla and white eye	<i>eeBBRRWwoJJ</i>	1	All	All
MJ-1	Magenta corolla and white eye	<i>eebbRRwwO^mO^mjj</i>	1	All	All
F ₁	Violet corolla and white eye	<i>eeBbRRWwO^moJj</i>	1	All	All
F ₂	Violet corolla and white eye	<i>eeB-RRW-O^m-J-</i>	81/256		
		<i>eeB-RRW-oo J-</i>	27/256		
		<i>eeB- RRW-O^m-jj</i>	27/256		
		<i>eeB-RRW-oojj</i>	9/256		
	Total		144/256	156	165.4
	Pink corolla and white eye	<i>eebbRRW-O^m-J-</i>	27/256		
		<i>eebbRRW-ooJ-</i>	9/256		
		<i>eebbRRW-O^m-jj</i>	9/256		
		<i>eebbRRW-oojj</i>	3/256		
	Total		48/256	56	55.1
	Magenta corolla and white eye	<i>eeB-RRwwO^m-jj</i>	9/256		
		<i>eebbRRwwO^m-jj</i>	3/256		
	Total		12/256	20	13.8
	Rose corolla and white eye	<i>eeB-RRwwO^m-J-</i>	27/256		
		<i>eebbRRwwO^m-J-</i>	9/256		
	Total		36/256	40	41.3
	White corolla	<i>eeB-RRwwooJ-</i>	9/256		
		<i>eeB-RRwwoojj</i>	3/256		
		<i>eebbRRwwooJ-</i>	3/256		
		<i>eebbRRwwoojj</i>	1/256		
	Total		16/256	22	18.4
	Total		1	294	294

$\Sigma\chi^2_{(d.f.4)}$ for the expected ratio of 144:48:12:36:16 = 4.077 (P = 0.50 – 0.30), $G_{(d.f.4)} = 3.706$ (P=0.50 – 0.30).

Backcross

F ₁ x MJ-1	Violet corolla and white eye	<i>eeBbRRWwO^m-Jj</i>	2/16		
		<i>eeBbRRWwO^m-jj</i>	2/16		
	Total		1/4	13	15
	Pink corolla and white eye	<i>eebbRRWwO^m-Jj</i>	2/16		
		<i>eebbRRWwO^m-jj</i>	2/16		
	Total		1/4	12	15
	Magenta corolla and white eye	<i>eebbRRwwO^m-jj</i>	2/16		
		<i>eeBbRRwwO^m-jj</i>	2/16		
	Total		1/4	16	15
	Rose corolla and white eye	<i>eebbRRwwO^m-Jj</i>	2/16		
		<i>eeBbRRwwO^m-Jj</i>	2/16		
	Total		1/4	19	15
Total			1	60	60

$\Sigma\chi^2_{(d.f.3)}$ for the expected ratio of 1:1:1:1 = 1.998 (P = 0.70 – 0.50), $G_{(d.f.3)} = 1.970$ (P=0.70 – 0.50)

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