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# Genetic inheritance of flower colour pattern and governing gene action in segregating population of pansy

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## ABSTRACT

***Viola wittrokiana* (Pansy) is the most popularly used garden and landscaping plant, due to its numerous bloom colours, long blossoming season (mid-autumn to late spring), and endurance to freezing temperatures. In addition to retaining other significant vegetative and reproductive characteristics of the crop, breeding programmes of floricultural crops place a strong emphasis on introducing a variety of colours to the existing gene pool. In the present study four inbred lines of pansy, namely Pa-11-1-3-7, Pa-24-4, Pa-64-1-5-14 and Pa-63-1-7-25, which were crossed to obtain two cross combinations. The results suggested that significant genetic variability exists in flower colour patterns indicating the involvement of two or more genes. However, the identified genes were not interacting with each other which gave rise to several modifications of the typical dihybrid ratio (9:3:3:1) in $F_2$ into several gene interactions. In Pa-11-1-3-7 and Pa-24-4 cross, 'V' gene masked the effect of gene 'W' and 'w' and the interactions were found to be inhibitory gene action (13:3). The cross Pa-64-1-5-14 and Pa-63-1-7-25 exhibited the complementary gene action (9:7) and gene 'V' was epistatic to gene P/p. It was concluded that inheritance of flower colour was not a simple trait, as divergent gene interactions with the involvement of different alleles were noticed.**

**Key words:** Dihybrid ratio, flower colour, genetic segregations, genetic variability, *Viola wittrokiana*.

## INTRODUCTION

The genus *Viola* comprises around 400-500 species widely distributed around the world. The modern pansy (*Viola* × *wittrockiana*) is a popular ornamental bedding plant and thought to have been derived from hybridization among *Viola lutea*, *V. tricolor* and *V. atalica*. Pansy behaves as a perennial in cold climates but under North Indian conditions, it is grown in the winter season annually under sub-tropical conditions of Punjab with peak blooming period in the months of March-April. The flowers of pansies are hermaphrodite, pedicellate, zygomorphic, and hypogynous with pentamerous structure, *i.e*., five petals. The flower size in pansy varies from 2.5 to 8.0 cm. Small-flowered types flower profusely and hence, are suitable for bedding purposes, in pots, borders, rockery, window boxes, hanging baskets or in landscapes (Pearson *et al*., 9). A sporophytic self-incompatibility exists and thus the flower buds at 3/4$^{th}$ stage of opening are hand-pollinated individually to obtain seed. Pansy possesses vibrant colours such as white, red, purple, yellow, and orange. Expression of genes required for anthocyanin biosynthesis is well documented (Chiou and Yeh, 1). Certain pansy varieties have huge cyanic spots on the petals of their flowers. Previous research has examined the anthocyanin concentration of the cyanic blotched regions of pansy corolla (Endo, 1959). An analogous of pansy, *viz*., *Viola cornuta* used for the isolation of DRF, ANS and CHS genes (Farzad *et al*., 2). $F_1$ hybrids of these flower crops are in great demand due to uniform plant vigour, floriferous and unique colour combinations. The information on the heritable control of traits and the function of non-allelic interactions is important for selecting breeding methods (Ravikumar and Dhatt, 12). Diallel crossing system allows the estimation of genetic and non-genetic components of variation and is used to assess genetic variability in each group as among the elected set of parents (Ravikumar and Dhatt, 10; Murray *et al*., 8). The structural variation of this pentamerous flower affects its flower shape, which is an important criterion for varietal registration in pansy (Yoshioka *et al*., 13; Krahl and Randle, 4). The current investigation helps to assess the gene action governing the flower colour and number of genes governing the flower colour inheritance in pansy.

## MATERIALS AND METHODS

The current investigation was performed at the Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana during 2020-2022. The present experimental plant materials have been generated by four to five generations of selfing. Four inbred lines of pansy which are distinguished by morphological traits (Table 1) were utilized for the

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**Table 1.** Flower colour of selected inbred pansy lines.

| Genotype | Flower colour |
|---|---|
| Pa-11-1-3-7 | Yellow with chocolate blotch |
| Pa-24-4 | Maroon |
| Pa-64-1-5-14 | Purple and yellow |
| Pa-63-1-7-25 | Purple and white |

study. The selected inbred lines possess variation in growth habit; flower colour and flowering duration. Two cross combinations were performed, *viz*., Pa-11-1-3-7×Pa-24-4 and Pa-64-1-5-14×Pa-63-1-7-25 by using 4 *Viola* inbred lines.

The seeds of selected pansy lines were transplanted in the main field and in October crosses were attempted to generate $F_1$ plants. The seeds of crosses ($F_1$) as well as selfed (parents) progenies were harvested manually. The collected $F_1$ seeds were kept for drying in cool and moisture-free conditions. The dried and viable seeds were utilized in the next season for further research. In the next season, the stored $F_1$ seeds along with their parents were sown in the nursery and transplanted in the main field in October. The seedlings were transplanted at a spacing of 30 cm × 30 cm within and between the plants and rows on a plot of 2.4 m × 2.4 m size. The $F_1$ along with parents were evaluated in Randomized Block Design (RBD). Simultaneously, each parent and cross ($F_1$) were also selfed from January-March to get sufficient seeds of parents and $F_2$ generation for use in the next season for further selection of desirable plant types. Recorded data on flower colour were subjected to statistical analysis using MS Excel to find the Chi-Square values.

## RESULTS AND DISCUSSION

To study the inheritance pattern of the flower colour, the $F_2$ progeny was raised by selfing the $F_2$ plants to obtain a sufficient population of two cross combinations. Variation was noticed in two different crosses for the flower colour. Chi-square test confirmed the expected deviation from the Mendelian segregation ratio among the $F_2$ population (Table 2). The results suggested that the involvement of two or more genes but they were not interacting with each other, which gave rise to several modifications in a typical di-hybrid $F_2$ ratio (9:3:3:1). These interactions were due to inhibitory gene action (13:3) and complementary gene action (9:7), concerning two different cross combinations.

The inheritance of colour in the cross combination, *viz*., Pa-11-1-3-7 × Pa-24-4 reveals a $F_2$ segregation ratio of 13:3 (Yellow with chocolate blotch: Maroon), indicating the involvement of two loci. Therefore, in an inhibitory gene action, one dominant inhibitory gene blocks the expression of second dominant gene activity. This modifies the typical 9:3:3:1 $F_2$ ratio into 13:3 ratio with a non-significant Chi square value of 0.110 (Table 2). Out of 170 plants of $F_2$ generation, 130 plants shown yellow with chocolate blotch colour and 40 plants had maroon colour (18 partially maroon + 22 maroon). The yellow flowers showed slightly maroon colour, this change is due to presence of modifier gene. Yellow with chocolate blotch governed by V gene, which is dominant over maroon colour. The results were in agreement with Kumar *et al*. (5) in chickpea. The maroon colour is controlled by a dominant gene W. The cross was made between yellow with chocolate blotch colour (VVww) and maroon colour flowers (vvWW) generated the yellow with chocolate blotch coloured flowers. Selfing of $F_1$ plants produced yellow with chocolate blotch and maroon in 13:3 ratio in the $F_2$ generation. Similarly, diallel analysis was followed in periwinkle to exploit flower colour in the segregated ratio of magenta and white coloured flowers. It has been earlier reported that magenta coloured flower partially dominated the white flowers (Ravikumar and Dhatt, 11). From the above Fig. 1, it can be inferred as the allele V is epistatic to allele W and w. Hence in $F_2$, plants with V-W (96), V-ww (32) and vvww (10) genotypes expressed yellow with chocolate blotch colour flowers because V gene masked the effect of W and w. The plants with vvW (32) possessed maroon colour due to absence of V gene (Fig. 2). These results were in agreement with the studies of Li *et al*. (6) on pansy.

The cross combination Pa-64-1-5-14 × Pa-63-1-7-25 shows distribution of plants in a 9:7 segregation ratio, which indicates the presence of complementary gene action. Out of 74 plants of $F_2$ generation, 45

**Table 2.** Chi-square test for inheritance of colour in $F_2$ population of pansy.

| Genotype | Total No. of plants | Observation frequencies in $F_2$ phenotypic classes | | Expected ratio | $X^2$ | *p*-Value |
|---|---|---|---|---|---|---|
| Pa-11-1-3-7 × Pa-24-4 | 170 | 130 (Yellow with chocolate blotch) | 40 (Maroon) | 13:3 | 2.55 | 0.110 |
| Pa-64-1-5-14 × Pa-63-1-7-25 | 74 | 45 (Purple and yellow) | 29 (Purple and white) | 9:7 | 0.625 | 0.429 |

| | | | | | |
|---|---|---|---|---|---|
| Parents | **VVww** (Yellow with chocolate blotch) | × | **vvWW** (Maroon) | | |
| | ↓ | | | | |
| $F_1$ | **VvWv** (Yellow with chocolate blotch) | | | | |
| $F_2$ | VwWv × VwWv | | | | |
| | ↓ | | | | |
| Gametes | **VW** | **Vw** | **vW** | **vw** | |
| Progeny of $F_2$ | V-W (Yellow) | V-ww (Yellow) | vvww (Yellow) | vvW (Maroon) | |

**Fig. 1.** Genotypic expression of cross Pa-11-1-3-7 × Pa-24-4.

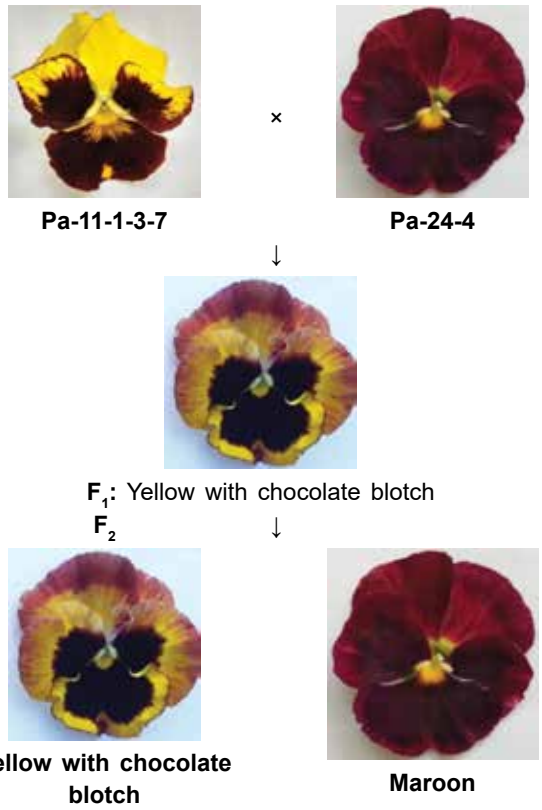


**Fig. 2.** Flower colour of parents and progenies in the cross Pa-11-1-3-7 and Pa-24-4, Pa-64-1-5-14.

plants possessed purple-yellow colour (35 purple +10 partially purple) and 29 plants had purple white colour. The Chi Square value against 9:7 ratio was non-significant with 0.625 (Table 2). When two genes governing a trait interact in this way, one phenotype can only be produced when dominant alleles of both of those genes are present. The present results were in agreement with the results of Gaur and Gour (3) in chickpea. Purple with yellow colour in pansy was governed by two dominant genes V and P. Cross between Pa-64-1-5-14 (Purple and yellow) × Pa-63-1-7-25 (Purple and white) produced the purple and yellow flowers in $F_1$ generation (Fig. 4). Selfing of $F_1$ plants resulted in production of purple and yellow and purple and white flowers in 9:7 ratio in $F_2$ generation.

According to experimental results the allele 'V' was epistatic to P/p genes and masks the expression of P/p. The allele p was epistatic to V/v and masks the expression of V/v genes. Hence in $F_2$ generation plants with V-P- (43) genotypes possessed purple and yellow colour flowers. The genotypes of vvP-(14), V-pp-(14) and vvpp (3) produced purple white colour flowers (Fig. 3). The present results were in

| | | | | |
|---|---|---|---|---|
| Parents | **VVPP** (Purple and yellow) | × | **vvpp** (Purple and white) | |
| | ↓ | | | |
| $F_1$ | **VvPp** (Purple and yellow) | | | |
| $F_2$ | **VvPp × VvPp** | | | |
| | ↓ | | | |
| Gametes | **VP** | **Vp** | **vP** | **vp** |
| $F_2$ progeny | V-P- (Purple and yellow) | vvP- (Purple and white) | V-pp (Purple and white) | vvpp (Purple and white) |

**Fig. 3.** Genotypic expression of cross Pa-64-1-5-14 × Pa-63-1-7-25

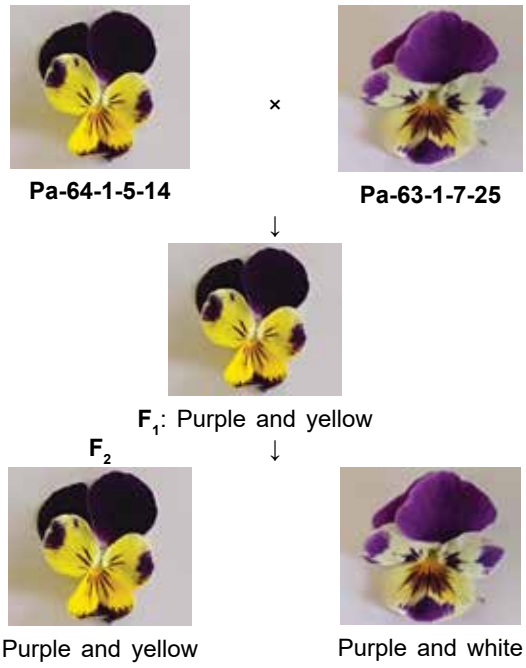


**Fig. 4.** Flower colour of parents and progenies in the cross Pa-64-1-5-14 and Pa-63-1-7-25.

accordance with the earlier reports by Li *et al*. (7) on pansy.

The above studies indicated that significant genetic variability was noticed in inheritance of flower colour pattern in pansy inbred lines. The yellow with chocolate blotch flower colour masks the effect of maroon colour flowers in pansy inbred lines and shown the inhibitory gene interactions by crossing yellow and chocolate blotch plants and maroon plants. The cross between purple and yellow and purple and white reported the complementary gene interaction and purple and yellow epistatic to purple and white. Further research is required to examine the precise causes of the variable expression of the genes governing the diversified flower colours in pansies to completely exploit the potentiality of flower colour patterns.

## AUTHORS' CONTRIBUTION

Conceptualization of research (Bolagam Ravikumar and K K Dhatt); Designing of the experiments (Bolagam Ravikumar and K K Dhatt); Contribution of experimental materials (Bolagam Ravikumar and K K Dhatt); Execution of field/lab experiments and data collection (Bolagam Ravikumar and K K Dhatt); Analysis of data and interpretation (Bolagam Ravikumar and K K Dhatt); Preparation of the manuscript (Bolagam Ravikumar and K K Dhatt).

## DECLARATION

Authors declare that they do not have any conflict of interest.

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