Indian Journal of Agricultural Sciences **93** (5): 495–500, May 2023/Article
https://doi.org/10.56093/ijas.v93i5.132433

Exploitation of heterosis in a diallel crosses of periwinkle (*Catharanthus roseus*) for morphological traits

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Received: 18 January 2023; Accepted: 6 April 2023

ABSTRACT

Despite the economic significance of periwinkle [*Catharanthus roseus* (L.) G. Don] the existence of genetic variation in traits have been largely unexplored and very little attempt has been done to improve its genetics for ornamental prospective. An experiment was conducted at the research farm of Punjab Agricultural University, Ludhiana, Punjab during 2019 to 2021 using 6 inbred lines of periwinkle to develop 30 F_1 populations in full-diallel fashion. The highly significant positive heterobeltiosis was recorded in the progeny of Vi-29 × Vi-14-3 for leaf breadth, follicle length, number of flowers per plant and number of seeds per follicle. The cross combination Vi-13-2 × Vi-29 was recorded significantly high heterobeltiosis for plant height and plant spread. The minimum heterobeltiosis was recorded for plant spread and follicle length in cross Vi-14-3 × Vi-15-1. The highest mean performance of the parents reported in Vi-15-1 for plant spread, internode length, flower diameter, number of flowers per plant, follicle length and number of seeds per follicle. As per the results among the cross combinations superior mean performance was observed in the Vi-15-1 × Vi-14-3 for leaf length and number of flowers per plant. These findings suggest that hybridization is an efficient way of contributing genetic variability in periwinkle for landscape utilization.

Keywords: Diallel cross, Heterobeltiosis, Heterosis, Mean performance, Periwinkle

Periwinkle [*Catharanthus roseus* (L.) G. Don] is a perennial semi-shrub, belongs to Apocynaceae family (Sreevalli *et al.* 2002). The genus comprises eight species being seven endemics to Madagascar, and one to the India (Van 1996). It has been cultivated as an ornamental plant all over the world and commonly grown in summer and rainy season for bedding and pot culture in the subtropical climatic conditions of Punjab and nearby areas. Under local conditions of Ludhiana region, it blooms year round except in December and January (Singh and Dhatt 2021). It grows well in various climatic conditions and soils of tropical and sub-tropical areas of northern India and resistance to drought and high temperature and water stress. Periwinkle is extensively used in landscaping as a border plant due to its delightful appearance, varied colour, size, shape and forms of flowers, etc. (Howe and Waters 1994).

Understanding of the plant's biology is an important step for breeding and maintaining germplasm integrity in a particular crop. The haploid chromosome number of periwinkle is 2n=16 and it is propagated by seeds or cuttings (Das 2010). Two to three circular shaped hermaphrodite and hypogynous flowers are borne in cymes in terminal and axillary clusters. The dehiscent fruit has two cylindrical follicles with sequentially arranged seeds (Rao 2007). It is treated as self-pollinated crop (Kullarni 1999). It is important for breeders to have information of the genetic control of characters and the role of non-allelic synergy for selection of breeding method (Esmail 2007, Hallauer 2007). Diallel analysis offers genetic information on quantitative characters and heterosis (Bhatt *et al.* 2001, Murray *et al.* 2003, Glover *et al.* 2005). The F_1 hybrids and varieties with varied morphological traits are an important aspect for landscaping purpose in periwinkle. For instance, heightest plants are being used for screening, whereas lowest plants are used as pot culture and edges. However, the information on heterosis studies in periwinkle is meager, in light of these considerations, the current research aimed to exploit heterosis in periwinkle for a variety of morphological traits.

MATERIALS AND METHODS

Present study was carried out at the research farm of Punjab Agricultural University, Ludhiana, Punjab during 2019–21. The experiment was designed in Randomized block design (RBD) with 3 replications with 2.4 m × 1.2 m plot size and the seedlings were planted at spacing of 60 × 40 cm^2. Morphologically different 6 inbred lines of periwinkle, viz. white centered magenta (Vi-15-1), white with red spot (Vi-13-2), dark purple (Vi-16), light purple

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(Vi-15-2), white (Vi-29) and dark pink colour (Vi-14-3) were utilized as parents in a diallel scheme (Table 1). These nearly homogeneous inbred lines were generated by continuous selfing of 4 to 5 generations. The 6 inbred lines were crossed in all possible combinations including reciprocals and developed 30 cross combinations. The fully matured and unopened flower buds were emasculated in the evening with forceps and next day morning pollinated by dusting desired pollen of the plant on emasculated buds. The matured F_1 seeds were harvested individually and sown in nursery bed in the next season. The observations were recorded from 5 randomly selected plants from each plot for various morphological characters.

Statistical analysis: The data recorded from the 30 cross combinations and their 6 different parents were calculated manually in MS Excel work sheet for mean performance. The estimates of heterosis over better parent (heterobeltiosis) were computed in MS Excel-2010 work sheet as (Hallauer and Mirinda 1988):

$$\text{Heterosis over better parent} = \frac{\overline{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

where BP, Better parent mean performance.

Furthermore, statistical significance of the heterobeltiosis was determined through the t-test proposed by Wynne *et al.* (1970).

Table 1 Morphological characters of inbred lines used in crossing programme of periwinkle

Inbred	As per RHS colour chart	
	Flower colour	Stem colour
Vi–15-1	Red purple group N74A	Red purple group 59C
Vi–13-2	White group N155D	Yellow green group N144B
Vi–16	Red purple group N74 A	Red purple group 60A
Vi–15-2	Red purple group 73 A	Red purple group 59B
Vi–29	White group 155D	Yellow green group 149A
Vi–14-3	Red purple group 63B	Yellow green group N144B

RESULTS AND DISCUSSION

Mean performance: The mean performance of the parents reported significant differences for the characters under study (Table 2). Vi-15-1 was recorded as wider plant spread (43.58 cm), internode length (2.02 cm), number of flowers/plant (447.02), flower diameter (4.83 cm), follicle length (3.39 cm) and number of seeds per follicle (31.90). The Vi-29 had lengthy leaf (5.07 cm), long corolla tube (2.32 cm) and early flowering (36.08 days). The early flowering in the parental lines might be due to additional dry matter accumulation during advantageous climatic situations. These results were in conjunction with the findings of Dhiman (2003) in chrysanthemum; Kumar *et al.* (2013) in cucumber; Sultan and Nassour (2019) in China aster. The Vi-13-2 was observed as more number of branches/plant (9.67) and widest leaf breadth (2.65 cm). The highest plant height (60.22 cm) and longer flowering duration (126.55) were observed in Vi-15-2 and Vi-16, respectively. The Vi-14-3 recorded lowest plant height (40.90 cm), narrow plant spread (30.00 cm), less number of branches/plant (5.72), short internode length (1.34 cm), short leaf length (3.99 cm), narrow leaf breadth (2.02 cm), small flowering duration (107.70 days), less number of flowers/plant (336.20) and small size flowers (4.22 cm). The less number of flowers/plant might be due to less number of branches/plant. These results were in concurrence with the findings of Tirakannanavar *et al.* (2015) in China aster. The parent Vi-29 had shortest follicle length (1.28 cm) and less number of seeds/follicle (12.40).

The results of 5 highly significant cross combinations for all the traits in mean performance are presented in the Table 3 (Supplementary Table 1, 2). As per the results the cross combination Vi-13-2 × Vi-29 indicated superior mean performance for plant height (84.05 cm) and plant spread (66.32 cm). The more number of branches/plant (12.47) and longer flowering duration (140.17 days) were recorded Vi-16 × Vi-15-1 progeny. The cross Vi-15-1 × Vi-14-3 had high mean value for leaf length (5.93 cm) and number of flowers per plant (569.60). The lengthy follicle (2.68 cm) and more number of seeds per follicle (35.30) were observed in

Table 2 Mean performances of parental lines for various traits in periwinkle

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13
Vi-15-1	60.22	43.58	8.75	4.88	2.34	2.02	1.94	36.83	116.75	447.02	4.83	3.39	31.90
Vi-13-2	58.71	35.56	9.67	4.71	2.65	1.97	2.20	40.67	124.33	404.10	4.53	2.46	28.92
Vi-16	57.85	38.02	8.98	4.36	2.12	2.01	2.22	42.63	126.55	365.27	4.46	2.72	30.27
Vi-15-2	68.44	35.05	8.82	4.78	2.53	1.97	2.24	38.13	118.02	396.68	4.63	2.52	25.78
Vi-29	58.63	33.51	6.43	5.07	2.16	1.98	2.32	36.08	120.77	340.38	4.64	1.28	12.40
Vi-14-3	40.90	30.00	5.72	3.99	2.02	1.34	2.15	38.43	107.70	336.20	4.22	1.90	15.33
Mean	57.46	35.95	8.06	4.63	2.30	1.88	2.18	38.80	119.02	381.61	4.55	2.38	24.10
Range	40.90-68.44	30.00-43.58	5.7-9.67	3.99-5.07	2.02-2.65	1.3-2.02	1.94-2.32	36.08-42.63	107.70-126.55	336.20-447.02	4.22-4.83	1.28-3.39	12.40-31.90
CD (P=0.05)	7.70	5.14	0.71	0.24	0.20	0.20	0.10	1.84	9.32	70.29	0.30	0.32	2.25

1, Plant height (cm); 2, Plant spread (cm); 3, Number of primary branches/plant; 4, Leaf length (cm); 5, Leaf breadth (cm); 6, Internode length (cm); 7, Corolla tube length (cm); 8, Days of flowering; 9, Duration of flowering (days); 10, Number of flowers per plant; 11, Flower diameter (cm); 12, Follicle length (cm); 13, Number of seeds per follicle.

Vi-15-1 × Vi-13-2. The progeny of Vi-15-1 × Vi-29, Vi-13-2 × Vi-16, Vi-15-1 × Vi-16 and Vi-29 × Vi-15-1 were recorded more internode length (4.11 cm), leaf breadth (3.14 cm), corolla tube length (2.65 cm) and flower diameter (5.13 cm), respectively. The variation in flower diameter might be due to availability of favourable environment to express the dominant gene in the genotypes. Similar findings were reported by Rai and Chaudhari (2016) in China aster. The early flowering was observed in the cross combination of Vi-15-1 × Vi-15-2 (27.23). The mean comparison between hybrids and parents revealed that several cross combinations were superior over better parents for the various traits. These results were in concurrence of Singh and Dwivedi (2017) in periwinkle.

Heterobeltiosis: The heterosis was measured as a percentage increase or decrease in F_1 over the better parent (heterobeltiosis) with respect to characters. Five best cross combinations were isolated with their significant and desirable estimates of heterobeltiosis for different characters studied (Table 4) (Supplementary Table 3).

According to the current heterosis investigation, none of the 30 crosses of periwinkle reported consistency in

Table 3 Top five cross combinations identified on basis of mean performance in different traits in periwinkle

Character	Range	Overall mean of 30 crosses	Cross combination	Mean values
Plant height (cm)	45.37–84.05	67.20	Vi-13-2 × Vi-29	84.05
			Vi-15-1 × Vi-29	82.33
			Vi-15-2 × Vi-16	80.31
			Vi-29 × Vi-15-1	79.31
			Vi-16 × Vi-15-1	77.75
Plant spread (cm)	28.80–66.32	47.03	Vi-13-2 × Vi-29	66.32
			Vi-29 × Vi-15-2	63.80
			Vi-16 × Vi-15-1	62.98
			Vi-15-2 × Vi-13-2	55.77
			Vi-15-2 × Vi-15-1	54.48
Number of primary branches/plant	6.03–12.47	8.82	Vi-16 × Vi-15-1	12.47
			Vi-15-2 × Vi-16	12.37
			Vi-16 × Vi-29	11.70
			Vi-29 × Vi-15-1	10.87
			Vi-13-2 × Vi-29	10.57
Leaf length (cm)	4.26–5.93	5.25	Vi-15-1 × Vi-14-3	5.93
			Vi-29 × Vi-14-3	5.91
			Vi-29 × Vi-15-1	5.84
			Vi-16 × Vi-15-2	5.73
			Vi-15-1 × Vi-29	5.69
Leaf breadth (cm)	2.29–3.14		Vi-13-2 × Vi-16	3.14
		2.54	Vi-15-1 × Vi-14-3	2.94
			Vi-13-2 × Vi-15-2	2.91
			Vi-29 × Vi-14-3	2.86
			Vi-16 × Vi-13-2	2.80
Internode length (cm)	2.14–4.11	2.54	Vi-15-1 × Vi-29	4.11
			Vi-15-1 × Vi-16	3.16
			Vi-15-2 × Vi-16	3.07
			Vi-15-1 × Vi-14-3	2.85
			Vi-13-2 × Vi-16	2.76
Corolla tube length (cm)	1.56–2.65	2.13	Vi-15-1 × Vi-16	2.65
			Vi-15-1 × Vi-13-2	2.40
			Vi-15-2 × Vi-15-1	2.32
			Vi-13-2 × Vi-29	2.31
			Vi-15-2 × Vi-13-2	2.30

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Table 3 (*Concluded*)

Character	Range	Overall mean of 30 crosses	Cross combination	Mean values
Days to flowering	27.23–39.70	32.94	Vi-15-1 × Vi-15-2	27.23
			Vi-15-1 × Vi-14-3	28.80
			Vi-29 × Vi-15-2	29.40
			Vi-29 × Vi-16	30.20
			Vi-29 × Vi-15-1	30.37
Duration of flowering (days)	115.03–140.17	132.28	Vi-16 × Vi-15-1	140.17
			Vi-15-1 × Vi-14-3	137.33
			Vi-15-2 × Vi-13-2	137.00
			Vi-15-2 × Vi-29	136.23
			Vi-15-2 × Vi-14-2	135.93
Number of flowers/plant	344.93–569.60	492.84	Vi-15-1 × Vi-14-3	569.60
			Vi-13-2 × Vi-14-3	556.40
			Vi-29 × Vi-14-3	546.57
			Vi-13-2 × Vi-15-2	546.17
			Vi-29 × Vi-13-2	545.00
Flower diameter (cm)	3.68–5.13	4.56	Vi-29 × Vi-15-1	5.13
			Vi-16 × Vi-29	5.12
			Vi-29 × Vi-16	5.03
			Vi-29 × Vi-14-3	5.02
			Vi-15-1 × Vi-15-2	5.01
Follicle length (cm)	1.47–2.68	2.10	Vi-15-1 × Vi-13-2	2.68
			Vi-16 × Vi-15-1	2.50
			Vi-13-2 × Vi-16	2.39
			Vi-13-2 × Vi-15-2	2.31
			Vi-15-1 × Vi-14-3	2.26
Number of seeds/follicle	11.37–35.30	22.99	Vi-15-1 × Vi-13-2	35.30
			Vi-16 × Vi-15-1	29.97
			Vi-15-1 × Vi-15-2	29.23
			Vi-15-2 × Vi-13-2	28.13
			Vi-29 × Vi-14-3	27.73

the direction and degree of heterosis for all of the traits studied. Some indicated positive heterosis, while others showed negative heterosis. It is might be due to varied extent of genetic diversity among parents of various cross combinations. The magnitude of heterosis was greater for the majority of the growth and flowering traits and the manifestation of negative heterosis for various characters may be due to the combination of the undesirable genes. Similar results were observed by Kulkarni (2015) and Kumari *et al.* (2018) in China aster who reported such heterosis variation for different characters. As per results, significantly maximum positive heterobeltiosis for plant height was recorded in progeny of Vi-13-2 × Vi-29 (43.17%). These results are in conjunction with the findings of Kumari *et al.* (2018) in China aster who reported 11 crossings exhibited positive heterosis over the mid parent and 9 crosses recorded positive heterosis over the better parent for plant height. In periwinkle plant spread is most important character because in many countries periwinkle is being utilized as a bedding plant. The progeny of Vi-13-2 × Vi-29 had highest positive heterobeltiosis for plant spread (86.51%). These findings were also in accordance with Panwar *et al.* (2013) in marigold. Heterosis for more number of branches/plant is desirable because more branches allow greater flower yield. Higher positive magnitudes for number of branches/plant recorded in Vi-16 × Vi-15-1 (38.82%). Similar findings were reported by Hassan *et al.* (2012) in petunia. There were 27 crosses that revealed significant positive heterosis over better parent for leaf length. The cross combination of Vi-29 × Vi-14-3 (32.56%), Vi-15-1 × Vi-14-3 (25.50%) have shown positive heterobeltiosis for leaf breadth. Photosynthesis is increased with increased leaf area and also enhances carbohydrate accumulation. Furthermore, it affects the physiological characteristics of the flower as

Table 4 Five crosses identified as high heterobeltiosis from 30 F_1 plants for various traits in periwinkle

Character	Parentage with high heterobeltiosis	Heterobeltiosis (%)
Plant height (cm)	Vi-13-2 × Vi-29	43.17
	Vi-15-1 × Vi-29	36.71
	Vi-29 × Vi-15-1	31.71
	Vi-29 × Vi-14-3	29.64
	Vi-16 × Vi-15-1	29.12
Plant spread (cm)	Vi-13-2 × Vi-29	86.51
	Vi-29 × Vi-15-2	82.03
	Vi-29 × Vi-14-3	58.06
	Vi-15-2 × Vi-13-2	56.82
	Vi-13-2 × Vi-14-3	49.04
Number of primary branches/plant	Vi-16 × Vi-15-1	38.82
	Vi-15-2 × Vi-16	37.71
	Vi-15-2 × Vi-14-3	30.29
	Vi-29 × Vi-14-3	27.53
	Vi-29 × Vi-15-1	24.18
Leaf length (cm)	Vi-15-1 × Vi-14-3	21.58
	Vi-16 × Vi-15-2	19.80
	Vi-13-2 × Vi-15-2	17.71
	Vi-15-2 × Vi-14-3	16.67
	Vi-16 × Vi-13-2	16.60
Leaf breadth (cm)	Vi-29 × Vi-14-3	32.56
	Vi-15-1 × Vi-14-3	25.50
	Vi-13-2 × Vi-16	18.36
	Vi-16 × Vi-14-3	13.52
	Vi-14-3 × Vi-16	10.38
Internode length (cm)	Vi-15-1 × Vi-29	103.47
	Vi-15-1 × Vi-16	56.27
	Vi-15-2 × Vi-16	52.90
	Vi-15-1 × Vi-14-3	41.25
	Vi-13-2 × Vi-14-3	38.91
Corolla tube length (cm)	Vi-15-1 × Vi-16	19.52
	Vi-15-1 × Vi-13-2	9.09
	Vi-13-2 × Vi-14-3	3.94
	Vi-15-2 × Vi-15-1	3.57
	Vi-15-2 × Vi-13-2	2.83
Days to flowering	Vi-15-1 × Vi-15-2	-26.06
	Vi-13-2 × Vi-16	-22.39
	Vi-15-1 × Vi-14-3	-21.80
	Vi-29 × Vi-15-2	-18.55
	Vi-16 × Vi-14-3	-18.51
Duration of flowering (days)	Vi-15-1 × Vi-14-3	17.63
	Vi-14-3 × Vi-15-1	15.37
	Vi-15-2 × Vi-14-3	15.18
	Vi-14-3 × Vi-15-2	13.29
	Vi-15-2 × Vi-29	12.80
Number of flowers/ plant	Vi-29 × Vi-14-3	60.58
	Vi-16 × Vi-29	46.76
	Vi-16 × Vi-14-3	43.84
	Vi-29 × Vi-16	38.60
	Vi-13-2 × Vi-14-3	37.69

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Table 4 (*Concluded*)

Character	Parentage with high heterobeltiosis	Heterobeltiosis (%)
Flower diameter (cm)	Vi-16 × Vi-29	10.42
	Vi-15-1 × Vi-29	9.20
	Vi-29 × Vi-16	8.47
	Vi-29 × Vi-14-3	8.12
	Vi-15-2 × Vi-13-2	7.99
Follicle length (cm)	Vi-29 × Vi-14-3	18.24
	Vi-14-3 × Vi-29	-6.53
	Vi-13-2 × Vi-15-2	-8.47
	Vi-15-2 × Vi-29	-11.64
	Vi-13-2 × Vi-16	-11.69
Number of seeds/ follicle	V-29 × Vi-14-3	80.91
	Vi-15-1 × Vi-13-2	10.66
	Vi-15-2 × Vi-13-2	-2.72
	Vi-29 × Vi-15-2	-6.00
	Vi-16 × Vi-15-1	-6.06

well as flower yield. These results are in concurrence with the findings of Azimi and Banijamali (2019) in gladiolus.

The progeny of Vi-15-1 × Vi-16 (19.52%) had maximum significant positive results for corolla tube length. These results were in conformity with the findings of Gupta (2001) in marigold. The progeny Vi-15-1 × Vi-15-2 (-26.06%), Vi-13-2 × Vi-16 (-22.39%) and Vi-15-1 × Vi-14-3 (-21.80%) were recorded desirable negative heterobeltiosis. Heterosis for early flowering is predominant in flower crops breeding. These findings were in concurrence with the findings of Lahkar (2020) in marigold who reported cross combination of MSC1 × JS2 (-22.87% to 19.98%) had desirable better parent heterosis for days to 50% flowering. Highly significant positive heterobeltiosis for duration of flowering was reported by Vi-15-1 × Vi-14-3 progeny (17.63%). Duration of flowering is major character for the hybrids in landscape gardening. These results were in conjunction with the findings of Bhargav *et al.* (2018) in China aster who reported relative heterosis varied from -37.15 (L4 × T4) to 63.94 (L5 × T1) among 30 crosses. The progeny of Vi-29 × Vi-14-3 (60.58%) had positive heterobeltiosis for number of flowers/plant followed by Vi-16 × Vi-29 (46.76%). These results were compatible with the findings of Lahkar (2020) in marigold who reported maximum heterobeltiosis 24.36% in the MSC1 × AAUM5 progeny for flower yield/plant. The increased flower diameter is a critical factor for better appearance. Heterobeltiosis for flower diameter varied from -23.65 to 10.42%. The cross combinations with significant positive heterosis over better parent for follicle length was recorded in the Vi-29 × Vi-14-3 (18.24%). The rest of the cross combinations have shown negative heterobeltiosis. These results were accordance with the findings of Singh (2021) in periwinkle who reported cross combinations P18 (53.22%) and P38 (37.87%) had higher positive heterobeltiosis for follicle length among 28 crosses. The more number of seeds per follicle over better parent recorded in progeny of V-29 × Vi-14-3 (80.91%).

The identification of morphological traits with high mean performance may present a successful method of improving characters in *Catharanthus* genotypes. The present investigation concluded that parents Vi-15-1, Vi-29 and Vi-13-2 were reported superior mean performance among 6 inbred lines. While cross combinations Vi-13-2 × Vi-29, Vi-16 × Vi-15-1 and Vi-15-1 × Vi-14-3 were recorded outstanding mean performance. Highly significant heterosis was present in the progeny of Vi-29 × Vi-14-3, Vi-13-2 × Vi-29 and Vi-15-1 × Vi-14-3 for most the traits when compared to the 30 F_1 plants over better parent lines. It is clearly evident that heterosis impacts are a valuable resource in periwinkle breeding for utilization from landscaping point of view.

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