



## GENETIC ANALYSIS AND MORPHOLOGICAL PROFILING OF RANUNCULUS GENOTYPES

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### SUMMARY

Ranunculus is a well-known flower having a great economic value for its bright colors and vibrant patterns. Given the importance of the ranunculus cut flower, conducting this study elucidated the genetic behavior and variability of various morphological attributes in ranunculus genotypes. Assessment of six ranunculus genotypes measured germination percentage (%), days to emergence, days to maturity, stem length, stem thickness, flowers per plant, flower size, and flower duration on the plant. Analysis of variance depicted significant variation in all the studied traits except days to emergence, number of stems, and germination percentage. Clustering ranunculus genotype with the Euclidean distances-based hierarchical clustering resulted in two clusters of the genotype. One genotype (red) did not join any group, indicating an early separation of this genotype during the evolution of *Ranunculus* species. Correlation analysis showed that most studied traits negatively correlated, with only a few positively correlating traits. The number of flowers per plant and stem length showed a d positive correlation (0.7437 and 0.8064, respectively). Overall, the results showed that the red, yellow, and rose genotypes are the best performers for cultivation to produce better-quality flowers. Genetic analysis using line × tester analysis revealed higher values for the SCA component than GCA, suggesting non-additive gene action for most traits under study. Moreover, the hybrids developed in the current study developed new color combinations/shades. These hybrids could further benefit ranunculus stable variant improvement.

**Keywords:** Ranunculus, color variance, general combining ability, specific combining ability, flower quality, gene action

**Key findings:** The red, yellow, and rose genotypes are the best performers. Most studied traits are under non-additive gene action control. Based on better GCA, the genotypes T1 (yellow) and T3 (white) could benefit future breeding programs for quality improvement.

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### INTRODUCTION

*Ranunculus asiaticus* is a perennial plant, belonging to the family *Ranunculaceae*. Its abundant distribution stretches through the Mediterranean basin and Asia. It is an annually

cultivated cut flower crop (Carillo *et al.*, 2021). Ranunculus flowers are well-known for their bright colors and vibrant patterns. These flowers are like an origami creation when packaged in multiple layers of delicate crepe paper with thin petal shapes. Ranunculus

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flowers are nectar-producing pollen flowers that bloom in the spring (Weryszko-Chmielewska *et al.*, 2017). Various insects visit the *Ranunculus* genus flowers, which help to pollinate them (Jürgens and Dötterl, 2004). In recent years, a notable substantial increase in *R. asiaticus* cultivation worldwide occurred (Beruto *et al.*, 2018). In Pakistan, Swat and Kashmir are home to some of the most untamed varieties of the ranunculus, harvested in Potohar during September and October and in Quetta, Rawalkot, Murree, and Swat, Pakistan, during October and November.

The flower stem quality varies greatly depending on the genotypes, the size of the tuberous roots, the procedure used to store and prepare the flower for planting, and the conditions in growing the flower (Benschop *et al.*, 2010; Aziz *et al.*, 2016; Mohsin *et al.*, 2023). The genotypes vary for the length of vegetative growth and the number of flowers, while tuber size considerably influences both these parameters. The plants/genotypes with larger tubers produce a higher flower number, coupled with early flowering and vice versa. Larger tubers (roots) have more reserve starch available for flower stem development; therefore, plants with massive roots generally flower earlier and produce more flowers than plants with smaller roots (Carillo *et al.*, 2019). Four different grades or sizes are available for the claw-like bulbs, which are tubers in their firmest form. *Ranunculus* genotypes vary for the length of the vegetative period and the number of flowers (Tayal, 2021). *R. asiaticus* is available in various sizes, three to six inches wide. Likewise, it has a variety of flower colors, including pale orange, pink, red, yellow, and white. Therefore, the morphological characterization of different genotypes of ranunculus is imperative to find out suitable genotypes for increasing the production of flowers to meet the demand of flower lovers.

In improving plant characteristics, it is critical to understand its genetic behavior. Among various techniques, combining ability analysis helps reveal the type of gene actions involved. It plays an essential role in the selection of parents and crosses. Moreover, it is also helpful to select what breeding methods to use (Salgotra *et al.*, 2009). General and specific combining abilities influence the selection of suitable parents for developing hybrid cultivars, especially in crops. Line × tester analysis is one of the most powerful tools for predicting the general combining

ability (GCA) of parents and selecting suitable parents and crosses with a high specific combining ability (SCA) (Rashid *et al.*, 2007). Line × tester is a mating design that provides information about combining ability effects of genotypes and also knowledge regarding genetic mechanisms controlling the performance of parents and crosses. Owing to the above-stated facts, the presented study engaged the variability comparison among the ranunculus genotypes to find the best parents and suitable crosses for hybrid development with improved *R. asiaticus* flower quality.

## MATERIALS AND METHODS

The experimental material comprises six ranunculus genotypes with different colors (Figure 1). Seeds of these genotypes came from Chanandin and Sons Nursery, Lahore. Growing the genotypes proceeded in the nursery research area of the Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi, at the end of September 2015. The prepared potting medium with high organic matter contained a mixture of 30% peat and 70% perlite. Then, earthen pots (14 inches) filled with the medium had 12–15 seeds sown per pot. At the four to five true leaves stage (after eight weeks), seedling transplanting was one seedling per pot. Planting 30 pots per genotype followed a Complete Randomized Design (CRD) in the experiment. Data recording for morphological and flowering parameters included days to emergence, floral stem length (cm), stem thickness (cm), number of flowers per plant, days to maturity and flowering (days), flower life on the plant (days), and number of petals and anthers.

The germination percentage calculation used the following formula:

$$\text{Germination \%age} = \frac{\text{No. of germinated seeds per treatment}}{\text{Total No of seeds planted per treatment}} \times 100$$

Artificial hybridization proceeded on six parent ranunculus genotypes to produce new hybrids with color variances following line × tester mating design with three lines (L1: orange, L2: pink, L3: rose) and three testers (T1: yellow, T2: red, T3: white). Then, the characterization of developed F<sub>1</sub> hybrids was for quality parameters.



**Figure 1.** Flower color of six ranunculus genotypes.

### Statistical analysis

The recorded data on the selected parameters underwent statistical analysis using the statistical software, Statistix 8.1, calculating the mean  $\pm$  SD for all observed parameters. Employing Pearson's linear correlation, analyzed quality traits of six various color ranunculus genotypes. Data obtained from parents and their respective hybrids underwent line  $\times$  tester analysis (Kempthorne, 1957) for the identification of specific combiners (parents) for various morphological parameters under study (Steel *et al.*, 1997).

### RESULTS

All the studied morphological characters under consideration in the current study showed a wide range of significant variation except days to emergence, number of stems, and germination percentage (Tables 1 and 2). Maximum germination percentage occurred in the yellow-color genotype (96%), whereas minimum germination percentage in the pink-color genotype (87%). The days of emergence were maximum in the orange-color genotype (17.67 days), with the minimum observed in the red-color genotype (8 days). A recorded maximum number of stems was in the pink-color genotype (9.34) and the minimum in the yellow-color genotype (7.0). The highest stem

length (34.70 cm) appeared in the yellow-color genotype, and the shortest in the white-color genotype (22.86 cm). The maximum stem thickness materialized in the rose-color genotype (0.687 cm) and the minimum (0.543 cm) in the white-color genotype.

The maximum number of flowers per plant emerged in the yellow-color genotype (10.34), with the minimum in the white-color and orange-color genotypes (7.0 each). Exhibiting the maximum flower size was the red-color genotype (8.86 cm), whereas the minimum was the white-color genotype (6.46 cm). The minimum number of days to maturity resulted in the white-color genotype (120.67 days), but the maximum was in the red-color genotype (129 days). For days to flowering, the maximum was with the rose-color genotype (113 days) and the minimum was with the red-color genotype (94.67 days). The minimum flower duration of the plant emerged for the orange-color genotype (12.25 days) and pink-color genotype (12.38 days), respectively. Conversely, the maximum duration of the flower intact to the plant showed in the red-color genotype (15.11 days). The maximum number of petals per flower was in the red-color genotype (113.67), non-significantly followed by the rose-color genotype (108.34). However, the lowest number of petals per flower came from the white-color genotype (82.34), non-significantly followed by the orange-color genotype (89.88).

**Table 1.** Analysis of variance (mean square) for various morphological traits in ranunculus genotypes.

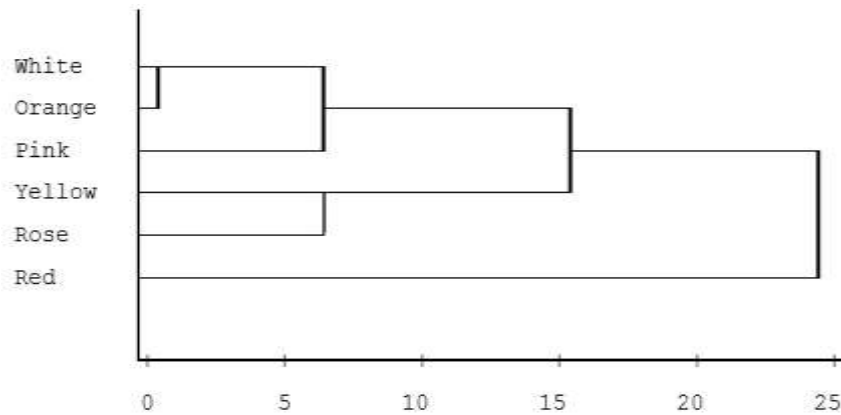
Source	Genotypes	Error
Df	5	12
Days to Flowering	157.522**	17.111
Days of Emergence	41.5222 <sup>ns</sup>	1.3333
Days of Maturity	655.156**	582.5
Flower Life on Plant	11.8333**	3.1111
No of Flowers Per Plant	4.88889**	0.55556
Flower Size (cm)	3.12222*	0.77778
Germination Percentage	42.0556 <sup>ns</sup>	31.0556
No of Anthers	286.989**	30
No of Petals	1144.46**	47
No of Stems	3.52222 <sup>ns</sup>	1.61111
Stem Length (cm)	11.6889**	1.0556
Stem Thickness (cm)	0.276**	0.04333

ns: Non-Significant; \* Significant  $P < 0.05$ ; \*\* Highly significant  $P < 0.01$ .

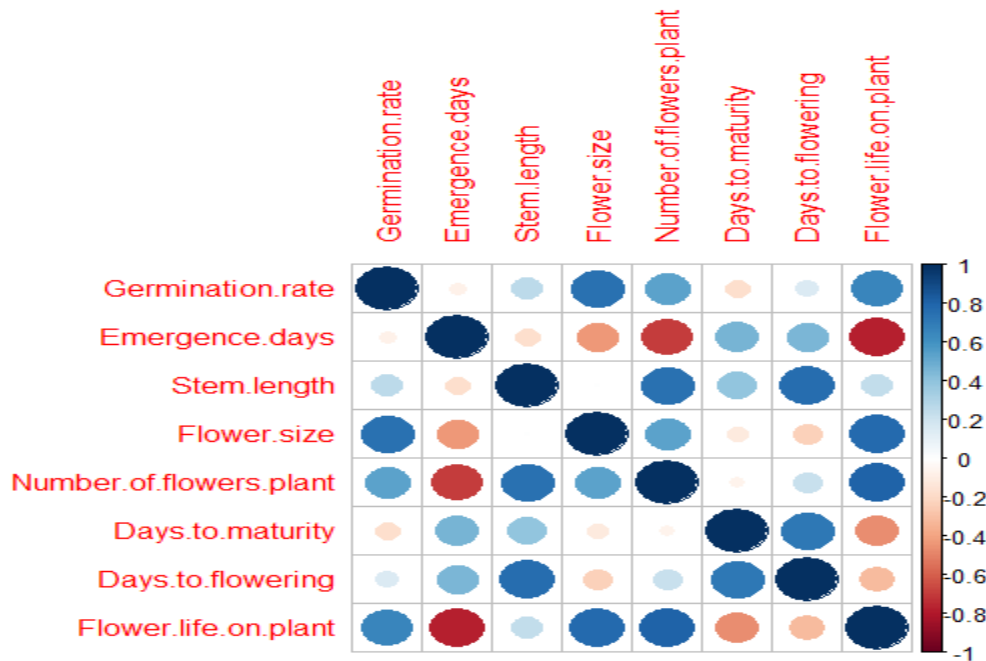
**Table 2.** Mean performance of ranunculus genotypes for various morphological traits.

Genotype	Yellow	Red	White	Orange	Pink	Rose
Days to Flowering	111 ± 4.58	94.66 ± 4.16	99.66 ± 3.05	110 ± 4.58	108 ± 3	113 ± 5
Days of Emergence	8.33 ± 0.57	8 ± 1	13 ± 1	17.66 ± 1.52	12 ± 1	14.66 ± 1.52
Days of Maturity	124.33 ± 4.16	85.66 ± 5.70	124.33 ± 3.51	118.33 ± 2.08	116.33 ± 2.51	121.67 ± 2.88
Flower Life on Plant	12.66 ± 2.0	13 ± 2.64	9.66 ± 2.08	8.66 ± 0.57	8.33 ± 0.57	10.66 ± 1.52
No of Flowers Per Plant	10.33 ± 0.57	8.66 ± 0.57	7 ± 1	7 ± 1	7.66 ± 0.57	8.66 ± 0.57
Flower Size (cm)	6.33 ± 0.57	5.66 ± 0.57	5.33 ± 1.52	5 ± 1	3.33 ± 0.57	4.66 ± 0.57
Germination Percentage	96 ± 5.29	95 ± 5	90 ± 5	95 ± 5	86.66 ± 7.63	95 ± 5
No of Anthers	71.66 ± 8.32	68.33 ± 3.05	77 ± 5.56	65 ± 5	57.66 ± 3.78	50 ± 5.56
No of Petals	115 ± 6.24	64 ± 5.56	101.67 ± 9.29	116 ± 3	110.33 ± 8.62	95.33 ± 6.50
No of Stems	7 ± 1	7 ± 1	8.33 ± 0.57	9 ± 2	9.33 ± 1.52	7 ± 1
No of Sepals	4.33 ± 0.57	4.66 ± 0.57	4.66 ± 0.57	5.33 ± 0.57	5.66 ± 0.57	4.66 ± 0.57
Stem Length (cm)	34.70 ± 3.86	24.54 ± 2.92	22.86 ± 2.54	25.4 ± 2.54	29.62 ± 1.45	33.56 ± 1.45
Stem Thickness (cm)	2.46 ± 0.15	2.96 ± 0.15	2.4 ± 0.26	2.16 ± 0.25	2.8 ± 0.2	2.8 ± 0.2

± Standard Deviation



**Figure 2.** Complete linkage Euclidean distances dendrogram for similarities among six parent ranunculus genotypes.



**Figure 3.** Linear correlation of various quality traits of ranunculus parent genotypes.

The maximum number of anthers per plant occurred in the red-color genotype (68.34), with the minimum in the white-color genotype (47.34), followed by the orange-color genotype (51.34).

The linear correlation analysis revealed that days to emergence were positively correlated to days to flowering (0.459), days to maturity (0.471), number of petals per flower (0.495), and number of stems per plant (0.550). The linear correlation analysis also showed that the number of flowers per plant is strongly positively correlated to stem length

(0.743) and flower life on the plant (0.806) while noting a weak positive correlation with flower size (0.534), germination rate % (0.537%), and stem thickness (0.327). The linear correlation also showed that the flower size of the ranunculus plant has a strong positive correlation to flower life on the plant (0.773) and germination rate % (0.745%) (Figure 3). Furthermore, UPGMA (Unweighted Pair Group Method with Arithmetic) cluster analysis divided the five genotypes into two clusters, while one genotype (red) did not cluster in any group (Figure 2).

Analysis of variance obtained from line  $\times$  tester analysis revealed that the effect of almost all factors was significant for all the traits (Table 3). The GCA effects of three lines (orange, pink, rose) and three testers (yellow, red, white) on the morphological traits of ranunculus genotypes revealed that the germination percentage of L2 showed a maximum positive value (2.185), whereas L1 and L3 showed negative values being minimum on L1 (-0.926) and maximum on L3 (-1.259), respectively. The only confirmed positive values in testers were in T2 (2.296), with T1 showing a minimum negative value (-0.815) and T3 the maximum negative value (-1.481). For several flowers, L1 gave the only positive value (2.704), whereas L1 provided the maximum negative value (-2.407), followed by the minimum (-0.296) in L2, respectively. For flower size, the noted maximum positive GCA values were in L1 (0.396) and T2 (0.525), with the maximum negative GCA values in L2 (-0.326) and T1 (-0.204), followed by the minimum negative GCA values in L3 (-0.070) and T3 (-0.048) (Table 4).

The SCA effects of  $F_1$  hybrids on the morphological traits of *the* ranunculus plant revealed that for germination percentage, the noted maximum positive SCA value was in  $L1 \times T1$  (4.481) and the minimum in  $L2 \times T1$  (0.704). However, the maximum negative SCA value showed in  $L3 \times T1$  (-5.185) while the minimum was in  $L1 \times T3$  (-1.519). For the number of stems, the observed maximum positive SCA value was in  $L2 \times T1$  and  $L1 \times T3$  (each 0.481), and the minimum in  $L3 \times T1$  and  $L1 \times T2$  having the same SCA value of 0.148. On the other hand, the maximum negative SCA value resulted in  $L2 \times T3$  (-0.741) and the minimum in  $L3 \times T2$  (-0.407). For days to flowering, the maximum positive SCA value occurred in  $L1 \times T2$  (2.407) and the minimum in  $L2 \times T3$  (0.519), while the maximum negative SCA value was in  $L1 \times T1$  (-3.037) and minimum in  $L3 \times T2$  (-0.704). For the number of flowers per plant, the maximum SCA positive value appeared in  $L3 \times T3$  (0.370) and the minimum in  $L3 \times T1$  (0.148). However, the noted maximum SCA negative value was in  $L3 \times T2$  (-0.519), with the minimum in  $L1 \times T1$  and  $L2 \times T1$  having the same negative values (each at -0.074). The maximum positive SCA value for flower size emerged in  $L2 \times T2$  (0.359), while the maximum negative SCA value was in  $L2 \times T1$  (-0.385) (Table 5).

## DISCUSSION

Ranunculus is a beautiful flower, famous in the cut flower industry, having various colors and sizes. Breeding for new shades always attracts breeders to the market. However, before breeding for color variation/shades or other parameters, it is critical to understand the genetic behavior of that particular specie (Hussain *et al.*, 2009). Thus the presented study progressed to understand the genetic behavior of various morphological parameters of the Ranunculus.

The results of the recent study indicated significant variation among genotypes for various studied parameters. These findings agree with previous studies where substantial variation occurred among ranunculus cultivars for most morphological parameters (Tayal, 2021). Understanding the correlation among parameters is a valuable tool for plant breeders, allowing them to make more informed decisions about which plants to select for their breeding programs. (Kishan, 2010; Hossain *et al.*, 2011; Malik and Pal, 2014). Correlation analysis of this work revealed a positive correlation between days to flowering, days to maturity and the number of petals per flower, and the number of stems per plant. These results align with previous findings (Lal and Pant, 1989; Mishra and Jha, 2000). Such a positive correlation can have important implications for plant breeding and horticulture. For example, if a breeder wants to develop a plant variety with more flowers per plant, they could select plants with longer stems and breed them together to create offspring with both traits. Similarly, horticulturists could manipulate stem length through cultural practices to increase flower production in ornamental plants (Malik and Pal, 2014).

The morphological attributes grouped the cultivars into two major clusters in the UPGMA tree, while the red genotype did not cluster in any group. It indicated significant diversity among genotypes regarding morphological parameters studied. Moreover, the red genotype showed distinct morphological features compared with other genotypes. Significant variations in morphological characters and notable diversity among cultivars found in the current study suggested that these genotypes may serve as breeding materials in genetic studies of the

**Table 3.** Analysis of variance (mean square) for various morphological traits in ranunculus genotypes obtained from the line x tester analysis.

Source of variation	Replications	Treatments	Parents	P vs C	Crosses	Lines	Testers	L x T	Error
Germination Rate	31.267	322.658***	42.055*	3906.404***	50.064*	32.481	36.592	65.595*	19.48
Days to Emergence	2.755	18.231***	41.522***	0.301	5.916**	4.333	12.111	3.611*	1.35
No of Stems	1.401	2.323*	3.522**	0.181	1.842*	4.592	0.148	1.314	0.852
Stem Length	0.422	7.269***	11.689***	7.1E-15	5.416***	13.778	2.111	2.889*	0.755
Stem Diameter	0.021	0.136**	0.276***	0.0094	0.0653	0.1337	0.0281	0.0498*	0.0368
Days to Maturity	342.755	381.879	655.155*	6.533	258.01	134.334	784.111*	56.777	232.684
Days to Flowering	26.601	114.104***	157.522***	0.0037	101.231***	59.37	307.703*	18.925	9.576
No of Flowers/Plant	0.288	3.736***	4.889***	2.903*	3.120**	5.148*	6.370*	0.481	0.526
Flower Size	0.286	1.565**	3.122***	1.511*	0.599	1.207	0.482	0.353	0.33
Flower Duration	0.716	9.235***	11.833**	0.133	8.750**	0.694	29.194*	2.555	1.609
No of Petals	18.15	704.27***	1144.45***	1.63	517.00***	120.44	1637.33*	155.11**	24.2
No of Anthers	60.2	176.38***	286.98***	113.42*	115.12***	125.81*	317.81**	8.42	15.93

\*Significant at  $p < 0.05$ ; \*\* Highly Significant at  $p < 0.01$ ; \*\*\* Highly Significant at  $p < 0.001$ .

**Table 4.** Estimates of GCA effects for morphological traits in ranunculus genotypes.

Lines/Testers	L1 (orange)	L2 (pink)	L3 (rose)	S.E (L)	T1 (yellow)	T2 (red)	T3 (white)	S.E (T)
Germination rate	-0.926	2.185	-1.259	1.471	-0.815	2.296	-1.481	1.471
Days to Emergence	0.778	-0.222	-0.556	0.387	-0.778	-0.556	1.333	0.387
No of Stems	0.296	0.519	-0.815	0.3077	-0.148	0.074	-0.815	0.3077
Stem Length	-0.222	-1.111	1.333	0.289	0.333	0.222	-0.556	0.289
Stem Diameter	0.141	-0.07	-0.07	0.064	-0.059	0.052	0.007	0.064
Days to Maturity	2.556	-4.444	1.889	8.806	5.333	-10.778	5.444	8.806
Days to Flowering	-2.407	-0.296	2.704	1.031	3.704	-6.741	3.037	1.031
No of Flowers per Plant	-0.593	-0.259	0.852	0.2419	0.963	-0.37	-0.593	0.2419
Flower Diameter	0.396	-0.326	-0.07	0.191	-0.204	0.252	-0.048	0.191
Flower Life	0	-0.278	0.278	0.4228	0.333	1.611	-1.944	0.4228
No of Petals per Plant	2.222	2	-4.222	1.6398	7.111	-15.556	8.444	1.6398
No of Anthers per Plant	3.519	0.407	-3.926	1.3307	6.741	-4.481	-2.259	1.3307

**Table 5.** Estimates of SCA effects for morphological traits in ranunculus genotypes.

F <sub>1</sub> hybrids	L1 x T1	L2 x T1	L3 x T1	L1 x T2	L2 x T2	L3 x T2	L1 x T3	L2 x T3	L3 x T3	S.E (SCA)
Germination rate	4.481	0.704	-5.185	-2.963	1.926	1.037	-1.519	-2.63	4.148	2.548
Days to Emergence	1.222	-1.111	-0.111	-1	1	0	-0.222	0.111	0.111	0.671
No of Stems	-0.63	0.481	0.148	0.148	0.259	-0.407	0.481	-0.741	0.259	0.533
Stem Length	0.667	-1.111	0.444	0.111	0	-0.111	-0.778	1.111	-0.333	0.501
Stem Diameter	0.048	-0.107	0.059	0.07	-0.052	-0.019	-0.119	0.159	-0.041	0.11
Days to Maturity	-2.778	1.556	1.222	5.667	-2	-3.667	-2.889	0.444	2.444	8.806
Days to Flowering	-3.037	1.185	1.852	2.407	-1.704	-0.704	0.63	0.519	-1.148	1.786
No of Flowers per Plant	-0.074	-0.074	0.148	0.259	0.259	-0.519	-0.185	-0.185	0.37	0.419
Flower Diameter	0.326	-0.385	0.059	-0.263	0.359	-0.096	-0.063	0.026	0.037	0.311
Flower Life	0.778	-0.111	-0.667	0.333	-0.056	-0.278	-1.111	0.167	0.944	0.732
No of Petals per Plant	5	1.222	-6.222	-5	-4.111	9.111	0	2.889	-2.889	2.841
No of Anthers per Plant	0.037	-0.185	0.148	-1.741	1.704	0.037	1.704	-1.519	-0.185	2.304

ranunculus germplasm. Given their genetic makeup, the variable number of flowers relates to their recurrent blooming habit (Debener and Mattiesch, 1999). Increasing plant height, leaves, and leaf area may refer to increasing productivity. The greater the leaf area, the greater the photosynthetic rate, resulting in an excessive dry matter accumulation (producing more flowers per plant). Sindhu and Kumar (2004) and Mantur *et al.* (2004) have previously investigated variations in flower production. The data on differences in the number of branches also agree with the findings of Silberbush and Lieth (2004), as well as, Manjula (2005). The cultivars with the highest plant height tend to produce longer flower stalks (Shafiq *et al.*, 2011). Genetic factors contribute to the level of variation between cultivars. Different cultivars may have different genetic backgrounds, which can lead to differences in traits. Flowering characteristics attain physiological control by environmental factors and light intensity (Kim and Lee, 2008). These findings are also consistent with Khan *et al.* (2011), who discovered that the number of flowers, the size of flowers, the number of petals per flower, and plant height increase when using treated water instead of fresh water. With their longer vase life and fresh appearance, cut flowers with tight buds open slowly and uniformly, creating an appealing effect. These variations could be due to varietal characteristics or the amount of time it takes from bud initiation to the full bloom stage, among other things (Bhattacharjee *et al.*, 1993).

Line × Tester analysis is a plant breeding method used to estimate the combining ability of parental lines in hybrid breeding programs. It involves crossing a set of inbred lines (referred to as "lines") with a set of tester lines (referred to as "testers") that are genetically unrelated to the inbred lines. The resulting hybrids then undergo evaluation for their performance in traits of interest, such as yield, disease resistance, or quality characteristics (Yadav *et al.*, 2013). Improvement in traits depends upon the nature and degree of gene action (Evans *et al.*, 2002). GCA and SCA values and GCA/SCA ratio indicate the degree of gene action and are helpful in the development of suitable breeding strategies (Fasahat *et al.*, 2016). In this study, the three lines achieved crossing with three testers. Germplasm diversity is beneficial in accurately assessing the inheritance of all

morphological traits (Pragya *et al.*, 2010). A significant amount of variation occurred for all studied traits. SCA components have higher values than GCA components in the current study. It is because of the larger genetic distances among the testers (Guimaraes *et al.*, 2012). The higher SCA values indicate the presence of non-additive gene actions controlling the studied traits (Kaushik *et al.*, 2018). Among the studied genotypes, one genotype showed significant improvement for the studied traits. GCA effects provide information about the genotypes which are suitable for selection and can serve to guide breeding program design and implementation. The SCA values indicated the importance of studied characters, as some traits have more positive values versus the negative required for their proper function. On planting height, minimum fluctuations emerged; the biggest resulted in fruit weight. Generally, research findings indicate that most studied traits gained control from non-additive gene action.

## CONCLUSIONS

Significant diversity occurred among ranunculus genotypes for most traits except days to emergence, number of stems, and germination percentage. Non-additive gene action showed for most features. It suggests a delayed selection while breeding for concerned parameters. The genotypes T1 (yellow) and T3 (white) gave better GCA for most of the parameters and thus could serve in future breeding programs.

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