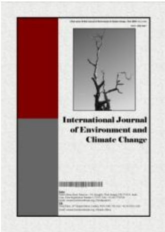

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Studies of Phenotypic and Genotypic Variation for Morphological Traits in Saffron (*Crocus sativus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MI, SN and UR did the conceptualization, data curation, methodology, statistical analysis, wrote and investigated the study. Authors MAW, MHK and RAB did the conceptualization, data curation and validation. Authors SAN, ZR, SB and SM performed the methodology, formal analysis and validation. Authors MI and UR did the compilation, investigation as well as reviewed and edited. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The "Saffron (*Crocus sativus* L.)" germplasm was evaluated, based on phenotypic and genotypic variability studies to identify elite divergent traits for economic yield enhancement.
Study Design: 140 germplasm lines were collected from different saffron growing areas of Kashmir and abroad viz., Afghanistan, Iran. The pedigree details of all samples were recorded. Saffron corms weighing 5g to 16g were planted in Augmented Block Design with a row length, width of 3m, 2m and inter and intra-row spacing of 20 and 10 cms, respectively.
Place and Duration of Study: The research was conducted at Advanced Research Station for Saffron and Seed Spices (ARSSSS), Pampore, SKUAST-Kashmir during cropping season 2017-18.

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Methodology: Observations were recorded for 15 morphological traits viz., number of flowers corm^{-1} line^{-1}, number of days to 50% flowering, fresh flower weight corm^{-1} line^{-1}, inner tepal length, outer tepal length, inner tepal width, outer tepal width, anther length, anther width, style length, stigma length, fresh pistil weight, dry pistil weight, leaf length, number of leaves corm^{-1}line^{-1}.
Results: Significant variation was observed for all traits like., number of flowers corm^{-1} line^{-1} (4.00-35.33), fresh pistil weight (104.84- 1207.38 mg), stigma length (2.55-4.18 cm), leaf length (16.12-37.10 cm), number of leaves corm^{-1} line^{-1} (6.00-11.33), fresh flower weight corm^{-1} line^{-1} (1.80-14.31 mg) indicating presence of high level of variability in germplasm.
Conclusion: The estimates of phenotypic variation were higher than equivalent estimates of genotypic variation except few traits indicating major impact of environment towards expression of a trait. Our findings contributes better understanding of genetic variation in saffron for both vegetative and yield attributes and indicates high level of variability in germplasm, therefore imply considerable scope for saffron improvement through clonal selection.

Keywords: Crocus sativus L.; divergence; phenotypic variability; genotypic variability; clonal selection.

1. INTRODUCTION

Saffron (*crocus sativus* L.) a perennial herb belongs to family *Iridaceae* is one of the most expensive spices in the world and is popularly known as the “golden condiment”. Dried stigmas of saffron flowers compose the most expensive spice which has been valuable since time immemorial for its odoriferous, coloring and medicinal properties [1]. The Kashmir saffron is famous across the world for its high crocin content and rich aroma; it commands a higher price than saffron from Spain or Iran. In India it is a legendary crop cultivated on well drained karewa soils of Kashmir and Kishtwar, where agro-climatic conditions are conducive for its quantitative and qualitative growth and development. It grows at an elevation of 1500-2000 m amsl. Photoperiod and temperature exerts a profound influence on the flowering of saffron. Saffron production in the globe is estimated to be around 300 tons per year [2]. The primary saffron producing countries are Iran, India, Spain, and Greece, with Iran covering the most territory and generating roughly 88 percent of global saffron production. Despite having the world's second-largest land area, India produces only about 7% of total global output [3]. The reason may be the use of traditional practices for saffron cultivation which ignore the importance of optimum resource management and also lack of quality planting material has led to poor performance in terms of the yield, quality and low Benefit Cost ratio. Saffron is solely grown in Jammu & Kashmir, India's only saffron-producing state. Spain is the world's third greatest producer; with 600 ha of land and an average yield of 8.33 kg/ha, the highest in the world. With intensive production processes, top saffron-growing countries like Iran, Spain, and Greece are able to reach higher production and productivity than we are, posing a serious threat to our saffron business as imports rise year after year as shown in (Fig. 1).

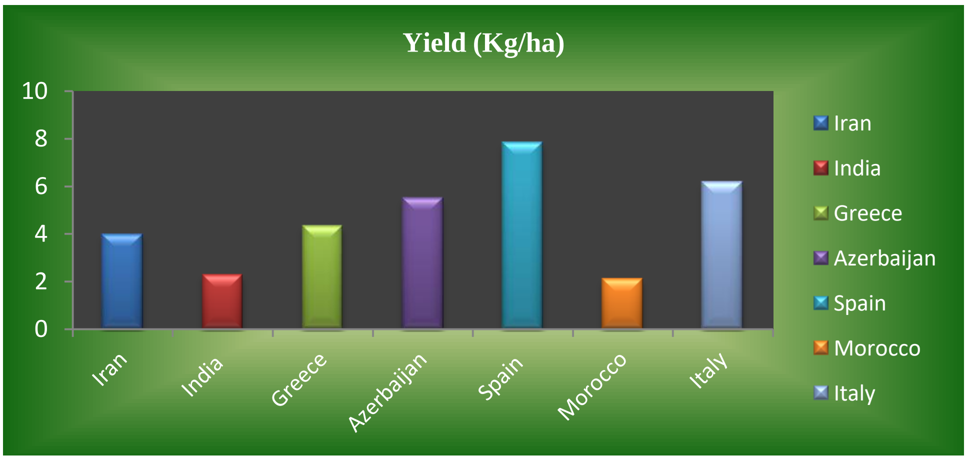


Fig. 1. This fig indicates that Spain is the largest producer of saffron (8.33 kg/ha) followed by Italy (6.16 kg/ha), Azerbaijan (5.43 kg/ha), Greece (4.3 kg/ha) and Iran (3.98 kg/ha) [4]

To make it globally competitive and remunerative to growers, there is a need to expand production by bringing more land under cultivation and double average productivity by adopting an intense production method, efficient processing, and marketing. Saffron farming covers 3715 hectares in J&K, with annual yield and productivity of 13.3 MT and 3.0–4.0 kg/ha, respectively [5]. Saffron is largely grown in four districts in J&K (Pulwama, Budgam, Srinagar, and Kishtwar), with an 86 percent saffron agricultural system at the Pampore heritage site covering 3200 hectares. Pampore, as a peri-urban location, is vulnerable to commercialization and colonization; consequently, expanding saffron cultivation to new prospective and non-traditional areas of J&K will ensure the saffron farming system's long-term viability. This would help to boost J&K's overall saffron production in order to meet the national demand of 100 M.T., as well as provide livelihood security to the state's moderately poor farmers. Prospect of saffron genetic improvement is through clonal selection from the available germplasm. Identification of elite genotypes with distinct superiority in quality and yield attributes can be used as a source for further improvement. Development of varieties from the identified germplasm exhibiting high yielding potential and quality will enhance the production and productivity of saffron to reduce the burden of costs on the import of priced commodity and improving the socio-economic status of the stakeholders associated with the production, processing and value chain of this important commercial crop.

2. MATERIALS AND METHODS

The present investigation was carried out at Advanced Research Station for Saffron and Seed Spices (ARSSSS), Dusso Konibal, Pampore, during cropping season 2017-18., a constituent Research Station of Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir. The experimental site is located at 34^{o}N latitude, 74^{o}E longitude at an altitude of 1650m amsl. The material for study comprised of 140 saffron germplasm lines collected from different saffron growing areas of Kashmir and abroad viz., Afghanistan, Iran. The pedigree details of all the 140 corm samples were recorded. Saffron corms weighing 5g to 16g were planted in Augmented Block Design with a row length of 3m, width 2m and inter and intra-row spacing of 20 and 10 cms, respectively for daughter corm production under annual planting cycle as shown in (Fig. 5). Recommended package of practice from SKUAST- Kashmir was followed for nutrition and intercultural operations in the crop planted. Observations were recorded on 10 randomly selected plants and tagged competitive plants as shown in (Figs. 2 & 6) from each line for all the traits during the period of experimentation.

2.1 Morphological Traits

Number of flowers corm^{-1}, number of days to 50% flowering, fresh flower weight corm^{-1} (mg), inner tepal length (cm), outer tepal length (cm), inner tepal width (cm), outer tepal width (cm), anther length (cm), anther width (mm), style length (cm), stigma length (cm), fresh pistil weight (mg), dry pistil weight (mg), leaf length (cm), number of leaves corm^{-1} line^{-1}.

Mean values for all the characters were estimated for analysis of variance [6,7] and character association at genotypic and phenotypic level [8]. The coefficient of variation for different characters was estimated by formula as suggested by Burton [9].

3. RESULTS AND DISCUSSION

Genetic variability assists a great deal in detecting the range of genetic diversity for various traits in a population [10]. Components of phenotypic and genotypic variability indicated that a wide range of variability existed for all the traits studied shown in (Table 2, Chart-1, Figs. 3 & 4, Pie Chart 1). Outer tepal length ranged from (3.13-4.55cm) with a mean value of (4.25cm), inner tepal length (3.46-3.96cm), with a mean value of (3.69cm), tepal width (3.45-3.95cm) with a mean value of (3.70cm), inner tepal width (1.55-2.03cm) with a mean value of (1.84cm), stigma length (2.55-4.18cm) with a mean value of (3.60cm), style length (2.34-7.35cm) with a mean value of (4.31cm), leaf length (16.12-37.10cm) with a mean value of (27.73cm), number of leaves corm^{-1} (6.00-11.33) with a mean value of (8.93), number of flowers corm^{-1} (4.00-35.33) with a mean value of (15.33), fresh flower weight corm^{-1} (1.80-14.31g) with a mean value of (7.23g), fresh pistil weight (104.84-1207.38mg) with a mean value of (483.91mg), dry pistil weight (25.95-976.25mg) with a mean value of (114.71mg), anther length (1.34-2.37cm) with a mean value of (1.90cm), anther width (1.26-3.74mm) with a mean value of (2.40mm) and 50% flowering (68.66-76.66) with a mean value of (73.10). Results are in conformity with the reports of Sheikh et al. [11-16] as they observed same results and reported wide range of variability exists in the natural population of saffron as indicated by the magnitude of *per se* performance, phenotypic and genotypic coefficient of variation, implying considerable scope for saffron improvement through clonal selection. The study suggests that there is an ample scope for saffron genetic improvement through selection of superior genotypes from the heterogeneous saffron populations. For floral attributes viz, outer tepal length, inner tepal length, outer tepal width, inner tepal width, anther length, anther width indicates less range of variability. The estimates of phenotypic variances were higher than the corresponding estimates of genotypic variances thereby revealing a strong influence of environment in the expression of the traits studied. Per se performance of genotypes under evaluation revealed a gross fresh pistil weight of 40.24mg per corm from 1.21 flowers corm^{-1} resulting in 3 kg laccha per hectare with planting density of 5 lakh corms per hectare showing an increase of 25% over state average. Estimates of phenotypic and genotypic correlation coefficient among the characters have been found useful in planning and evaluating breeding programme [17,8] Genotypic correlation coefficient provide a measure of genetic association among the characters and gives an indication of characters that could be useful as to identify more important ones during a selection programme. At phenotypic and genotypic level, dry pistil weight (economic product) exhibited a significant positive correlation with all the traits. The results clearly reveal a scope for simultaneous improvements of this important trait through selection. Interrelationship among various floral, vegetative and yield contributing traits was observed to be significant both at genotypic and phenotypic levels. Yield is influenced by environmental conditions and is a complex mode for inheritance and low heritability. However, most of the yield components which naturally leads to use of some other trait that is highly correlated with yield and has a much higher heritability through selection of the best progenies for more reliability. In this study, to assess the phenotypic and genotypic relationships between studied traits, correlation coefficients based on phenotypic and genotypic values were calculated (Table 1, Chart 2). It was notable in this study that in most of the cases the genotypic correlation coefficients were larger than the phenotypic correlation coefficients. This finding is in agreement with the results of previous studies [18,10,19] Saffron under temperate conditions of Kashmir Valley reported wide spectrum of variability for floral & vegetative attributes and the results implied a great scope for saffron improvement. Flower size, number of flowers per corm and saffron recovery per flower were observed to help as selection index for increasing saffron yield. Valley exhibited highest range of variability in saffron germplasm. From natural population flowers completely devoid of style and anthers, some flowers with 4 and 5 stigmas were observed. The presence of higher number of stigmas per flower results from physiological or developmental irregularities leading to 4-5 stigmas. These results are in conformity with Nehvi et al. [20-22]. The phenotypic and genotypic variations between different saffron ecotypes for yield and yield attributing traits like Fresh pistil Weight, Dry pistil Weight and Stigma Length were measured and the results showed that phenotypic variances were much higher than genotypic variances for all traits except stigma length, fresh flower weight corm^{-1} line^{-1}, number of flowers corm^{-1} line^{-1}, fresh pistil weight, dry pistil weight in which the genotypic variance were higher than phenotypic variance. In general, with the purpose of the study and assessments to genotypic coefficient of variation and other genetic parameters, we conclude that traits like, number of flowers corm^{-1} line^{-1}, fresh flower weight corm^{-1} line^{-1}, fresh pistil weight were the most effective traits and had the highest positive impacts on the saffron yield. Therefore, with the phenotypic selection of saffron ecotypes in respect of these traits, the saffron yield can be increased and these findings are in agreement with the results of previous studies of Bayat et al. [19,23]. They reported that with regard to significant genotypic coefficient of variation and high positive direct effects, the selection might be based on these two characters and must be focused on most effective traits for the direct improvement of yield.

Table 1. Estimate of Phenotypic variance (δ^2p), genotypic variance (δ^2g), phenotypic coefficient of variation (PCV), Genotypic coefficient of variation (GCV) for morphological traits of Saffron (*Crocus sativus* L.) year 2017-2018

Characters	Phenotypic Variance (δ^2 P)	Genotypic variance (δ^2g)	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)
Outer tepal length (cm) (OTL)	0.015	0.058	5.65	4.63
Inner tepal length (cm) (ITL)	0.013	0.015	3.11	3.01
Outer tepal width (cm) (OTW)	0.013	0.014	3.16	3.09
Inner tepal width (cm) (ITW)	0.012	0.013	6.08	5.19
Stigma length (cm) (SL)	0.18	0.25	12.24	11.94
Style length (cm) (STL)	0.51	0.51	16.65	16.52
Leaf length (cm) (LL)	11.59	12.69	12.94	12.76
Number of leaves corm^{-1} line^{-1}(L/C/L)	0.95	2.971	19.72	14.28
Number of flowers corm^{-1} line^{-1} (F/C/L)	31.83	33.663	35.88	39.72
Fresh Flower weight corm^{-1} line^{-1} (FW/C/L)	9.055	9.353	40.87	43.61
Fresh pistil weight (g) (FPW)	47967.17	48544.88	42.67	45.83
Dry pistil weight (g) (DPW)	5556.44	10101.17	64.97	87.61
Anther length (cm) (AL)	0.092	0.092	15.98	15.79
Anther width (mm) (AW)	0.33	0.360	27.07	25.06
50% flowering (50% F)	2.29	4.680	2.98	2.91

Fig. 2. Saffron flower clearly depicting leaves, tepals, anther and stigma

Table 2. Magnitude of variability for different morphological traits in saffron (*Crocus sativus* L.)

Characters	Range	Mean	C. V.	S.E	C.D. @5%
Outer tepal length (cm) (OTL)	3.13-4.55	4.25	4.85	0.11	0.33
Inner tepal length (cm) (ITL)	3.46-3.96	3.69	0.55	0.01	0.03
Outer tepal width (cm) (OTW)	3.45-3.95	3.70	0.48	0.02	0.02
Inner tepal width (cm) (ITW)	1.55-2.03	1.84	1.14	0.01	0.03
Stigma length (cm) (SL)	2.55-4.18	3.60	4.08	0.08	0.23
Style length (cm) (STL)	2.34-7.35	4.31	0.70	0.02	0.04
Leaf length (cm) (LL)	16.12-37.10	27.73	3.77	0.60	1.68
Number of leaves corm^{-1}line^{-1} (L/C/L)	6.00-11.33	8.93	16.02	0.82	2.30
Number of flowers corm^{-1} line^{-1} (F/C/L)	4.00-35.33	15.33	8.81	0.78	2.17
Fresh Flower weight corm^{-1}line^{-1} (FW/C/L)	1.80-14.31	7.23	7.55	0.31	0.87
Fresh pistil weight (g) (FPW)	104.84-1207.38	83.91	4.96	13.87	38.63
Dry pistil weight (g) (DPW)	25.95-976.25	114.71	58.76	38.92	108.35
Anther length (cm) (AL)	1.34-2.37	1.90	1.01	0.01	0.03
Anther Width (cm) (AW)	1.26-3.74	2.40	6.97	0.09	0.26
50% flowering (50% F)	68.66-76.66	73.10	1.87	0.78	2.19

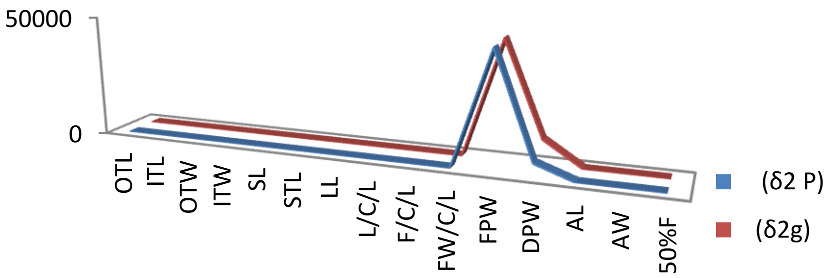


Chart 1. Estimates of δ^2 p & δ^2 g for morphological traits

Based on the results of the investigation, wide range of variability exists in the natural population of saffron as indicated by the magnitude of performance, phenotypic and genotypic coefficient of variation, implying considerable scope for saffron improvement through clonal selection. Number of flowers corm^{-1} and stigma length are the important traits for which due emphasis should be given while selecting for both quality and yield. The motive of this study was to find out the variability present in available germplasm and identification of elite genetic resources showing distinct superiority in certain traits can act as a source for further improvement and development of high yielding lines which will boost both the production and productivity of saffron, which may be beneficial to improve the socio-economic well being of the people associated with this important commercial crop.

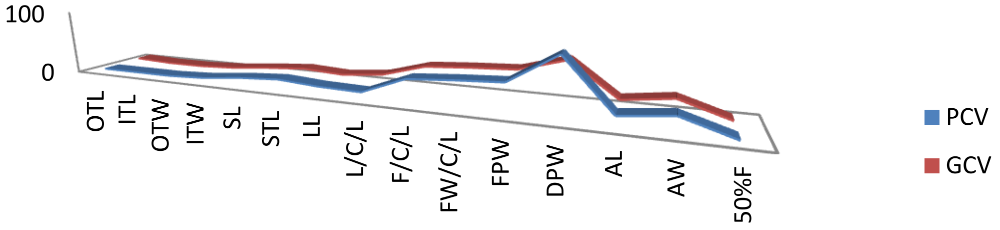


Chart 2. Estimates of PCV & GCV for morphological traits

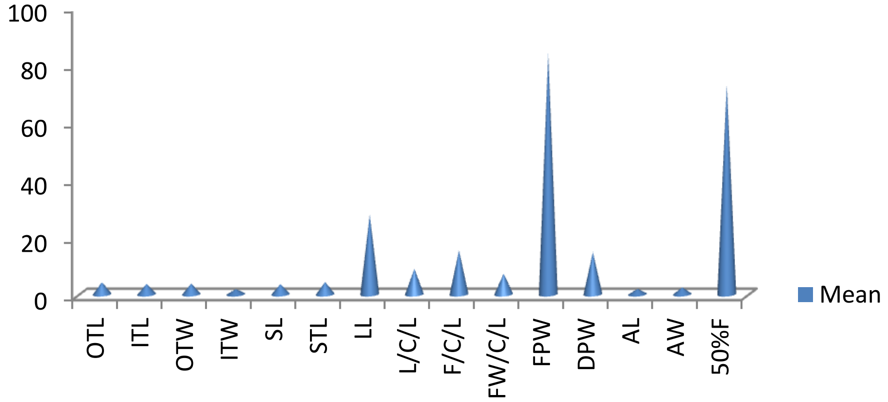


Fig. 3. Mean for magnitude of variability for different morphological traits

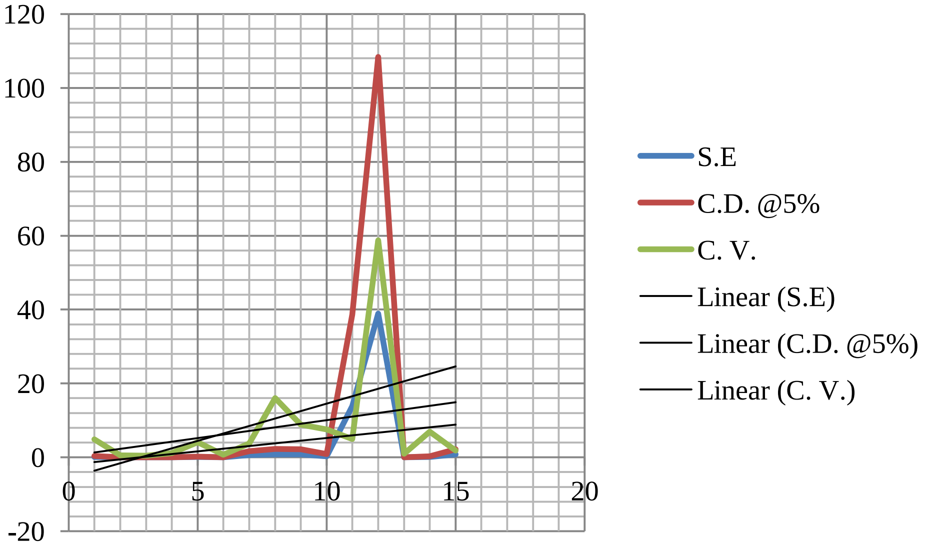
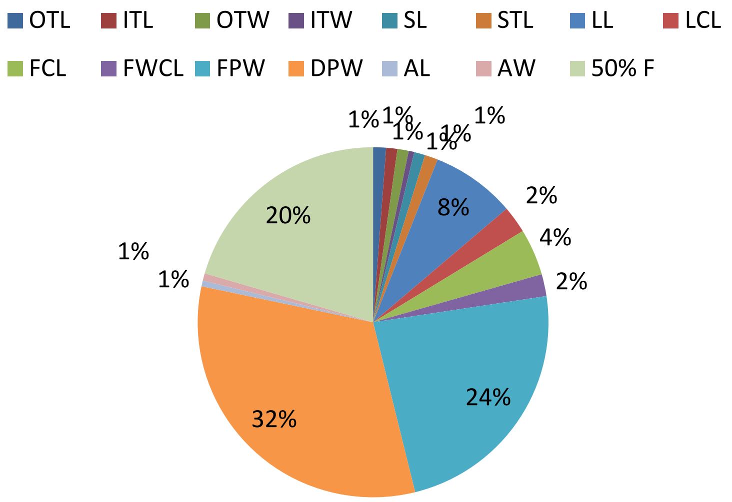


Fig. 4. Magnitude of variability for different morphological traits



Pie Chart 1. Magnitude of variability for different traits in saffron

Fig. 5. Layout of Research trial at ARSSSS, SKUAST-K Pampore

Fig. 6. Randomly selected and tagged saffron flower for evaluation

4. CONCLUSION

The diversity found in saffron germplasm suggests that saffron has a lot of room for development in terms of morphological features and therefore divergence studies are critical for determining the level of variability and the potential for its future use in breeding.

Our findings also contributes a better understanding of genetic variation in saffron for vegetative and yield contributing traits indicating high level of variability in germplasm, therefore imply considerable scope for saffron improvement through clonal selection.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author up on request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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