Electronic Journal of Plant Breeding

Research Article

Genetic divergence of saffron germplasm for morphological and corm attributes

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Abstract
Fifty diverse genotypes of saffron (*Crocus sativus* L.) were evaluated for genetic divergence with respect to morphological and corm attributes at Saffron Research Station Pampore, SKUAST-K during *rabi* 2017-18. Ten randomly and tagged competitive plants were selected for the recording of observations for eighteen (18) morphological, floral and corm traits. All the genotypes were grouped into eleven clusters with the highest number of genotypes (28) in cluster I, followed by cluster II with 7 genotypes and cluster IV with 5 genotypes based on divergence analysis. The percentage contribution towards total divergence by various characters indicated that fresh pistil weight contributes 21% followed by multiplication index (18%), total flower weight, corm^{-1} (13.52%) and the number of leaves corm^{-1} line^{-1} (13.27%) and big corm index (5%), respectively. High cluster mean for floral attributes was observed to be on account of the grouping of genotype SSR/SD-30, SSR/SD-6, SSR/SD-15, SSR/SD-29, SSR/SD-2, SSR/SD-1, and SSR/SD-24 for yield attributes which provides sufficient scope for saffron crop improvement through clonal selection.

Keyword
Crocus sativus L., clusterring, Mahalanobis D^2 analysis.

INTRODUCTION

Saffron (*Crocus sativus* L.) is part of the *Iridaceae* and is the world's most expensive spice. In India, it is a legendary crop of Jammu and Kashmir, produced on well drained karewa soils, where ideal climatic conditions are available for good growth, growth of the shoot and production of flowers. It is a legendary crop of Jammu and Kashmir, India grown on a well drained *karewa* soils, where suitable climatic conditions are accessible for good growth shoot growth of the shoot and production of flowers. The *Iridaceae* family comprises about 60 genera and 1,500 species. The genus *Crocus* is particularly well represented in arid south-eastern Europe, Western and Central Asian countries. Saffron (*C. sativus* L.) is the most enthralling species among the 85 species belonging to the *Crocus* genus (Fernandez,2004). Dried stigmas of saffron flowers make up the most costly spice herb and

is a rich source of proteins, vitamins especially riboflavin and thiamine, potassium, iron, zinc, copper, sodium and manganese thereby providing antioxidants and treating multiple diseases (Yasmin *et al.*, 2018). Moreover, it contains carotenoids which impute an anticancer, antitumour and immunomodulatory properties. However, some noteworthy characteristics of saffron are fairly low water use, growth and development during fall and winter and a very small harvesting index.

The Kashmir province of UT Jammu & Kashmir produces between 16.45 Metric tonnes of saffron mostly dedicated to self consumption by India (Anonymous, 2018). Owing to triploidy imposed sterility, clonal selection as one of the better approaches which offer an ample scope to increase production and productivity gains in the saffron crop. The

saffron crop is being grown in Jammu and Kashmir for so many centuries. Genetic and environmental influences are responsible for the variability observed in the natural population of saffron for morphological, developmental and yield component traits. Instigation of strategical development breeding programmes on sound scientific lines includes the generation and implementation of specific knowledge on different genetic parameters to achieve plant breeding goals pragmatically. Moreover, the corm multiplication in saffron does not induce genome variation except for those natural mutations that are not easily detectable in triploid saffron population and as such genetic divergence studies are very critical in comprehending the degree and scale of variability and possibilities of its prospective use and future utilization in subsequent breeding programmes. Studies of the divergence are of great importance in the variation estimation and the potential of its future utilization in saffron crop improvement programs (Ahmad *et al.,* 2013; Salwee and Nehvi, 2014). Development of a variety from the established germplasm resources, exhibiting a better yielding ability and quality will enhance the production and productivity of saffron and also would improve the socio-economic well being of the people associated with this significant commercial crop of Jammu and Kashmir. Therefore the present study involving 50 germplasm lines was undertaken to attain a meaningful grouping of saffron genotypes in relation to the contribution of each character towards divergence using Mahalanobis D^2 statistics, which will help in the effective germplasm management and its further utilisation in future breeding programs.

MATERIAL AND METHODS
The present research work was carried out during 2017-18 at Saffron Research Station, Konibal Pampore, which is a major Research and Development Station of saffron of Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir. The experimental site is situated on 34^0N latitude, 74^0E longitude and around 1650 m a.s.l. The research material comprised of 50 saffron germplasm genotypes collected from different saffron growing areas

of Kashmir and other saffron growing areas of the world. Each saffron corm weighing 5g to 16g were planted in randomized block design in three replications, with a row length and width of 3m and 2m and a plant geometry of 20x10cm (inter and intra-row spacing) respectively. Corms were planted under each category supplemented with adequate nutrients as per the recommendations of SKUAST-K, Shalimar. Agronomic practices recommended by SKUAST-K were followed to produce a successful crop. Observations for different traits were recorded on 10 randomly selected and tagged competitive plants from each line during the crop year 2017-2018.

The observations were recorded for different traits detailed below.

Floral attributes: Number of flowers corm^{-1}line^{-1}, Number of flowers corm^{-1} line^{-1}, Total flower weight corm^{-1}, Outer tepal length, Inner tepal length (cm), Outer tepal width (cm), Inner tepal width (cm), Anther length (cm), Anther width (mm), Style length (cm), Stigma length (cm), Fresh pistil weight per line (mg), Dry pistil weight per line (mg).

Vegetative parameters: Leaf length (cm), no. of leaves per line

Corm attributes: No. of days to 50% sprouting, Big Corm Index, multiplication index, mean values for all the characters have been estimated for analysis of variance (Verma *et al.* 1987; Singh and Chaudhary, 1985) and character association at genotypic and phenotypic level (Al Jibouri, 1958).

RESULTS AND DISCUSSION
Fifty (50) saffron germplasm lines were evaluated for genetic divergence as per the Mahalanobis D^2 analysis employing Tocher's method (Rao, 1952). Based on the performance, the genotypes were grouped into 11 clusters. Cluster I contains the maximum number of genotypes (28). Rest of genotypes were grouped in 10 clusters irrespective of the geographical area (**Table1**.). Cluster

Table 1. Clustering pattern of saffron (*Crocus sativus* L.) genotypes based on D^2 statistics

Cluster	Number of genotypes	Name of germplasm lines SSR/SD-
I	28	28, ,32, 9, 45, 47, 34, 48, 12, 20, 50, 4, 16, 44, 35, 46, 21, 19, 25, 18, 49, 33, 31, 27, 26, 40, 42, 37,38,
II	7	41, 43, 23, 3, 22, 8, 5
III	1	24
IV	5	17, 11, 10, 36, 14
V	1	7
VI	1	2
VII	3	6, 15, 29
VIII	1	1
IX	1	30
X	1	13
XI	1	39

mean of different characters revealed that the number of flowers corm^{-1} line^{-1} had the maximum cluster mean. Cluster means were generally associated with divergence pattern (**Table 2**). Studies by means of D^2 statistics in a number of crop species with divergent breeding systems indicate that genetic diversity need not be directly related with enough geographical diversity. Sufficient knowledge regarding genetic diversity is currently not available for the development of high yielding saffron varieties although the technique has been extensively used by multiple workers to understand the nature of genetic divergence and to select diverse parents for successful hybridization in the breeding populations. Similarly in saffron, the fundamental aim is to examine the group of sub populations for their

genetic diversity and consequent utilization of diverse genotypes in the development of high yielding varieties. Analysis of variance for divergence studies indicated that the value of statistics measuring Wilk's criterion was high and significant suggesting the existence of considerable genetic diversity in the present set of the research material. All the genotypes were classified into 11 clusters, with cluster I accommodated a maximum number of genotypes irrespective of geographical location.

The same pattern of findings of diversity analysis has also been revealed by Desh and Misra (1993), Desh Raj and Misra (1999), Arya *et al*., (1999), and Nimbalkar *et al*., (2002) in gladiolus.

Table 2. Cluster means of 18 different morphological and corm traits in saffron (*Crocus sativus* L.)

Cluster	OTL	ITL	OTW	ITW	STGL	STYL	LFL	L/C/L	F/C/L	FWT/C/L	BCI	MI	FPW	DPW	AL	AW	50%F	50%S
I	4.24	3.71	3.73	1.83	3.63	4.26	27.11	8.69	14.16	6.58	9.94	3.77	441.42	100.26	1.88	2.31	73.25	128.43
II	4.30	3.68	3.72	1.89	3.72	5.32	29.60	9.52	18.10	8.98	11.65	3.91	605.76	166.34	2.11	2.88	72.36	134.01
III	4.13	3.65	3.63	1.85	3.36	3.96	31.20	10.13	10.33	5.67	9.33	3.00	314.87	74.65	1.38	1.87	72.00	133.00
IV	4.20	3.67	3.66	1.78	3.19	3.73	26.39	10.87	27.11	5.63	12.75	3.69	361.85	84.91	1.55	1.93	73.74	129.28
V	4.45	3.77	3.76	1.97	4.14	3.55	31.29	10.63	22.67	11.78	14.0	4.66	674.17	157.93	2.35	3.25	73.67	127.33
VI	4.27	3.74	3.52	1.67	3.66	3.96	29.14	9.33	14.67	4.63	10.67	3.00	405.27	99.94	2.36	1.76	73.00	132.00
VII	4.30	3.71	3.73	1.84	3.89	3.14	30.13	9.75	24.21	11.53	14.23	4.73	855.37	173.69	2.24	2.74	73.33	127.17
VIII	4.43	3.64	3.95	2.03	4.13	6.34	29.07	9.33	20.33	7.88	12.67	4.00	624.22	142.53	2.26	3.26	69.67	125.00
IX	4.23	3.93	3.57	1.96	4.15	7.36	34.13	10.67	26.00	13.30	14.96	4.87	904.67	188.64	2.37	3.19	70.67	132.00
X	4.22	3.69	3.65	1.75	2.83	2.34	26.16	10.00	14.00	7.21	9.00	3.00	418.10	98.18	1.37	1.84	72.67	129.33
XI	4.33	3.74	3.86	1.99	3.85	4.13	32.11	11.00	21.33	9.35	13.33	4.65	539.91	132.88	2.16	3.15	72.00	122.00

OTL (Outer tepal length), ITL (Inner tepal length), OTW (Outer tepal width), ITW (Inner tepal width), STGL (Stigma length), STYL (Style length), LFL (Leaf length), L/C/L (Number of leaves per corm per line), F/C//L (Number of flowers per line), FWT/C/L (Total flower weight per line), BCI (Big corm index), MI (Multiplication index), FPW (Fresh pistil weight), DPW (Dry pistil weight), AL (Anther length), AW (Anther width), 50% F (50 percent flowering), 50% S (50 percent sprouting).

Data analysis revealed a maximum number of genotypes were accommodated in cluster I (28) followed by cluster II (7) and cluster IV (5). The level of divergence as depicted by mean intra-cluster and inter-cluster distances revealed that maximum divergence within a cluster between genotypes was recorded for cluster VII (415.78) which accounted for the maximum genetic distance between genotypes SSR/SD-28 and SSR/SD-38 (**Table 3**). Maximum inter cluster distance of 10923.91 was recorded between cluster X and cluster XI accommodating genotypes SSR/SD-13 and SSR/SD-39 followed by cluster VIII and cluster X (7598.09) which accommodates genotypes SSR/SD-1 and SSR/SD-13, cluster VII and cluster XI (6977.24) accommodating genotypes SSR/SD-6, SSR/SD-15, SSR/SD-29, and SSR/SD-39. The maximum inter cluster distance of 10923.91 was observed between cluster X and cluster XI accommodating genotypes SSR/SD-13 and SSR/SD-39 followed by cluster VIII and cluster X (7598.09) which accommodates genotypes SSR/SD-1 and SSR/SD-13, cluster VII and cluster XI (6977.24) accommodating genotypes SSR/SD-6, SSR/SD-15, SSR/

SD-29, and SSR/SD-39. The research findings clearly showed that there is potential scope for identification of diverse genotypes which can serve as a source of allelic resources for the development of new saffron varieties. Cluster means indicates that there was a significant variation in both morphological and corm attributes revealing substantial variability existed for all these traits (**Table 2**). The high cluster means for saffron yield, big corm index and stigma length wereobserved in Cluster IX on account of a grouping of high yielding genotype SSR/SD-30. The cluster IX exhibited superiority in terms of saffron yield and big corm index. Moreover, the cluster IX was observed to record maximum cluster means for multiplication index. Similarly, the highest cluster mean for saffron and corm yield was attributed to SSR/SD-30 genotypes grouped in cluster IX, while high cluster means for floral attributes was observed to be on account of the grouping of genotype SSR/SD-30, SSR/SD-6, SSR/SD-15, SSR/SD-29, SSR/SD-2, SSR/SD-1, and SSR/SD-24 for yield attributes which provides sufficient scope for saffron crop improvement through clonal selection.

Table 3. Inter-cluster and Intra-cluster distances (D² values) between different saffron (*Crocus sativus* L.) germplasm lines

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI
I	**329.27**	889.36	501.82	562.74	649.79	504.75	1021.07	2188.17	677.06	1990.91	4090.82
II		**366.39**	1671.70	1806.54	1411.63	1037.13	2174.90	692.93	961.59	4512.35	1808.75
III			**0.00**	197.35	1192.37	1099.73	1345.27	3439.47	1385.45	1030.92	5548.56
IV				**300.69**	998.41	946.74	1095.23	3694.55	1294.69	958.77	5976.76
V					**0.00**	304.56	313.40	3036.28	283.14	1904.69	5499.87
VI						**0.00**	673.98	2516.83	327.60	2308.87	4611.44
VII							**415.78**	4194.76	884.75	1491.67	6977.24
VIII								**0.00**	2226.13	7598.09	429.36
IX									**0.00**	2845.20	4110.82
X										**0.00**	10923.91
XI											**0.00**

Genotypes SSR/SD-30, SSR/SD-6, SSR/SD-15, SSR/SD-29, SSR/SD-2, SSR/SD-1, and SSR/SD-24 exhibited high mean performance for saffron yield associated with high mean performance for corm yield also.   Similar results of contribution have been observed by Makhdoomi (2007). The percentage contribution of different characters (**Table 4**) towards divergence revealed that fresh pistil weight (21%) followed by a multiplication index (18%), total flower weight corm⁻¹ (13.52%) and the number of leaves corm⁻¹ line⁻¹ (13.27%) and big corm index (5%) contributed the maximum towards divergence. The contribution of each character towards genetic divergence, Kanawjia and Saravanan, (2016) found that spike length (33.16%) contributed highest, followed by the number of cormels per plant (21.05%), corm diameter (8.95%), days to spike emergence (11.05%), the diameter of floret (4.74%) in gladiolus respectively . Sardana *et al*., (1997) also observed that cluster mean and coefficient of variation represent the picture of diversity. Maximum contribution of fresh pistil weight, stigma length, fresh flower weight and fresh style weight has also been reported by Makhdoomi (2007). Similarly, fresh stamen weight (20.86%) followed by plant height (17.77%), fresh flower weight (15.31%) and pistil length (9.98%) had contributed significantly towards diversity (Qadri *et al*., 2012).

There is a great possibility of saffron improvement through clonal selection from the available germplasm resources. The current research findings lead to the identification of elite genotypes with distinct saffron yield superiority and

Table 4. Percent contribution of individual trait towards total divergence in saffron (*Crocus sativus* L.)

Traits	Contribution of individual trait towards whole divergence (%)
Fresh pistil weight	21.42
Multiplication index	18.0
Total flower weight per line	13.52
Dry pistil weight	13.27
Big corm index	5.0
Leaf length	4.0
Outer tepal width	3.75
Stigma length	3.57
Number of flowers per line	3.22
Inner tepal length	2.99
Inner tepal width	2.21
Outer tepal length	2.11
Number of leaves per corm per line	1.97
50 per cent flowering	1.36
50 per cent sprouting	1.31
Style length	1.24
Anther length	0.64
Anther width	0.42

corm attributes which may serve as a basis for further improvement and development of high yielding varieties, that could serve the saffron industry of Jammu and Kashmir, especially to the marginal and small farmers associated with saffron cultivation by increasing their net returns from saffron, besides encouraging farmers from nontraditional areas to brought up saffron cultivation.

Based on divergence studies, all the 50 saffron genotypes were grouped into eleven clusters with a maximum of 28 in cluster I, 7 in cluster II, 5 in cluster IV and 7 in a cluster (VII) whereas, remaining clusters were monogenotypic. The highest intra-cluster distance of 415.78 was observed in a cluster I between genotypes SSR/SD-28 and SSR/SD-38. The inter-cluster distance of 10923.91 recorded was highest between cluster X and cluster XI comprising of genotypes SSR/SD-13 and SSR/SD-39 followed by cluster VIII and cluster X (7598.09), which accommodates genotypes SSR/SD-1 and SSR/SD-13, cluster VII and cluster XI (6977.24) accommodating genotypes SSR/SD-6, SSR/SD-15, SSR/SD-29, and SSR/SD-39. The cluster mean of various traits revealed a wide range of variability present in the set of germplasm lines.

For stigma length, big corm index and saffron yield, cluster IX revealed high cluster mean on account of a grouping of high yielding genotype SSR/SD-30. Similarly for floral attributes viza viz yield attributes, high cluster mean was observed to be on account of a grouping of genotype SSR/SD-30, SSR/SD-6, SSR/SD-15, SSR/SD-29, SSR/SD-2, SSR/SD-1, and SSR/SD-24 which provides a wider scope for improving saffron crop by clonal selection.

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