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EXTENT AND PATTERN OF DIVERSITY IN SAFFRON GERMPLASM OF INDIAN KASHMIR

ANIL KUMAR SINGH*[1], MANIBHUSHAN, NARESH CHANDRA AND NARAYAN BHAKTA

National Bureau of Plant Genetic Resources, Regional Station, Srinagar 190 005 J&K

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Abstract

Keeping in view the dearth of well-defined saffron germplasm, an exploration trip was conducted to collect saffron germplasm from the growing tracts of Kashmir valley. A total of 28 diverse accessions were collected and evaluated. Data recorded was subjected to basic statistical analysis *viz.*, minimum, maximum, mean and coefficient of variance as well as Shannon-Weaver Diversity Index (SDI) was also worked out. Correlation study was done to know the extent and pattern of contribution among the traits and saffron yield. An attempt was made to quantify the extent of diversity present among the accessions explored and evaluated with the help of NTSYSpc software; dissimilarity coefficient and a dendogram was drawn. Presence of good variability in respect of vegetative, agro-morphological traits as well as reproductive traits like days to anthesis (35 - 44 days), duration of flowering (15 - 21 day), No. of flowers per plant (2 - 4), style length (26 - 32 mm) and saffron (style) weight, confirmed that there is tremendous scope of improvement in saffron production. The Shannon-Weaver Diversity Index (SDI) ranged from 0.08 to 0.25. Style length and dry saffron weight showed good amount of diversity as compared to the other parameter. Correlation study confirmed that corm size is a single trait which influences vegetative growth (plant height and number of leaves) positively. This genetic stock tagged with known potential could be utilized for current need as well as for future hybridization programme with its close relatives *viz., C. thomasii* and *C. cartwrightianus.*

Introduction

Saffron (*Crocus sativus* L.) is flowering autumnal geophyte, an ancient plant species, a member of large family Iridaceae, comprising some 85 species as reported by Dhar *et al.* 1988 and Herbert 1847. The word 'saffron' probably derived from Arabic word 'Zafran' where it means spice. In India, it is a crop of the poor and marginal farmers residing mainly in the Kashmir valley. Due attention has not been paid to improve saffron production technology to make it economically sustainable. It is the most expensive spice and valuable herbal medicine known for at least 3,500 years. Saffron has been originated in eastern Greece and presently it is being cultivated from the western Mediterranean, i.e. Spain to India (Harlan 1974). No other country than India can produce quality saffron worldwide, we are second largest producer of saffron after Iran. Filaments or threads are actually the dried style of the saffron flower, containing *'crocine'* the source of its strong colouring properties, which offer, distinctive aroma and bitter taste and essential oils that are responsible for its therapeutic properties (Basker and Negbi 1983, Singh *et al.* 2005). Saffron sector is struggling with declining profitability, which is dragging its performance. Singh *et al.* 2005, Singh *et al.* 2010 reported that cultivated land area under saffron has gone down from about 5,707 ha to just 3,785 ha and the productivity has gone down from 3.13 to 2.50 kg/ha in the last few years. Keeping in view the above situation, for revitalizing the saffron culture in Kashmir, Government of India has approved a plan under National Saffron Mission Programme for four years for the revival of saffron production in Jammu and Kashmir. Producing enough saffron from

*Author for correspondence: <anil.icarpat@gmail.com >.
[1]ICAR Research Complex for Eastern Region, Patna (800 014) Bihar, India.

4.50 to 5.50 kg against the existing level for increased demand against the background of changing climate scenario is a challenging task for agencies involved in saffron research and development. Singh *et al.* (2005) and Molina *et al.* (2004), reported that the optimal temperature requirement for emergence of saffron flower is 17°C. Being vegetatively propagated crop through corms or cormlet; the corm selection is as old as its cultivation centuries ago. Corm selection has played a vital role in improving population. Fascinatingly the long red stigmas are the result of such evaluation and evolution procedure, an event gradually taking place. The triploid *C. sativus* is mainly male sterile and seed setting is rare event but not impossible. In the light of future technological breakthrough in the reproductive biology of saffron, as suggested by results of crossing of saffron and its close relatives *C. thomasii* and *C. cartwrightianus* had revealed that diploid *C. thomasii*, *C. cartwrig htianus* are selfsterile but fortunately cross fertile, though the pollen of saffron *Crocus sativus* has very low percentage of germinating pollen grain. Research revealed that saffron *C. sativus* can be fertilized by the pollens of C. *thomasii* or *C. cartwrigtianus* as reported by Chichriccoo 1996 and Grilli 1995. To harness the gift of frontier sciences, *viz*., molecular biology, biotechnology, nanotechnology, which provide ample scope for saffron genetic improvement. It will be channelized with the increasing possibility of crossability with its close allies to produce superior saffron hybrid in future.

To select the super quality of saffron lines by selecting quality corms, for their current requirements as high yielding, improved varieties cultivars clones, plant exploration is one of the fastest methods to collect the existing genetic resource of the region. Therefore, evaluation process to know the extent and pattern of diversity present in these germplasms which could be utilized in the further crop improvement work (Verma *et al*. 2015).

Materials and Methods

Saffron (*C. sativus* L.) is propagated asexually through corms or cormlets. In Kashmir valley, it is produced in highly localized pockets at Pampore, Khannabal, Charare-e-Sharif and Rangreth. This survey based exploration was design to collect the existing landraces and primitive cultivars in the saffron-growing pockets. Plant exploration and collection of germplasm is quickest way to collect modest variability. The geographical areas surveyed for collection of saffron diversity were Pampore, Khannabal, Charare-e-Sharif and Rangreth of Kashmir valley. Every possible care was taken to collect only diverse type of corm tagged for collection based on agro-morphological distinctness. To eliminate possibility of repeated collection/selection, obvious importance was given on agro-morphological traits, collection number was further reduced and only 28 accessions were collected after crop cycle was over. Maximum (10 accessions) and minimum (4 accessions) were collected from Pampore and Rangreth sites.

All saffron accessions were planted at KD Research Farm Old Airfield, National Bureau of Plant Genetic Resources, Regional Station, Srinagar, Jammu and Kashmir, which is located at 34^0 N 6 latitude and 74^0 E 48 longitude, for agro-morphological evaluation, and were grown in experimental field in the plot size of 2 m × 2 m and replicated thrice. Observations were taken and simultaneously data recorded on agro-morphological traits, *viz.*, plant height (cm), leaves per mother corm, leaf length (cm), leaf width (mm), days to anthesis (days), flowering duration (days), flowers per plant, fresh saffron weight (decagram), dry saffron weight (decagram), corm weight (g) and style length (mm). Statistical analysis was carried out to have minimum, mean, maximum, coefficient of variance (CV), and correlation (r^2) etc. Coefficient of variance (CVs) and correlation (r^2) are useful in estimating diversity. High CV and low correlation values indicate presence of more diverse germplasm as suggested by Hayward and Breese 1993. Diversity index of saffron germplasm was also workout with help of Shannon-Weaver Diversity Index (SDI) for

individual accessions, location-wise as well as for total collection by using formula given by Poole 1974 based upon frequency distribution. To measure the diversity with the help of dissimilarity coefficient within the collected germplasm data were subjected to NTSYSpc software; dissimilarity coefficient and a dendogram have been also drawn.

Results and Discussion

The collected accessions were evaluated and subjected for basic statistic especially maximum, minimum, means and coefficient of variations (CV%) to know the presence of nature and pattern of diversity (Table 1). Maximum diversity in terms of range was recorded in case of corm weight, range between 12 and 28 g with mean value of 20.14 g for all collections. The plant height is one of the important agro-morphological features of any autotrophic plant to synthesize food and efficient translocations to the economic part. Plant height was recorded in between 14 and 30 cm and both accessions with lone stand highest weight were collected from Pampore. The mean plant height of all collections was recorded to 22.25 cm. Maximum and minimum diversity was noticed germplasms Pampore and Khannabal collections with CV of 19.96 and 12.11%, respectively. Not only for corm weight and plant height but also for other recorded agro-morphological traits *viz.*, no. of leaves per mother corm, leaf length, leaf width, Pampore collection has got maximum variability with maximum CV (%) among other location collections and it was recorded 16.27, 19.85 and 12.05, respectively. Flowering duration was range from 15 to 21 days with mean value of 17.8 days for all collection. Pampore and Rangreth collections have accessions taking maximum and minimum flowering duration 15 - 21 days. Number of flowers per plant is one of the most important traits contributing directly to style production. It ranged from minimum 2.0 to maximum 4.0 flowers per plant with an average of 2.6; suggest majority of accessions had low flowers per plant. Rangreth collection site has got maximum diversity as indicated by maximum CV (30.6%) as compared to other collection sites (Table 1). Since style is produced in extremely less number of collections hence its production ranged 3.10 to 3.80 g with the mean of 3.40. Dry saffron style length (mm) also exhibit considerable amount of diversity, and ranged from 25 - 32 mm with mean value 27.9 mm for whole collections. Longest style was produced in Pampore collection, whereas smallest belonged to Khannabal site (25 mm). The style weight (Decagram) ranged between 61 and 71 dg with average value of 65. Maximum dried style weight (71 dg) was recorded with the accessions collected from Rangreth.

The corresponding values of Shannon-Weaver Diversity Index (SDI) for all parameters ranged from 0.08 to 0.25. Fortunately style length and dry saffron weight exhibited fairly good diversity as compared to the trend in diversity exist in other parameters (Table 2). Location-wise Pampore had got the maximum diversity index in relation to corm weight, days to anthesis, style length, fresh and dry saffron weight (Bali and Sagwal 1987, Nahvi *et al.* 2007, Singh *et al*. 2010). Grilli1 (1995), Karihaloo (2003) and Singh *et al.* 2010 also reported similar results.

Correlation studies (Table 3) revealed that corm weight had positive influence on all parameters under study and *vice versa*. It was proved significant (1% level) for plant height and number of leaf however; it was marginally and negatively influenced by dry saffron weight only. Plant height was positively correlated with number of leaves per plant, leaf width, duration of flowering, number of flowers per plant, fresh saffron weight and style length but it was negatively correlated with days to anthesis and dry saffron weight. Number of leaves per plant was significantly positively correlated with plant height and other parameters influenced marginally either positive or negative side. Leaf lengths were positively correlated while the corm weight, plant height, leaf width and flowering duration were marginally negatively correlated with other parameters. Leaf width was marginally positively influenced by corm weight, plant height, leaf

Table1. Agromorphological parameters for saffron (*Crocus sativus* L.) germplasm.

Parameters	Pampore					Khannabal					Charar-e-sharif					Rengreth					Total collection				
	Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV (%)	Min	Max	Mean	SD	CV (%)	Min	Max	Mean	SD	CV (%)	Min	Max	Mean	SD	CV (%)
Corm wt. .(g)	12.00	28.00	20.73	4.94	23.84	16.00	22.00	18.33	2.07	11.27	18.00	23.00	20.40	2.30	11.29	18.00	24.00	20.67	2.16	10.45	12.00	28.00	20.14	3.52	17.50
Plant ht. (cm)	14.00	30.00	21.64	4.32	19.96	17.00	23.00	21.17	2.56	12.11	19.00	26.00	22.60	2.88	12.75	20.00	29.00	24.17	3.31	13.70	14.00	30.00	22.25	3.56	15.98
Leaves / mother corm	9.00	15.00	11.45	1.86	16.27	10.00	14.00	11.33	1.51	13.28	10.00	14.00	11.80	1.79	15.16	10.00	12.00	11.00	0.89	8.13	9.00	15.00	11.39	1.55	13.59
Leaf length (cm)	14.00	25.00	18.91	3.75	19.85	18.00	22.00	19.50	1.64	8.43	17.00	23.00	19.20	3.03	15.80	14.00	25.00	20.00	3.69	18.44	14.00	25.00	19.32	3.13	16.19
Leaf width (cm)	1.70	2.50	2.10	0.25	12.05	1.90	2.40	2.13	0.18	8.21	1.80	2.10	2.04	0.13	6.58	1.90	2.40	2.17	0.18	8.08	1.70	2.50	2.11	0.20	9.42
Days to anthesis (days)	35.00	44.00	39.64	3.01	7.59	37.00	42.00	39.17	1.72	4.40	38.00	41.00	39.80	1.10	2.75	36.00	40.00	38.33	1.37	3.56	35.00	44.00	39.29	2.17	5.54
Flowering duration (days)	15.00	21.00	17.82	1.83	10.29	16.00	18.00	16.83	0.98	5.84	17.00	20.00	18.40	1.14	6.20	15.00	21.00	18.33	2.16	11.78	15.00	21.00	17.82	1.68	9.42
Flowers per plant	2.00	4.00	2.45	0.69	28.01	2.00	3.00	2.67	0.52	19.36	2.00	3.00	2.60	0.55	21.07	2.00	4.00	2.67	0.82	30.62	2.00	4.00	2.57	0.63	24.66
Fresh saffron weight (g)	3.10	3.80	3.42	0.21	6.25	3.10	3.80	3.36	0.29	8.57	3.10	3.80	3.36	0.29	8.57	3.10	3.60	3.40	0.17	4.92	3.10	3.80	3.41	0.19	5.61
Dry saffron weight (g)	0.61	0.69	0.65	0.03	4.28	0.61	0.68	0.65	0.03	3.99	0.61	0.68	0.65	0.03	3.99	0.61	0.71	0.65	0.03	3.93	0.61	0.71	0.65	0.03	3.93
Style length (mm)	26.00	32.00	28.64	1.96	6.86	26.00	30.00	27.40	1.67	6.11	26.00	30.00	27.40	1.67	6.11	26.00	30.00	28.00	1.55	5.53	25.00	32.00	27.89	1.77	6.35

number, leaf length, days to anthesis, flowering duration, flowers per plant and style length and numerically negative for other parameters taken into account. Days to anthesis was marginally positively correlated with corm weight, leaf length, flowering duration, flowers per plant, style length and dry saffron weight. However other parameters were negative. Number of flowers does not influence significantly any parameters. Fresh saffron weight is positively correlated with corm weight, plant height, style length and dry saffron weight. Dry saffron weight has influenced

Table 2. Shannon-Weaver diversity indices (SDI) for different quantitative parameters in saffron (*Crocus sativus*) germplasm.

Parameters	Pampore	Khannabal	Charer-e-sharif	Rengreth	Total collection
Plant height (cm)	0.17	0.15	0.01	0.21	0.13
Leaf length (cm)	0.16	0.15	0.17	0.25	0.14
Leaf width (cm)	0.20	0.21	0.14	0.25	0.13
Leaves per mother corm	0.19	0.17	0.20	0.15	0.14
Corm weight (g)	0.19	0.07	0.05	0.11	0.14
Days to anthesis (days)	0.19	0.14	0.07	0.11	0.11
Flowering duration (days)	0.19	0.17	0.05	0.25	0.13
Flowers/plant	0.16	0.15	0.18	0.25	0.14
Fresh saffron weight (g)	0.16	00	0.09	0.11	0.12
Dry saffron weight (g)	0.16	00	0.05	0.11	0.10
Style length (mm)	0.18	0.11	0.18	0.17	0.13

Table 3. Correlation studies of different parameters recorded for (*Crocus sativus* L.) germplasm collected from Kashmir valley (India).

Parameter	Corm weight (g)	Plant height (cm)	Leaves per mother corm	Leaf length (cm)	Leaf width (cm)	Days to anthesis (days)	Duration of flowering (days)	Flowers per plant	Fresh saffron weight (g)	Dry saffron weight (g)	Style length (mm)
Corm weight (g)	1.000	0.857**	0.546**	0.207	0.188	0.096	0.117	0.211	0.042	–0.083	0.086
Plant height (cm)		1.000	0.533	0.269	0.174	–0.038	0.200	0.279	0.023	–0.070	0.140
Leaves per mother corm			1.000	–0.073	0.215	–0.068	0.057	–0.049	–0.015	–0.145	–0.079
Leaf length (cm)				1.000	–0.202	0.253	0.082	0.333	–0.155	–0.078	0.234
Leaf width (cm)					1.000	–0.084	–0.005	0.185	–0.296	–0.022	–0.239
Days to anthesis (days)						1.000	0.126	0.253	–0.034	0.089	0.143
Flowering duration (days)							1.000	–0.144	–0.121	–0.055	0.205
Flowers per plant								1.000	–0.052	0.201	0.057
Fresh saffron weight (g)									1.000	0.807**	0.036
Dry saffron weight (g)										1.000	0.000
Style length (cm)											1.000

*, **, significant at 5 and 1% level. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

significantly positively with fresh saffron weight and style length, however, other parameters correlated either marginally positive or negative. Overall correlation studies revealed that corm size is single major parameter to influence vegetative growth, i.e. plant height and numbers of leaves. However, style length and fresh saffron weight only influenced the economic produce, i.e. dry weight of saffron style significantly as reported by Bali and Sagwal 1987, and Singh *et al.* 2010.

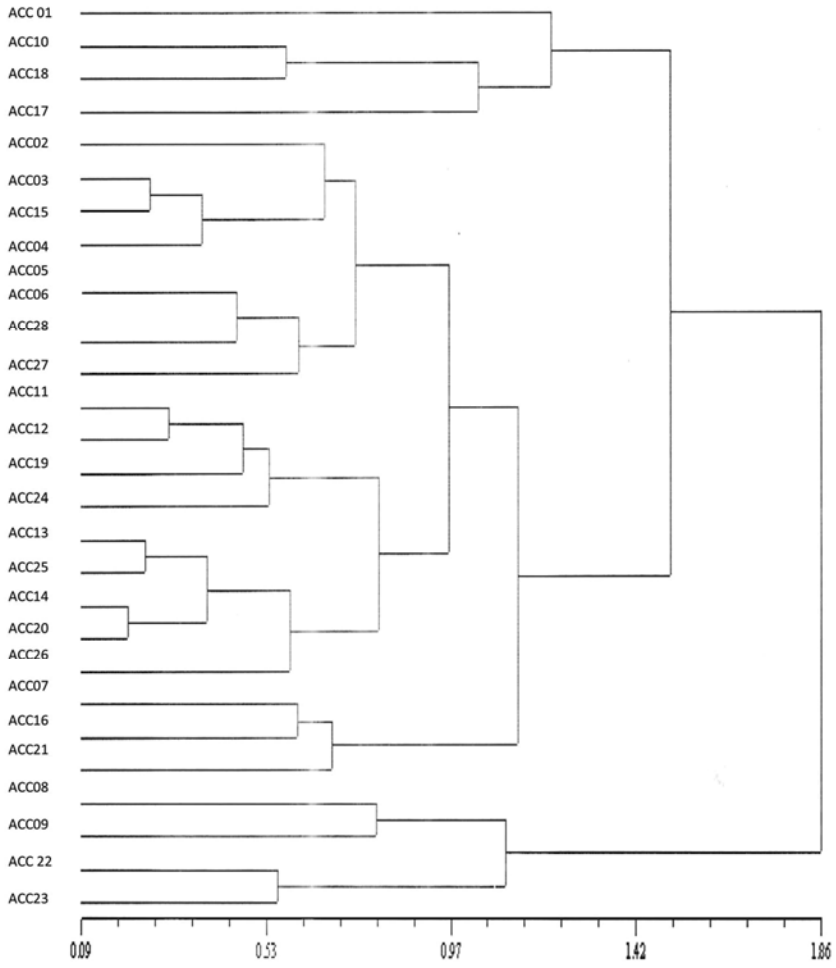

Fig. 1. Dendogram showing variability in saffron germplasm of Kashmir valley.

An attempt has been made to quantify the extent of diversity present among the saffron accessions explored and evaluated with the help of NTSYSpc software; dissimilarity coefficient and a dendogram have been drawn. Dissimilarity is expressed as a measure of diversity. Based on dissimilarity coefficient dendogram (graphical representation) of 28 saffron accessions has been drawn and presented in Fig. 1. When phenon line was drawn at 50% of dissimilarity index, it divided total collection into seven cluster groups out of which tow cluster group are represented by

just one individual accession each (Fig. 1) are more related to each other than the accessions of other cluster fell in same. Phenon line at 60% dissimilarity indicates that accessions of clusters IV and V are more related to each other than other clusters. Similarly, cluster VI had closer evolutionary relation with cluster VII. Cluster III is closer to cluster II. Interestingly cluster I is completely diversified. Phenon line at 40% dissimilarity increased the number of clusters to eight where in individual accessions of each cluster was more closely related. Variability and diversity though the crop is purely vegetative propagated. These results are in conformity with the results as reported by Karihaloo 2003 and Singh *et al*. 2010.

Intra-cluster analysis at different level of dissimilarity indicated that most of the accessions fell in cluster IV. Clusters I and III had single accession each. Accession Nos. 5 and 6 were most closely related to each other (Fig. 1). Accessions falling in cluster IV were more common having more or less same diversity and are more frequent. At 50% dissimilarity >60% accessions came in a particular cluster grouping, whereas most distantly related accessions of clusters I, II, III, V and VII.

Present study proves that considerable amount of variability is present that can be utilized for saffron improvement programme. As results of this study confirmed that there is tremendous scope of improvement in production of saffron style length due to presence of good variability in respect of vegetative, agro-morphological traits as well as reproductive traits like days to anthesis (35 - 44 days), duration of flowering (15 - 21 days), No. of flowers per plant (2 - 4), style length (26 - 32 mm) and saffron (style) weight. Selection of suitable corms is the easiest and most effective way to improve its productivity of desired quality right now to fulfill growing demand of tailor-made saffron cultivar of desired trait. Keeping in view of its nature of propagation, very limited scope exists for its improvement through sexual means still technological breakthroughs for hybridization realizes in near future.

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