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Studies of Phenotypic and genotypic variation for morphological traits in saffron (*Crocus sativus* L.)

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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. **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.

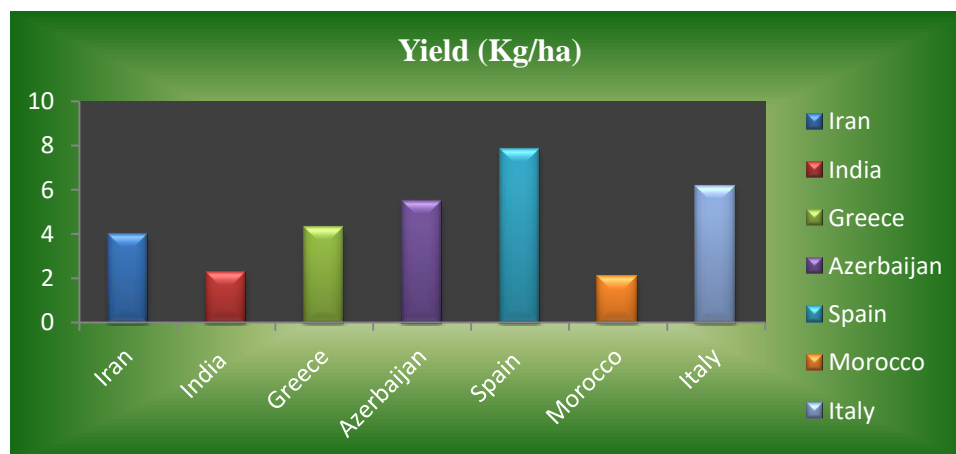
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11 *Keywords: Crocus sativus* L., divergence, phenotypic variability, genotypic variability, clonal selection.

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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

31 to reach higher production and productivity than we are, posing a serious threat to our saffron
32 business as imports rise year after year as shown in (Fig.1).
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36 **Fig. 1:** This fig indicates that Spain is the largest producer of saffron (8.33kg/ha) followed by Italy
37 (6.16kg/ha), Azerbaijan (5.43kg/ha), Greece (4.3kg/ha) and Iran (3.98kg/ha) [4].
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40 To make it globally competitive and remunerative to growers, there is a need to expand production
41 by bringing more land under cultivation and double average productivity by adopting an intense
42 production method, efficient processing, and marketing. Saffron farming covers 3715 hectares in
43 J&K, with annual yield and productivity of 13.3 MT and 3.0–4.0 kg/ha, respectively [5]. Saffron is
44 largely grown in four districts in J&K (Pulwama, Budgam, Srinagar, and Kishtwar), with an 86
45 percent saffron agricultural system at the Pampore heritage site covering 3200 hectares. Pampore,
46 as a peri-urban location, is vulnerable to commercialization and colonization; consequently,
47 expanding saffron cultivation to new prospective and non-traditional areas of J&K will ensure the
48 saffron farming system's long-term viability. This would help to boost J&K's overall saffron production
49 in order to meet the national demand of 100 M.T., as well as provide livelihood security to the state's
50 moderately poor farmers. Prospect of saffron genetic improvement is through clonal selection from
51 the available germplasm. Identification of elite genotypes with distinct superiority in quality and yield
52 attributes can be used as a source for further improvement. Development of varieties from the
53 identified germplasm exhibiting high yielding potential and quality will enhance the production and
54 productivity of saffron to reduce the burden of costs on the import of priced commodity and
55 improving the socio-economic status of the stakeholders associated with the production, processing
56 and value chain of this important commercial crop.
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58 MATERIAL AND METHODS

59 The present investigation was carried out at Advanced Research Station for Saffron and Seed
60 Spices (ARSSSS), Dusso Konibal, Pampore, during cropping season 2017-18., a constituent
61 Research Station of Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir.
62 The experimental site is located at 34°N latitude, 74°E longitude at an altitude of 1650m amsl. The
63 material for study comprised of 140 saffron germplasm lines collected from different saffron growing
64 areas of Kashmir and abroad viz., Afghanistan, Iran. The pedigree details of all the 140 corm
65 samples were recorded. Saffron corms weighing 5g to 16g were planted in Augmented Block Design
66 with a row length of 3m, width 2m and inter and intra-row spacing of 20 and 10 cms, respectively for
67 daughter corm production under annual planting cycle as shown in (Fig. 5). Recommended package
68 of practice from SKUAST- Kashmir was followed for nutrition and intercultural operations in the crop
69 planted. Observations were recorded on 10 randomly selected plants and tagged competitive plants
70 as shown in (Fig. 2 & 6) from each line for all the traits during the period of experimentation.
71

72 Morphological traits

73 [Number of flowers corm⁻¹, number of days to 50% flowering, fresh flower weight corm⁻¹ (mg), inner
74 tepal length (cm), outer tepal length (cm), inner tepal width (cm), outer tepal width (cm), anther
75 length (cm), anther width (mm), style length (cm), stigma length (cm), fresh pistil weight (mg), dry
76 pistil weight (mg), leaf length (cm), number of leaves corm⁻¹ line⁻¹].

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

80 81 RESULTS AND DISCUSSION

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83 Genetic variability assists a great deal in detecting the range of genetic diversity for various traits in a
84 population [10]. Components of phenotypic and genotypic variability indicated that a wide range of
85 variability existed for all the traits studied shown in (Table 2, Chart-1, Fig. 3 & 4, chart 3). Outer tepal
86 length ranged from (3.13-4.55cm) with a mean value of (4.25cm), inner tepal length (3.46-3.96cm),
87 with a mean value of (3.69cm), tepal width (3.45-3.95cm) with a mean value of (3.70cm), inner tepal
88 width (1.55-2.03cm) with a mean value of (1.84cm), stigma length (2.55-4.18cm) with a mean value
89 of (3.60cm), style length (2.34-7.35cm) with a mean value of (4.31cm), leaf length (16.12-37.10cm)
90 with a mean value of (27.73cm), number of leaves corm⁻¹ (6.00-11.33) with a mean value of (8.93),
91 number of flowers corm⁻¹ (4.00-35.33) with a mean value of (15.33), fresh flower weight corm⁻¹ (1.80-
92 14.31g) with a mean value of (7.23g), fresh pistil weight (104.84-1207.38mg) with a mean value of
93 (483.91mg), dry pistil weight (25.95-976.25mg) with a mean value of (114.71mg), anther length
94 (1.34-2.37cm) with a mean value of (1.90cm), anther width (1.26-3.74mm) with a mean value of
95 (2.40mm) and 50% flowering (68.66-76.66) with a mean value of (73.10). Results are in conformity
96 with the reports of Sheikh *et al.* [11-16] as they observed same results and reported wide range of
97 variability exists in the natural population of saffron as indicated by the magnitude of *per se*
98 performance, phenotypic and genotypic coefficient of variation, implying considerable scope for
99 saffron improvement through clonal selection. The study suggests that there is an ample scope for
100 saffron genetic improvement through selection of superior genotypes from the heterogeneous
101 saffron populations. For floral attributes viz, outer tepal length, inner tepal length, outer tepal width,
102 inner tepal width, anther length, anther width indicates less range of variability. The estimates of
103 phenotypic variances were higher than the corresponding estimates of genotypic variances thereby
104 revealing a strong influence of environment in the expression of the traits studied. *Per se*
105 performance of genotypes under evaluation revealed a gross fresh pistil weight of 40.24mg per corm
106 from 1.21 flowers corm⁻¹ resulting in 3 kg laccha per hectare with planting density of 5 lakh corms
107 per hectare showing an increase of 25% over state average. Estimates of phenotypic and genotypic
108 correlation coefficient among the characters have been found useful in planning and evaluating
109 breeding programme [17,8] Genotypic correlation coefficient provide a measure of genetic
110 association among the characters and gives an indication of characters that could be useful as to
111 identify more important ones during a selection programme. At phenotypic and genotypic level, dry
112 pistil weight (economic product) exhibited a significant positive correlation with all the traits. The
113 results clearly reveal a scope for simultaneous improvements of this important trait through
114 selection. Interrelationship among various floral, vegetative and yield contributing traits was
115 observed to be significant both at genotypic and phenotypic levels. Yield is influenced by
116 environmental conditions and is a complex mode for inheritance and low heritability. However, most
117 of the yield components which naturally leads to use of some other trait that is highly correlated with
118 yield and has a much higher heritability through selection of the best progenies for more reliability. In
119 this study, to assess the phenotypic and genotypic relationships between studied traits, correlation
120 coefficients based on phenotypic and genotypic values were calculated (Table 1, Chart 2). It was
121 notable in this study that in most of the cases the genotypic correlation coefficients were larger than
122 the phenotypic correlation coefficients. This finding is in agreement with the results of previous
123 studies [18,10 & 19] Saffron under temperate conditions of Kashmir Valley reported wide spectrum
124 of variability for floral & vegetative attributes and the results implied a great scope for saffron
125 improvement. Flower size, number of flowers per corm and saffron recovery per flower were
126 observed to help as selection index for increasing saffron yield. Valley exhibited highest range of
127 variability in saffron germplasm. From natural population flowers completely devoid of style and
128 anthers, some flowers with 4 and 5 stigmas were observed. The presence of higher number of
129 stigmas per flower results from physiological or developmental irregularities leading to 4-5 stigmas.
130 These results are in conformity with Nehvi *et al.* [20-22]. The phenotypic and genotypic variations
131 between different saffron ecotypes for yield and yield attributing traits like Fresh pistil Weight, Dry
132 pistil Weight and Stigma Length were measured and the results showed that phenotypic variances
133 were much higher than genotypic variances for all traits except stigma length, fresh flower weight
134 corm⁻¹ line⁻¹, number of flowers corm⁻¹ line⁻¹, fresh pistil weight, dry pistil weight in which the
135 genotypic variance were higher than phenotypic variance. In general, with the purpose of the study
136 and assessments to genotypic coefficient of variation and other genetic parameters, we conclude
137 that traits like, number of flowers corm⁻¹ line⁻¹, fresh flower weight corm⁻¹ line⁻¹, fresh pistil weight

138 were the most effective traits and had the highest positive impacts on the saffron yield. Therefore,
 139 with the phenotypic selection of saffron ecotypes in respect of these traits, the saffron yield can be
 140 increased and these findings are in agreement with the results of previous studies of Bayat *et al.* [19,
 141 23]. They reported that with regard to significant genotypic coefficient of variation and high positive
 142 direct effects, the selection might be based on these two characters and must be focused on most
 143 effective traits for the direct improvement of yield.
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145 **Table 1. Estimate of Phenotypic variance (δ^2p), genotypic variance (δ^2g), phenotypic**
 146 **coefficient of variation (PCV), Genotypic coefficient of variation (GCV) for morphological**
 147 **traits of Saffron (*Crocus sativus* L.) year 2017-2018**

Characters	Phenotypic Variance ($\delta^2 P$)	Genotypic variance ($\delta^2 g$)	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

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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

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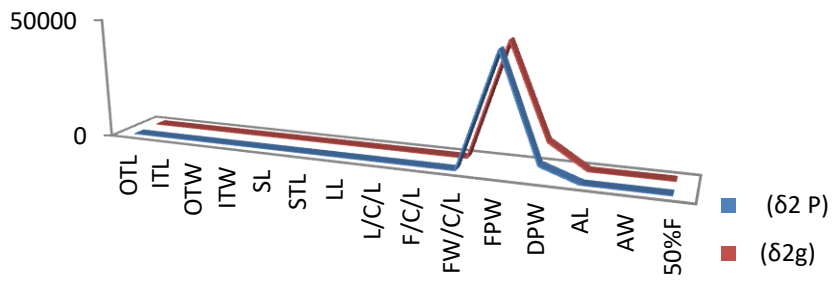


chart 1 . Estimates of $\delta^2 p$ & $\delta^2 g$ for morphological traits

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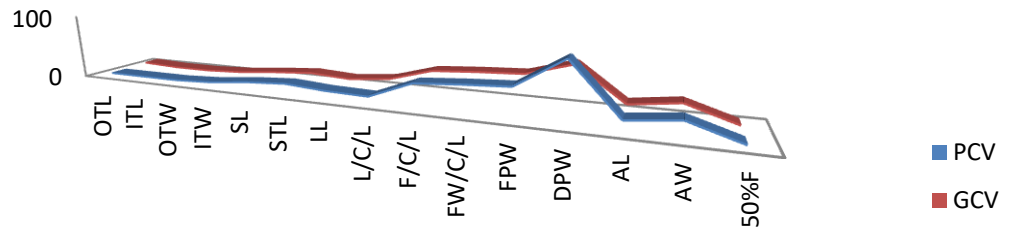


chart 2. Estimates of PCV & GCV for morphological traits

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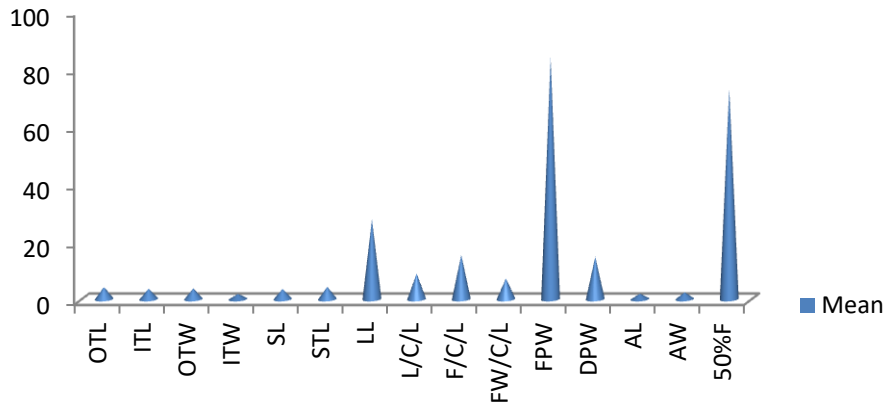


Fig. 3. Mean for magnitude of variability for different morphological traits

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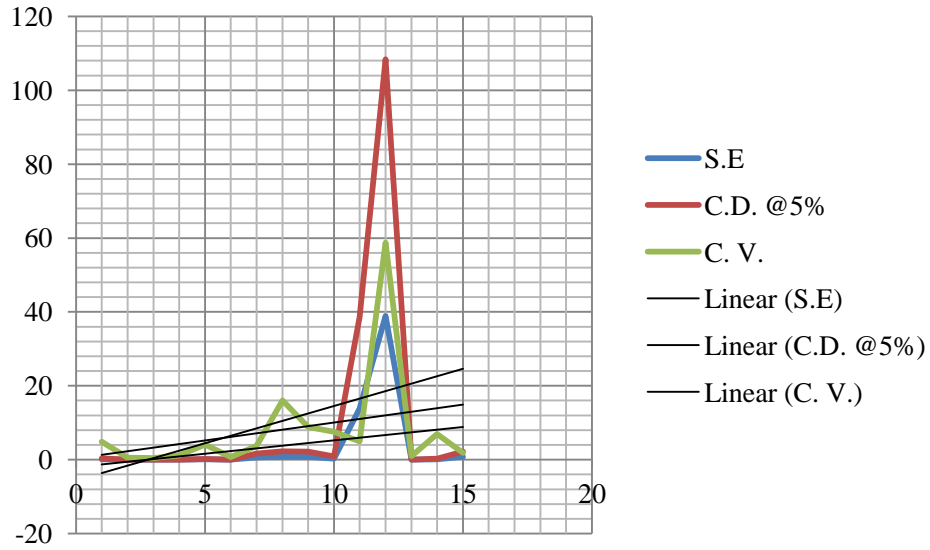


Fig. 4: Magnitude of variability for different morphological traits

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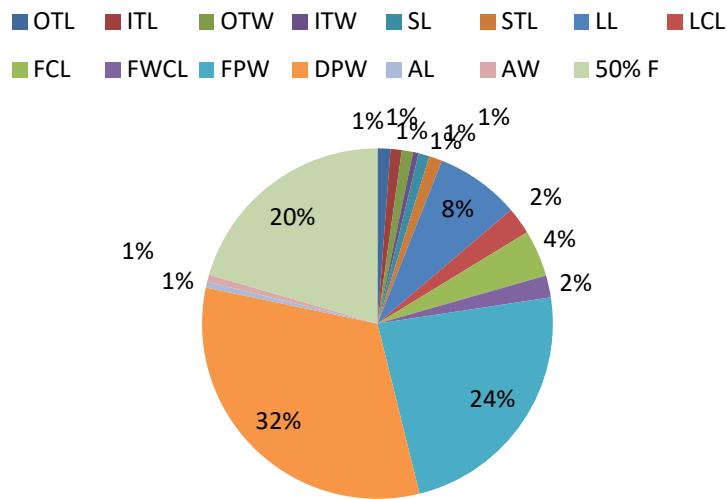


chart 3: Magnitude of variability for different traits in saffron

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

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

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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.

CONCLUSION

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

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

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240 **Data availability statement**

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

243
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245
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253 **COMPETING INTERESTS**

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255 "Authors have declared that no competing interests exist."

256
257 **AUTHORS' CONTRIBUTIONS**

258
259 Mohammad Irfan, Uzma Rashid: conceptualization, data curation, methodology, statistical
260 analysis, writing, investigation; Sabeena Naseer, Mohd Ashraf Bhat, M. Altaf Wani:
261 conceptualization, data curation, validation; M. H. Khan, Gowhar Ali: methodology, formal
262 analysis, validation; Mohammad Irfan, Uzma Rashid, compilation, investigation, review and
263 editing. All authors read and approved the final manuscript.

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