Original Research Article

Estimation of Genetic Variability in Saffron (*Crocus sativus* L.)Germplasm for Morphological and Quality Traits

Abstract

Crop improvement relies heavily on the genetic variability present within the gene pool. Understanding the nature and extent of genetic variations within germplasm is essential for breeders to design effective breeding programs. The present investigation comprised of 35 germplasm lines ofsaffron (Crocus sativus L.)conducted in a RCBD with three replications at ARSSSS, SKUAST-K, Pampore. Observations were recorded on morphological, yield, and qualitytraitsfor the assessment of genetic variability and other parameters. The findings revealed significant differences among germplasm lines, indicating substantial variability across all traits. Phenotypic coefficient of variation consistently exceeded the genotypic coefficient of variation (GCV) for all characters, with stigma length exhibiting the highest PCV (25.24%) and GCV (24.08%), while the number of days taken to first flush demonstrated the lowest PCV (1.84%) and GCV (1.66%). The study reported high broad-sense heritability for all traits, ranging from 75.1% for corm diameter to 99.2% for the number of leaves. Notably, safranal content, stigma length, and fresh weight of pistil displayed the highest heritability and genetic advance over mean. Correlation analysis revealed significant associations, with dry weight of stigma exhibiting positive correlations with various traits, including pistil length, stigma length, and safranal content, while displaying negative correlations with outer tepal width, inner tepal width, and number of leaves per corm/plant. Path coefficient analyses underscored the importance of traits such as pistil length, fresh weight of pistil, stigma length, and style length and the UPGMA clustering method categorized saffron genotypes into five clusters.The study reports the morphological characterization and genetic variability of diverse saffron genotypes, which could be used for future saffron breeding programs especially clonal selection from the available germplasm resources is of prime importance.

Keywords: *Crocus sativus*L., characterization, variability, association, heritability, quality analysis

Introduction

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Saffron, also known as *Crocus sativus* L., is a spice derived from the dried stigmas of the saffron crocus flower. Etymologically, the term "crocus" originates from the Greek word "croci," signifying the weft, a thread used in loom weaving (Anjum, 2015). Historically, saffron characterization dates back to the first century AD, emphasizing its enduring importance in diverse fields (Wenger, 2022). Its cultivation spans from 0° to 90°E longitude (Spain to Kashmir) and 30° to 45°N latitude (Persia to England), with Iran, Spain, and India contributing over 80% of global production (Sameer *et al*., 2012). In India, Pampore, Jammu and Kashmir, has a historical association with saffron cultivation, with 84.5% of the total saffron area (3715 ha) is concentrated in this region (Naseer et al., 2018; Nehvi *et al*., 2022) and is characterized having intrinsic quality in terms of crocin content (Nehvi *et al*., 2012), which has been well recognized by the GI authority of India (Nehvi *et al*., 2022) by conferring with Geographical Indication tag.

Saffron plant is a herbaceous geophyte belongs to Iridaceae family (Kumar *et al*., 2008), which emerges from the soil in October for flowering and remains in active vegetative growth until April (Iqbal *et al*., 2012; Nehvi *et al*., 2022). The flower of the saffron plant is bisexual, with three purple-coloured tepals, each housing three stamens and three anthers. Saffron being sterile, propagates vegetatively through corms (Fernandez, 2004; Petersen *et al.,* 2008; Agayev *et al.,* 2009; Khan *et al.,* 2022; Nehvi *et al.,* 2022) which is globular in shape, ranging from 1.5- 2.5 mm in diameter. Each corm includes 1-4 apical bud(s) where the flower and leaves originate. There are also many small brown spots between the horizontal lines of corms, which are the origins of cormlets (Ali *et al*., 2012). Saffron, exhibits sub-hysteranthous behaviour (Yasmin *et al*., 2018) and is a prized, low-growing perennial with captivating flowers and a pleasant fragrance and is known aromatic properties (Hussain *et al*., 2019) and has been widely used for its medicinal properties since ancient times (Abdullah, 2002; Premkumar *et al*., 2006; Gutheil *et al*., 2012; Kim *et al*., 2014). Functioning as a medicinal plant with low toxicity, saffron has demonstrated various therapeutic effects, including antioxidant, anti-inflammatory, anti-cancer, and neuroprotective properties (Abdullah, 2002; Qadri *et al*., 2012;Qadri *et al*., 2017, Khan et al, 2021, Anaeigoudari *et. al.,* 2023).

The chemical composition of saffron has attracted the interest of several research groups during the last decades and among the estimated more than 150 volatile and several non-volatile compounds of saffron, approximately 40-50 constituents have already been identified (Winterhalter and Straubinger, 2000). Among them, the pharmacologically active

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compounds, crocin, picrocrocin, and safranal (Aung *et al*., 2007; Yasmin et al., 2017 Spinelli *et al*., 2023) determine color intensity, flavour, and aroma strength, respectively (Menia *et al*., 2018). The levels of each pigment in saffron vary due to the different origins of the plant and the overall processing conditions and storage length (Ordoudi *et al.*, 2004). Various analytical methods, including the ISO 3632 standardization system, have been developed for quality control.

In recent years, global saffron production has been facing difficult challenges in productivity, with a decline observed in almost all producing countries, due to the use of ?subpar planting material (Khan *et al*., 2022). In this pursuit, genetic variations serve as a fundamental tool for developing new cultivars with improved traits related to yield and quality (Ali *et al*., 2018). The breeding for improved nutritional quality has played a pivotal role in solving the problem of malnutrition especially for the community, including animals (Qureshi et al,2019). Saffron landraces cultivated in different regions exhibit distinct genetic signatures, reflecting centuries of selection by local farmers for traits suited to specific agroecological conditions. Population genetic analyses suggest that Saffron's genetic diversity is influenced by factors such as geographical isolation, historical migration patterns, and local adaptation. Morphological traits such as corm size, flower color, stigma length, and leaf morphology display notable variability among different Saffron populations. Similarly, the morphological diversity in flower characteristics, petal shape, colour intensity, number of style branches, stamens, and stigmas in saffron obtained from various regions due to environmental effects, postharvest processing of stigmas and basal genetic variation (Nehvi *et al*., 2006; Agayev *et al*., 2009; Siracusa *et al*., 2013; Babaei *et al*., 2014). Likewise, the characterization, systematic documentation and conservation and developing a distinctive descriptor profile to explore an existing saffron biodiversity is an essential component for delineating the uniqueness of global and niche-specific saffron germplasm and for the genetic improvement of saffron (Iqbal et al., 2023).Therefore, for the successful establishment of the crop on a commercial scale, the availability of adapted cultivars with desired characteristics is key to improving the current scenario (Nehvi *et al*., 2009). Additionally, the creation of varieties from the identified germplasm that have high yielding potential and quality will increase saffron production and productivity, lowering the cost of expensive imports and enhancing the socioeconomic status of those involved in the production, processing and value chain of this significant commercial crop.

Materials and Methods

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The material for the present study comprised of 35 germplasm lines (*Crocus sativus* L.) planted in a Randomized Complete Block Design with three replications at the Experimental Farm of Advanced Saffron Research Station for Saffron & Seed Spices (ARSSSS), Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir at Dussu, Pampore, during *Kharif*, 2022. All the germplasm lines of saffron (*Crocus sativus* L.) were collected from primitive saffron cultivation regions of Pampore, Pulwama (31 accessions) and Kishtwar districts (4 accessions) of Jammu & Kashmir and the pedigree details of the germplasm lines are presented in Table-S1. Corms weighing more than 8g were used in the current study. Each genotype was planted with a plant geometry of 20x10cm with 20 cm between rows and 10 cm between corms. The standard agronomic practices were employed to ensure optimal crop growth. Observations were recorded at flowering, post-flowering, and corm harvesting stages, including the number of days taken to first flush (days), fresh weight of pistil (mg), dry weight of pistil (mg), pistil length (cm), stigma length (cm), style length (cm), veining pattern of tepals, tepal shape, outer tepal length (cm), inner tepal length (cm), outer tepal shape, inner tepal shape, outer tepal width (cm), inner tepal width (cm), number of leaves per corm (No.), leaf length (cm), corm diameter (cm), weight of corm (g), aggregate number of daughter corms per corm, aggregate weight of bigger corms per mother corm (g), corm tunic and texture and shape of naked corms. For DUS characterization of germplasm, the descriptor of saffron of PPV and FRA, New Delhi were used. Following harvest, stigmas were carefully separated by hand and subjected to drying in a forced-air oven at a temperature of 60°C for 25-30 minutes.Moreover, the quality parameters crocin ($E^{1\%}_{1cm}$at 440 nm), picrocrocin ($E^{1\%}_{1cm}$ at 257 nm) and safranal ($E^{1\%}_{1cm}$ at 330 nm) were estimated using a UV-Vis spectrophotometer following the ISO 3632-2 trade standard (ISO/TS 3632-2, 2010/2011). The chlorophyll a (mg/F.W),chlorophyll b (mg/F.W) and total chlorophyll (mg/F.W) were estimated using dimethyl sulfoxide (DMSO method) proposed by Barnes *et al*. (1992).

The observations were recorded from 10 plants and averaged over the 10 plants except the number of days taken to first flush which was recorded on the whole plot. The data generated were analyzed using R software (version 4.0.5) for genetic variability, and "DARWIN" software program (Perrier and Jacquemoud-Collet, 2006) estimated genetic diversity using the for UPGMA hierarchical clustering.

Results

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Characterization Studies for Morphological and Yield Traits in Saffron

The thirty-five germplasm lines of *Crocus sativus* L. assessed in this study were categorized into various classes based on character classification, which revealed substantial variation in morphological characters, offering valuable insights into the genetic diversity and relationships among saffron germplasm lines(Table-S2). The frequency distribution of genotypes for various characters demonstrated a broad spectrum of variation (Fig. 1).The investigation into flower attributesrevealed that flowering primarily occurred within a medium number of days (94%), while some exhibited early flowering (6%). Regarding pistil characteristics, a majority of germplasm lines displayed a high fresh weight(63%),with the remaining possessing a medium weight (27%). Similarly, for dry weight of pistil, most exhibited a high weight (57%), while others had a medium weight (43%). Pistil length (Fig. 2) predominantly fell into the category of medium length (69%), followed by short (42%) and long length was seen in few germplasm lines (3%). Stigma length exhibited the highest frequency for medium length (51%), followed by long (20%) and short length (29%). Notably, style length was predominantly long (71%), followed by medium (23%) and short length (6%).

The veining pattern of tepals (Fig. 2)was observed mostly as discontinuous (64%) and continuous (29%). A small percentage also had parallel veining (7%). Tepal shape was predominantly elliptical (53%), followed by oblanceolate (31%) and obovate (16%). For outer tepal length and inner tepal length, the maximum frequency was observed for medium length (71%, 86%), followed by long length (29%, 14%).The shapes of outer tepals and inner tepals were mostly elliptical (55%, 52%), oblanceolate (31%, 30%), and obovate (14%, 18%), respectively. Outer tepal width exhibited the highest frequency for medium width (40%), short (43%) and long (17%). Inner tepal width recorded the maximum frequency for short width (77%), followed by medium width (23%). Sparse-type leaves and long leaf lengths were observed for the total number of leaves per plant and leaf length, respectively, which hinted at a low variation of these characters in these germplasm lines.

The study on corm traitsrevealed that corm diameter and weight were predominantly of medium size. Concerning the aggregate number of daughter corms per corm(Fig. 2), the majority of germplasm lines recorded a medium count (74%), then many (20%) and lastly few (6%) daughter corms. The aggregate weight of bigger corms per plant was recorded

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mostly as low. Corm tunic and texture were majorly wholly parallel (63%), then smooth and splitting (31%), and interwoven fibres (6%) in the germplasm lines. Regarding the shape of naked corms, the highest frequency was observed for flattened (86%), and a portion of germplasm lines also had flattened globose (14%) corms.

Genetic variability analysis

Analysis of variance for 22 morphological, yield and quality traits was performed (Table-S3 and Table-S4). Analysis of variance indicated that the mean sum of squares due to germplasm lines were significant for all the characters which revealed that there was considerable genetic variability among the material under study, which can be exploited through selection. The saffron germplasm lines exhibited noteworthy variations across all assessed traits (Table 1). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) exhibited high values (> 30), evident for stigma length, safranal content, aggregate number of daughter corms per cormand fresh weight of pistil. Moderately substantial PCV and GCV (10-20) were observed for chlorophyll a, total chlorophyll, chlorophyll b, aggregate weight of bigger corms per mother corm, pistil length, number of leaves per corm/plant, dry weight of pistilpicrocrocin content, leaf length, and outer tepal width. Corm diameter inner tepal length, weight of corm, inner tepal width, and number of days taken to the first flush exhibited low PCV and GCV (<10), respectively. However, style length displayed high PCV but moderate GCV and crocin content, and outer tepal length showed moderate PCV and low GCV.

A comprehensive assessment of broad-sense heritability(Table 1) revealed uniformly high estimates for all examined traits. The heritability values for the traits were notably high (>60), ranging from 75.1% (corm diameter) to 99.2% (number of leaves per corm/plant).Substantial high (>20) genetic advances as a percentage of the mean were observed for safranal content, stigma length, fresh weight of pistil, aggregate number of daughter corms per corm, style length, chlorophyll a, chlorophyll b, total chlorophyll, number of leaves per corm/plant, pistil length, dry weight of pistil, picrocrocin content, leaf length, and outer tepal width. Moderately (10-20) genetic advances were observed for outer tepal length, crocin content, corm diameter, aggregate weight of bigger corms per mother corm, weight of corm, inner tepal length, and inner tepal width. Conversely, a low (<10) magnitude of genetic advance as a percentage of the mean was noted for the number of days taken to the first flush.Boxplots (Fig. 3) displaying descriptive statistics give a detailed account of the

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distribution, spread, and central tendency of genetic traits within the saffron population. The length of the box (interquartile range) indicates the spread or dispersion of the genetic variability. A wider box suggests greater variability among the genotypes for the different traits.

To assess genetic divergence, a dendrogram (Fig. 4) was created to represent the data relationships. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering utilizing Euclidean distance as the dissimilarity measure, was applied. The thirty-five germplasm lines were categorized into five clusters. Cluster I stood as the largest, comprising 10 germplasm lines i.e., SRS-2022-29, SRS-2022-28, SRS-2022-31, SRS-2022-33, SRS-2022-35, SRS-2022-20, SRS-2022-26, SRS-2022-23, and SRS-2022-16, followed by Cluster V (9 germplasm lines) i.e., SRS-2022-3, SRS-2022-2, SRS-2022-1, SRS-2022-6, SRS-2022-22, SRS-2022-15, SRS-2022-13, SRS-2022-11 and SRS-2022-4, Cluster III (6 germplasm lines) i.e., SRS-2022-21, SRS-2022-10, SRS-2022-25, SRS-2022-27, SRS-2022-14 and SRS-2022-17, Cluster IV (6 germplasm lines) i.e., SRS-2022-24, SRS-2022-8, SRS-2022-30, SRS-2022-34, SRS-2022-19 and SRS-2022-18, and Cluster II (4 germplasm lines) i.e., SRS-2022-9, SRS-2022-7, SRS-2022-12 and SRS-2022-5. The presence of the clusters indicates that significant diversity can be found in saffron germplasm lines.

Correlation and path coefficient analysis

The analysis of correlation coefficients among various morphological, yield, and biochemical traits revealed significant associations (Table 2). Notably, DWP exhibited a positive and highly significant correlation with FWP (0.98), which is also the highest correlation observed for all the traits.Several noteworthy positive correlations included the association between PC and SC (0.98), CA and TC (0.93), CB and TC (0.93), DWP andPL (0.84), FWP and PL (0.84), FWP and STGL (0.81), STGL and PL (0.81), CW and AWBC (0.77) DWP and STGL (0.75), CD and CW (0.71), STYL and SC (0.65), STYL and PC (0.63), DWP and ITL (0.59), FWP and ITL (0.59), FWP and SC (0.57), ITL and PC (0.57), ITL and OTL (0.53) DWP and SC (0.56), FWP and PC (0.55), FWP and LL (0.54), LL and PC (0.54), LL and SC (0.54) DWP and PC (0.53), OTL and ITL (0.53) DWP and LL (0.52), DWP and STYL (0.51). However, the highest negative significant correlation was exhibited between OTL and CD (-0.55) and OTW and LL (-0.51).

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Path coefficient analysis, a method elucidating both direct and indirect influences of key independent variables (component traits) on the dependent variable, was employed for partitioning of genotypic correlation coefficients and provided insights into the significant direct effects (Table-S5) of the dependent trait which was dry weight of pistil.Pistil length showed a considerable direct effect with the dry weight of pistil (0.430), emerging as having the most influential effect, followed by fresh weight of pistil (0.399), stigma length (0.289) and style length (-0.397). These results show that pistil length and fresh weight of pistil have maximum effect on the yield of saffron which can be further improved by selecting for such traits.

Discussion

The exploration of saffron variability in diverse saffron-growing regions of Jammu and Kashmir was a primary objective of this study. An in-depth analysis of morphological, yield, and biochemical traits across saffron germplasm lines revealed a noteworthy degree of variability. The analysis of variance demonstrated highly significant mean squares for all examined traits, aligning with findings from Bayat *et al*. (2015), Ghanbari *et al*. (2019), and Irfan *et al*. (2022). These studies, focus on flower-related traits, corm characteristics, and picrocrocin and safranal contents, respectively, validating the substantial differences among distinct saffron genotypes Utilizing the saffron descriptor of Plant Varieties and Farmers' Rights Authority (PPV & FRA), the characterization study uncovered prevalent frequencies among germplasm lines. Medium days for the first flush, high fresh and dry pistil weights, medium pistil and stigma lengths, long style length, discontinuous veining pattern of tepals, elliptical tepal shape, medium outer and inner tepal lengths, elliptical outer and inner tepal shapes, medium outer tepal width, and short inner tepal width were the predominant characteristics. This aligns with findings by Yasmin and Nehvi (2018) for days taken to the first flush and by Cardone *et al*. (2021) for fresh and dry pistil weights. However, discrepancies were noted, such as early flowering in Italian, Iranian, and Spanish accessions reported by Torricelli *et al*. (2019). Mitsopoulou and Tsimidou (2003) and Ozdemir *et al*. (2006) highlighted variations in stigma length and morphological features within the Crocus genus, respectively.

Notably, leaf characteristics predominantly exhibited a sparse type and elongated leaf length, consistent with observations by Kothari *et al*. (2021) and Irfan *et al*. (2022). Corm traits indicated a medium corm diameter, corm weight, aggregate number of daughter corms,

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aggregate weight of bigger corms, parallel corm tunic, and a flattened shape of naked corms. These findings resonated with studies by Hassan-Beygi*et al*. (2009) on corm diameter and weight, Khan *et al*. (2011) on the number of daughter corms, and Mir *et al*. (2021) on stigma length. Comparable results were also found in the characterization of gladiolus cultivars by Hiremath *et al*. (2020).

The comprehensive exploration of saffron variability in this study contributes valuable insights into the distinctive traits prevalent in the diverse saffron germplasm lines of Jammu and Kashmir. These findings serve as a foundational reference for further research endeavours in saffron breeding, conservation, and cultivation strategies.The exploration of phenotypic and genotypic coefficient of variation (PCV and GCV) in various saffron characters has revealed substantial variability, providing valuable insights into the potential for improvement through clonal selection. Our findings align with previous studies by Nehvi *et al*. (2006), Sheikh *et al*. (2014) and Iqbal *et al*. (2023), demonstrating higher PCV compared to GCV, indicative of significant variation within the natural population of saffron.This observed variation is reflected not only in the marked differences in performance but also in the coefficients of variation, underscoring a considerable opportunity for enhancing saffron traits through clonal selection. The identification of superior genotypes from the diverse saffron populations holds promise for advancing the genetics of this valuable crop.

The intra-specific variability observed in agro-morphological traits can be attributed to a complex interplay of genetic and environmental factors, as noted by Barro-Kondombo *et al*. (2008, 2010). Stigma length, safranal content, aggregate number of daughter corms per corm, and fresh weight of pistil emerge as key discriminating traits with high magnitudes of both PCV and GCV. Comparable results have been documented by Singh *et al*. (2000), Balaram *et al*. (2000) in gladiolus, and Zargar *et al*. (2001), Cardone *et al*. (2021) in saffron.Moreover, our study reveals a high magnitude of heritability coupled with substantial genetic advance for traits like safranal content, stigma length, fresh weight of pistil, aggregate number of daughter corms per corm, style length, number of leaves per corm/plant, pistil length, dry weight of pistil, picrocrocin content, leaf length, and outer tepal width. This implies that effective selection practices can be employed for these traits, aligning with similar observations by Nehvi *et al*. (2006) for fresh flower weight, perianth tube length, and stigma length.In the context of gladiolus, Lepcha *et al*. (2007) and Kispotta *et al*. (2017) have also reported high heritability coupled with significant genetic advance for various traits,

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showcasing the potential for effective selection strategies. The collective evidence from these studies underscores the dynamic interplay of genetic and environmental factors influencing saffron traits, paving the way for targeted improvements and advancements in saffron cultivation practices.

This investigation into the correlation structure of saffron traits unveils meaningful insights for the identification of superior clones and the potential enhancement of breeding programs. The positive correlations observed between the dry weight of pistil and pistil length, fresh weight of pistil, and stigma length underscore the prospect of selecting these traits for the identification of superior saffron clones. This strategic selection could be instrumental in advancing future breeding initiatives and enhancing the overall quality and yield of saffron.The correlation patterns observed in our study align with findings in similar studies on saffron, as reported by Zargar (2001), Parviz *et al*. (2004) and Sheikh *et al*. (2010), further establishing the robustness and consistency of these relationships across different investigations. Additionally, parallels can be drawn with gladiolus studies conducted by Lal *et al*. (1985), Anuradha and Gowda (1994), Hedge *et al*. (1997), Deshraj *et al*. (1997, 1998), Sirohi *et al*. (2000), Nazir *et al*. (2003), and Balaram *et al*. (2009), where agro-morphological traits exhibited similar correlation magnitudes.A noteworthy positive correlation between corm weight and key yield-related traits, including stigma length, average number of daughter corms per plant, and average weight of daughter corms, implies the potential for increased yield in subsequent sowing. This finding is consistent with results reported by Iqbal *et al*. (2012) for corm weight, reinforcing the significance of this trait in predicting and influencing saffron yield. Path coefficient analysis reveals that pistil length and fresh weight of pistil emerge as major determinants of saffron yield, corroborating earlier studies by Sheikh *et al*. (2014) and Irfan *et al*. (2020) where stigma length exhibited the highest correlation with saffron yield. These consistent findings underscore the pivotal role of specific traits in influencing saffron yield, providing valuable guidance for targeted breeding efforts and the continued improvement of saffron cultivation practices.

In our study, we employed the Agglomerative Hierarchical Clustering (AHC) with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) procedure to unravel the genotypic clustering patterns within saffron germplasm lines. This method allowed us to classify the germplasm lines into distinct groups based on their genotypic similarities, providing valuable insights into the underlying genetic relationships among these lines. The AHC-UPGMA analysis identified five distinct groups, with entities within each cluster

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sharing higher genetic similarities compared to those in other clusters. Surprisingly, our findings indicated that the clustering pattern did not exhibit any discernible correlation with the geographical origin of the germplasm lines. This intriguing observation challenges the conventional notion that geographical proximity necessarily results in genetic similarity among saffron populations.Comparable results were reported by Izadpanah *et al*. (2015), who conducted a cluster hierarchical analysis of 29 saffron germplasm accessions and assigned them to three clusters. Similarly, Singh *et al*. (2015) identified seven major clusters in gladiolus cultivars, emphasizing the versatility of genotypic clustering techniques across different plant species. Soukrat *et al*. (2019), in their study on saffron, obtained five distinct groups through cluster analysis, further supporting the notion that genetic diversity is not solely dictated by geographical origin.Intriguingly, the study by Qadri *et al*. (2013) aligns with our findings, emphasizing that geographical diversity may not be the sole determining factor in grouping genotypes from a specific source. This broader perspective challenges traditional assumptions about the influence of geography on genetic clustering and highlights the complex interplay of various factors shaping the genetic diversity observed in saffron germplasm lines.

Conclusion

The comprehensive assessment of saffron germplasm lines conducted in this study revealed that the all the characterized saffron germplasm lines based on DUS descriptor shall provide a basic population for the traits specific improvement in the saffron breeding programme. Wide range of variability existed in the population under study as indicated by the magnitude of per se performance, phenotypic and genotypic coefficient of variation, implying considerable scope for saffron improvement through clonal selection. High heritability in broad sense coupled with high genetic advance for most of the traits contributing to saffron yield revealed that these traits could be utilized for improvement for saffron yields. Pistil length, stigma length, style length, fresh weight of pistil, corm diameter, aggregate weight of bigger corms per mother corm, etc. had high significant positive association with dry weight of pistil. Hence, selection based on such component characters may be rewarding for yield improvement. The best identified germplasm lines in terms of dry weight of pistil were SRS-2022-8, SRS-2022-10, SRS-2022-19, SRS-2022-28, and SRS-2022-31. The study concluded that selection of superior saffron germplasm lines based on their performance for safranal content, stigma length, fresh weight of pistil, aggregate number of daughter corms per corm,

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style length, number of leaves per corm/plant, pistil length, dry weight of pistil, crocin , picrocrocin, and safranal will be effective in future breeding programmes.

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Table- S1: Geographical distribution of saffron germplasm lines

S. No	Germplasm line	Source	Place of Collection	Coordinates
01	SRS-2022-1	Farmers Field	Shar, Pampore, District Pulwama	34.01238455082422, 75.0088042101665
02	SRS-2022-2	Farmers Field	Lethipora, Pampore, District Pulwama	33.96772530196106, 74.9648450466155
03	SRS-2022-3	Farmers Field	Dussu, Pampore, District Pulwama	33.998293824420855, 74.9667762799316
04	SRS-2022-4	Farmers Field	Chandhara, Pampore, District Pulwama	33.98691332282017, 74.949742480349
05	SRS-2022-5	Farmers Field	Wuyan, Pampore, District Pulwama	34.02631047040069, 74.96628191729188
06	SRS-2022-6	Farmers Field	Nambal bal, Pampore, District Pulwama	33.99421724690634, 74.9496810898887
07	SRS-2022-7	Farmers Field	Patal bagh, Pampore, District Pulwama	33.984280344981585, 74.92338414821428
08	SRS-2022-8	Farmers Field	Kisthwar	33.3114037348526, 75.76578870089446
09	SRS-2022-9	Farmers Field	Khrew, Pampore, District Pulwama	34.02099174802852, 74.9998635032671
10	SRS-2022-10	Farmers	Gundbal, Pampore,	33.9835779254693,

		Field	District Pulwama	75.00474161678946
11	SRS-2022-11	Farmers Field	Gundbal, Pampore, District Pulwama	33.994637114019916, 74.98176194460237
12	SRS-2022-12	Farmers Field	Gundbal, Pampore, District Pulwama	33.994512581555036, 74.98262293369136
13	SRS-2022-13	Farmers Field	Sombur, Pampore, District Pulwama	34.02506769642144, 74.93207025441052
14	SRS-2022-14	Farmers Field	Sombur, Pampore, District Pulwama	34.022884663438845, 74.9313407344849
15	SRS-2022-15	Farmers Field	Dussu, Pampore, District Pulwama	33.998293824420855, 74.9667762799316
16	SRS-2022-16	Farmers Field	Ladhou, Pampore, District Pulwama	33.99650102985633, 74.996344947432
17	SRS-2022-17	Farmers Field	Ladhou, Pampore, District Pulwama	33.99562042282745, 74.99415090047005
18	SRS-2022-18	Farmers Field	Munpora, Pampore, District Pulwama	34.018660451628755, 74.96426582368662
19	SRS-2022-19	Farmers Field	Munpora, Pampore, District Pulwama	34.02048342607026, 74.96413171323647
20	SRS-2022-20	Farmers Field	Chandhara, Pampore, District Pulwama	33.98420420902539, 74.94776861831568
21	SRS-2022-21	Farmers Field	Chandhara, Pampore, District Pulwama	33.9865616856758, 74.9529935614476
22	SRS-2022-22	Farmers Field	Patal bagh, Pampore, District Pulwama	33.9833606897786, 74.92363359365125
23	SRS-2022-23	Farmers Field	Wuyan, Pampore, District Pulwama	34.0263638214965, 74.96856715935982
24	SRS-2022-24	Farmers Field	Wuyan, Pampore, District Pulwama	34.02713296273406, 74.9644419219179
25	SRS-2022-25	Farmers Field	Wuyan, Pampore, District Pulwama	34.02705293680309, 74.96582057734388
26	SRS-2022-26	Farmers Field	Wuyan, Pampore, District Pulwama	34.026448293996296, 74.9651124741679
27	SRS-2022-27	Farmers Field	Wuyan, Pampore, District Pulwama	34.02726633911799, 74.96737625856387
28	SRS-2022-28	Farmers Field	Wuyan, Pampore, District Pulwama	34.026306024474486, 74.96733334321986
29	SRS-2022-29	Farmers	Shar, Pampore,	34.01238455082422,

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		Field	District Pulwama	75.0088042101665
30	SRS-2022-30	Farmers Field	Shar, Pampore, District Pulwama	34.011857620566, 75.00902951569697
31	SRS-2022-31	Farmers Field	Munpora, Pampore, District Pulwama	34.02005214053724, 74.96409416231045
32	SRS-2022-32	Farmers Field	Munpora, Pampore, District Pulwama	34.019251811061324, 74.96423363717844
33	SRS-2022-33	Farmers Field	Kishtwar	33.32035449272348, 75.76689310045646
34	SRS-2022-34	Farmers Field	Kishtwar	33.32033600210333, 75.77036924332438
35	SRS-2022-35	Farmers Field	Kistwar	33.31871329505941, 75.7742852685032

Table- S2: Frequency distribution of morphological traits

S.NO.	Character	State of expression	Number of germplasm lines	Frequency Distribution (%)
1.	Number of days taken to first flush (days)	Early	2	6%
		Medium	33	94%
2.	Fresh weight of pistil (mg)	Medium	13	27%
		High	22	63%
3.	Dry weight of pistil (mg)	Medium	15	43%
		High	20	57%
4.	Pistil length (cm)	Short	10	42%
		Medium	24	69%
		Long	1	3%

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5.	Stigma length (cm)	Short	10	29%
		Medium	18	51%
		Long	7	20%
6.	Style length (cm)	Short	2	6%
		Medium	8	23%
		Long	25	71%
7.	Veining pattern of tepals	Discontinuous veining	24	64%
		Continuous veining	10	34%
		Parallel veining	1	2%
8.	Tepal Shape	Obovate	5	16%
		Oblanceolate	11	31%
		Elliptical	19	53%
9.	Outer tepal length (cm)	Medium	25	71%
		Long	10	29%
10.	Inner tepal length (cm)	Medium	30	86%
		Long	5	14%
11.	Outer tepal shape	Obovate	5	14%
		Oblanceolate	11	31%
		Elliptical	19	55%

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12.	Inner tepal shape	Obovate	6	18%
		Oblanceolate	11	30%
		Elliptical	18	52%
13.	Outer tepal width (cm)	Short	15	43%
		Medium	14	40%
		Long	6	17%
14.	Inner tepal width (mg)	Short	27	77%
		Medium	8	23%
15.	Total number of leaves / plant (No.)	Sparse	35	100%
16.	Leaf length (cm)	Long	35	100%
17.	Corm diameter (cm)	Medium	35	100%
18.	Weight of corm (g)	Medium	35	100%
19.	Aggregate number of daughter corms per corm	Few	2	6%
		Medium	26	74%
		Many	7	20%
20.	Aggregate weight of bigger corms per corm (g)	Low	35	100%
21.	Corm tunic and texture (coat)	Smooth and splitting	11	31%
		Interwoven fibres	2	6%

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		Wholly parallel	22	63%
22.	Shape of naked corms	Flattened	30	86%
		Flattened globose	5	14%

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Table-S3:Analysis of variance (mean squares) for morphological and yield traits in saffron (*Crocus sativus* L.)

Source of variance	d.f	D1stF (days)	FWP (mg)	DWP (mg)	PL (cm)	STGL (cm)	STYL (cm)	OTL (cm)	ITL (cm)	OTW (cm)	ITW (cm)	NL	LL (cm)	CD (cm)	CW (g)	ANDC	AWBC (g)
Replication	2	16.83	7.04	0.018	0.02	0.012	0.013	0.12	0.07	0.013	0.024	1.34	4.72	0.01	0.04	0.02	0.01
Treatment	34	7.58**	308.83**	10.89**	1.69**	0.81**	0.59**	0.42**	0.16**	0.08**	0.01**	5.13**	76.24**	0.15**	2.11**	1.44**	1.37**
Error	68	0.534	3.018	0.088	0.015	0.025	0.018	0.02	0.01	0.002	0.0005	0.013	2.046	0.015	0.08	0.056	0.05

D1stF (days) = No. of days taken to firstflush, **FWP (mg) =** Fresh weight of pistil (mg), **DWP (mg)** = Dry weight of pistil (mg), **PL (cm)** = Pistil length (cm), **STGL (cm)** = Stigma length (cm), **STYL (cm)** = Style length (cm), **OTL (cm)** = Outer tepal length (cm), **ITL (cm)** = Inner tepal length (cm), **OTW (cm)** = Outer tepal width (cm), **ITW (cm)** = Inner tepal width (cm), **NL =** Number of leaves per corm/plant (No.), **LL (cm)** = Leaf length (cm), **CD(cm) =** Corm diameter (cm), **CW (g) =** Weight of corm (g), **ANDC =** Aggregate number of daughter corms per corm, **AWBC (g) =** Aggregate weight of bigger corms per mother corm (g)

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Table-S4:Analysis of variance (mean squares) for biochemical traits in saffron (*Crocus sativus* L.)

Source of variation	d.f	PC	SC	CC	CA	CB	TC
Replication	2	12.67	7.18	736.82	2.36	2.13	4.91
Treatment	34	238.75**	249.07**	857.41**	23.22**	64.81**	27.82**
Error	68	1.55	2.15	56.41	4.77	0.60	0.79

PC ($E^{1\%}_{1cm}$) = Picrocrocin content ($E^{1\%}_{1cm}$), **SC ($E^{1\%}_{1cm}$) =** Safranal content ($E^{1\%}_{1cm}$), **CC ($E^{1\%}_{1cm}$) =** Crocin content ($E^{1\%}_{1cm}$), **CA** = Chlorophyll A, **CB =** Chlorophyll B, **TC =** Totalchlorophyll

** = significant at 0.05 levels

Table-S5:Path coefficient analysis among yield traits in saffron (*Crocus sativus* L.) germplasm lines

	FWP	PL	STGL	LL	CW	ANDC
FWP	**0.399**	0.110	0.156	0.162	-0.014	-0.029
PL	0.315	**0.430**	0.154	0.072	-0.010	-0.058
STGL	0.214	0.214	**0.289**	0.259	-0.024	0.005
LL	0.025	0.134	0.060	**0.186**	0.096	-0.033
CW	0.017	0.037	0.048	-0.092	**-0.022**	0.053
ANDC	-0.008	-0.097	0.008	-0.055	-0.021	**0.011**

FWP (mg) = Fresh weight of pistil (mg), **PL (cm)** = Pistil length (cm), **STGL (cm)** = Stigma length (cm), **LL (cm)** = Leaf length (cm), **CW (g)** = Weight of corm (g), **ANDC** = Aggregate number of daughter corms per corm

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Table 1: Estimation of genetic parameters for morphological, yield and biochemical traits in saffron (*Crocus sativus*L.)

S.no	Characters	Mean	Range		GCV (%)	PCV (%)	h^2 (BS)	GA % Mean
			Min.	Max.				
1.	**D1stF (days)**	92.06	89	95	1.66	1.84	81.5	3.1
2.	**FWP (mg)**	48.92	28.33	65.33	20.64	20.94	97.1	41.89
3.	**DWP (mg)**	11.22	7.85	13.93	16.91	17.12	97.6	34.42
4.	**PL (cm)**	4.36	2.84	6.25	17.18	17.41	97.4	34.93
5.	**STGL (cm)**	2.13	1.08	3.09	24.08	25.24	91.1	47.31
6.	**STYL (cm)**	2.23	1.16	3.52	19.68	20.57	92.6	38.79
7.	**OTL (cm)**	3.73	3.19	4.54	9.73	10.5	85.9	18.57
8.	**ITL (cm)**	3.19	2.57	3.64	7.14	7.65	87.2	13.74
9.	**OTW (cm)**	1.33	1.1	1.77	12.28	12.80	91.9	24.26
10.	**ITW (cm)**	1.10	1	1.27	5.49	5.92	86.3	10.52
11.	**NL**	7.62	10.5	9.93	17.12	17.19	99.2	35.13
12.	**LL (cm)**	35.13	26.93	44.31	14.16	14.73	92.4	28.03
13.	**CD (cm)**	2.66	2.21	2.96	8.05	9.29	75.1	14.36
14.	**CW (g)**	11.51	10.04	12.88	7.13	7.58	88.3	13.8
15.	**ANDC**	3.20	1.93	4.6	21.23	22.49	89.1	41.29
16.	**AWBC (g)**	9.31	9.90	10.05	17.12	17.51	90.1	13.9
17.	**PC**	57.02	41.73	69.75	15.59	15.75	98.1	31.81
18.	**SC**	37.46	21.73	54.5	24.22	24.53	97.5	49.257
19.	**CC**	168.19	142.66	197.24	9.72	10.69	82.6	18.19
20.	**CA**	14.60	7.92	21.62	18.63	19.5	90.9	36.61
21.	**CB**	26.02	14.63	36.45	17.78	18.03	97.3	36.13
22.	**TC**	16.81	8.05	24.71	17.85	18.61	91.9	35.26

D1stF (days) = No. of days taken to firstflush, **FWP (mg) =** Fresh weight of pistil (mg), **DWP (mg)** = Dry weight of pistil (mg), **PL (cm)** = Pistil length (cm), **STGL (cm)** = Stigma length (cm), **STYL (cm)** = Style length (cm), **OTL (cm)** = Outer tepal length (cm), **ITL (cm)** = Inner tepal length (cm), **OTW (cm)** = Outer tepal width (cm), **ITW (cm)** = Inner tepal width (cm), **NL =** Number of leaves per corm/plant (No.), **LL (cm)** = Leaf length (cm), **CD(cm) =** Corm diameter (cm), **CW (g) =** Weight of corm (g), **ANDC =** Aggregate number of daughter corms per corm, **AWBC (g) =** Aggregate weight of bigger corms per mother corm (g), **PC =** Picrocrocin content, **SC** = Safranal content, **CC** = Crocin content, **CA** = Chlorophyll A, **CB =** Chlorophyll B, **TC =** Totalchlorophyll

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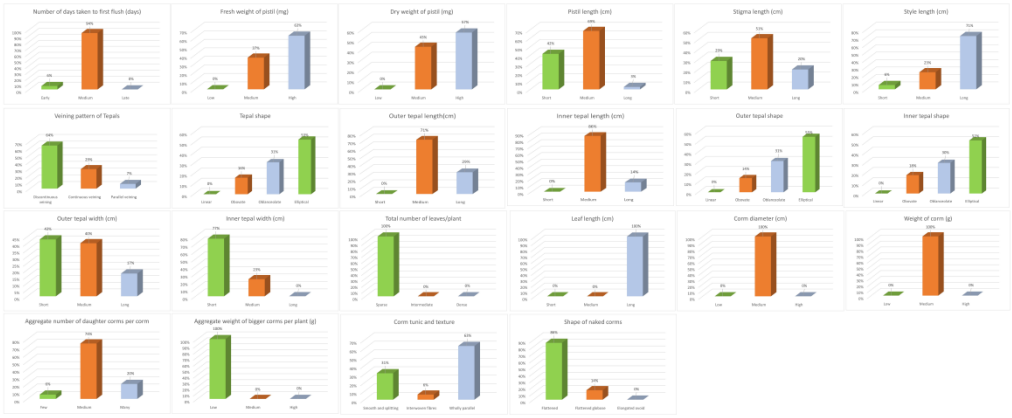

Figure 1: Frequency distribution of saffron germplasm lines

Figure 2: Morphological characterization (floral and corm traits) of saffron germplasm lines

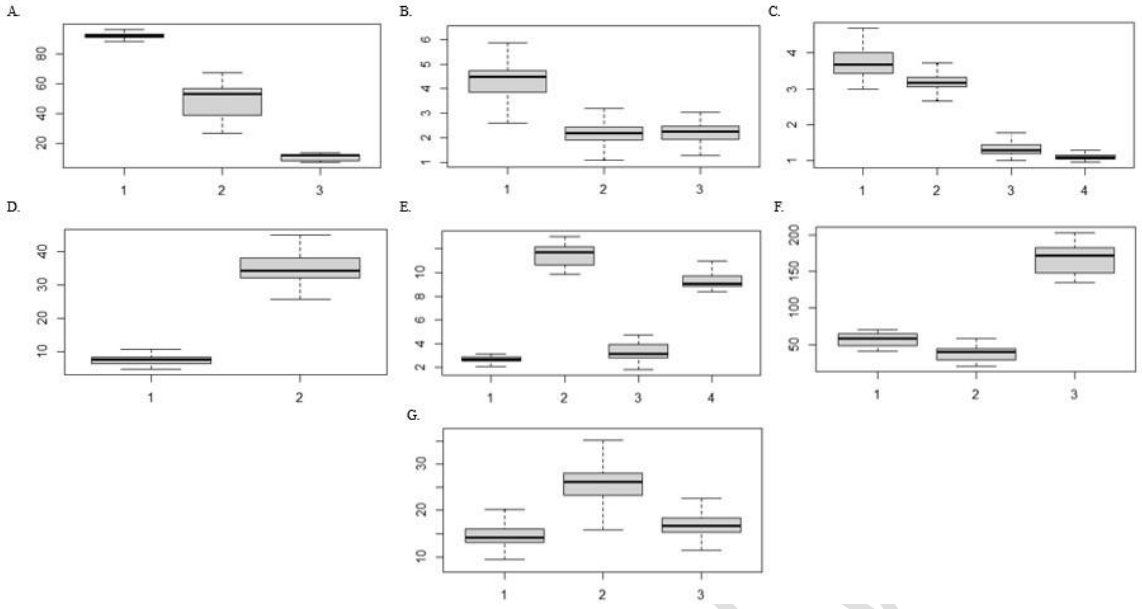

Figure 3: Boxplot showing distribution, spread, and central tendency of genetic traits within the saffron population

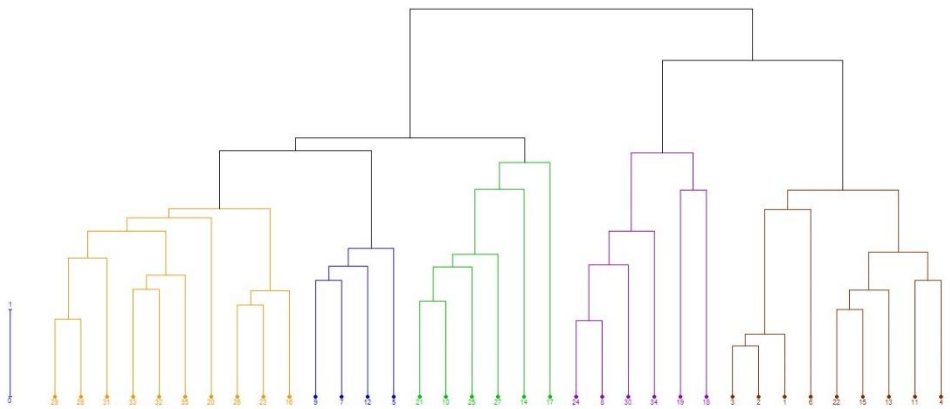

Fig-4: Dendrogram representing genetic diversity of saffron germplasm

Table 2: Estimation of genotypic correlation coefficients for morphological, yield and quality traits in saffron (*Crocus sativus*L.) germplasm lines

	D1stF	FWP (mg)	DWP (mg)	PL (cm)	STGL (cm)	STYL (cm)	OTL (cm)	ITL (cm)	OTW (cm)	ITW (cm)	NL	LL (cm)	CD (cm)	CW (mg)	ANDC	AWBC (g)	PC	SC	CC
1stF	**1**	0.01	-0.04	-0.05	-0.24**	0.14	0.24**	0.11	-0.16*	0.16*	0.02	0.04	-0.05	-0.21**	-0.28**	0.02	0.44**	0.45**	0.07
P(mg)		**1**	0.98**	0.84**	0.81**	0.45**	0.01	0.59**	-0.47**	-0.39**	-0.15	0.54**	0.26**	-0.01	-0.01	0.19*	0.55**	0.57**	0.28**
P(mg)			**1**	0.84**	0.75**	0.51**	0.01	0.59**	-0.47**	-0.42**	-0.42**	0.52**	0.26**	0.01	-0.03	0.19*	0.53**	0.56**	0.31**
(cm)				**1**	0.81**	0 71**	0.18*	0.55**	-0.24**	-0.37**	-0.28**	0.38**	0.15	0.10	-0.07	0.16*	0.63**	0.64**	0.16*
L(cm)					**1**	0.18*	-0.13	0.36**	-0.43**	-0.47**	-0.23**	0.31**	0.34**	0.25**	0.04	0.28**	0.36**	0.36**	0.11
L(cm)						**1**	0.41**	0.46**	0.05	-0.10	-0.21**	0.38**	-0.11	-0.11	-0.18*	-0.06	0.63**	0.65**	0.18*
L(cm)							**1**	0.53**	0.33**	0.46**	-0.07	0.13	-0.55**	-0.38**	-0.01	-0.34**	0.34**	0.28**	-0.21**
(cm)								**1**	-0.16*	0.01	-0.03	0.45**	-0.31**	-0.29**	-0.01	-0.14	0.57**	0.57**	0.18*
W(cm)									**1**	0.47**	0.22**	-0.51**	-0.28**	0.05	0.28**	-0.22**	-0.31**	-0.36**	-0.42**
W(cm)										**1**	0.20*	-0.23*	-0.25**	-0.11	0.23**	-0.28**	-0.13	-0.15	-0.41**
NL											**1**	-0.35**	-0.09	0.12	0.40**	0.08	-0.47**	-0.41**	-0.21**
(cm)												**1**	0.12	-0.49**	-0.29**	-0.29**	0.54**	0.54**	0.46**
D(cm)													**1**	0.71**	0.19*	0.71**	0.04	0.07	0.35**
W(mg)														**1**	0.47**	0.77**	-0.31**	-0.36**	-0.24**
NDC															**1**	0.19*	-0.28**	-0.26**	-0.07
BC(g)																**1**	-0.15	-0.06	0.15
PC																	**1**	0.98**	0.40**
SC																		1	0.36**
CC																			**1**
CA																			
CB																			
TC																			

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D1stF (days) = No. of days taken to firstflush, **FWP (mg) =** Fresh weight of pistil (mg), **DWP (mg)** = Dry weight of pistil (mg), **PL (cm)** = Pistil length (cm), **STGL (cm)** = Stigma length (cm), **STYL (cm)** = Style length (cm), **OTL (cm)** = Outer tepal length (cm), **ITL (cm)** = Inner tepal length (cm), **OTW (cm)** = Outer tepal width (cm), **ITW**

(cm) = Inner tepal width (cm), **NL =** Number of leaves per corm/plant (No.), **LL (cm)** = Leaf length (cm), **CD(cm) =** Corm diameter (cm), **CW (g) =** Weight of corm (g), **ANDC =** Aggregate number of daughter corms per corm, **AWBC (g) =** Aggregate weight of bigger corms per mother corm (g), **PC =** Picrocrocin content, **SC** = Safranal content, **CC** = Crocin content, **CA** = Chlorophyll A, **CB =** Chlorophyll B, **TC =** Totalchlorophyll

**1% level of significance and *5% level of significance

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