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ETHYL METHANE SULPHONATE - A POTENT MUTAGEN FOR INDUCING GENETIC VARIABILITY IN SAFFRON (*Crocus sativus* L.)

Salwee Yasmin and Firdos A. Nehvi

Saffron Research Station, S.K. University of Agricultural Sciences and Technology of Kashmir, Konibal – 192 121, Pulwama, Jammu & Kashmir (India)
e-mail: salwees@yahoo.com*

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ABSTRACT

Mutagenic effects of three concentrations of ethyl methane sulphonate [EMS] (0.1, 0.15 and 0.20%) to improve floral, foliar, corm and quality attributes in saffron was evaluated. A considerable genetic variability was observed among the mutagenic lines created with 0.15 and 0.20% EMS dose with significant shift in mean values in positive direction for almost all the traits studied. Identification of 64 mutant lines from available population of 660 lines confirmed highest mutagenic frequency of 43.75% by 0.15% EMS followed by 0.2% EMS (42.18%); whereas 0.10% EMS showed least mutagenic frequency. Component analysis showed medium to high GCV values for most of the traits confirming variability of genetic nature thereby implying that clonal selection has paramount significance in the identification of elite lines. Divergence analysis grouped 92% mutagenic lines in cluster 1 followed by 6.1% in cluster 2 and 1.5% in cluster 3. Maximum contribution towards divergence of most of the traits was recorded by lines 'SS/EMS-3/113', 'SS/EMS-3/132', 'SS/EMS-3/130' and 'SS/EMS-1/219'. *Per se* performance studies identified 18 extra-ordinary lines which showed significant superiority over base population for floral, foliar, corm and quality attributes and thus can be harnessed for commercial exploitation for enhancement in factor productivity per unit area.

Key words: Divergence, ethyl methane sulphonate, mutation, saffron, variability

INTRODUCTION

Genetic variations are the basic tools to develop new cultivars with better traits like tolerance against various environmental stresses, resistance against pests and diseases and improved yield and quality. The mutagenesis technology has been applied to plant breeding comprehensively which allow the crop to produce beneficial varieties with good traits (Maluszynski *et al.*,1995; Gu *et al.*, 2003). The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency, efficient mutagenesis being the product of the maximum desirable changes accompanied by the least possible undesirable changes (Tamina and Tabash, 2010). Mutations may be gross, resulting in large-scale deletions of DNA or only involve point mutations. Induced mutation is one of the most widely used techniques for creating additional variability in flower character. Mutagens introduce random changes throughout the genome generating a wide variety of mutations in all target genes and a single plant can contain a large number of different mutations resulting in manageable population sizes (Parry *et al.,* 2009). Exploiting natural or induced genetic diversity is a proven strategy to create novel variation particularly valuable in crops with restricted genetic variability due to inherent problem of reproductive sterility. Chemical mutagens usually cause point mutation but loss of a chromosome

segment or deletion can also occur (Thilagavathi and Mullainathan, 2011). Mutagens such as EMS act primarily on base pairs of DNA molecule and yield a higher number of gene mutations. Because of the basic mechanistic difference between these two groups of mutagens (physical and chemical), chemical mutagens are generally considered to be superior to physical mutagens for induction of mutation (Nilan and Konzak, 1961). Chemical mutagens most often only affect single nucleotide pairs. EMS creates a larger proportion of non-sense mutations involving the introduction of novel stop codons due to the specificity in creating G-A and C-T transitions and any individual mutation is therefore more likely to have a phenotypic effect (Parry *et al.,* 2009).

Iran, Kashmir, Spain, Morocco, Italy, Greece, Azerbaijan, Turkey and Afghanistan are the major traditional saffron (*Crocus sativus* L.) producers in the world. During 2013-2014, Jammu and Kashmir state produced 15 metric tonnes from 3715 ha with an average productivity of 4.1 kg ha^{-1} compared to 296.94 metric tonnes contributed by Iran (Sourthern Khorasan Province), Spain (Castile-La-Mancha region), Greece (Krokos Kozani region), Morocco (Taliouine), Turkey (Saffron Bolo), Italy (Aquila), Afghanistan (Hirat Province), Azerbaijan (Apsheron near Baku) with more than 89% contribution by Iran (Salwee *et al*., 2017). As against the global export value of 1,69,940 thousand $ during 2014, India's contribution was 1,850 thousand $ (Salwee *et al*., 2017). Saffron is a crop of great economic importance for J&K state. The improvement in saffron production can be brought about by following non-conventional methods which can help in the development of high yielding saffron varieties as hybridization is restricted in saffron due to triploidy (Salwee and Nehvi, 2014). Mutation breeding helps in the selection of elite lines from existing natural population and can create genetic blocks of great promise (Akhund-Zade. and Muzaferova, 1975). Therefore, the present study was carried out to induce genetic variability in a set of homozygous lines of natural population available with SKUAST-K using ethyl methane sulphonate (EMS) as a mutagen.

MATERIALS AND METHODS

The present investigation was carried out during the years 2012-13 and 2013-14 at Saffron Research Station, Pampore (Kashmir). A set of 220 homozygous lines were subjected to 3 doses of EMS *viz.,* 0.1, 0.15 and 0.2% during July 2012. The resultant 660 created lines were subjected to preliminary evaluation for variability among floral attributes and confirmed superiority of 64 created lines with proportion of 9 lines from 0.1% EMS created lines, 28 lines from 0.15% EMS created lines and 27 lines from 0.2% EMS created lines. These elite lines were evaluated along with base population of homozygous lines over two years in a randomized block design in plots of 0.18 m^2 size accommodating 20 corms in 5 ditches (4 corms $ditch^{-1}$) with inter- and intra-row spacing of 25 x 15 cm. Each treatment was replicated twice. All the recommended package of practices were adopted to raise a good crop (Nehvi *et al*., 2011).

Observations were recorded on plot basis in each replication for 4 floral attributes (fresh flower weight $plot^{-1}$, number of flowers $plot^{-1}$, fresh pistil weight $plot^{-1}$ and dry pistil weight $plot^{-1}$ in October-November 2012 (Y1) and 2013 (Y2). Three morphological attributes (number of radical leaves $plot^{-1}$, leaf length and leaf thickness were recorded in February; while four corm attributes (final corm weight $plot^{-1}$, big corm index $plot^{-1}$ (recorded as per cent weight of big corms ($\geq$ 8 g) from the total weight of all corms of a given line in May), multiplication index $plot^{-1}$ (recorded as the number of times the weight of initial planting material of a given line increased during each year on plot basis in May), flower raising index $plot^{-1}$ (recorded as total weight of corms necessary for the formation of maximum number of flowers at a given planting cycle of the line on plot basis in May) were recorded. Quality attributes *viz.,* picrocrocin, safranal and crocin were estimated in November as per ISO method (2010) using UV-VIS Spectrophotometer (model: UV5704SS of Electronic Corporation of India). The absorbance (D) at three wavelengths were recoded as follows:

$E1^{\%}$257 nm: absorbance at about 257 nm (maximum absorbance of picrocrocin)
$E1^{\%}$ 330 nm: absorbance at about 330 nm (maximum absorbance of safranal)
$E^{1\%}$ 440 nm: absorbance at about 440 nm (maximum absorbance of crocin)

$$E1\% = \frac{D \times 10000}{m \times (100 - H)}$$

Where, D is the specific absorbance; M is mass of the saffron sample (g); H is the moisture content of sample expressed as mass fraction

Statistical analysis was carried using standard statistical procedures (Johnson *et al*., 1955) for estimates of components of genetic variability and genetic divergence (Rao, 1952; Singh and Choudhary, 1985).

RESULTS AND DISCUSSION

Induced mutation is one of the most widely used techniques for creating additional variability in flower character. Exploiting natural or induced genetic diversity is a proven strategy for improvement of all major food crops and the use of mutagenesis to create novel variation is particularly valuable in crops with restricted genetic variability due to inherent problem of reproductive sterility. It is the technique utilized to improve the qualitative and quantitative traits in plants against various biotic and abiotic stresses. Chemical or physical mutagenesis have a number of advantages over such approaches owing to the fact that mutagens introduce random changes throughout the genome generating a wide variety of mutations in all target genes and a single plant can contain a large number of different mutations resulting in manageable population sizes (Parry *et al.*, 2009). Since clonal selections offer opportunity for the development of elite varieties; therefore, it is imperative to have a wide variability in a population under study so as to make selections effective. The present study over 2 years confirmed highest mutagenic frequency of 43.75% by 0.15% EMS mutagenic dose, followed by 0.2% EMS (42.18), whereas 0.1% EMS recorded least mutagenic frequency. Higher average effectiveness of EMS than γ-rays have also been reported (Tamina and Tabash, 2010). Among the floral attributes a desirable change was observed in 0.15% and 0.2% EMS created lines. Maximum number of flowers (47.290 $plot^{-1}$) associated with maximum fresh flower weight (1496.72 mg $plot^{-1}$), maximum fresh pistil weight (752.14 mg $plot^{-1}$) and maximum dry pistil weight (194.95 mg $plot^{-1}$) was observed in 0.15% EMS mutagenic lines confirming the effectiveness of this dose in saffron (Table 1). Important change in the mean values of various traits in treated group as compared to the base population indicates a lot of variability among created lines and thus fulfils the question of genetic conditionality of analyzed attributes to make selection effective (Agayev *et al*., 2009). Superiority of floral characters is attributed to maximum mean values for big corm index $plot^{-1}$ (32.61%), flower raising index (12.75 g $plot^{-1}$) and multiplication index (3.54) exhibited by 0.15% EMS created lines. However, lower dose of EMS (0.1%) resulted in maximum number of longer and thicker leaves.

On an average EMS created lines exhibited 28.34, 38.32, 39.96, 44.24, 9.81, 7.81, 3.06, 27.83, 46.02, 0.16 and 27.86% increase over base population with respect to the number of flowers, fresh flower weight, fresh pistil weight, dry pistil weight, number, length and thickness of leaves, final corm weight, multiplication index, big corm index and flower raising index, respectively. The study revealed that EMS acts primarily on base pairs of DNA and yields higher number of gene mutations leading to superior variants. Because of the basic mechanistic difference between these two groups of mutagens, chemical mutagens are generally considered superior to physical mutagens for induction of mutation. Similar results regarding the creation of new saffron variants for economic characters have previously been reported (Nehvi *et al*., 2007; Khan *et al*., 2011). The study indicates the presence of elite lines in the material created through exposure of saffron corms to EMS doses

Table 1: Estimates of mean and range for floral, foliar corm and quality attributes over years in base and selected elite lines of saffron (*Crocus sativus* L) at different concentrations of EMS

Characters	EMS treatments				EMS pooled	Increase (%)
	0.1%	0.15%	0.2%	Base population		
Flowers plot^{-1} (No.)	40.3±1.00 (32.7-48.5)	47.2±2.2 (23.0-62.2)	42.5±2.3 (27.2-64.0)	33.1± 1.3 (24.2-44.2)	42.5±2.1 (23.0-64.0)	28.3
Flower weight plot^{-1} (mg)	12238±345 (8172-16426)	14966±714 (4631-25645)	13584±847 (6511-2449)	9620±359 (5418-14921)	13307±701 (4631-25645)	38.3
Fresh pistil weight plot^{-1} (mg)	604.3±17.3 (454.0-841.2)	752.1±32.2 (161.2-1328.7)	630.9±26.9 (151.2-1289.7)	460.9±26.4 (236.0-938.0)	645.6±28.2 (151.2-1328.7)	39.9
Dry pistil weight plot^{-1} (mg)	158.0±4.1 (120.2-214.7)	194.9±7.2 (42.4-335.3)	167.3±6.3 (39.1-332.7)	116.7±5.0 (58.9-236.6)	168.4±6.2 (39.1-335.3)	44.2
Leaves plot^{-1} (No.)	320±6.0 (271.0-369.0)	317.6±8.1 (245.0-372.0)	286.7±7.5 (162.0-433.0)	272.9±11.0 (159.0-324.0)	299.9±8.4 (159.0-433.0)	9.8
Leaf length plot^{-1} (cm)	38.3±1.7 (31.0-43.7)	39.6±1.8 (29.7-48.0)	36.1±1.6 (28.2-49.2)	34.7±1.2 (27.5-40.7)	37.5±1.7 (27.5-49.2)	7.8
Leaf thickness plot^{-1} (mm)	1.1±0.0 (0.9-1.1)	1.0±0.0 (0.5-1.2)	0.9±0.0 (0.3-1.4)	0.9±0.0 (0.5-1.5)	1.0±0.0 (0.3-1.5)	3.0
Final corm weight plot^{-1} (g)	508.2±18.8 (354.4-696.5)	566.8±39.2 (352.0-747.8)	478.3±42.2 (274.0-755.2)	391.7±24.1 (256.4-545.6)	500.8±36.8 (256.4-755.2)	27.8
Big corm index plot^{-1} (%)	19.94±0.6 (15.0-25.6)	32.6±1.1 (14.4-58.9)	27.3±1.7 (13.4-59.8)	18.4±0.3 (14.5-25.7)	27.0±1.2 (13.4-59.8)	46.0
Flower raising index plot^{-1} (g)	12.4±0.6 (9.2-17.6)	12.7±1.3 (6.5-30.3)	11.2±1.1 (7.0-18.8)	12.0±0.8 (7.3-15.0)	12.0±1.1 (6.5-30.3)	0.1
Multiplication index plot^{-1}	3.17±0.1 (2.2-4.3)	3.54±0.3 (2.2-4.6)	2.9±0.2 (1.71-4.1)	2.4±0.1 (1.6-3.4)	3.1±0.2 1.6-4.7	27.8
Picrocrocin ($E^{1\%}$)	135.9±1.0 (113.5-164.8)	131.7±0.9 (94.9-169.0)	136.9±2.0 (98.5-189.2)	135.0±1.0 (111.6-166.9)	134.9±1.4 (94.9-18.5)	-
Safranal ($E^{1\%}$)	46.2±0.6 (34.7-77.2)	55.0±0.7 (31.8-84.5)	60.3±1.9 (34.7-161.6)	45.6±0.6 (33.6-76.5)	56.3±1.2 (31.8-161.6)	21.8
Crocin ($E^{1\%}$)	367.5±0.4 (326.5-441.0)	381.7±0.9 (340.5-458.9)	370.4±2.0 (344.8-434.8)	364.3±0.7 (323.5-440.6)	373.3±1.4 (326.5-458.9)	1.5

*Data within parenthesis depicts range associated with the character

@ 0.15% and 0.2%. The lines could be harnessed for development of high yielding varieties for commercial use. Among the quality attributes 0.2% created EMS lines recorded highest population mean for picrocrocin (136.96 $E^{1\%)}$) and safranal (60.34 $E^{1\%}$) with a range of 98.6-189.0 and 34.7-161.6, respectively; whereas for crocin 0.15% EMS lines showed highest population mean (381.7) with a range of 340.6-458.9. Base population was statistically at par with EMS created lines for picrocrocin, whereas for safranal and crocin EMS created lines showed an increase of 21.9 and 1.58% over base population for safranal and crocin, respectively. Similar findings have also been reported for temporal sub-population of saffron at global level (Nehvi *et al.*, 2006; Nehvi *et al.*, 2010; Khan *et al.*, 2011). Similar findings have been reported for temporal sub-population of saffron at global level (Nehvi *et al.*, 2010; Khan *et al.*, 2011). It is important to assess the relative magnitude of components of variability so as to use such information along with other selection parameters for improvement of plant types through adoption of effective breeding methods (Briggs and Knowels, 1967). Higher estimates of phenotypic variance than the corresponding genotypic variance were observed for all the traits, thereby revealing importance of environmental variance in the expression of the traits under study. An insight into Table 2 revealed that GCV was high (>20.0) for all EMS created lines and base population, except for number of flowers, number and length of leaves, final corm weight, big corm index, flower raising index, multiplication index, picrocrocin and crocin that recorded medium values of GCV. Similar medium values of GCV (10-20%) was recorded by 0.10%

Table 2: Estimates of genotypic coefficient of variation (GCV), heritability (h^2) and expected genetic gain (percent of mean) for floral, foliar, corm and quality attributes in base and EMS created lines of saffron (*Crocus sativus* L.) [data pooled over years]

Characters	GCV (%)					Heritability					Genetic gain (% of mean)				
	B.P.	0.1% EMS	0.15% EMS	0.2% EMS	Mean	B.P.	0.1% EMS	0.15% EMS	0.2% EMS	Mean	B.P.	0.1% EMS	0.15% EMS	0.2% EMS	Mean
Flowers plot^{-1}	18.7	12.18	24.2	28.7	26.82	0.90	0.92	0.92	0.93	0.93	36.8	24.1	47.9	57.1	53.46
Av. flower wt. (mg plot^{-1})	28.9	22.26	39.6	40.7	40.15	0.96	0.96	0.97	0.95	0.96	58.7	45.1	80.4	82.0	81.32
Fresh pistil wt. (mg plot^{-1})	42.3	21.6	43.7	48.7	46.28	0.96	0.96	0.98	0.98	0.98	85.7	43.97	89.3	99.7	94.50
Dry pistil wt. (mg plot^{-1})	40.3	22.3	43.8	47.9	46.05	0.97	0.97	0.98	0.98	0.98	82.2	45.4	89.5	98.1	94.25
No of leaves plot^{-1}	15.4	11.0	10.0	23.1	17.08	0.88	0.94	0.88	0.97	0.94	29.8	22.1	19.4	46.9	34.28
Av. leaf length plot^{-1} (cm)	10.4	11.1	11.4	16.2	13.79	0.80	0.74	0.75	0.86	0.80	19.3	19.8	20.5	31.1	25.55
Av. leaf thick-ness (mm plot^{-1})	26.7	5.2	16.5	32.0	23.70	0.97	0.81	0.76	0.98	0.93	54.3	9.6	29.9	65.5	47.11
Final corm wt. (g plot^{-1})	18.3	31.7	22.6	32.1	29.18	0.81	0.97	0.84	0.86	0.88	34.0	64.5	42.9	61.7	56.62
Big corm index (% plot^{-1})	19.2	19.2	43.4	53.6	49.62	0.97	0.94	0.98	0.97	0.98	39.2	38.4	88.8	109.1	101.34
Flower raising index (g plot^{-1})	18.3	31.7	22.6	32.1	29.18	0.81	0.97	0.84	0.86	0.88	34.0	64.5	42.9	61.7	56.63
Multiplication index plot^{-1}	18.3	31.7	22.6	32.1	29.20	0.81	0.97	0.84	0.87	0.88	34.0	64.5	42.8	61.8	56.66
Picrocrocin ($E^{1\%}$	13.5	8.8	14.5	13.4	13.28	0.99	0.98	0.99	0.97	0.98	27.7	18.2	29.8	27.4	27.19
Safranal ($E^{1\%}$)	27.8	22.8	27.1	42.3	34.50	0.99	0.99	0.99	0.98	0.99	57.2	46.9	55.6	86.6	70.77
Crocin ($E^{1\%}$)	10.7	8.0	8.7	6.4	8.32	0.99	0.99	0.99	0.98	0.99	22.0	16.5	18.0	13.2	17.11

B.P. = Base population

EMS created lines for the number of flowers, number and length of leaves and big corm index associated with low value of GCV (<10) for average leaf thickness, picrocrocin and crocin. The 0.15% EMS created lines depicted medium GCV values for the number, length and thickness of leaves while picrocrocin associated with low GCV values for crocin. The 0.2% EMS created lines recorded high values of GCV for all the traits, except for length of leaves, picrocrocin and crocin. High values of GCV for most of the attributes revealed that variability observed among mutagenic created lines was heritable in nature which was further confirmed by high heritability values (>60) for all these traits. High values of GCV have also been reported in saffron (Misra and Saini, 1988; Maqhdoomi, 2006; Nehvi *et al.*, 2007; Sheikh *et al.*, 2012). Medium to high GCV values recorded for base population for most of the traits revealed substantial variability already available which has been improved further through EMS mutagenic treatments. Results confirmed that clonal selection has a paramount significance for the identification of elite lines as most of the characters exhibited variability due to genetic causes and thus offers ample scope for improvement despite the fact that the two years could not be representative of random environment required to remove G x E interaction exclusively. The results are in agreement with the findings of Khan (2004), Maqhdoomi (2006) and Nehvi *et al.* (2006). High GCV values for most of the attributes exhibited by EMS created lines has contributed to high values of genetic gain as percent of mean (>20.0). Floral attributes exhibited by EMS created lines showed genetic gain ranging from 53.46% (No. of flowers plot^{-1}) to 94.5% (fresh pistil weight); whereas EMS created lines recorded genetic gain of 25.5 (average length of leaves) to 47.1% (average leaf thickness) for morphological attributes. For corm attributes, EMS lines exhibited genetic gain ranging from 56.6 (final corm weight) to 101.3% (big corm index). Highest value of genetic gain exhibited for big corm index revealed the importance of

Table 3: Distribution of elite mutant lines of saffron (*Crocus sativus* L.) created through different doses of EMS into clusters based on D^2 statistics

Cluster No.	No. of lines	Nomenclature
1	60	SS/EMS-3/96, SS/EMS-3/197, SS/EMS-3/75, SS/EMS-3/111, SS/EMS-3/82, SS/EMS-3/27, SS/EMS-4/82, SS/EMS-2/145, SS/EMS-4/7, SS/EMS-2/65, SS/EMS-4/149, SS/EMS-2/32, SS/EMS-4/140, SS/EMS-2/80, SS/EMS-2/19,SS/EMS-4/194, SS/EMS-3/99, SS/EMS-3/144, SS/EMS-3/19, SS/EMS-4/182, SS/EMS-3/68, SS/EMS-4/95, SS/EMS-3/107, SS/EMS-2/4, SS/EMS-4/93, SS/EMS-4/104, SS/EMS-4/142, SS/EMS-4/116, SS/EMS-3/93, SS/EMS-4/114, SS/EMS-4/103, SS/EMS-4/121, SS/EMS-2/156, SS/EMS-2/102, SS/EMS-4/129, SS/EMS-4/52, SS/EMS-4/16, SS/EMS-3/138, SS/EMS-3/165, SS/EMS-3/35, SS/EMS-3/103, SS/EMS-3/23, SS/EMS-4/102, SS/EMS-4/180, SS/EMS-3/171, SS/EMS-4/84, SS/EMS-3/187, SS/EMS-3/29, SS/EMS-3/133, SS/EMS-4/5, SS/EMS-3/104, SS/EMS-2/153, SS/EMS-4/173, SS/EMS-3/196, SS/EMS-4/40, SS/EMS-4/46, SS/EMS-4/98, SS/EMS-4/156, SS/EMS-3/168, SS/EMS-3/163
2	4	SS/EMS-3/113, SS/EMS-3/132, SS/EMS-3/130, SS/EMS-1/219
3	1	SS/EMS-4/32

this trait towards clonal selection. Similar results have been reported by Agayev *et al.* (2009). Low to medium GCV values for quality attributes, except safranal, contributed to medium to high genetic gain for quality attributes ranging from 17.1 (crocin) to 70.8% (safranal) [Table 2].

Divergence analysis grouped 65 lines including base population into 3 clusters with maximum number of lines in cluster-1 (60), followed by 4 lines in cluster-2 whereas cluster-3 accommodated EMS-4/32 as monogenotypic **(**Table 3). Most of the identified elite lines were grouped in cluster-1, except SS/EMS-4/32 that was grouped in cluster-3 and EMS-3/113, EMS-3/132, EMS-3/130 and EMS-1/219 grouped in cluster-2 recorded highest intracluster distance (610.5), followed by cluster-1 (307.3). Lines accommodated in cluster-2 (EMS-3/113, EMS-3/132, EMS-3/130 and EMS-1/219) and cluster-3 (EMS-4/32) depicted maximum intercluster distance (2753.1), followed by lines accommodated in cluster-1 and cluster-3 (1511.1) confirming their maximum genetic divergence (Table 4). EMS created lines accommodated in cluster-2 contributed towards maximum divergence for number of flowers, average flower weight, fresh and dry pistil weight, leaf biomass, final corm weight, big corm index, multiplication index and crocin; whereas cluster-1 and cluster-3 recorded maximum divergence for flower raising index, picrocrocin and safranal (Table 5).

Table 4: Average intercluster distance (above diagonal) amongst elite mutant lines of Saffron (*Crocus sativus* L.) created through different doses of EMS

Clusters	1	2	3
1	307.3	876.4	1511.1
2		610.5	2753.1
3			0

Table 5: Cluster mean for floral, morphological, corm and quality attributes in elite mutant lines of saffron created through different doses of EMS

Characters	Cluster-1	Cluster-2	Cluster-3
No of flowers plot^{-1}	42.39	48.31	31.75
Av. flower weight plot^{-1} (mg)	13219.2	16078.6	8523.0
Fresh pistil weight plot^{-1} (mg)	646.04	718.38	324.75
Dry pistil weight plot^{-1} (mg)	168.41	187.18	95.00
No of leaves plot^{-1}	299.90	316.0	237.00
Av. length of leaves plot^{-1} (cm)	37.38	42.19	28.25
Av. leaf thickness plot^{-1} (mm)	1.02	1.02	0.37
Final corm weight plot^{-1} (g)	499.55	558.21	362.00
Big corm index plot^{-1} (%)	26.67	36.06	14.50
Flower raising index plot^{-1} (g)	12.13	11.50	11.23
Multiplication index plot^{-1}	3.12	3.49	2.26
Picrocrocin ($E^{1\%}$)	135.06	129.33	151.30
Safranal ($E^{1\%}$)	55.55	45.17	161.60
Crocin ($E^{1\%}$)	369.37	446.75	362.43

The highest consideration needs to be given to those lines that produce largest number of flowers (basic crop) and simultaneously have bigger corms. These two attributes are most important selection criteria for elite lines. Based on these criteria, 12 EMS created lines *viz.*, SS/EMS-3/23, SS/EMS-3/27, SS/EMS-3/132, SS/EMS-3/138, SS/EMS-3/163, SS/EMS-3/165, SS/EMS-3/171, SS/EMS-3/187, SS/EMS-4/5, SS/EMS-4/46, SS/EMS-4/84 and SS/EMS-4/98 were identified as extraordinary lines on the basis of maximum values for the number of flowers, saffron yield and

Table 6: Contribution of different elite lines towards increase in floral, morphological, corm and quality attributes over base population in saffron

Characters	Per cent Increase	Lines
Floral and foliar		
No of flowers plot^{-1}	79.1	SS/EMS-3/23, SS/EMS-3/27, SS/EMS-3/132,
Average flower weight plot^{-1} (mg)	78.2	SS/EMS-3/138, SS/EMS-3/163, SS/EMS-
Fresh pistil weight plot^{-1} (mg)	57.8	3/165, SS/EMS-3/171, SS/EMS-3/187,
Dry pistil weight plot^{-1} (mg)	34.4	SS/EMS-4/5, SS/EMS-4/46, SS/EMS-4/84,
No of leaves plot^{-1}	18.1	SS/EMS-4/98
Av. length of leaves plot^{-1} (cm)	13.1	
Av. leaf thickness plot^{-1} (mm)	12.6	
Corm		
Final corm weight plot^{-1} (g)	42.5	SS/EMS-3/82, SS/EMS-3/96, SS/EMS-3/113
Big corm index plot^{-1} (%)	40.6	
Flower raising index plot^{-1} (g)	35.9	
Multiplication index plot^{-1}	29.0	
Quality		
Picrocrocin ($E^{1\%}$)	28.9	SS/EMS-2/35, SS/EMS-3/35, SS/EMS-4/156,
Safranal ($E^{1\%}$)	12.5	SS/EMS-4/180
Crocin ($E^{1\%}$)	11.8	

higher biomass recording a saffron yield ranging from 15.42 to 18.48 kg ha^{-1}. Similarly, lines SS/EMS-3/82, SS/EMS-3/96 and SS/EMS-3/113 were identified as elite on the basis of corm attributes. For quality attributes, lines SS/EMS-2/153, SS/EMS-3/35, SS/EMS-4/156 and SS/EMS-4/180 were identified elite for quality traits (Table 6). Amongst the floral and foliar attributes, elite lines on an average showed 79.1, 78.2, 57.8, 34.4, 18.1 and 12.6% superiority over base population for the number of flowers, average flower weight, fresh pistil weight, dry pistil weight, number and length of leaves and average leaf thickness, respectively. For corm attributes, elite lines showed superiority over base population for final corm weight (42.5%), big corm index (40.6%), flower raising index (35.9%) and multiplication index (29%). For quality attributes, superiority of elite lines was maximum to the tune of 28.9% for picrocrocin followed by safranal (12.46%) and crocin (11.8%).

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