



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(12): 275-279
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www.thepharmajournal.com
Received: 22-10-2019
Accepted: 24-11-2019

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Variability and genetic parameters evaluation for fertility restoration traits in safflower (*Carthamus tinctorius* L.) hybrids

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Abstract

The present investigation was undertaken with a view of identification of the putative restorers in safflower for the exploitation of high yielding hybrids. In cross combinations two cytoplasmic male sterile lines were tested with fifteen restorers in line x tester mating design. Restorers GMU-2952, GMU-3420 and GMU- 3715, exhibited high fertility restoration ability and per cent pollen fertility and could be incorporated in the hybridization programme for the development of high yielding safflower hybrids. The genotypic and phenotypic coefficient of variation for the number of flowers with normal type opening was 12.72 and 13.77, for character number of flowers with good anther dehiscence estimated as 12.54 and 14.06 and for the *per cent* pollen fertility was 10.95 and 11.60, respectively. These three traits used for the present study showed high heritability. Genetic components indicated the predominant role of the genotype of parents for governing the traits studied. Therefore, from the present experimental investigation it was inferred that, the three traits studied were found stable in performance due to minor role of environment in its expression.

Keywords: Safflower, CMS lines, fertility restoration, pollen fertility, GCV, PCV, heritability

Introduction

Groundnut, rapeseed-mustard, sesame, sunflower, safflower, Niger, castor, linseed and soybean are the most prime oilseed crop being grown in India. Amongst them, safflower has high potential value due to nutritional and pharmaceutical properties of seed oil and petals. Safflower oil is rich in PUFA (Linoleic acid 78%) which plays an important role in reducing blood cholesterol level, has good dyeing property and therefore, used in the manufacturing of paints, varnishes and linoleum. Safflower oil contains 55-81 percent linoleic acid, 7.42 percent oleic acid, 1-10 percent stearic acid and 1-10 percent palmitic acid with 90 percent unsaturated and 10 percent saturated fatty acids [3]. The seeds of safflower contain almost 27.5 percent oil, 15 percent protein, 41 percent crude fibre and 2.3 percent ash [6].

Major safflower producers are India, United States, Mexico, Kazakhstan, Argentina, China, Ethiopia and Australia. India has legitimate pride of being largest producer of safflower in the world. More than 60 countries of the world grow safflower, but over half is produced in India mainly for vegetable oil market [13]. Safflower acreage and production around the world has been witnessing wide fluctuations since last two decades. Hence, there is an urgent need not only to stabilize it but also to enhance the productivity of safflower, which can be achieved by developing high yielding hybrids. Heterosis breeding is an important way for improving crop productivity and quality in order to feed the ever-increasing human population, particularly in developing countries.

With the availability of male sterile sources in safflower [5], exploitation of heterosis on a commercial scale has become feasible and economical. The dominant and recessive male sterility system offered a feasible approach to hybrid development [11]. Five GMS based high yielding hybrids were released in India. DSH-129 developed at Directorate of Oilseeds Research, Hyderabad and MKH-11 of MAHYCO was first two hybrids released for commercial cultivation in 1997. The first non-spiny hybrid in India NARI-NH-1 was developed at NARI and released in 2001. For the easy and cost effective seed production, stable cytoplasmic male sterile lines are most important. Cytoplasmic genetic male sterility has been developed in USA and exploited for hybrid development. In India, a private seed company 'MAHYCO', released CGMS based hybrid MRSA-521 for commercial cultivation in 2006. However, there are certain problems in seed production in this hybrid.

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The attempt was made to develop CMS system at Oilseed Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. P.D.K.V.), Akola and succeeded in development of two CMS lines viz., AKS CMS 2A and AKS CMS 3A [4], which increases the ease of hybrid seed production considerably because, there is no need to rogue out 50 percent plants from female rows and hence, it is of great importance to plant breeders for exploitation of heterosis by developing high yielding hybrids involving CMS lines. However, there is an urgent need to search for a stable restorer giving perfect fertility in the CMS background. Hence, the present study was conducted to identify superior male parents with the high

genetic potential for fertility restoration in the newly developed CMS background so that new high yielding hybrids can be developed using CGMS system of safflower.

Material and Methods

Plant Material and population development

Genetically diverse fifteen plant materials were deliberately selected on the basis of their distinguishing characters to cross with two CMS lines (AKS CMS 2A and AKS CMS 3A developed at Oilseeds Research Unit, Dr. P.D.K.V., Akola) as shown in Table 1.

Table 1: Genotypes (parental material) and checks along with their source and general features

SN	Genotypes	Source	General features
A			Females
1	AKS CMS 2A/B	Dr. PDKV, Akola	Yellow petals turn to orange, spiny, 100 percent sterile, good floral opening
2	AKS CMS 3A/B	Dr. PDKV, Akola	White petals, spiny, 100 percent sterile, good floral opening
B			Males
1	GMU 3420	IIR, Hyderabad	Non spiny, bicapitulum, tall
2	GMU 5711	IIR, Hyderabad	Less spiny, tall, more numbers of seeds per capitulum
3	GMU 801	IIR, Hyderabad	Less branching, tall
4	GMU 3715	IIR, Hyderabad	Less branching, medium height, dark yellow flowers
5	GMU 5609	IIR, Hyderabad	Less branching, medium height, creamy colour flowers
6	GMU 2900	IIR, Hyderabad	Tall, highly spiny, white flowers
7	GMU 3164	IIR, Hyderabad	Short height, initially yellow flower convert into red at maturity, good branching
8	GMU 3923	IIR, Hyderabad	Medium height, more branching, initially yellow flower convert into light red at maturity
9	GMU 2952	IIR, Hyderabad	Dwarf, very low branching, white flowers
10	GMU 3638	IIR, Hyderabad	Good branching, medium height
11	AKS S/41	Dr. PDKV, Akola	Dwarf with basal branching
12	AKS 207 [also check]	Dr. PDKV, Akola	Early maturity with good yield potential
13	AKS 322	Dr. PDKV, Akola	Aphid tolerant genotype
14	AKS 325	Dr. PDKV, Akola	Tall, aphid tolerant, flower colour like 'Bhima'
15	AKDOR 1	Dr. PDKV, Akola	Tall, high yielding
C			Checks
1	AKS 207	Dr. PDKV, Akola	Early maturity with good yield potential
2	PKV Pink	Dr. PDKV, Akola	Late maturity, pink colour flowers at maturity, high oil content

IIR- Indian Institute of Oilseed Research, Hyderabad
PDKV-Panjabrao Deshmukh Krishi Vidyapeeth, Akola

The crosses were made based on a line x tester scheme for obtaining F₁ seeds of 30 crosses at Oilseeds Research Unit, Dr. P.D.K.V., Akola, during *rabi* 2013-14. Utilizing CMS system, only hand pollination using pollens from protected flowers of male parents was done in the protected flowers of CMS based females in the morning hours.

Experimental condition and data collection:

A field trial of 30 crosses along with 17 parents and standard check was conducted with three replications in Randomized Complete Block Design during *rabi* 2014-15. Each genotype was planted in a single row of 4 m length with 45 cm spacing between rows and 20 cm within rows. Complete package of practices was followed as per recommendations for safflower cultivation to raise healthy crop.

The observations were recorded on all the plants per plot per replication on the following traits related to fertility restoration.

1. A number of flowers with normal type opening: Visual observations for the floral opening were taken from fresh flowers of each plant i.e. "good type (normal type)" floral opening and "pinch type" floral opening which provides information about plant fertility or sterility, respectively and hence, was used as criteria.

2. A number of flowers with good anther dehiscence: An

equal number of flowers on each hybrid plant were screened for quality of dehiscence during the onset of flowering, full flowering and at end of flowering and plants were categorized as fertile and sterile on the basis of dehiscent and non-dehiscent, respectively.

3. Percent pollen fertility: The pollen fertility of every individual hybrid plants was determined, using 1 percent Potassium Iodide Iodine (KII) stain under a microscope. Pollen fertility from the dehiscent anther of fresh fully developed but un-opened flower bud was studied during 8.00 a.m. to 11.00 a.m. The anthers were squashed in 1 percent KII stain. The dark stained (orange colored) pollens were considered as fertile one. Whereas, unstained (yellow colored) and shrivelled pollen grains were considered as sterile. The pollen fertility was worked out on percent basis as per the formula is given below.

$$\text{Pollen fertility (\%)} = \frac{\text{Number of dark stained pollen grains}}{\text{Total number of pollens in microscopic field}} \times 100$$

Statistical analysis

Data were subjected to analysis of variance for mean performance as per the procedure [12], after its proper transformation. Further, the genotypic, phenotypic and environmental coefficient of variance were estimated [10],

whereas, genetic advance and heritability were estimated as per [8,7], respectively.

Results and Discussion

Analysis of variance for three different traits revealed highly significant differences suggesting the presence of considerable genetic variation in respect of all the three traits

studied (Table 2). The *per se* performance of each cross was worked out by taking the average of the observations recorded over the replications (Table 3). The plant fertility of different hybrids was assessed on the basis of floral opening and anther dehiscence. The restoration potential of different restorers was assessed based on the criteria of plant fertility and pollen fertility.

Table 2: Analysis of variance for the traits associated with fertility restoration in CMS backgrounds in safflower

Sources of variation	d.f.	Mean sum of squares		
		No. of flowers with 'normal type' opening	No. of flowers with good anther dehiscence	Per cent pollen fertility
Replications	2	12.5306	8.4966	3.6619
Treatments	29	231.6364**	238.501**	141.832**
Error	58	12.5176	18.7605	5.5656

(** Significant at P=0.01 probability level)

Table 3: Mean performance for various traits related with fertility restoration in CMS backgrounds of safflower

SN	Crosses	No. of flowers with 'normal type' opening (%)	No. of flowers with good anther dehiscence (%)	Per cent pollen fertility
1	AKS CMS 2A x GMU 3420	94.81 (76.90)	95.76 (78.36) ^{lm}	86.94 (68.82) ^{kl}
2	AKS CMS 2A x GMU 5711	67.78 (55.44) ^{ab}	71.48 (57.74) ^{abcd}	60.98 (51.36) ^{ab}
3	AKS CMS 2A x GMU 801	84.86 (67.77) ^{hij}	85.48 (67.72) ^{fghij}	78.89 (62.67) ^{ghi}
4	AKS CMS 2A x GMU 3715	94.56 (76.61) ^k	95.46 (77.77) ^{klm}	84.97 (67.29) ^{jkl}
5	AKS CMS 2A x GMU 5609	81.39 (64.53) ^{efghi}	82.94 (65.62) ^{efghi}	76.68 (61.23) ^{fghi}
6	AKS CMS 2A x GMU 2900	77.34 (61.61) ^{cdefg}	79.09 (63.01) ^{defg}	72.52 (58.45) ^{def}
7	AKS CMS 2A x GMU 3164	83.18 (65.84) ^{fghij}	84.14 (66.55) ^{efghi}	76.23 (60.88) ^{fgh}
8	AKS CMS 2A x GMU 3923	69.69 (56.72) ^{abc}	71.04 (57.61) ^{abcd}	62.71 (52.37) ^{abc}
9	AKS CMS 2A x GMU 2952	97.51 (81.05) ^{kl}	98.09 (82.05) ^{mn}	91.14 (72.79) ^m
10	AKS CMS 2A x GMU 3638	74.31 (59.61) ^{bcde}	75.56 (60.49) ^{bcde}	67.64 (55.36) ^{cd}
11	AKS CMS 2A x AKS S/41	85.99 (68.08) ^{hij}	86.54 (68.48) ^{fghij}	79.62 (63.31) ^{ghi}
12	AKS CMS 2A x AKS 207	88.50 (70.37) ^j	89.10 (70.80) ^{ijk}	68.89 (56.10) ^{cde}
13	AKS CMS 2A x AKS 322	75.93 (60.84) ^{bcdef}	77.24 (61.65) ^{cdef}	72.39 (58.31) ^{def}
14	AKS CMS 2A x AKS 325	84.82 (67.21) ^{ghij}	89.70 (74.57) ^{ijkl}	66.07 (54.39) ^{bc}
15	AKS CMS 2A x AKDOR 1	88.87 (70.73) ^j	90.04 (71.77) ^{ijkl}	82.09 (65.02) ^{ijk}
16	AKS CMS 3A x GMU 3420	97.63 (81.28) ^{kl}	98.19 (82.27) ^{mn}	91.29 (73.11) ^m
17	AKS CMS 3A x GMU 5711	67.66 (55.37) ^{ab}	68.53 (55.89) ^{abc}	61.06 (51.41) ^{ab}
18	AKS CMS 3A x GMU 801	61.36 (51.62) ^a	62.09 (52.01) ^a	74.49 (59.68) ^{efg}
19	AKS CMS 3A x GMU 3715	95.56 (77.90) ^k	96.02 (78.75) ^{lm}	89.51 (71.11) ^{lm}
20	AKS CMS 3A x GMU 5609	95.04 (77.18) ^k	95.40 (77.69) ^{klm}	88.79 (70.64) ^{lm}
21	AKS CMS 3A x GMU 2900	78.67 (62.52) ^{defgh}	79.72 (63.29) ^{defgh}	71.58 (57.79) ^{def}
22	AKS CMS 3A x GMU 3164	82.82 (65.55) ^{fghij}	83.55 (66.19) ^{efghi}	76.42 (60.97) ^{fgh}
23	AKS CMS 3A x GMU 3923	69.95 (56.84) ^{abcd}	71.48 (57.87) ^{abcd}	63.63 (52.91) ^{abc}
24	AKS CMS 3A x GMU 2952	98.76 (84.78) ^l	98.81 (86.37) ⁿ	91.97 (73.61) ^m
25	AKS CMS 3A x GMU 3638	86.15 (68.16) ^{hij}	87.47 (69.39) ^{ghij}	79.85 (63.34) ^{ghi}
26	AKS CMS 3A x AKS S/41	86.19 (68.53) ^{ij}	85.85 (68.34) ^{fghij}	79.19 (62.94) ^{ghi}
27	AKS CMS 3A x AKS 207	86.18 (68.23) ^{hij}	87.05 (69.39) ^{ghij}	78.55 (62.46) ^{ghi}
28	AKS CMS 3A x AKS 322	87.88 (69.72) ^{ij}	87.97 (70.10) ^{hij}	81.53 (64.56) ^{hij}
29	AKS CMS 3A x AKS 325	63.92 (53.12) ^a	65.17 (53.88) ^{ab}	58.04 (49.63) ^a
30	AKS CMS 3A x AKDOR 1	88.06 (70.43) ^j	89.42 (71.19) ^{ijk}	81.47 (64.51) ^{ghij}
General mean		83.18 (67.15)	84.28 (68.23)	76.50 (61.57)
Range		51.62 to 84.78	52.01 to 86.37	49.62 to 73.61
SE (m) ±		2.04	2.50	1.36
LSD Value		5.78	7.08	3.86

*Values shown in parenthesis are transformed values

The range for the trait number of flowers with normal type opening was 61.30 (AKS CMS 3A x GMU 801) to 98.75 (AKS CMS 3A x GMU 2952) *per cent* (Table 3). Out of 30 crosses, the floral opening of seven crosses was more than 90 percent, from which the crosses i.e. AKS CMS 3A x GMU 2952, AKS CMS 3A x GMU 3420 and AKS CMS 2A x GMU 2952 showed the highest number of flowers with normal type opening. Whereas, 13 crosses exhibited 'normal type' flower opening with a range of 80 to 90 percent and remaining crosses exhibited less than 80 percent 'normal type' floral opening. Out of the remaining crosses six crosses

showed very poor floral opening i.e. below 70 percent i.e. AKS CMS 2A x GMU 5711, AKS CMS 2A x GMU 3923, AKS CMS 3A x GMU 5711, AKS CMS 3A x GMU 801, AKS CMS 3A x GMU 3923 and AKS CMS 3A x AKS 325. The cross AKS CMS 3A x GMU 2952 was significantly superior for the trait number of flowers with normal type opening over rest of the crosses except two crosses *viz.*, AKS CMS 2A x GMU 2952 and AKS CMS 3A x GMU 3420. Out of these three crosses *viz.*, AKS CMS 3A x GMU 2952, AKS CMS 2A x GMU 2952 and AKS CMS 3A x GMU 3420, two crosses involved same male parent i.e. GMU 2952. It

indicates high genetic potential of male parent *viz.*, GMU 2952 for the trait number of flowers with normal type opening in CMS background followed by other two male parents *viz.*, GMU 3420 and GMU 3715. Though these three male parents were found to be significantly superior to others, but they are at par with each other.

Range of the character, number of flowers with dehiscent anther was 62.14 (AKS CMS 3A x GMU 801) to 98.72 (AKS CMS 3A x GMU 2952) percent. Out of 30 crosses, the anther dehiscence of eight crosses was more than 90 percent, out of which the crosses AKS CMS 3A x GMU 2952 followed by AKS CMS 3A x GMU 3420, AKS CMS 2A x GMU 2952 and AKS CMS 2A x GMU 3420 showed significantly superior performance for anther dehiscence capacity, but, they were found to be at par with each other. Further, 12 crosses exhibited good anther dehiscence (range 80 % to 90 %) and remaining crosses exhibited less than 80 percent anther dehiscence, out of the remaining crosses six crosses showed very poor anther dehiscence (below 75 %) *i.e.* AKS CMS 2A x GMU 5711, AKS CMS 2A x GMU 3923, AKS CMS 3A x GMU 5711, AKS CMS 3A x GMU 801, AKS CMS 3A x GMU 3923 and AKS CMS 3A x AKS 325. The male parent GMU 2952 and GMU 3420 were found to possess the good genetic potential to express the trait of anther dehiscence in CMS backgrounds.

The trait pollen fertility was ranged from 58.14 (AKS CMS 3A x AKS 325) to 92.00 (AKS CMS 3A x GMU 2952) percent. Out of 30 crosses, pollen fertility of three crosses were more than 90 percent *i.e.* AKS CMS 3A x GMU 2952 followed by AKS CMS 2A x GMU 2952 and AKS CMS 3A x GMU 3420 which were found to be significantly superior over the rest of other crosses, however, at par with each other. Seven crosses exhibited pollen fertility (range 80 % to 90 %), remaining crosses exhibited less than 80 percent pollen fertility, out of the remaining crosses five crosses showed very poor pollen fertility (below 65 %) *viz.*, AKS CMS 2A x GMU 5711, AKS CMS 2A x GMU 3923, AKS CMS 3A x GMU 5711, AKS CMS 3A x GMU 3923 and AKS CMS 3A x AKS 325.

The crosses exhibited more than 90 percent 'normal type' flower opening along with very good anther dehiscence capacity (above 90 %) were considered as fertile with high pollen fertility. Four strong restorers *viz.*, GMU 2952, GMU 3420, GMU 3715 and GMU 5609 were identified with high fertility restoration capacity on the basis of best floral opening and anther dehiscence potential under both CMS backgrounds. Further, the crosses exhibited more than 85

percent pollen fertility was considered as fertile with highest pollen fertility. Strong restorer male parents that contain good fertility restoration capacity were identified *viz.*, GMU 2952 (91.21 % & 92.00 %), GMU 3420 (86.83 % & 91.18 %) and GMU 3715 (85.16 % & 89.51 %) on the basis of percent pollen fertility of the crosses under both the CMS backgrounds *i.e.* AKS CMS 2A and AKS CMS 3A. The 77 restorer lines (R-lines) identified, which could restore 100 percent male fertility on various CMS backgrounds in safflower [1, 2]. The 8 fertility restorer lines (R lines) were identified and evaluated by producing the hybrid combinations and found that the restorer lines *i.e.* AKS 11-2 R, AKS 8 R and AKS 9-1 R restored the fertility 92 percent, 88.23 percent and 85 percent, respectively, in CMS line *i.e.* AKS CMS 2A [4]. In the study conducted by earlier workers, various characters associated with fertility restoration have not been studied. However, in the present investigation, different traits associated with fertility restoration have been studied. On the basis of all the traits studied, another three suitable restorers have been identified having more than 95 percent fertility in the CMS background. Three common restorers for CMS line derived from different sources was identified in pigeon pea [14] also, identified good restorers for CMS lines in pigeon pea [9]. Studies for the identification of restorers in safflower indicated the availability genes for fertility restoration in CMS background. However, it is necessary to identify such a fertility restorers which should result in to perfect fertility whenever exploited development of hybrids for commercial cultivation in variable environmental conditions.

The estimates of the genetic coefficient of variance (GCV) were maximum for a number of flowers with normal type opening (Table 4) followed by number of flowers with good anther dehiscence and percent pollen fertility. However, the phenotypic coefficient of variance (PCV) was found maximum for number of flowers with good anther dehiscence followed by number of flowers with normal type opening and percent pollen fertility. High heritability was recorded for all the traits studied. The magnitude of heritability was maximum for the trait percent pollen fertility followed by number of flowers with normal type and number of flowers with good anther dehiscence. The estimates of genetic advance for the trait number of flowers with normal type opening were highest followed by number of flowers with good anther dehiscence and percent pollen fertility. Further, the correlations between these traits were found to be highly significant (Table 5).

Table 4: Genetic parameters for the traits related with fertility restoration in CMS backgrounds of safflower

SN	Crosses	No. of flowers with 'normal type' opening (%)	No. of flowers with good anther dehiscence (%)	Per cent pollen fertility
1	Genotypic variance (σ^2_g)	73.03	73.24	45.42
2	Phenotypic variance (σ^2_p)	85.55	92.01	50.99
3	Environmental variance (σ^2_e)	12.51	18.76	5.57
4	Genotypic Coefficient of Variance (GCV) (%)	12.72	12.54	10.95
5	Phenotypic Coefficient of Variance (PCV) (%)	13.77	14.06	11.60
6	Environmental Coefficient of Variance (ECV) (%)	5.27	6.34	3.83
7	Heritability (h^2) % (Broad Sense)	85.40	79.60	89.10
8	Genetic Advance (5% Selection Intensity) (%)	16.27	15.73	13.10

Table 5: Correlations among the traits related with fertility restoration in CMS backgrounds of safflower

Crosses	No. of flowers with 'normal type' opening (%)	No. of flowers with good anther dehiscence (%)	Per cent pollen fertility
No. of flowers with 'normal type' opening (%)	--	0.99**	0.88**
No. of flowers with good anther dehiscence (%)	--	--	0.85**

** Significant at 1 % level of significant "r" value from table at n-2 d.f. = 0.6785

The genetic components suggest that all the traits studied are mostly governed by genotype of an individual and have the minor role of environment in determining its phenotype; hence, the restorers are expected to give a stable performance in the CMS background for fertility restoration.

Fifteen male parents were used in two different CMS backgrounds (females) viz., AKS CMS 2A and AKS CMS 3A. However, only three male parents viz., GMU 2952, GMU 3420 and GMU 3715 with highest restoration capacity in both CMS backgrounds have been identified on the basis of floral opening, anther dehiscence and percent pollen fertility. Hence, it is suggested that these three male parents can be used for diversification of different superior male parent in the development of hybrids based on CMS lines. Further, these restorers may be used for further breeding programme for development of hybrids based on CMS backgrounds.

Conclusion

The present investigation revealed that three restorers, GMU 2952, GMU 3420 and GMU 3715, exhibited high fertility restoration ability and percent pollen fertility and could be incorporated in further breeding programme for the development of high yielding hybrids in safflower. The genotypic and phenotypic variation revealed high heritability among the genotypes for the studied characters. Further study needs the molecular investigation of fertility restorer genes (*Rf*) which plays important role in fertility restoration.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical standards: This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions: IA and VLG conceived and designed the study. IA acquired the data and wrote the primary draft of the manuscript. VLG analysed and interpreted the data and wrote the final manuscript draft.

Acknowledgement

The authors are grateful to Dr. PDKV, Akola and Indian Institute of Oilseed Research, Hyderabad for providing safflower seed material to undertake the present investigation. We are grateful to all research scientists of Agricultural Botany Department for their cooperation and support during this investigation.

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