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GENETIC VARIABLITY STUDIES IN M_5 GENERATION OF MUSTARD

Prakash Hosur[1], Beena Nair[2], Shailendra Sagar Prajapati[3], and Pavan Chavan[4]

ABSTRACT

The present study was conducted with the objectives to estimate genetic variability of the selected mutants based on morphological characters and selection of superior mutants from M_5 generation of mustard at AICRP on Linseed and Mustard farm of College of Agriculture Nagpur during *rabi* 2018 in M_5 generation. In *rabi* 2017, 26 mutants along with four checks (Bio 902, Pusa bold, Kranti and Shatabdi) were evaluated in M_5 generation in three replications. Data were recorded on days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seed yield plant^{-1}, 1000 seed weight, number of seeds siliqua^{-1} and length of siliqua. The data on the analysis of variance resulted in highly significant mean squares due to between family for nine characters studied. Estimation of genetic parameters revealed the importance for selection of superior mutants based on GCV, PCV, h^2 and genetic advance. Thus, 8 mutants were selected from 26 mutants of M_4 generation. All these mutants will be forwarded to multilocational trials to identify the best mutants. Based on diversity studies using SSR markers five mutants were found diverse from the check variety, Bio 902 and Kranti.

(Key words: *Brassica juncea*, variability, mutation, heritability)

INTRODUCTION

Indian mustard (*Brassica juncea*) is called as "rai", "raya" or "laha" is one of the important oilseed crops belonging to family Cruciferae *(Syn. Brassicaceae)* and genus *Brassica.* Indian mustard or brown mustard *[Brassica juncea* Czern & Coss] genome content AABB is a natural amphidiploid (2n=36) of *Brassica campestris* (2n = 20) and *Brassica nigra* (2n = 16). Mustard is largely self pollinated crop but certain amount (5 -18%) of cross pollination may take place. *Brassica juncea* is second most important edible oilseed crop in India after groundnut and accounts for about 30% of the total oilseeds produced in the country. Indian mustard is cultivated in the states of Punjab, Rajasthan, Uttar Pradesh (UP), Assam, Gujarat, Haryana, Madhya Pradesh (MP), and West Bengal (WB) as a *rabi* crop.

Homozygosity is attained in M_5 generation predominantly self pollinated crops. The present study was thus undertaken to estimate the variability in M_5 generation, using the mutants selected in M_4 generation of Bio 902 and Pusa bold variety of mustard using two mutagenic agents i.e gamma rays alone and gamma rays in combination with EMS. The M_1 generation was raised during 2015-16 and individual plants in each treatment were harvested separately. The harvested seeds were used to raise M_2 generation. The mutants identified in M_2 were harvested separately to grow M_3 generation in 2017-18.

MATERIALS AND METHODS

Dry healthy seed of *Brassica juncea,* Pusa bold and BIO 902 treated with gamma rays and EMS. The gamma rays treatment of 900, 1000, 1100, 1200, 1300 Gy (^{60}Co) was done at BARC Trombay, Mumbai. Each of these treatments were treated with 0.5% aqueous solution of EMS.

26 high yielding mutants from M_4 generation were raised in 2018-19 in randomized block design with three replications for assessment along with 4 checks (Pusa Bold, BIO-902, Kranti, Shatabdi) at AICRP on linseed and mustard farm, College of Agriculture. Nagpur (Table 1).

Observations were recorded on days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seed yield plant^{-1}, 1000 seed weight, number of seeds siliqua^{-1} and length of siliqua for estimation of coefficient of variation, standard error, critical difference, mean, range, genotypic variance, phenotypic variance, genotypic and phenotypic coefficient of variation, heritability percentage and genetic advance. SSR marker polymorphism was conducted using 20 SSR markers. DNA extraction was done using CTAB method. DNA purification was done using phenol:chloroform and

1, 3 and 4. P.G. Students, Botany section, College of Agriculture, Nagpur
2. Linseed breeder, AICRP on Linseed and Mustard, College of Agriculture, Nagpur

DNA quantification using spectrophotometer. PCR was performed in the thermo cycler. All SSR fragments were scored manually. Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of DARwin (Perrier and Jacquemoud-Collet, 2006). The robustness of each dendrogram was evaluated by bootstrap analysis.

RESULTS AND DISCUSSION

The data on the analysis of variance resulted in highly significant mean squares due to between family for nine characters studied i.e. plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seed yield plant^{-1} 1000 seed weight, siliqua length and seed siliqua^{-1}, days to maturity and days to 50% flowering (Table 2). Significant differences were observed between the genotypes for all nine characters studied. In accordance to these results, significant variability between the families were also reported by Pawar *et al.* (2018) in mustard.

The coefficient of variation (CV) ranged from 2.17% to 38.21% for various characters (Table 3). The low coefficient of variation (d" 20%) was observed for the characters seed siliqua^{-1} (13.44%), plant height (12.12%), siliqua length (11.10%), 1000 seed weight (10.24%), days to 50% flowering (4.61%), days to maturity (2.17%), and which showed the best genetic potential and its genetic influence. High coefficient of variation (> 20%) was observed for seed yield plant^{-1} (38.21%), number of siliqua plant^{-1} (33.83%), number of primary branches plant^{-1} (24.58%) which indicated more influence of environmental fluctuation.

The grand mean recorded for various characters of 26 genotypes including 4 checks were found to be 49.99 days for days to 50% flowering, 104.17 days for maturity, 180.03 cm for plant height, 5.76 for number of primary branches plant^{-1}, 192.64 for number siliqua plant^{-1}, 8.43 for seed yield plant^{-1} and 5.24 for 1000 seed weight, 4.50 for length of siliqua, 9.19 for number of seeds siliqua^{-1} (Table 2). Wide range of variation was exhibited for number of siliqua plant^{-1} (444), plant height (140), seed yield plant^{-1} (18.8), days to maturity (14) and days to 50% flowering (13) whereas number of primary branches plant^{-1} (8), 1000 seed weight (3.4), number of seeds siliqua^{-1} (3), length of siliqua^{-1} (2.43) exhibited low range of variation.

High genotypic coefficient of variation was observed for seed yield plant^{-1} (27.48%). Number of siliqua plant^{-1} (15.79%) and 1000 seed weight (11.27%) possessed moderate genotypic coefficient of variation while number of primary branches plant^{-1} (7.45%), plant height (5.78%), length of siliqua (3.89%) and number of seeds siliqua^{-1} (3.15%),days to 50% flowering (3) and days to maturity (1.59) exhibited low genotypic coefficient of variation. In accordance with these results, high and low genotypic coefficient of variation for seed yield and days to maturity were also reported by Rameeh *et al. (*2015).

High phenotypic coefficient of variation was observed for seed yield plant^{-1} (47.07%), number of siliqua plant^{-1} (36.14%) and number of primary branches plant^{-1} (25.68%). Moderate phenotypic coefficient of variation were observed for 1000 seed weight (15.23%), number of seeds siliqua^{-1} (13.80%), plant height (13.42%) and length of siliqua (11.77%), and low phenotypic coefficient of variation for days to 50% flowering (5.49%) and days to maturity (2.69%).

Heritability per cent ranged from 54.80% (1000 seed weight) to 5.21% (number of seeds siliqua^{-1}). High heritability was observed for 1000 seed weight (54.80%), moderate heritability was observed for days to maturity (35.07%) seed yield plant^{-1} (34.09%), and low heritability was observed for days to 50% flowering (29.75%), plant height (18.52%), number of siliqua plant^{-1} (12.35%), length of siliqua (10.96%), number of primary branches plant^{-1} (8.41%) and number of seeds siliqua^{-1}(5.21%). High heritability for 1000 seed weight, yield plant^{-1}, days to maturity and 50% flowering were reported by Kumar *et al.* (2012) in mustard.

Genetic advance as a percentage of mean was observed highest for seed yield plant^{-1} (28.24%), moderate for 1000 seed weight (14.69%) and low for number of siliqua plant^{-1} (7.85%), days to maturity (7.82%), plant height (4.37%), number of primary branches plant^{-1} (3.80%), days to 50% flowering (2.88%), length of siliqua (2.27%), number of seeds siliqua^{-1} (1.27%) and days to maturity (1.66%). Similar to these results, moderate to low genetic advance as a percentage of mean was also reported by Kumar *et al.* (2012) and Anup *et al.* (2018) in mustard.

When the genetic parameters for all nine characters were considered, it was found that seed yield plant^{-1}exhibited high genotypic coefficient of variation and phenotypic coefficient of variation, moderate heritability along with high genetic advance as a percentage of mean. Number of siliqua plant^{-1} exhibited moderate genotypic coefficient of variation and high phenotypic coefficient of variation, low heritability with low genetic advance as a percentage of mean. Number of primary branches plant^{-1} exhibited low genotypic coefficient of variation and high phenotypic coefficient of variation, low heritability with low genetic advance as a percentage of mean. Low genotypic and moderate phenotypic coefficient of variation along with low heritability and low genetic advance was observed for plant height, number of seeds siliqua^{-1} and length of siliqua. Low GCV, PCV with moderate heritability and low genetic advance was observed for days to maturity. Moderate GCV, PCV, heritability and genetic advance was observed for 1000 seed weight where as low GCV, PCV, heritability and genetic advance was observed for days to 50% flowering. This indicated that seed yield plant^{-1} exhibited moderate heritability with moderate genetic advance and were influenced by additive gene action in their traits in M_5 generation and helps as a criteria for selection. A character exhibiting high broad sense heritability might not necessarily give high genetic advance. Therefore, selection should not be based solely on heritability (broad sense) but due consideration should be given to genetic advance as well.

Table 1. Pedigree of advanced mutants lines used in the study

Sr.No.	Name of mutant	Pedigree of mutant	Character of mutant
1.	ACNMM1	1000 GY M-2-7-5-1	Bold seed
2.	ACNMM2	1000 GY M-2-7-5-2	Bold seed
3.	ACNMM3	1100 GY M-3-104-23-3	High yield
4.	ACNMM4	1100 GY M-3-104-23-4	High yield
5.	ACNMM5	1200 GY M-4-12-41-1	Bold seed
6.	ACNMM6	1300 GY M-5-17-11-1	High yield
7.	ACNMM7	1300 GY M-5-17-3-5	Long siliqua
8.	ACNMM8	1300 GY M-5-18-31-1	High yield
9.	ACNMM9	1300 GY M-5-18-31-4	High yield
10.	ACNMM10	1300 GY M-5-107-28-5	High yield
11.	ACNMM11	1300 GY M-5-107-34-3	High yield
12.	ACNMM12	(900 GY+EMS)M-6-109-12-8	Appressed
13.	ACNMM13	(1200 GY+EMS)M-9-35-60-1	More branches
14.	ACNMM14	(1200 GY+EMS)M-9-38-38-4	Bold seed
15.	ACNMM15	(1300 GY+EMS)M-10-44-34-5	Bold and early
16.	ACNMM16	900 GY M-11-47-14-5	Early
17.	ACNMM17	900 GY M-11-51-36-1	High yield
18.	ACNMM18	900 GY M-11-51-36-2	High yield
19.	ACNMM19	900 GY M-11-51-36-6	High yield
20.	ACNMM20	1100 GY M-12-60-23-12	High yield
21.	ACNMM21	1300 GY M-15-70-47-15	Appressed
22.	ACNMM22	1300 GY M-15-68-51-5	Bold seed
23.	ACNMM23	1300 GY M-15-68-51-7	Bold seed
24.	ACNMM24	1300 GY M-15-68-51-12	Bold seed
25.	ACNMM25	(900 GY+EMS)M-16-74-34-11	Appressed
26.	ACNMM26	(1300 GY+EMS)M-19-126-60-14	Bold seed
27.	BIO-902	Check	
28.	Pusa Bold	Check	
29.	Kranti	Check	
30.	Shatabdi	Check	

Table 2. Analysis of variance for different characters in M_5 generation

Source of variation	df	Mean sum of square								
		Days to 50% flowering	Days to maturity	Plant height (cm)	Number of primary branches plant^{-1}	Number of siliqua plant^{-1}	Length of siliqua (cm)	Number of seeds siliqua^{-1}	Seed yield plant^{-1} (g)	1000 seed weight (g)
Between families	29	12.03**	13.36**	2097.83**	4.77**	13223.59**	0.71**	2.78**	90.96**	0.98**
Within families	420	5.30	5.10	475.85	2.0	4248.30	0.25	1.52	10.38	0.29
Intra class correlation(t)	0.110	0.298	0.351	0.185	0.084	0.123	0.110	0.052	0.548	

*significant at 5% level, **significant at 1% level

Table 3. Genetic parameter estimates for different characters in M_5 generation

Parameter	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of primary branches plant^{-1}	Number of siliqua plant^{-1}	Length of siliqua (cm)	Number of seeds siliqua^{-1}	Seed yield plant^{-1} (g)	1000 seed weight (g)
CV (%)	4.61	2.17	12.12	24.58	33.83	11.10	13.44	38.21	10.24
S.E(m) ±	1.63	1.60	15.42	1.0	46.09	0.35	0.87	2.28	0.38
C.D (5%)	4.60	4.52	42.87	2.78	128.10	0.98	2.43	6.33	1.09
Mean	49.99	104.17	180.03	5.76	192.64	4.50	9.19	8.43	5.24
Range	59 - 46 (13)	107 - 93 (14)	268 – 128 (140)	11 – 3 (8)	492 - 48 (444)	5.43 – 3 (2.43)	11 – 8 (3)	19.8 - 1 (18.8)	6.4 - 3 (3.4)
Genotypic variance	2.24	2.75	108.13	0.18	598.35	0.03	0.08	5.37	0.35
Phenotypic variance	7.54	7.85	583.98	2.19	4846.65	0.28	1.61	15.76	0.64
GCV (%)	3	1.59	5.78	7.45	12.70	3.89	3.15	27.48	11.27
PCV (%)	5.49	2.69	13.42	25.68	36.14	11.77	13.80	47.07	15.23
Heritability(%)	29.75	35.07	18.52	8.41	12.35	10.96	5.21	34.09	54.80
G.A	1.44	1.73	7.88	0.22	15.13	0.10	0.12	2.38	0.77
G.A (as % of mean)	2.88	1.66	4.37	3.80	7.85	3.80	1.27	28.24	14.69

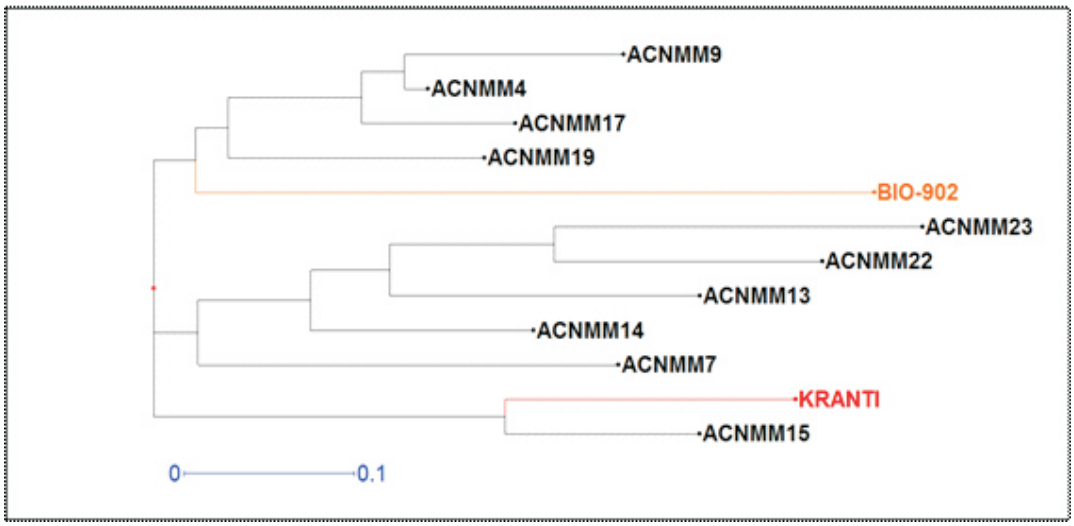
Ten most promising mutants out of 26 mutants of M_4 generation were utilised for molecular analysis to investigate the genetic relationships and diversity among them.

Assessment of diversity was done for 10 selected mutants. Result suggested that there are 3 clades. First clade consists of four mutant lines along with Bio 902. Second clade consists of 5 mutant lines without any checks, whereas third clade consists of one mutant with the check variety Kranti (Fig. 1).

The mutants were grouped into three major clusters at 0 similarity coefficient. Cluster 1 had the genotypes viz., ACNMM 9, ACNMM 4, ACNMM 17 and ACNMM 19 along with check variety BIO-902 indicating these mutant lines were closely related to BIO-902. Similarly, genotypes ACNMM 23, ACNMM 22, ACNMM 13, ACNMM 7 and ACNMM 14 were separated into individual clusters indicating that these mutant were neither related to any of the given parents and checks considered and were found independent. The cluster 3 consisted of one check variety Kranti and ACNMM 15 which indicates ACNMM 15 is closely related to check variety Kranti. Similar work was also conducted by Fayyaz *et al.* (2014). They depicted eight main groups using similarity coefficient of 0.70 through UPGMA clustering of F_2 progenies.

In the present study nine characters viz., seed yield plant^{-1}, number of siliqua plant^{-1}, number of branches plant^{-1}, 1000 seed weight, plant height, days to flowering and days to maturity, number of seeds siliqua^{-1}, length of siliqua were showed varying GCV, heritability and genetic advance as percentage of mean were considered for selection in M_5 generation. Eight superior mutants were selected from 26 mutants in M_5 generation.

Fig.1. Dendrogram derived from banding pattern of SSR marker analysis of 10 mutants and 2 parents



Selection of superior mutants was one of the main objectives of this experiment. Mutants were selected on the basis of seed yield and number of siliqua plant^{-1} as selection only on the basis of seed yield may not be effective and selection on the basis of large number of component characters become cumbersome. 8 superior mutants were selected from 26 mutants of M_4 generation which will be forwarded in multilocation yield trials to identify the best mutants. All these eight mutants which were selected from M_5 generation will be forwarded for one or more generation so that homozygosity will be attained and the superior genotypes can be selected for forwarding to yield trials in further generation.

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