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Research Article

Mutation induced phenotypic variation for yield traits in sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract
Sorghum is an important cereal crop, cultivated both in *kharif* and *rabi* seasons. Especially *rabi* sorghum is known for their grain quality and used exclusively for human consumption. The present study was undertaken to induce variability through physical and chemical mutagens for morphological and yield-contributing traits. A popular *rabi* sorghum variety, Parbhani Moti was initially irradiated with gamma rays (100-400 Gy), ethyl methane sulphonate (0.1- 0.4% EMS) and their combinations. Based on the univariate analysis, mutants with early flowering (63 days), mid tall (90 cm) and long panicles (21 cm) were identified across various mutagenic treatments in M_2 generation. Gamma ray and EMS combined treatment showed the highest grain yield (85 g/plant) with high SPAD values compared to parent. High heritability value of 74.6 per cent was observed for grain yield with genetic advance and ranged from 1.22 to 19.96 per cent across different treatments. Significant positive correlation between SPAD values and grain yield and panicle length were observed. Cluster analysis based on Euclidean distance grouped all the mutants into four clusters with those derived from gamma rays (400 Gy) emerging as highly diverse. Promising mutants identified in this study would serve as genetic resources for recombination breeding.

Keywords: EMS, Grain yield, Gamma rays, Genetic variability, Induced mutations

INTRODUCTION

Sorghum is a member of Poaceae family with five known genetic races cultivated in arid and semiarid regions worldwide. Globally sorghum is cultivated on an area of 41 million ha and production of 58 million tonnes with major contributions from the USA, Nigeria, Ethiopia, Mexico and India. In India, it is cultivated over of 4.48 million ha with an annual production of 4.38 million tonnes of grains with productivity of 1018 kg/ha (IIMR, 2019). Sorghum grain is valuable staple food for human consumption and fodder is used for livestock feed. Grain is rich in carbohydrate, protein, vitamins and minerals. It is mainly cultivated as dual crop for food and fodder purpose with major cultivation in *rabi* season. Mainly landraces, historical

varieties and improved cultivars are being cultivated in the *rabi* season. However, the cultivation is often challenged by shootfly infestation, terminal drought stress and charcoal rot disease incidence. Therefore, there is need to improve genetic variability for yield contributing traits and resistance to biotic and abiotic stresses.

Genetic variability plays an important role in selection of ideal plants based on yield contributing traits. Narrow genetic basis of the germplasm lines used for the development of inbred lines and hybrids would lead to drastic reduction in the yield levels of post rainy sorghum (Kebede *et al.,* 2001). In order to improve the genetic

diversity for yield contributing traits in crop plants, various breeding methods have been used. Among them, induction of mutations using physical and chemical mutagens which would help in enhancing the variability and improve defects in one or two traits of a well adapted variety (Chopra and Sharma, 1985). Although spontaneous mutations occur in nature but their frequency is very low (10^{-5} to 10^{-8}). Therefore induced mutations are valuable in increasing mutation frequency in economically important field crops (Maluszynski *et al.,* 2000). Among the various mutagens used, X-rays, gamma rays, fast neutrons, thermal neutrons, electron beam and ion beams (physical mutagens) and ethyl methane sulphonate and sodium azide (chemical mutagens) are important ones. Mutation breeding has been used in various cereal, oil seed and legume crops to improve genetic variability for quantitative traits. Successful application of mutagens has led to commercial release of over 3320 varieties in 228 different field crops worldwide (Suprasanna *et al.,* 2015), which are mainly dominated by seed propagating crops (80 %) and cereals in specific (48 %). Several agronomic traits have been improved using gamma rays and chemical mutagens including dwarfness, early flowering, high grain and fodder yields, high protein and lysine content, low lignin content (bmr) and resistance to biotic and abiotic stresses (Oria *et al*., 2000). Genetically characterized mutants with distinct phenotypes can be used directly as varieties or as parents in hybridization programs.

In order to get high frequency of mutations, selection of mutagen is essential in mutation breeding experiments. A highly effective mutagen may not lead to high mutation frequency but show less biological damage (Shah *et al.,* 2008). Various factors including biological, environmental and chemical would determine mutation rate of any mutagen (Kodym and Afza, 2003). Owing to the toxicity of the chemical mutagens, methylating agents are highly toxic and have to be used at lower concentrations than ethylating agents (Khan *et al*., 2005). Combination of physical and chemical mutagens would lead to increased mutation frequency among the field crops. Ideally, a mutagen causing less biological damage is highly useful and lead to more frequency of favourable mutations (Khan and Tyagi, 2010). Since there are no conclusive studies to reflect on these aspects, the present study was undertaken to know the induced genetic variation in *rabi* sorghum for qualitative and quantitative traits.

MATERIALS AND METHODS
A popular variety Parbhani Moti from Maharashtra was subjected to mutation breeding to improve earliness, grain and fodder yields. Selfed seeds with optimum moisture content were exposed to 100, 200, 300 and 400 Gy doses of gamma rays (Co^{60}) with a dose rate of 23.9 Gy/min at Nuclear Agriculture & Biotechnology Division, BARC, Mumbai, India. The chemical treatment was given on selfed seeds pre-soaked in water for 8 hrs and then treated with EMS @ 0.1%, 0.2%, 0.3% and 0.4% for 6 hrs and later thoroughly washed in tap water to remove the traces of mutagen before field sowing. Combination of treatments comprising gamma rays and EMS (100 Gy+0.1%/0.2% EMS; 200 Gy+0.1%/0.2% EMS) were also considered to study the combined effect of both mutagen.

Mutagenized seeds were grown following plant to row progenies method at Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra during post rainy season of 2016. Irradiated seeds were space planted at a distance of 45 cm between rows and 15 cm between each plant. Recommended package of practices were followed to raise successful crop. Any dominant mutations observed in the M_1 generation in comparison with control/ parental plants were documented. Further selfed seeds of individual treatments from M_1 generation were harvested and forwarded to M_2 generation. In M_2 generation, sowing was taken up as plant to row progenies with parental line repeated after every 25th row for comparison. Morphological traits *viz.,* days to 50 per cent flowering, plant height (cm), panicle length (cm), panicle width (cm), seed yield per plant (g/plant) and SPAD values were recorded on putative mutants.

The mean values obtained for various traits in the M_2 generation were used for further statistical analysis (Panse and Sukhatme, 1954). Genetic parameters were estimated among putative mutants and determined genetic and environmental effects on different traits. Genotypic, phenotypic and error variances were calculated for each trait. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated as per the formulae given by Burton (1952). Knowledge of heritability is essential for crop improvement based on selection, as it indicated the extent of transmissibility of a character into new generations (Arunkumar *et al.*, 2004). In present investigation, broad sense heritability was computed for all the traits under study using formula ($σ^2P = σ^2G + σ^2E$).

To determine genetic relationships among the different variables, Pearson correlation coefficients were calculated for each pair of traits using the PROC CORR statement of SAS. A cluster analysis of the morphological data was performed using Euclidean distances and a dendrogram was constructed using PAST software.

RESULTS AND DISCUSSION
The present study was carried out to estimate genetic variation in *rabi* sorghum mutants for yield contributing traits as effect of gamma rays and EMS mutagenic agents. Desirable mutations for plant growth and leaf traits were considered as one of the most dependable indices for evaluation of genetic effects of mutagenic treatments in M_2 generation. Such mutations in the Parbhani Moti population is summarized in the **Table 1**. Number of

Table 1. Desirable mutants for plant growth and leaf traits identified in M$_2$ generation of Parbhani Moti mutant population

Mutagens	Gamma rays				EMS				Gamma rays+EMS			
Dose	100Gy	200 Gy	300 Gy	400 Gy	0.10%	0.20%	0.30%	0.40%	100 Gy + 0.1%	100 Gy + 0.2%	200 Gy + 0.1%	200 Gy + 0.2%
I. Mutations for plant habit and growth												
Tall mutants	2	1	1	1	1	0	0	0	0	1	2	3
Semi dwarf	2	2	3	1	1	1	1	2	3	2	1	1
Dwarf	0	0	0	1	0	1	0	0	0	0	0	1
Thick stem	0	2	2	0	1	2	1	1	1	2	2	3
Long internodes	1	2	0	1	1	0	0	0	0	2	1	1
Short internodes	1	0	1	0	1	1	1	1	0	0	1	1
Early flowering	0	0	0	0	0	0	1	1	1	2	2	2
Total	**6**	**7**	**6**	**4**	**5**	**5**	**4**	**5**	**5**	**9**	**9**	**12**
II. Mutation affecting leaf characteristics												
Broad leaf	2	1	2	3	4	3	2	2	4	3	2	1
Narrow leaf	0	1	1	2	1	1	0	1	1	0	3	1
Brown midrib	0	0	0	0	1	0	0	-	0	0	0	1
White midrib	1	1	3	1	1	2	1	0	1	1	0	1
Thick leaf	0	2	3	0	0	2	3	1	0	1	0	1
More flag leaf area	0	0	1	0	0	0	0	0	0	0	1	2
Total	**3**	**5**	**9**	**6**	**7**	**8**	**6**	**4**	**6**	**5**	**6**	**7**

mutations for plant growth and habit varied from 4 (400 Gy gamma rays and 0.3% EMS) to 12 (200 Gy gamma rays + 0.2%EMS). Specifically, early flowering mutants (63 days) were identified from combined dose treatments and dwarf mutant (88 cm) was isolated from 200 Gy + 0.2% EMS treatment. Other phenotypes observed in the population were tall, thick and with short internodes, thin and with short internodes and semi dwarf mutants across different treatments. In addition, mutations affecting leaf traits varied from 3 (100 Gy) to 9 (300 Gy). Specifically, brown midrib (0.1% EMS and 200Gy + 0.2% EMS) and increased flag leaf area (300 Gy, 200 Gy + 0.1/0.2% EMS) mutants were identified. Other phenotypes such as broad, narrow and thick leaf mutants were also observed in different treatments. Previously, mutants showing reduced intermodal length leading to dwarfness were also reported in sorghum (Veenakumari, 1994). In addition, viable mutations affecting plant height, maturity, panicle size, midrib color, seed weight and type of spikelet were also reported in sorghum (Ramulu, 1975). Such viable mutations with altered plant habit isolated in M$_2$ generation will be confirmed for their genetic inheritance in M$_3$ generation.

Mean, range and genetic parameters for all the traits are presented in **Table 2**. Based on univariate analysis, Parbhani Moti mutants in M$_2$ generation showed wide range values for yield contributing traits across different

treatments of gamma rays and EMS. The putative mutants showed early flowering (63 V/s 75 days in control), mid tall (90 cm V/s 210 cm), long (21 cm V/s 13cm) and wide panicles (12 V/s 7.9 cm) across all the gamma rays and EMS treatments. Among the treatments, EMS and gamma rays + EMS treatments recorded wide variation for yield contributing traits as compared to gamma rays alone. Grain yield was maximum for gamma rays + EMS treatment (20-85 g/plant) as compared to control (45-54 g/plant). Similarly, gamma rays + EMS showed the highest SPAD values of 37-72 as against 48-59 in the control plants. Similar to this study, mutations leading to dwarf, early and photo insensitivity in sorghum genotypes were also evident from the earlier study (Reddy and Rao, 1981). Using mutation breeding, Indonesia has developed three sorghum mutant varieties which recorded higher grain yield (38-52%) with earliness (89 days) and resistance to lodging (Soeranto *et al.*, 2020).

The co-efficient of variation was the highest for grain yield (25.9%) and lowest for days to 50% flower (9.38%). The presence of wide range of variation for these quantitative traits indicated large genetic variation among the mutants and will aid in better selection of the mutants in M$_2$ generation. High values of phenotypic and genotypic co-efficient of variation (PCV and GCV) were observed among gamma rays and EMS treatments for the traits studied (**Table 1**). Among the traits, the highest GCV and PCV

Table 2. Mean, range and genetic parameters for Gamma rays, EMS and their combined treatments for yield traits and SPAD values in Parbhani Moti

Treatment	Range	Mean	CV (%)	PCV (%)	GCV (%)	H² (%)	GA (%)	GAM
Days to flower								
Gamma rays	65-88	72.00	13.32	5.73	3.89	46.07	3.99	5.46
EMS	63-90	75.00	13.41	5.83	4.31	55.64	4.94	6.65
Gamma+EMS	63-94	75.00	13.44	5.97	4.39	55.11	4.95	6.68
Control	75-79	76.81	9.38	1.44	0.90	39.22	0.89	1.17
Plant height (cm)								
Gamma rays	90-262	219.00	15.93	7.43	5.56	60.97	18.43	8.68
EMS	90-209	221.00	17.65	5.32	4.42	62.23	16.50	7.65
Gamma+EMS	88-209	218.00	16.86	6.89	5.35	51.03	18.96	8.79
Control	210-237	222.30	11.99	3.50	2.88	67.61	10.85	4.88
Panicle length (cm)								
Gamma rays	8.1-18	14,67	14.14	8.04	5.37	43.85	1.00	7.42
EMS	9.1-21	15.67	14.61	8.79	5.70	43.27	1.16	7.66
Gamma+EMS	8.1-21	15.33	19.09	9.59	6.32	44.54	1.25	8.84
Control	13-16	14.33	10.35	3.17	1.99	39.29	0.36	2.57
Panicle width (cm)								
Gamma rays	5-12	7.13	20.36	13.03	10.57	65.77	1.22	17.83
EMS	5.4-12	8.04	21.75	17.61	12.06	50.63	1.23	17.61
Gamma+EMS	5-12.1	9.14	21.29	19.92	16.90	73.36	2.32	29.68
Control	6-7.90	6.57	9.51	8.88	6.85	59.42	0.71	10.87
Grain yield (g/plant)								
Gamma rays	22.1-78	52.67	23.79	18.38	12.91	48.34	8.36	23.13
EMS	21-83	53.00	24.56	12.22	9.56	57.75	7.58	15.51
Gamma+EMS	20-85	55.00	25.90	11.43	9.98	74.60	8.75	18.05
Control	45-54	48.67	8.47	2.85	1.89	43.87	1.25	2.98
SPAD values								
Gamma rays	41-67	56.00	16.39	9.90	7.98	54.36	6.45	13.60
EMS	37-67	60.00	14.83	12.14	9.57	67.76	8.34	15.97
Gamma+EMS	37-72	52.00	14.62	12.04	9.15	58.85	7.14	14.56
Control	48-59	50.40	11.07	8.97	8.22	83.96	7.82	15.53

values were recorded for panicle width (20.36 – 21.75% and 13.03 – 19.92% ,respectively) followed by grain yield (23.79 – 25.9% and 11.43 – 18.38%, respectively). Low values of GCV were observed for days to flower (3.89%) and plant height (4.42%), which indicated that any improvement for these traits would be less effective during selection process. Earlier reports have also shown high GCV and PCV values for plant height, stalk yield and grain yield, while low values for days to flower and maturity (Yaqoob *et al*., 2015). Generally, GCV values were smaller than PCV values for quantitative traits indicating the potential contribution of environmental variance to the total phenotypic variance. Narrow variation between GCV and PCV values for panicle length, SPAD and days to flower showed less influence of environmental factors

and better scope for selection (Shivaprasad *et al*., 2019).

High heritability values were observed for grain yield (74.6%) and panicle width (73.36%) in gamma rays and EMS combined treatment. Moderate heritability was observed for days to flower, plant height and SPAD values. Whereas, panicle length registered lowest heritability (43 - 44.5%). Similar to this study, Anand and Kajjidoni (2014) also observed wide genetic variability and high heritability for plant height and grain yield among sorghum mutant lines. Among the forage sorghum landraces, high heritability with high genetic advance was evident for days to flower, plant height and leaf length. This trend was mainly contributed by additive component and would lead to better selections of the trait
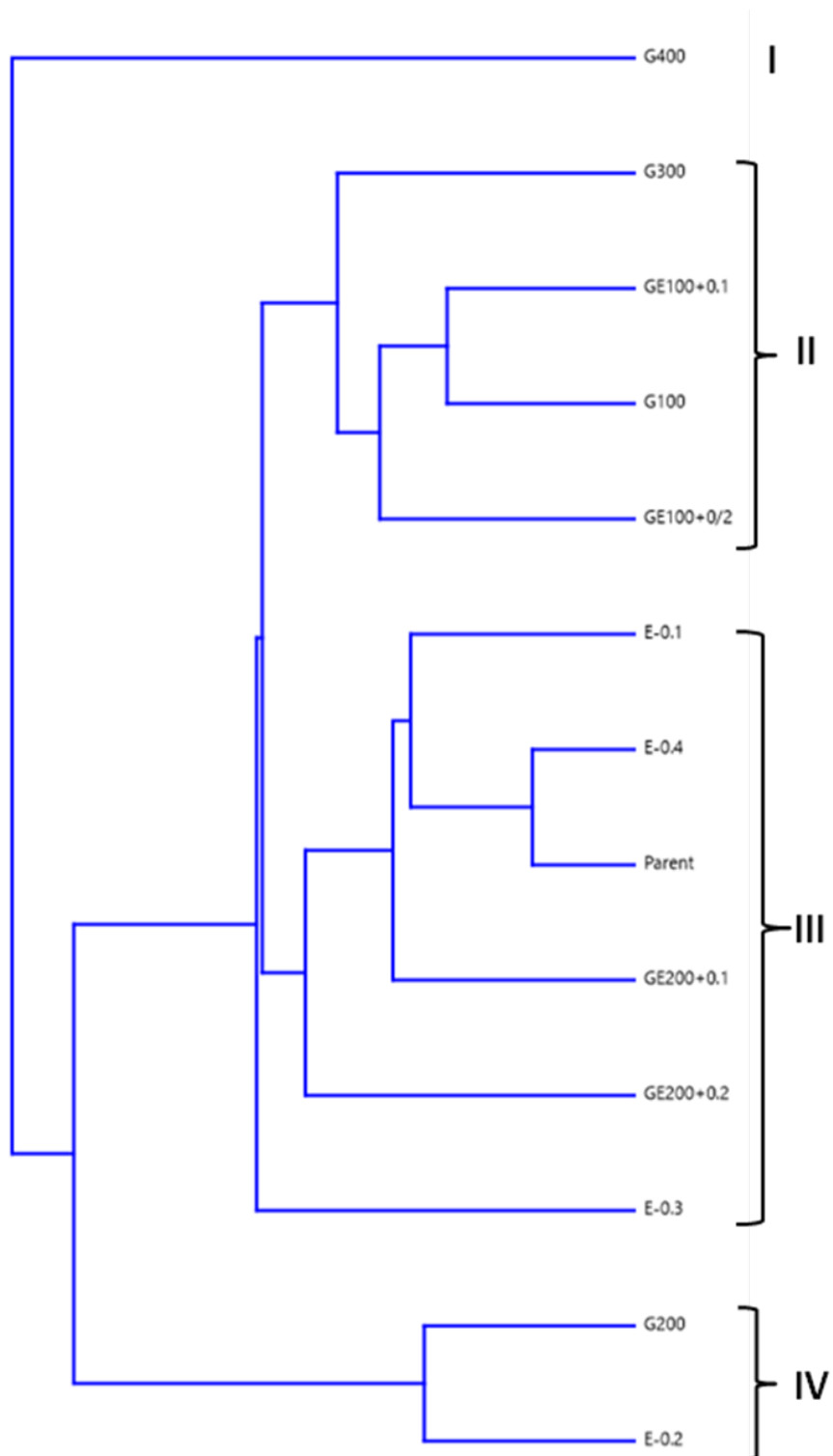
Fig. 1. Cluster analysis based on the Euclidean distances among Parbhani Moti mutants derived from various treatments in M$_2$ generation (G- Gamma rays; E-EMS)

Table 3. Correlation coefficient for yield contributing and SPAD traits in Parbhani Moti derived mutants

Traits	DF	PH	PL	PW	GY
DF	1.0				
PH	0.049	1.0			
PL	-0.342*	0.352*	1.0		
PW	-0.446*	0.177	0.072	1.0	
GY	0.070	0.064	0.250*	0.380*	1.0
SPAD	0.044	-0.238	0.411*	-0.007	0.594*

DF-Days to flower; PH-Plant height; PL-Panicle length; PW-Panicle width; GY- Grain yield

* significant at 5% level

in the segregating generations (Tariq *et al*., 2012). In the present study, genetic advance varied from 1.22 to 19.96 per cent for different traits across gamma rays and EMS treatments. Plant height recorded high genetic advance with combined treatment showed 18.96 per cent, while panicle length had shown the lowest values of 1.22 per cent.

The association between morphological and contributing traits can be estimated by the correlation coefficients. In Parbhani Moti population, significant positive correlations were evident between panicle length and plant height (0.352*); panicle width and grain yield (0.380*); grain yield with SPAD values (0.594*) **(Table 3)**. Similarly, significant negative correlations were also observed between days to flower and panicle length (-0.342*)/ panicle width (-0.446*). In the previous studies also significant positive correlations were observed between grain yield and number of leaves, plant height, stems girth and panicle area (Deepalakshmi and Ganeshmurthy, 2007). Significant positive correlations were observed between seed yield with plant height, panicle length and seed weight among the *rabi* sorghum landraces (Girish *et al*., 2016) and F_6 derived lines (Shinde *et al*., 2014).

Morphologically and genetically similar genotypes can be grouped based on the Euclidean distances for identifying potential parents for recombination breeding (Souza and Sorrels, 1991). In the present study, cluster analysis based on the Euclidean distances grouped all the mutants into four clusters (**Fig. 1**). Mutants derived from gamma rays at 400 Gy were found highly diverse, while mutants derived from EMS at 0.1 and 0.4% had similar diversity levels compared to control. Cluster analysis resolved indigenous and exotic germplasms into four major clusters (Badigannavar *et al*., 2017), while accessions derived from Etria region for 15 traits were grouped into different clusters (Ayana and Bekele, 1999). Those clusters which are contributing maximum to the divergence could be further used for selection and considered as potential donors for recombination breeding.

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