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Genetic Variability Studies in M_5 Generations of Determinate Early Maturing Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub] (MDU-1) Mutants for Yield and Yield Attributing Characters

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ABSTRACT

Background: MDU-1 is a high yielding, indeterminate cluster bean variety. In order to develop determinate mutant with high yield, MDU-1 cluster bean variety was irradiated using gamma rays, electron beam and combination of gamma rays and EMS with different doses and the variability induced in M_5 generations were studied.

Methods: Twenty one best individual plants from M_4 generation were selected based on plant height and higher yield and forwarded to M_5 generation where they were evaluated in RBD with three replications. Fourteen non-segregating mutant lines with desirable traits were identified in M_5 generation and were evaluated for variability along with the parent MDU1.

Result: Five best mutant lines namely ACMC-020-04, ACMC-020-11, ACMC-020-08, ACMC-020-10 and ACMC-020-11 were selected from the 14 accessions based on *per se* performance and variability analysis. The selected mutants needs to be forwarded for stability testing in different environments.

Key words: Cluster bean, Electron beam, Gamma rays, Genetic variability, Heritability, Yield.

INTRODUCTION

Cluster bean, also known as guar, is a leguminous annual plant cultivated under resource constraint conditions of arid and semi-arid zones. The deep tap root system of the crop makes its survival possible in drought prone areas with soils having low water holding capacity. It is a multipurpose crop used as a vegetable, forage crop, green manure and also as an important industrial crop. The industrial value of the crop is mainly due to the presence of the polysaccharide galactomannan in its seeds. India is the leading guar producing country in the world with 90% of world's total production share and the country has exported 3,81,880.16 MT of the polysaccharide to the world for a value of Rs. 3,261.60 crores/456.96 USD Millions during the year 2019-20 (APEDA, 2019-2020). Traditional cluster bean cultivating belts of India are the western dry zones of Rajasthan, Haryana and Punjab. Rajasthan alone accounts for 72% of the cluster bean produced in the country. Recently, the crop is expanding its cultivation to the south India *i.e.* in the states of Tamil Nadu, Andhra Pradesh and Karnataka. MDU-1 is an important variety of cluster bean with higher yield and medium maturity duration. But the indeterminate nature of the variety makes its cultural operations (especially pod picking activity) difficult. Hence, an attempt was made to develop determinate mutants of MDU-1 without compromising the crop productivity.

Variability is a prerequisite in plant breeding as its amount decides the degree of response to any selection. The spontaneous variation through natural processes takes time and is of unpredictable nature. Hence, hybridization and mutation can be used as tools to create variability. Cluster bean is a self-pollinated crop with tiny complicated floral structure making hybridization tedious. Hybridization is also a long process and sometimes it is difficult to find parents with the desirable genes. Hence, mutation breeding can be considered as an ideal alternative approach in cluster bean improvement, where the target is to improve some of the easily identifiable useful characters like reduced plant height and increased pod yield in an otherwise well adapted variety.

In cluster bean, the improvement of polygenetically controlled characters like plant height, pod yield and number

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of clusters per plant can be generated through micro mutations which are highly localized and cause alteration at a single gene locus and are generally seen in M_3 and M_4 generations. The conformation of the genetic nature could be done only in the future generation. Hence, more attention has to be given for micro mutations over macro mutations taking into consideration of its higher frequency of beneficial variants (Shinde *et al*. 2017). The quantitative characters are controlled by multiple gene interactions, each having its smaller share in inducing variations. Hence, it is probable that the desirable trait is accompanied by one or more undesirable traits, unlike the single gene controlled specific characters. But this is considerably balanced by the increased frequency of mutations due to the large number of genes involved.

Keeping all these is mind, our objective was to study the genetic variability in M_5 generations of cluster bean (MDU-1) and to identify the determinate mutants with high yield and early maturity.

MATERIALS AND METHODS

Genetic material and mutagens used

MDU-1 cluster bean was used as the parent material taking into account its higher yielding potential (250 to 350 g/plant) along with other desirable characters. It is an indeterminate variety selected from a germplasm accession IC 432117. This variety is rich in dietary fibre and vegetable protein developed from Agricultural College and Research Institute, Madurai (TNAU) during 2015.

Experimental method

The experiment was conducted during the *rabi* seasons of 2020-2021 at the college main orchard, Department of Horticulture, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai.

Seeds of MDU-1 were mutagenized using different doses of gamma rays, electron beam and combination of gamma rays and EMS. Initially, doses of irradiation were determined based on the lethal dose (LD50) which was 406.69 Gy for gamma rays, 302.46 Gy for electron beam and 202.45 Gy for combination of EMS and gamma rays. LD50 was calculated from the germination and survival test of the cluster bean seedlings. M_1 generation was raised and the fertile plants were harvested individually. Healthy seeds (M_2 seeds) were collected randomly from M_1 plants to raise M_2, M_3 and M_4 plants as plant to row progenies and observations on yield and yield attributing characters were recorded.

The present study concentrated on the M_5 generation only. Twenty one best performing individual mutant plants were selected and forwarded from M_4 generation based on the observations recorded for further selection process in M_5 generation (Table 1). Here plants were planted at a spacing of 45×30 cm and were provided with proper package of practices throughout the crop growth period.

The forwarded 21 best performers from M_4 generation were evaluated under randomized block design with three replications along with the control parent MDU-1. Fourteen non- segregating families were identified and observations were recorded on ten random plants from each family for determinate plant height with higher yield, early maturity and contributing characters. Thirteen quantitative characters *viz*. plant height (cm), days to first flowering, number of clusters per pod, number of pods per cluster, total number of pods per plant, pod length (cm), pod girth (cm), pod weight (g), number of seeds per pod, days to maturity, protein content (%), galactomannan content (%) and pod yield per plant (g) were recorded.

Statistical analysis

The statistical parameters like mean, standard error and coefficient of variation were calculated by using the standard methods of analysis by Panse and Sukhatme (1954). Estimation of genetic parameters of variation *viz*, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability broad sense (H^2) and genetic advance as percentage of mean (GAM) were estimated using TNAUSTAT (2014).

RESULTS AND DISCUSSION

Estimating genotypic variability in an irradiated population would tell how much of the total variation is heritable. Johnson *et al.* (1955) stated that the GCV provided the mean to study the genetic variability generated in quantitative traits. However, the GCV alone isn't enough to determine the level of heritable variability. In addition, to determine the heritable part of total variation and the level of genetic gain expected for effective selection, an assessment of heritability and genetic advance as a percentage of mean is necessary. Since heritability in broad sense encompasses both additive and epistatic gene effects, it will be reliable if it is accompanied by a high level of genetic advancement (Burton, 1952). Heritability estimations combined with genetic advance, according to Johnson *et al*. (1955), are more useful than heritability values alone. This is because genotype–environment interactions affect heritability estimates, according to Kaul and Kumar (1983). Furthermore, under a suitable selection system, genetic advance indicates the level of stability and genetic advancement for a particular trait.

Here, thirteen polygenically controlled traits were recorded in the progenies raised in M_5 generation.

The analysis of variance for all the characters studied is given in the Table 2. It indicated high significant difference for all the thirteen characters studied when 15 genotypes were evaluated. Hence, there is wider genetic variation for all the characters studied on the cluster bean mutant genotypes along with the parent. Since the genetic variability is secured, the nest task is the separation of heritable portion of variability from the non- heritable part for better planning of the breeding program. This gives an opportunity to improve the traits with high heritability through selection.

The *per se* performances of the accessions were calculated (Table 3). Among the 14 non-segregating lines, ACMC-020-10 (500 Gy gamma rays) was identified as the best determinate accession with respect to pod yield per plant (253.83 gm), number of pods per cluster (10.22), total number of pods per plant (117.70), pod length (16.35 cm), pod girth (2.94 cm) and pod weight (2.09 g). ACMC-020-04 (200 Gy electron beam) was the tallest of the accessions (75.86cm), whereas, ACMC-020-014 (500 Gy gamma rays) was the shortest (46.26 cm) one. The earliest flowering mutant was ACMC-020-11 (200 Gy electron beam) which took approximately 22 days for opening of the first flower, whereas ACMC-020-09 (400 Gy gamma rays) was identified as the late flowering genotype which took 25 days to initiate flowering. Also, ACMC-020-11 (200 Gy electron beam) along with ACMC-020-08 (500 Gy electron beam) completed their life cycle within the shortest time period (8.40) when compared to the parent MDU-1 which took 100.8 days to complete the life cycle. Number of clusters per plant were highest in 500 Gy gamma irradiated treatments, ACMC-020-07 (11.50) and ACMC-020-10 (11.10), while, ACMC-020-14 (500 Gy gamma rays) produced only 5.80 clusters on an average. Even if ACMC-020-04 (200 Gy electrons beam) had the highest per pod weight (2.30 g) and protein content (2.83%), the galactomannan content (28.05%) was observed to be the least in it. ACMC-020-08 (500 Gy gamma rays) exhibited superiority with respect to number of seeds per pod (10.10) and also produced the pods with maximum weight (2.30 g) along with ACMC-020-10 and ACMC-020-04.

Magnitude of genetic variance for all the characters studied is given in Table 4. GCV and PCV observe the amount of variation in the available population. PCV value

Table 1: Data of selected 21 mutants of cluster bean in M_4 generation forwarded for evaluation in M_5 generation.

Treatments	Original mutagen dosage	PH	DFF	NOC/P	TNP/P	PL	PW	PY
5M1	500 Gy (gamma rays)	82.60	23.00	26.00	112.00	13.00	3.60	217.19
5M2	500 Gy (gamma rays)	78.50	24.00	27.00	104.00	14.50	3.62	213.25
5M3	500 Gy (gamma rays)	76.50	24.00	26.00	94.00	12.30	3.71	205.00
5M4	500 Gy (gamma rays)	78.00	24.00	26.00	93.00	13.20	3.70	200.50
5M5	500 Gy (gamma rays)	74.50	23.00	26.00	89.00	13.12	3.79	200.02
5M6	500 Gy (gamma rays)	76.80	23.00	28.00	99.00	13.40	3.68	211.25
5M7	500 Gy (gamma rays)	75.00	25.00	23.00	89.00	15.94	3.80	198.47
5M8	500 Gy (gamma rays)	67.50	25.00	19.00	69.00	15.92	3.89	173.26
5M9	500 Gy (gamma rays)	66.00	25.00	13.00	60.00	12.96	3.90	159.01
5M10	500 Gy (gamma rays)	75.60	25.00	13.00	65.00	12.94	3.40	161.99
5M11	200 Gy (electron beam)	77.00	25.00	19.00	96.00	14.08	3.41	192.99
5M12	200 Gy (electron beam)	76.50	25.00	14.00	62.00	13.02	3.45	145.85
5M13	200 Gy (electron beam)	62.50	24.00	12.00	55.00	13.10	3.60	137.05
5M14	200 Gy (electron beam)	63.00	24.00	14.00	53.00	13.02	3.59	136.00
5M15	200 Gy (electron beam)	58.50	24.00	11.00	52.00	13.06	3.63	134.99
5M16	200 Gy (electron beam)	67.00	24.00	14.00	57.00	13.04	3.69	146.92
5M17	200 Gy (electron beam)	67.00	24.00	11.00	58.00	13.06	3.69	144.33
5M18	400 Gy (gamma rays)	67.00	24.00	11.00	55.00	12.84	3.65	145.42
5M19	400 Gy (gamma rays)	76.50	24.00	13.00	87.00	15.96	3.70	197.13
5M20	400 Gy (gamma rays)	73.60	25.00	13.00	60.00	12.90	3.73	154.87
5M21	400 Gy (gamma rays)	70.50	25.00	13.00	61.00	12.90	3.73	153.24

PH: Plant height (cm), DFF: Days to first flowering, NOC/P: Number of clusters per plant, TNP/P: Total number of pods per plant, PL: Pod length (cm), PW: Pod weight (g), PY: Pod yield per plant (g).

Table 2: Analysis of variance for thirteen characters studied in M_5 generation.

Sources of variation	PH	DFF	NOC/P	NOP/C	TNP/P	PL	PG	DTM	PW	NOS/P	PRN	GUM	PY
Replication	0.16	0.11	0.97	0.06	8.32	0.00	0.01	0.00	0.03	0.07	0.00	0.00	18.55
Genotype	1150.89*	1.23*	25.93*	9.96*	2065.53*	8.96*	0.15*	48.61*	0.19*	0.58*	0.09*	0.42*	16268.83*
Error	1.24	0.11	0.30	0.03	1.21	0.01	0.00	0.09	0.03	0.05	0.00	0.00	5.17

PH: Plant height (cm), DFF: Days to first flowering, NOC/P: Number of clusters per plant, NOP/C: Number of pods per cluster, TNP/P: Total number of pods per plant, PL: Pod length (cm), PG: Pod girth (cm), DTM: Days to maturity, PW: Pod weight (g), NOS/P: Number of seeds per pod, PNR: Protein content (%), GUM: Galactomannan content (%), PY: Pod yield per plant (g).

Significant at 5% level of significance.

Table 3: *Per se* performance of 14 selected mutant accessions for yield and attributing characters in M_5 generation along with the parent MDU-1.

Accessions	Mutagen dose	PH	DFF	NOC/P	NOP/C	TNP/P	PL	PG	DTM	PW	NOS/P	PRN	GUM	PY
ACMC-020-01	500 Gy gamma rays	71.85	23.80	6.90	4.66	39.40	9.75	1.75	81.60	1.42	9.10	2.28	28.43	100.59
ACMC-020-02	500 Gy gamma rays	71.00	24.20	7.30	4.68	42.20	9.20	1.98	84.40	1.55	8.60	2.09	28.58	104.91
ACMC-020-03	500 Gy gamma rays	74.35	23.50	10.30	6.90	78.00	10.07	2.13	81.90	1.87	8.70	2.04	29.25	122.61
ACMC-020-04	400 Gy gamma rays	69.50	24.90	10.40	8.00	87.50	14.10	2.41	85.10	2.30	10.00	2.83	28.05	200.24
ACMC-020-05	400 Gy gamma rays	67.51	23.90	9.00	8.84	81.80	15.10	2.27	85.50	2.25	9.70	2.28	28.29	166.42
ACMC-020-06	400 Gy gamma rays	75.86	23.80	10.70	8.90	88.60	15.13	2.49	84.60	2.27	9.30	2.17	29.85	179.81
ACMC-020-07	400 Gy gamma rays	73.35	22.90	11.50	10.04	110.90	14.29	2.50	81.10	2.29	9.70	2.52	28.66	226.25
ACMC-020-08	500 Gy gamma rays	65.95	22.90	9.90	9.66	104.60	13.07	2.36	80.40	2.30	10.10	2.53	28.63	225.76
ACMC-020-09	500 Gy gamma rays	68.30	25.20	10.20	7.90	86.80	13.31	2.34	85.70	1.98	9.10	2.39	29.24	153.86
ACMC-020-10	500 Gy gamma rays	72.22	23.20	11.10	10.22	117.70	16.35	2.94	81.90	2.30	9.80	2.19	29.08	253.83
ACMC-020-11	200 Gy electron beam	73.95	22.30	10.10	9.10	95.90	15.41	2.56	80.40	2.29	9.70	2.24	29.15	211.53
ACMC-020-12	200 Gy electron beam	66.68	23.30	10.10	7.60	76.20	12.88	2.34	84.20	1.66	8.30	2.52	29.11	123.91
ACMC-020-13	200 Gy electron beam	52.14	23.70	6.90	5.22	45.60	12.94	2.32	83.40	1.96	9.50	2.13	29.00	113.60
ACMC-020-14	200 Gy electron beam	46.26	24.60	5.80	4.80	36.70	12.38	2.14	81.20	1.84	9.10	2.20	28.83	105.10
MDU-1	-	155.10	24.10	21.50	3.50	150.80	12.95	2.17	100.80	2.23	9.80	2.29	28.51	332.68
General mean		73.60	23.75	10.11	7.33	82.85	13.13	2.31	84.15	2.03	9.37	2.31	28.84	174.74
CV%		1.50	1.42	5.42	2.50	1.33	0.52	1.71	0.46	8.90	2.41	1.15	0.10	2.04
CD(5%)		2.39	0.73	1.18	0.39	2.37	0.15	0.09	0.83	0.39	0.49	0.06	0.06	7.68
SE(±)		0.79	0.24	0.39	0.13	0.78	0.05	0.03	0.27	0.13	0.16	0.02	0.02	2.53

PH: Plant height (cm), DFF: Days to first flowering, NOC/P: Number of clusters per plant, NOP/C: Number of pods per cluster, TNP/P: Total number of pods per plant, PL: Pod length (cm), PG: Pod girth (cm), DTM: Days to maturity, PW: Pod weight (g), NOS/P: Number of seeds per pod, PNR: Protein content (%), GUM: Galactomannan content (%), PY: Pod yield per plant (g).

was observed to be higher that GCV value for all the studied characters, indicating the environmental effect on the characters studies. The environmental effect was negligible in all the characters studied as difference between GCV and PCV was not very prominent. Both the GCV and PCV values were found to be higher in characters like plant height (35.60%, 35.60%), number of clusters per plant (35.40%, 35.80%), total number of pods per plant (38.80%, 38.80%), number of pods per cluster (30.40%, 30.50%) and pod yield (38.40%, 38.50%), indicating higher variation in the studied genotypes for all these characters. All the studied characters expressed high broad sense heritability coupled with high genetic advance, indicating the dependence of these characters on additive genes, which made selection possible in the early generations. Similar observation was in mutants of cluster bean variety Pusa Navbahar, irradiated using gamma rays and EMS for plant height, number of pods per plant, number of pods per cluster, number of clusters per plant and pod yield per plant by Babariya *et al.* (2008). Days to first flowering and galactomannan content in the seed endosperm reported high heritability (83.09%, 99.59%) but PCV (3.45%, 1.59%), GCV (3.15%, 1.59%) and genetic advance as percentage of mean (5.91%, 3.26%) for these traits were low, indicating that these characters were under the control of non-additive genes and selection was not rewarding. The characters like pod length, pod girth and pod weight expressed moderate values for PCV and GCV and had a high heritability and genetic gain values. Hence these characters were controlled by additive genes and could be used for forwarding these traits to next generations. Number of seeds per pod, days to maturity and seed protein content had low PCV and GCV and genetic gain values even if heritability values were high. Hence these traits could not be improved through simple selection methods. Similar observations in cluster bean were reported by Santhosha *et al.* (2017), Meghana *et al.* (2019), Deepashree *et al.* (2021) and Singh *et al.* (2021) and in black gram by Kurularasan *et al.* (2018).

Based on the studies in M_5 generation, five determinate mutant accessions *viz.* ACMC-020-04, ACMC-020-07, ACMC-020-08, ACMC-020-10 and ACMC-020-11 with higher yield were identified and forwarded to future generation for

Table 4: Estimates of variance and other genetic parameters in 14 homogenous mutant accessions of cluster bean along with the parent MDU-1.

Characters	Minimum	Maximum	Mean	GCV(%)	PCV(%)	Heritability(%)	GAM(%)	CV%
PH	46.26	155.10	73.60	35.57	35.60	99.82	73.212	1.50
DFF	22.30	25.20	23.75	3.15	3.45	83.09	5.91	1.50
NOC/P	5.80	11.50	21.50	35.39	35.81	97.71	72.07	5.42
NOP/C	3.50	10.22	7.33	30.37	30.47	99.33	62.35	2.50
TNP/P	36.70	117.70	82.85	38.78	38.80	99.88	79.84	1.33
PL	9.20	16.35	13.13	16.11	16.12	99.90	33.18	0.52
PG	1.75	2.94	2.31	11.76	11.88	97.93	23.97	1.71
DTM	80.4	100.80	84.15	5.90	5.92	99.40	12.11	0.46
PW	1.42	2.30	2.03	13.69	16.32	70.28	23.63	8.90
NOS/P	8.30	10.10	9.37	5.49	5.99	83.80	10.34	2.41
PRN	2.09	2.29	2.31	9.03	9.10	98.40	18.45	1.15
GUM	28.05	29.85	28.84	1.59	1.59	99.59	3.26	0.10
PY	100.59	332.68	174.74	38.44	38.49	99.72	79.07	2.04

PH: Plant height (cm), DFF: Days to first flowering, NOC/P: Number of clusters per plant, NOP/C: Number of pods per cluster, TNP/P: Total number of pods per plant, PL: Pod length(cm), PG: Pod girth(cm), DTM: Days to maturity, PW: Pod weight(g), NOS/P: Number of seeds per pod, PNR: Protein content(%), GUM: Galactomannan content(%), PY: Pod yield per plant (g).

Table 5: Best five mutant accessions selected from the homogenous accessions (M_5) based on *per se* performance and variability analysis along with the parent MDU-1.

Accession name	Mutagen dose	PH (cm)	NOC/P	TNP/P	DTM	PY(g)	PDP (kg/ha/day)
MDU-1	-	155.10	21.50	150.80	100.80	332.68	210.85
ACMC-020-10	500 Gy Gamma rays	72.22	11.10	117.70	81.90	253.83	216.93
ACMC-020-07	400 Gy Gamma rays	73.35	11.50	110.90	81.10	226.25	202.29
ACMC-020-08	500 Gy Gamma rays	65.95	9.90	104.60	80.40	225.76	200.56
ACMC-020-04	200 Gy Electron beam	73.95	10.10	87.50	85.10	211.53	194.70
ACMC-020-11	200 Gy Electron beam	69.58	10.40	95.90	80.40	200.24	183.17

PH- Plant height(cm), NOC/P- Number of clusters per plant, TNP/P-Total number of pods per plant, PY-Pod yield per plant (g), PDP (kg/ha/day): Per day productivity.

stability check (Table 5). These five mutants were evaluated for per day productivity with the parent MDU-1. ACMC-020-10 had per day productivity of 216.93 kg/ha/day against 210.85 kg/ha/day of MDU-1, which is a strong indication of the better performance of the mutant over the parent MDU-1.

CONCLUSION

The current research revealed the role of gamma rays and electron beam in developing determinate cluster bean mutants with higher yield from the indeterminate parent, MDU1. 200 Gy electron beam was reported to be the best in producing early maturing determinate mutant with maximum height, whereas 500 Gy gamma rays was more effective in increasing the total number of pods per plant and total pod yield per plant. ACMC-020-10 was identified as the best determinate mutant taking into consideration of its higher per day productivity when compared to the parent MDU-1.

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