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Comparative mutagenic efficiency, effectiveness and induced polygenic variability in mothbean (*Vigna acontifolia* L.)

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Abstract

Induced biological damage, polygenic variability and comparative mutagenic effectiveness and efficiency of EMS, MMS, SA and HA were studied in four varieties of moth bean, namely, RMO-40, RMO-257, Jwala and CZM-1. Gradual reduction in germination, pollen fertility and survival percentage was observed with increased doses of mutagens. The frequency of chlorophyll mutants was less and were of two types i.e., *albino* and *xantha* in which albino type was less frequent. The spectrum of morphological mutations was defined in ten different classes (revertant, bushy, bifurcated stem, bolted, sterile, miniature, branched, increased peduncle length, spreading habit, and increased primary branches). The most effective treatment appeared to be 0.05% MMS, whereas least were 50mM hydroxylamine. The effectiveness of various mutagen treatments was generally higher on RMO-40 and lower on CZM-1. The effectiveness of treatments was decreased in the order of MMS > SA> EMS > HA. The highest mutagenic efficiency was observed with 1.0 mM Sodium azide in the Mothbean variety RMO-40. On the basis of estimates of pooled over genotypes and doses with in a mutagen, SA was the most efficient mutagen followed by MMS, EMS and HA. In M_3 generation, 15 progenies showed significantly superior seed yield per plant with higher magnitude of yield contributing traits then the best check, RMO-225. Branches per plant and pods per plant exhibited high genetic variability in M_3 generation.

Key words: Moth bean, mutagenic efficiency, effectiveness, methyl methane sulphonate, sodium azide, ethyl methane sulphonate, hydroxyl amine

Introduction

The moth bean (*Vigna acontifolia* L.) belonging to family Leguminoceae subfamily Papilionaceae is also called as mat bean or Turkish gram in some parts of the world. It has numerous and adapts to extremes or uncongenial ecological niches particularly, in areas receiving fewer rains with erratic distribution of India and Pakisthan. Moth bean is a hot weather, drought resistant legume. The crop is generally grown in northwestern deserts regions of India and Pakistan especially in area where green gram suffers from drought. Production of moth bean varies greatly within India, and all production is consumed within the country as a rich source of protein. It is mostly consumed by low-income consumers in rural areas and in the tribal belts. Despite its multifarious importance, the systematic efforts to improve the yielding ability of this crop are limited. Genetic variation among genotypes and relationship between major yield contributing traits/characters is of vital importance to breeding programmes that aim to produce important cultivars [1]. Use of mutagens for inducing genetic variation is well established [2, 3]. Therefore the present investigation was undertaken to understand the response of different types of moth bean varieties to more than one type and treatment of chemical mutagens and evaluate the mutagenic progenies in respect to yield and yield attributes in M_2 and M_3 generation.

Materials and methods

The material used in the present study was comprised of four released varieties of mothbean i.e., RMO-40 (early and mutant) RMO-257 (early and mutant), Jwala (Normal, parent of RMO-40) and CZM-1 (medium maturity). One hundred dry seed with a moisture

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content of 10-12% (approx) were used for treatment with different chemical mutagens with three concentrations i.e., EMS (0.2, 0.5* and 0.7%), MMS (0.005, 0.1*, 0.05%), sodium azide (1, 2*, 3 mM) and hydroxylamine (10, 30* and 50 mM) alone and in combination (0.2% EMS + I mM SA, 0.2% EMS + 10 mM HA, 1 mM SA + 10 mM HA) in each variety. Thus 16 treatment combinations (including control) were used irrespect of each variety for treatment with mutagens. The treatments were given in 0.1M phosphate buffer adjusted at pH 3.0 (for sodium azide) and pH 7.0 (for EMS, MMS and HA). The seeds were pre soaked in distilled water for 6 hours and then immersed in freshly prepared mutagen solution for 6 hours (with periodic shaking), followed by post treatment washing in gentle flow of tap water to remove the traces of chemical from the seed surface and then air dried on blotting paper at room temperature. The treated seeds were (100 seeds/treatment/variety) were immediately sown in R.B.D. in 2 x 2 meter bed with 40 cm row spacing and 10 cm plant spacing in three replication during the *kharif* 2002-03.

Each M_1 plant was harvested individually and M_2 progeny raised in separate rows in modified single seed bulk design (only those M_1 progenies were considered which bore sufficient quantity of seed). The M_2 progenies were sown in plots consisting of two rows each 2m long and spaced 40 cm apart during *kharif* 2003-04. Observation were recorded on germination, plant survival and pollen sterility in M_1 and chlorophyll deficient mutants along with plants showing gross morphological changes in M_2 generation. Chlorophyll and other macro-mutations were scored and classified as per Singh *et al*. [4] and Singh *et al*. [5]. Mutation frequency was calculated as percentage of M_2 progenies and mutagenic effectiveness and efficiency were also calculated as per Kharkwal [6, 7]. On the basis of significantly higher yield per plant (as compared to their respective parents) and coefficient of variation (CV) individual M_2 plant were selected from elite M_2 progenies. The M_3 progenies so selected were evaluated in Augmented Randomized Block Design with 9 blocks during *kharif* 2004-05. In each block, 35 M_3 progenies and 5 checks *viz*., RMO-40, RMO-257, CZM-1, Jwala and RMO-225 were assigned. Each progeny was grown in single row plot of 3 m length with row to row distance of 40 cm and interplant distance of 10 cm. Recommended agronomical practices were adopted. Ten randomly selected plants from each progeny were tagged for recording observations on days to flowering, maturity, yield and yield attributes including harvest index. Statistical analyses were performed according to Federer [8] and Sharma [9].

*The dose being LD_{50}

Results and discussion

The immediate effects of mutagenic treatments in the four varieties were recorded in terms of reduction in germination, plant survival and pollen sterility as compared to the respective parents in M_1 generation. With the increasing doses of the mutagens, a steady decrease in germination, pollen fertility and plant survival were observed in all the four varieties of mothbean. In general, there was no fixed pattern in reduction of germination percentage (%), pollen viability and survival. Sodium azide was found most potent to cause more biological damage on the basis of germination and survival percentage, whereas pollen fertility was affected by hydroxylamine in all the four varieties. The mutagens could be in the order sodium azide > HA> MMS> EMS on the basis of germination and survival per cent. Among the varieties the maximum plant survived in M_2 generation in CZM-1 followed by Jwala RMO-257 and RMO-40. These findings are consistent with the earlier reports in different pulse crops [10-12].

The M_2 progenies were evaluated for both macro and micro mutational changes. These changes were characterized on the basis of occurrence of chlorophyll deficient and morphological variants in the M_2 progenies of the four genotypes. In the present study, both RMO-40 and RMO-257 were early, erect and short statured type whereas Jwala was spreading, tall and late maturity type and CZM-1 being semi spreading, medium maturity and medium tall with compact plant canopy. The spectrum of morphological mutations is defined using ten different classes *viz*., revertant (mutant genotypes revert to their parent like appearance in respect of growth habit, tall and susceptibility to YMV etc. Fig. 1a), bushy (no main stem, Fig. 1i), bifurcated stem (main stem bifurcates into two, Fig. 1h), bolted (no leaf incision, Fig. 1 b&d), sterile (flowers are male sterile Fig. 1 c&d), miniature (Fig. 1e,f&g), branched, increased peduncle length (Fig. 1a), spreading habit (habit to spread on ground Fig. 1j) and increased primary branches (Fig. 1i&j). Mutant genotypes (RMO-40 and RMO-257) showed more than one fourth progenies reverting to their respective parents. The mutation frequency calculated on population basis ranged from 6.94 per cent (CZM-1)

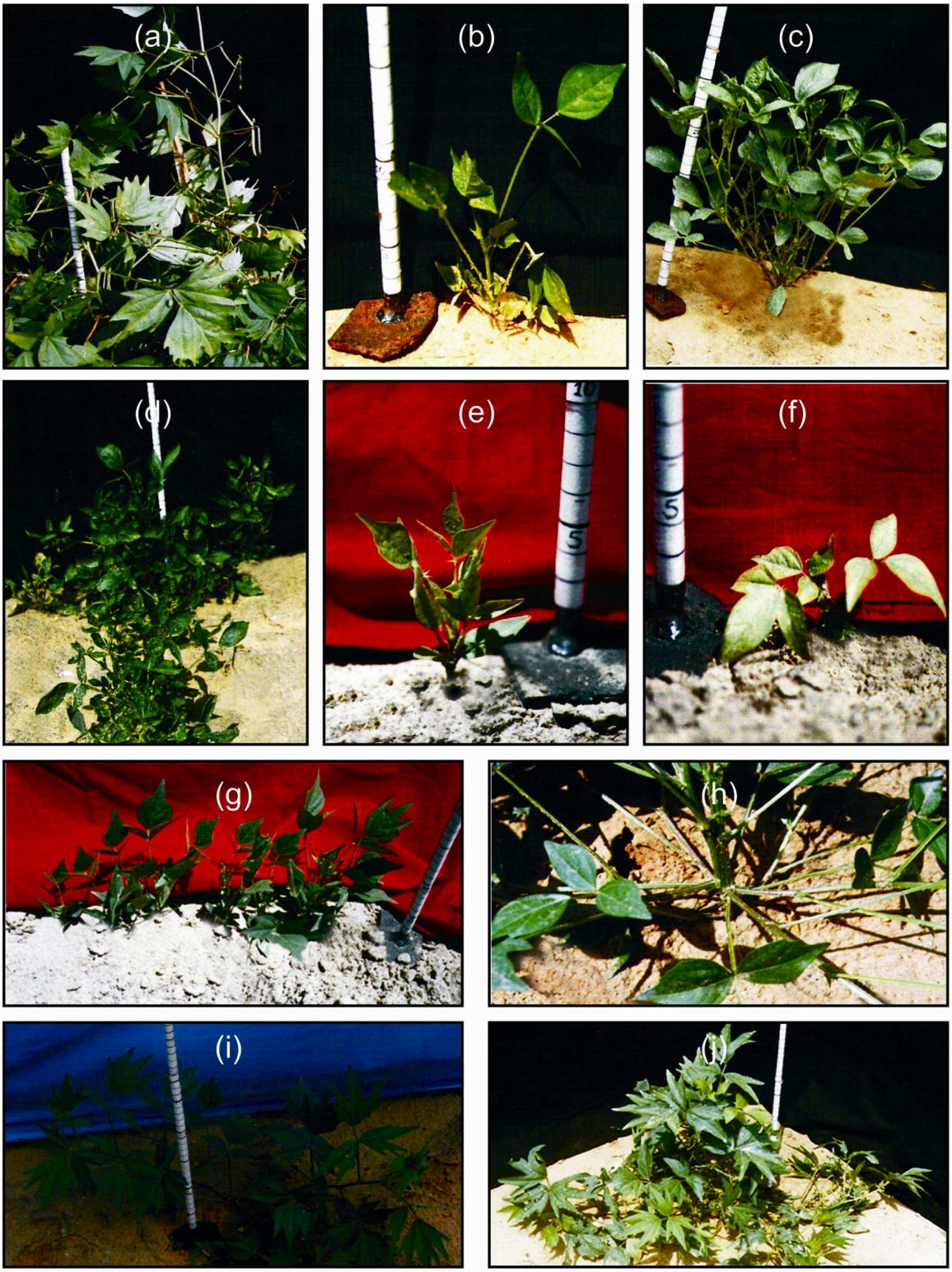

Fig. 1. A view of different morphological mutants in M2 generation moth bean. (a) increases in peduncle length in RMO-257, (b) bolted type mutant in variety Jwala, (c) sterile plant in variety RMO-257, (d) bolted and sterile mutant in variety RMO-257, (e) miniature mutant in CZM-1, (f) miniature and bolted type mutent in variety Jwala, (g) miniature and bolted mutant in variety RMO-257, (h) bifurcated stem in RMO-257, (i) increases in length of primary branches and bushy type mutant in RMO-40 and (j) increases in length of primary branches and spreading type mutant in variety RMO-257

to 25.76 per cent (RMO-40) whereas on family basis it ranged 35.86 per cent (CZM-1) to 81.32 per cent (RMO-40). The genotype RMO-40 showed more mutagenic frequency followed by RMO-257, Jwala and CZM-1 (Table 1).

Three types of chlorophyll mutations were observed *viz.*, xantha (a lethal mutation with entirely yellow leaves), chlorina (yellowish green coloured leaves with majority of them died after 30-40 days), virscent (yellow green coloured leaves which later transformed into green and survived). The frequency of chlorophyll mutants were less in all the genotypes and mutant genotypes showing least chlorophyll mutants. In general, we found less chlorophyll mutants in our study (Table 1). Higher frequency of certain plant types at higher doses might be due to considerable changes at DNA level by the mutagens while lack of any pattern of certain morpho-types or chlorophyll mutants might be due to random nature of mutations. It was observed that all the four cultivars manifested differential reactions to the treatment with EMS, MMS and HA. Differential behavior of various mutagens and selective response of cultivars might be due to the mode of action of the mutagens employed and genetic variability among the cultivars used in the study. These findings are consistent with the findings in different pulse crop [13-15].

Pollen sterility was used as the criteria of biological damage and the mutagenic effectiveness and efficiency have been determined for the four genotypes (Table 2). The mutagenic effectiveness is a measure of the factor mutations induced by a unit

Table 1. Frequency of morphological and chlorophyll mutants in M_2 generation of different genotypees of mothbean

Treatments	RMO-40			Jwala			RMO-257			CZM-1		
	Total plant observed	Total progenies observed	M_2 family segregating for mutations (Me)%	Total plant observed	Total progenies observed	M_2 family segregating for mutations (Me)%	Total plant observed	Total progenies observed	M_2 family segregating for mutations (Me)%	Total plant observed	Total progenies observed	M_2 family segregating for mutations (Me)%
0.2% EMS	360	9	66.67^{abcde}	840	21	57.14^{cdhij}	440	11	72.73^{bcdfj}	1160	29	41.38^{acdh}
0.5% EMS	400	10	80.00^{bcdeh}	360	9	55.56^{cd}	120	3	66.67^{cdf}	1240	31	32.26^{cgj}
0.7% EMS	280	7	71.43^{de}	840	21	47.62^{acdi}	40	1	100.00^{i}	840	21	19.05^{cdg}
0.005%MMS	120	3	66.67^{de}	1080	27	$55.56^{acdfhij}$	440	11	81.82^{bcdfg}	1960	49	32.65^{cdhg}
0.01% MMS	120	3	66.67^{bc}	280	7	85.71^{cd}	280	7	71.43^{bdi}	280	7	28.57^{c}
0.05% MMS	640	16	87.50^{bc}	280	7	28.57^{adg}	440	11	72.73^{cdgijk}	160	4	25.00^{h}
1.0 mM SA	40	1	100.00^{c}	80	2	50.00^{d}	160	4	50.00^{d}	200	5	80.00^{cdg}
2.0 mM SA	440	11	$72.73b^{cdegh}$	520	13	46.15^{cdij}	360	9	77.78^{cdfij}	200	5	80.00^{cdi}
3.0 mM SA	80	2	100.00^{cdh}	40	1	100.00^{i}	240	6	66.67^{ad}	160	4	25.00^{c}
10 mM HA	80	2	100.00^{bc}	480	12	33.34^{cdj}	280	7	71.43^{bcdfj}	1000	25	36.00^{cdghj}
30 mM HA	80	2	100.00^{de}	80	2	50.00^{c}	160	4	50.00^{di}	760	19	31.00^{cdi}
50 mM HA	200	5	80.00^{bfi}	200	5	60.00^{adi}	80	2	50.00^{d}	920	23	4.3^{ad}
0.2% EMS + 1 mM SA	240	6	83.33^{bcdh}	520	6	53.84^{acd}	120	3	100.00^{cf}	1640	41	26.83^{acdh}
1 mM SA + 10 mM HA	240	6	83.33^{bd}	840	6	52.38^{acdfhj}	560	14	57.14^{bcdfj}	2040	51	35.29^{acdhi}
0.2% EMS + 10 mMHA	520	13	61.54^{bdh}	560	14	64.28^{acdfhj}	200	5	80.00^{bd}	400	10	40.00^{cd}

a = Chlorophyll mutants, b = Revertants, c = Bushy, d = Miniature, e = Branched, f = increased peduncle length, g = Bifurcated stem, h = Spreading type , i = bolted, j = increased primary branched and k = Flower sterile

Table 2. Estimation of effectiveness and efficiency of the different mutagenic treatments for different genotypes

Treatments	Mutagenic effectiveness (Me/t.c)				Mutagenic efficiency (Me/S)			
	RMO-40	Jwala	RMO-257	CZM-1	RMO-40	Jwala	RMO-257	CZM-1
0.2% EMS	55.55	47.61	60.60	34.48	7.87	8.34	6.37	2.63
0.5% EMS	26.66	18.52	22.22	10.75	8.31	7.60	5.12	1.91
0.7% EMS	17.01	11.34	23.80	4.64	6.91	4.24	7.15	1.09
0.005%MMS	2222.30	1852.00	2727.33	1088.33	8.31	8.36	9.27	2.59
0.01% MMS	1111.16	1428.50	1190.50	476.17	7.38	12.43	7.29	2.13
0.05% MMS	291.66	95.23	242.43	83.33	7.24	2.57	6.88	1.53
1.0 mM SA	256.41	128.20	128.20	205.13	11.21	7.91	5.53	8.21
2.0 mM SA	93.24	59.16	99.72	102.37	6.76	6.23	6.27	7.34
3.0 mM SA	85.47	85.47	56.98	21.37	4.5	7.00	5.16	2.21
10 mM HA	50.50	16.83	36.07	18.18	7.35	4.40	6.53	3.28
30 mM HA	16.83	8.42	8.42	5.32	6.08	5.40	3.55	2.29
50 mM HA	8.08	6.06	5.05	0.44	4.65	4.52	3.41	0.30
0.2% EMS + 1 mM SA	52.41	33.86	62.89	16.87	4.07	7.16	9.61	2.06
1 mM SA + 10 mM HA	35.16	22.10	24.11	14.89	5.59	6.69	5.87	3.04
0.2% EMS + 10 mM HA	19.35	20.21	25.16	12.58	4.68	9.07	7.74	2.24

Me= M_2 family segregating for mutations per cent, c=concentration of mutagen, S= pollen sterility per cent, t=duration of treatment.

dose of mutagen. A number of chemical mutagens have been found to be equally and even many times more effective and efficient mutagens [16]. In the present investigation the highest mutagenic efficiency was observed with 0.01 per cent MMS in the mothbean genotype Jwala. Further, in all the genotypes, the efficiency of various treatments was reduced with increase in the dose of mutagens. Efficiency of mutagenic treatments indicated that SA was most efficient followed by MMS, EMS and HA. More or less similar results with different chemical mutagens were also observed by the earlier reporters in different pulses [17-19]. The relative higher efficiency at lower concentration/dose of the mutagen could be ascribed to the lesser percentage of injury at such doses and difference in effects of mutagen on different materials might be due to seed metabolism, onset of DNA synthesis and differential sensitivity within crop [20, 21]. The efficiency of a mutagenic agent is of complex nature, as it not only depends on reactivity of agent with the material and on its applicability through which physiological damage, chromosomal aberrations and pollen sterility gets induced in addition to mutation [22]. However, the effectiveness of various mutagenic treatments was generally higher on RMO-40 and lower on CZM-1. The effectiveness of treatments decreased in the order of MMS>SA>EMS>HA. For all the mutagens, effectiveness increase with the increases dose of mutagen. One remarkable observation was the relative instability of both mutant varieties i.e. RMO-40 and RMO-257. The progenies of RMO-40 frequently reverted bake to its parents Jwala which is late maturing and spreading type.

The selected 315 M_3 progenies in respect of yield and yield traits were evaluated in augmented block design. The M_3 progenies were also significantly different for the all characters from the checks except seed per pod, pod length and seed yield per plant. Fifteen M_3 progenies exhibiting superior yield per plant than the best check i.e., RMO-225 were identified. A perusal of data revealed that the higher yield was generally associated with significantly higher magnitude of plant height, branches per plant, pods per plant and clusters per plant but coupled with significantly lower (or at par with the control) values of seed per pod, pod length, seed index and harvest index. It is further seen that out of these fifteen progenies, one progeny belonged to RMO-40, four to Jwala, two to RMO-257 and eight progenies belonged to CZM-1. The M_3 progeny 100 derived from 0.005 % MMS of Jwala genotype recorded highest yield of 5.22 g/plant, which is 50.43% increase over the best check i.e., RMO-225 and 81.25 % increase over its parent Jwala. The selection of progenies on the basis of desirable mean and greater variance in the early generation was found to be highly useful, leading to the desirable improvement of yield and its components in the M3 generation [23].

Table 3. Magnitude of yield and yield attributes of 15 M3 progenies of different genotypes of moth bean showing significantly higher yield than their parents

M3 accession no.	M2 pedigree	Seed yield/ plant (g)	Days to 50% flowe-ring	Days to matu-rity	Plant height (cm)	Br./ plant	Pods/ plant	Clusters/ plant	Seeds/ pod	Pod length	Seed index (g)	Harvest index (%)
100	121-9B	5.22	51.07	70.73	20.83	3.07	53.99	11.92	5.88	3.75	2.593	46.71
78	716-6D	4.84	49.47	69.13	22.23	2.67	42.15	11.82	5.90	3.95	2.575	48.90
95	413-1D	4.70	50.47	69.13	20.63	2.47	45.95	12.92	5.60	3.83	2.607	47.50
89	242-4B	4.66	50.07	70.73	22.73	2.17	46.09	10.32	5.98	3.79	2.613	41.61
183	129-7B	4.53	48.27	69.53	23.65	2.81	45.13	11.64	5.44	3.68	2.691	38.50
110	457-5D	4.50	49.07	70.73	24.43	2.47	48.19	10.42	5.58	3.67	2.623	42.41
45	4-2A	4.47	49.47	73.33	17.71	3.51	30.77	13.10	5.98	3.81	2.575	32.97
161	701-3D	4.32	50.87	71.53	24.63	2.23	63.83	13.00	5.22	3.64	2.621	40.06
230	668-6D	4.30	50.27	67.53	25.05	2.61	30.33	9.14	5.64	3.88	2.541	52.20
366	516-5D	4.28	50.87	70.93	21.21	2.69	52.39	15.86	5.58	3.51	2.699	44.18
66	716-3D	4.28	46.47	69.13	20.03	2.37	44.55	10.92	5.20	3.68	2.515	46.00
124	261-3B	4.27	49.87	72.53	26.13	3.03	53.73	12.90	5.22	3.59	2.561	36.26
328	377-1C	4.24	50.87	69.93	26.71	2.29	57.39	13.26	5.58	3.55	2.609	39.58
365	424-4D	4.22	50.87	71.93	23.01	3.29	48.19	14.36	5.68	3.56	2.569	42.98
195	346-1C	4.20	39.67	59.33	13.19	1.73	30.99	8.56	6.54	3.90	2.841	41.82
Mean of checks												
RMO-40		3.10	37.44	55.78	15.17	0.40	25.71	6.78	6.12	3.85	2.85	50.43
RMO-257		3.05	37.89	55.44	15.39	0.40	24.54	7.20	6.01	3.78	2.90	50.56
Jwala		2.88	51.11	73.67	20.18	2.09	23.40	9.24	5.43	3.64	2.60	35.36
CZM-1		3.18	48.11	70.89	21.01	2.17	26.97	8.93	5.59	3.68	2.67	42.79
RMO-225		3.47	37.73	55.89	15.81	0.49	28.31	6.97	6.22	3.84	2.95	51.82
C.D. (P= 0.05%)												
Between check varieties		0.36	1.39	1.07	2.17	0.47	2.92	1.41	0.27	0.11	0.030	5.94
Between accessions within a block		1.13	4.39	3.39	6.88	1.49	9.22	4.45	0.86	0.35	0.095	18.77
Between accessions between blocks		1.26	4.90	3.79	7.69	1.67	10.31	4.98	0.96	0.39	0.106	20.98
Between check varieties and accessions		0.92	3.58	2.77	5.60	1.22	7.52	3.63	0.71	0.28	0.077	15.30

A,B,C & D stands for genotype, RMO-40, Jwala, RMO-257 and CZM-1, respectively.

Estimates of phenotypic variance were relatively higher than genotypic variance for harvest index and clusters per plant indicating the greater influence of environmental factors on these two characters in particular. The variances of various characters were compared on the basis of coefficient of variation. It was observed that branches per plant and pods per plant exhibited relatively high genotypic as well as phenotypic coefficient of variation. Estimates of heritability were moderate to high for all the characters. The magnitude of genetic advance expressed as percentage of mean were variable among different characters being lowest for seed index and highest for branches per plant followed by pods per plant. Though, heritability coupled with genetic advance was high for branches per plant and pods per plant. High phenotypic and genotypic variances in the quantitative traits in all the mutagenic treatments indicate better chances for selection to be successful. However, with the genotypic coefficient of variation alone it is not possible to determine the amount of variation that is heritable. The heritable portion of the variation was

determined with the aid of heritability estimates. The values of heritability increased and differed from trait to trait. The high estimates of heritability in the quantitative traits has been found to be useful from the point of view of plant breeding, as this enables selection to be based on phenotypic performance [24] suggested that heritability estimates along with genetic advance is usually more helpful than the heritability value alone in predicting the resultant effect of selecting the best individuals. In the present study, high heritability coupled with high genetic advance was noticed for most of the traits. Therefore, these traits have high selection value and can be exploited for the improvement of moth bean.

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