Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 1685-1696

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.191

M_1 Biological Injuries: Indicators for M_2 Macro- and Micro-mutation in Mungbean [*Vigna radiata (L.)* Wilczek]

Digbijaya Swain[1], Bhabendra Baisakh[1], Devraj Lenka[1] and Swapan K. Tripathy[2*]

[1]Department of Plant Breeding and Genetics, [2]Department of Agricultural Biotechnology, OUAT, Bhubaneswar, Odisha-751003, India

**Corresponding author*

Keywords

Mutagen, M1 generation, M2 macro-mutation, M2 micro-mutation, mungbean

Article Info

Accepted:
18 August 2019
Available Online:
10 September 2019

A B S T R A C T

Seed treatment with gamma rays, EMS, NG and their combinations in two mungbean genotypes, BKG-1 and OUM 11-5, significantly reduced germination, seedling growth including fresh weight and dry weight, pollen fertility, seed fertility and survival at maturity in M_1 over the parents. The biological damage in M_1 generation showed a dose dependent linear relationship. NG in both single and combination treatments resulted in more pronounced biological damage than other treatments. Five types of chlorophyll mutations (albina, xantha, chlorina, viridis and sectorial) and nineteen different morphological macro-mutations were recorded in M_2. Population variance in M_2 increased in each mutagenic treatment of both the varieties over the respective control for six quantitative traits including seed yield. The M_1 parameters e.g., germination, survival and seedling characters showed negative correlation with M_2 macro-mutation frequencies and M_2 population variance (micro-mutation), while pollen and seed sterility in M_1 showed positive association with M_2 macro-mutation frequencies and M_2 population variance. Such a relationship may be useful for effective selection of mutagenic populations at even M_1 generation to achieve wider genetic variability in M_2 and later generations.

Introduction

Pulses have a pivotal position in meeting the protein needs of the people in developing countries like India. Amongst the pulses, greengram (*Vigna radiata* (L.) Wilczek) is an important crop of India owing to its feasibility for year round cultivation due to short duration and better adaptability to varied environments. But the average national productivity of this crop is very low (472 Kg/ ha) and almost has been stagnant over the years. It has very narrow genetic variability as large part of genetic variation has been eroded due to its cultivation in marginal and sub-marginal land and its adaptation to survival fitness rather than yield. This led to limited scope for conventional breeding. Further, hybridization in this crop is difficult due to its small cleistogamous flower and frequent

flower drop. Induced mutagenesis has been proved as a potential tool to widen the base of the genetic variation and has been successfully utilised to improve yield and yield components in various crops. The recent database of FAO/ IAEA (August, 2019) indicates that 3304 varieties with improved characters have been released officially in over 70 countries for more than 232 crops and plant species through induced mutation. The present investigation is an attempt to assess the effect of gamma rays, EMS, NG and their combinations on M_1 and its relation with M_2 macro and micro-mutation frequency in two mungbean genotypes.

Materials and Methods

Dry, uniform and well-filled seeds of two mungbean genotypes (BKG-1 and OUM 11-5) were treated with gamma rays, EMS, NG and their combinations. BKG-1 is a pureline selection from a local cultivar collected from Keonjhar district of Odisha and OUM 11-5 is a promising OUAT variety released through CVRC in 2004. Seeds were irradiated with gamma rays (200 Gy, 400 Gy and 600 Gy) using the ^{60}Co source in Gamma chamber at Bhabha Atomic and Research Centre (BARC), Mumbai. For chemical mutagenesis, seeds were pre-soaked in distilled water for six hours followed by treatment with freshly prepared aqueous solution of Ethyl methane sulphonate (EMS: 0.2%, 0.4%, and 0.6%) and N-nitro-N-nitrosoguanidine (NG: 0.005%, 0.010% and 0.015%) for six hours. Besides, 400 Gy gamma-ray irradiated seeds were pre-soaked in distilled water for six hours followed by treatment with above mentioned three different concentrations of EMS and NG for six hours. In addition, seeds were treated with 0.4% of EMS and 0.01% NG aqueous solutions separately for three hours each to serve as chemical mutagen combination treatment. All the treatments were carried out at room temperature (22 ± 1^{o}C) with intermittent shaking. The seeds treated with chemical mutagens were thoroughly washed under tap water for two hours to leach out residual chemicals absorbed to the treated seeds and then the seeds were dried on the blotting paper. Ninety treated seeds from each treatment of both the genotypes including parents were sown in earthen pots filled with sterilized sand in three replications and were kept at room temperature to assess extent of germination, seedling shoot length, root length, seedling fresh weight and dry weight on 7th day after sowing. Five hundred seeds from every treatment along with the parental genotypes were sown in two trials in a completely randomized block design with two replications in 10 rows of 2.5 m length with spacing of 30 x 10 cm^2 at EB-II Section, Department of Plant Breeding and Genetics, OUAT to raise the M_1 generation. Standard agronomic practices and recommended doses of fertilizer (20-40-20 Kg N; P_20_5 and K_20/ ha) were followed to raise the crop. Extent of germination on 7th day, survival at maturity, pollen fertility and seed fertility were recorded in the field. Mean values for these traits in different treatments were used for statistical analysis. Bulk seeds harvested from all the surviving M_1 plants of sixteen mutagenic treatments along with control for the parent varieties were sown in two separate trials in a completely randomized block design with three replications. In M_2 generation, observations on macro-mutations (chlorophyll & morphological) and variation in polygenic traits (micro-mutations) were recorded. The macro-mutation frequency was calculated following Gaul (1960). Micro-mutation in $M_2$was assessed for six quantitative traits (pant height, clusters/ plant, pods/ plant, pod length, seeds/ pod and yield/ plant) based on twenty normal looking randomly selected plants of each treatment per replication to study induced variability. Observations recorded on 60 randomly selected plants per treatment were subjected to statistical analysis for estimation

of mean and variance. Besides, the population mean and variance of each character for 16 treatments including control for were subjected to analysis of variance.

Results and Discussion

Effect of mutagens on seedling growth, survival at maturity, pollen and seed fertility in M_1

The analysis of variance of M_1 seedling characters in the laboratory experiment and characters recorded in the field experiment revealed significant differences among all the mutagenic treatments in both the genotypes.

In M_1 population of all mutagenic treatments of both the genotypes, there was significant reduction in germination percentage in both laboratory and field experiment, seedling shoot and root length, seedling fresh and dry weight and survival at maturity except G1 in BKG-1 for both seedling shoot and root length, G1 and G2 in OUM 11-5 for root length and in G1 for seedling fresh weight in OUM 11-5 in comparison to their respective parents (Table 1 and Fig. 1). Germination percentage ranged from 48.9% (G2N3) to 81.1% (G1) in the treatments of BKG-1 and 42.2% (G2N3) to 85.6% (G1) in OUM 11-5 as against 92.2% and 95.6% in their respective parents in laboratory experiment and 40.0% (G2N3) to 82.6% (G1) in M_1 population of BKG-1 and 42.2% (E3) to 80.8% (G1) in OUM 11-5 as against 87.4% and 92.8% in their respective parents in the field experiment. The mean shoot length ranged from 11.03 cm (G2N3) to 19.80 cm (G1) in BKG-1 as against 21.16 cm in its parent. In case of OUM 11-5, the shoot length ranged from 7.80 cm (G2N3) to 13.48 cm (G1) as against 16.51 cm of its parent. The mean root length range was from 2.82 cm (G2N3) to 10.88 cm (E1) in BKG-1 treatments, while in OUM 11-5, it ranged from 3.06 cm (G2N3) to 7.12 cm (G2) as against 11.32cm and 7.27 cm, respectively in the parents. The range of variation seedling fresh weight was 2.82 g (G2N3) to 4.40 g (E1) and 1.64 g (G2N3) to 2.79 g (G1) in different treated population of BKG-1 and OUM 11-5, respectively as against 4.82 g and 3.09 g in respective parents. The range of variation in seedling dry weight of the treated population in BKG-1 was 0.332 g (G2N3) to 0.429 g (G2E1) and 0.142 g (G2N3) to 0.189 g (G2E1) in OUM11-5, while those in respective parents were 0.484 g and 0.237 g. With regards to survival at maturity, maximum mortality was observed at G2N3 (53.5%) followed by G2E3 (52.2%) in M_1 population of BKG-1, while in OUM 11-5, it was observed at E3 (62.8%) followed by G2N3 (62.2%). In the treatments of BKG-1 the pollen fertility and seed fertility varied from 75.5% (G2E3) to 94.1% (E1) and 85.4% (N3) to 92.8% (G1) as against control means of 97.8% and 96.6%, respectively. In OUM 11-5, the treatment means for pollen fertility and seed fertility ranged from 81.7% (G2E3) to 94.9% (E1) and 90.0% (G2N3) to 96.2% (G1) as against the control means of 98.2% and 98.6%, respectively. All the treatments in both the genotypes showed significant reduction over control for pollen sterility and seed sterility.

In general, a dose dependant reduction in M_1 parameters was observed in all the mutagenic treatments in both the genotypes. The biological injury as observed in the present study may be explained due to three possible effects of physical and chemical mutagens, viz., physiological damage (primary injury), factor mutation (gene mutation) and chromosomal mutation (chromosomal aberrations) in M_1 generation (Singh and Mohapatra, 2004). The physiological effects are generally sieved off in the M_1 generation and are not inherited, while both gene and chromosomal mutations are carried forward from M_1 to the following generations. In most

of cases meiotic abnormalities are responsible for pollen and seed sterility. Similar biological damages in M_1 generation with dose dependent linear relationship following mutagen treatments in mungbean have been reported earlier (Sujay *et al.,* 2001; Wani, 2004; Khan and wani, 2006; and Mori Vaishali, 2016). The drastic reduction in shoot length as compared to germination percentage observed in OUM 11-5 may be due to delay in onset of cell division and slowing down of the mitotic cycle of cell (Gaul, 1977). Chromosomal aberrations, particularly deficiencies, may also lead to loss of important genes leading to stunted growth. In the present investigation the reduction as compared to the respective parental genotypes was more pronounced in both single and combination treatments involving NG which confirmed its description as 'Super mutagen' (Swaminathan *et al.,* 1968). The pronounced biological damage observed in the combination treatments in the present study may be due to synergistic effect of combination treatments over single treatments.

Macro-mutation in M_2

Observation on different types of macro-mutations (chlorophyll and viable morphological) were recorded in M_2 population for both the genotypes. Chlorophyll mutations in each treatment of M_2 were recorded daily from emergence of seedlings to 15th days after sowing. Different chlorophyll mutations viz., albina, xantha, chlorina, viridis and sectorial were observed in M_2 generation of both the genotypes.

The viable morphological mutations were recorded from germination to physiological maturity of the crop. Nineteen and eighteen types of morphological macro-mutations affecting cotyledonary leaf (mono/ tri/ tetra-cotyledonary), leaf (unifoliate, bifoliate, quadrifoliate, pentafoliate, lobed leaf, serrated leaf), stem (fasciated stem), hypocotyl pigmentation, fertility (sterile plant), plant type (tall, dwarf, trailing), seed size, pod size, flowering duration (early, late) and pod numbers (profuse podded) were recorded in M_2 of the treated population of BKG-1 and OUM 11-5, respectively. The frequencies of chlorophyll and viable morphological mutations are presented in Table 2.

Micro-mutation in M_2

The estimates of variance of different treatments in both the genotypes for six quantitative traits indicated increase in population variance (Table 3) over the parents and such expanded range for different characters are due to induced variability in the quantitative characters. Analysis of variance of M_2 population means and variances of different treatments revealed significant differences among treatments of both the genotypes for six quantitative traits studied.

Relationship of M_1 parameters with induced macro and micro-mutation of M_2 generation

The effect of the M_1 parameters on induction of macro and micro-mutations in M_2 generation was ascertained from the estimates of correlation coefficient of M_1 parameters in different mutagenic treatments with chlorophyll, morphological, total macro-mutation frequency and M_2 population variance (Table 4 and 5).

In both the genotypes, all the M_1 generation parameters of the laboratory and field experiment except pollen sterility and seed sterility showed negative correlation with chlorophyll, morphological and total mutation frequency as well as M_2 population variance in M_2, while the correlation of M_1 pollen and seed sterility showed positive correlation with M_2 frequencies in both the genotypes.

Table.1 Effect of mutagens on M_1 generation of greengram variety BKG-1 and OUM 11-5 in laboratory experiment

Sl. No.	Treatment	Treatment symbol	BKG-1					OUM 11-5				
			Germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
Gamma rays												
1.	200 Gy	G_1	81.1↓ (87.9)	19.80 (93.6)	10.83 (95.7)	4.13↓ (85.7)	0.373↓ (77.1)	85.6↓ (89.6)	13.48↓ (81.6)	6.95 (95.7)	2.79 (90.4)	0.189↓ (79.7)
2.	400 Gy	G_2	75.6↓ (81.9)	17.42↓ (82.3)	10.23↓ (90.4)	4.35↓ (90.1)	0.382↓ (78.9)	84.4↓ (88.4)	12.54↓ (76.0)	7.12 (98.0)	2.31↓ (74.7)	0.158↓ (66.7)
3.	600 Gy	G_3	70.0↓ (75.9)	17.28↓ (81.7)	9.83↓ (86.8)	4.06↓ (84.2)	0.375↓ (77.5)	72.2↓ (75.6)	11.24↓ (68.1)	5.15↓ (70.9)	2.15↓ (69.6)	0.169↓ (71.3)
EMS												
4.	0.2%	E_1	76.7↓ (83.1)	18.48↓ (87.3)	10.88 (96.2)	4.40↓ (91.3)	0.399↓ (82.4)	83.3↓ (87.2)	11.00↓ (66.6)	5.72↓ (78.8)	2.43↓ (78.6)	0.174↓ (73.4)
5.	0.4%	E_2	77.8↓ (84.3)	18.29↓ (86.4)	9.73↓ (86.0)	4.08↓ (84.6)	0.351↓ (72.5)	62.2↓ (65.1)	10.81↓ (65.5)	4.55↓ (62.7)	2.05↓ (66.6)	0.157↓ (66.2)
6.	0.6%	E_3	74.4↓ (80.7)	17.92↓ (84.7)	9.97↓ (88.1)	3.82↓ (79.3)	0.367↓ (75.8)	56.7↓ (59.3)	10.42↓ (63.1)	3.65↓ (50.2)	2.02↓ (65.4)	0.154↓ (65.0)
NG												
7.	0.005%	N_1	67.8↓ (73.5)	18.33↓ (86.6)	7.09↓ (62.6)	3.98↓ (82.6)	0.415↓ (85.7)	80.0↓ (83.8)	13.47↓ (81.6)	5.81↓ (79.9)	2.39↓ (77.3)	0.180↓ (75.9)
8.	0.010%	N_2	62.2↓ (67.4)	16.18↓ (76.5)	4.37↓ (38.6)	3.74↓ (77.7)	0.379↓ (78.3)	70.0↓ (73.3)	11.80↓ (71.5)	5.36↓ (73.8)	2.49↓ (80.7)	0.182↓ (76.8)
9.	0.015%	N_3	57.8↓ (62.6)	15.35↓ (72.5)	3.57↓ (31.5)	3.32↓ (69.0)	0.345↓ (71.3)	44.4↓ (46.5)	11.88↓ (72.0)	5.03↓ (69.3)	2.16↓ (69.8)	0.165↓ (69.6)

Table 1. (contd…..)

Sl. No.	Treatment	Treatment symbol	BKG-1					OUM 11-5				
			Germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
Gamma rays + EMS												
10.	400 Gy + 0.2%	G_2E_1	74.4↓ (80.7)	18.32↓ (86.6)	6.95↓ (61.4)	4.30↓ (89.2)	0.429↓ (88.6)	78.9↓ (82.6)	9.76↓ (59.1)	5.67↓ (78.0)	2.32↓ (75.3)	0.189↓ (79.7)
11.	400 Gy + 0.4%	G_2E_2	66.7↓ (72.3)	13.91↓ (65.7)	6.55↓ (57.8)	4.06↓ (84.2)	0.395↓ (81.6)	62.2↓ (65.1)	9.56↓ (57.9)	5.48↓ (75.4)	2.29↓ (74.1)	0.166↓ (70.0)
12.	400 Gy + 0.6%	G_2E_3	58.9↓ (63.8)	13.16↓ (62.2)	6.69↓ (59.1)	3.90↓ (80.9)	0.374↓ (77.3)	63.3↓ (66.3)	9.36↓ (56.7)	4.64↓ (63.8)	1.85↓ (60.0)	0.161↓ (67.9)
Gamma rays + NG												
13.	400 Gy + 0.005%	G_2N_1	66.7↓ (72.3)	15.38↓ (72.7)	5.95↓ (52.6)	3.81↓ (78.9)	0.396↓ (81.8)	62.2↓ (65.1)	10.61↓ (64.2)	5.60↓ (77.0)	2.12↓ (68.6)	0.176↓ (74.3)
14.	400 Gy +0.010%	G_2N_2	60.0↓ (65.0)	15.23 (72.0)	3.20↓ (28.2)	3.69↓ (76.6)	0.355↓ (73.3)	61.1↓ (64.0)	9.10↓ (55.1)	3.77↓ (51.9)	2.04↓ (66.1)	0.171↓ (72.2)
15.	400 Gy +0.015%	G_2N_3	48.9↓ (53.0)	11.03↓ (52.1)	2.82↓ (24.9)	2.82↓ (58.5)	0.332↓ (68.5)	42.2↓ (44.2)	7.80↓ (47.3)	3.06↓ (42.1)	1.64↓ (53.1)	0.142↓ (59.9)
EMS + NG												
16.	0.4% + 0.010%	E_2N_2	65.6↓ (71.1)	14.75↓ (69.7)	4.75↓ (42.0)	3.93↓ (81.5)	0.361↓ (74.6)	60.0↓ (62.8)	10.70↓ (64.8)	4.33↓ (59.5)	2.38↓ (76.9)	0.170↓ (71.7)
Control/ Parent												
17.	In distilled water	C	92.2 (100.0)	21.16 (100.0)	11.32 (100.0)	4.82 (100.0)	0.484 (100.0)	95.6 (100.0)	16.51 (100.0)	7.27 (100.0)	3.09 (100.0)	0.237 (100.0)
		CD (5%)	**7.99**	**1.81**	**0.83**	**0.33**	**0.04**	**6.31**	**1.24**	**1.15**	**0.32**	**0.02**

Figures in parentheses indicate percentage of the control

↓ Significant decrease from control at p = 0.05

Table.2 Frequency of macro-mutations in M2 generation

Sl. No.	Mutagenic treatments	Macro-mutation frequency					
		BKG-1			OUM 11-5		
		Mf_C	Mf_M	Mf_T	Mf_C	Mf_M	Mf_T
1.	G_1	0.79	2.18	2.98	0.78	1.96	2.75
2.	G_2	0.61	3.06	3.67	0.81	2.63	3.44
3.	G_3	1.03	4.96	5.99	1.21	3.43	4.64
4.	E_1	1.03	3.70`	4.72	0.77	3.07	3.83
5.	E_2	1.26	4.40	5.66	1.26	4.21	5.47
6.	E_3	1.25	5.42	6.67	1.58	4.75	6.34
7.	N_1	1.33	4.89	6.22	1.52	4.33	5.84
8.	N_2	1.67	5.44	7.11	1.83	5.48	7.31
9.	N_3	1.72	4.53	6.25	2.20	3.52	5.71
10.	G_2E_1	1.35	5.86	7.21	1.28	5.56	6.84
11.	G_2E_2	2.12	6.60	8.73	2.39	6.94	9.33
12.	G_2E_3	1.92	8.65	10.58	2.41	7.71	10.12
13.	G_2N_1	1.80	6.08	7.88	1.97	5.26	7.24
14.	G_2N_2	2.37	9.01	11.37	2.89	8.00	10.89
15.	G_2N_3	2.71	7.92	10.63	3.08	7.47	10.55
16.	E_2N_2	1.43	10.45	11.89	1.58	4.34	5.92
Mean		**1.50**	**5.75**	**7.25**	**1.69**	**4.86**	**6.55**

Mf_C: Chlorophyll mutation frequency
Mf_M: Morphological mutation frequency
Mf_M: Total macro-mutation frequency

Table.3 Relationship of M_1 parameters with induced macro-mutation of M_2

Sl. No.	M_1 Parameters	Correlation coefficient of M_1 parameters with macro-mutational frequencies			Correlation coefficient of M_1 parameters with macro-mutational frequencies		
		BKG-1			OUM 11-5		
		Chlorophyll	Morphological	Total	Chlorophyll	Morphological	Total
Laboratory Experiment							
1.	Germination (%)	-0.905**	-0.764**	-0.821**	-0.836**	-0.646**	-0.711**
2.	Seedling shoot length	-0.883**	-0.796**	-0.843**	-0.792**	-0.877**	-0.870**
3.	Seedling root length	-0..847**	-0.732**	-0.782**	-0.761**	-0.723**	-0.747**
4.	Seedling fresh weight	-0.824**	-0.572*	-0.646**	-0.779**	-0.764**	-0.782**
5.	Seedling dry weight	-0.612**	-0.543*	-0.577*	-0.627**	-0.574*	-0.600*
Field Experiment							
1.	Germination (%)	-0.902**	-0.812**	-0.860**	-0.848**	-0.809**	-0.835**
2.	Survival (%)	-0.772**	-0.646**	-0.696**	-0.827**	-0.784**	-0.811**
3.	Pollen Sterility (%)	0.529*	0.526*	0.545*	0.769**	0.827**	0.826**
4.	Seed sterility (%)	0.592*	0.485*	0.525*	0.792**	0.803**	0.815**

*Significant at 5% level ** Significant 1% level

Table.4 M_2 population variance for different characters

Sl. No.	Mutagenic treatments	M_2 population variance											
		BKG-1						OUM 11-5					
		Plant height	Clusters/ plant	Pods/ plant	Pods length	Seeds/ pod	Grain yield/ plant	Plant height	Clusters/ plant	Pods/ plant	Pods length	Seeds/ pod	Grain yield/ plant
1.	G_1@	8.51	0.34	3.00	0.57↑	1.97↑	0.68	11.14	0.41	8.59	0.14	0.66	0.44
2.	G_2	16.64↑	0.48↑	3.95↑	0.64↑	3.28↑	1.71↑	18.64↑	0.60↑	13.94↑	0.26↑	0.84↑	0.64↑
3.	G_3	20.12↑	0.67↑	5.18↑	0.40↑	3.51↑	1.99↑	20.44↑	0.77↑	14.99↑	0.28↑	1.01↑	0.91↑
4.	E_1	8.71	0.34	3.41	0.34	1.74	0.67	11.24	0.40	10.19	0.17	0.66	0.48
5.	E_2	16.34↑	0.49↑	5.29↑	0.66↑	4.63↑	1.68↑	24.46↑	0.68↑	17.16↑	0.25↑	1.33↑	0.89↑
6.	E_3	20.44↑	0.61↑	6.00↑	0.58↑	4.37↑	1.99↑	33.00↑	0.85↑	17.88↑	0.29↑	1.21↑	0.94↑
7.	N_1	18.77↑	0.53↑	4.07↑	0.61↑	3.86↑	2.64↑	23.79↑	0.54	13.78↑	0.27↑	1.15↑	0.88↑
8.	N_2	21.25↑	0.70↑	5.40↑	0.70↑	4.32↑	3.87↑	15.97↑	0.90↑	18.13↑	0.25↑	1.15↑	1.17↑
9.	N_3	23.39↑	0.54↑	5.28↑	0.64↑	4.59↑	3.20↑	32.14↑	0.95↑	17.66↑	0.27↑	1.38↑	1.08↑
10.	G_2E_1	15.30↑	0.64↑	3.94↑	0.34	3.07↑	0.74	10.02	0.76↑	12.96↑	0.22↑	0.94↑	0.75↑
11.	G_2E_2	18.92↑	0.67↑	6.31↑	0.70↑	4.41↑	3.50↑	27.34↑	0.81↑	16.70↑	0.34↑	1.14↑	1.06↑
12.	G_2E_3	20.50↑	0.67↑	6.08↑	0.78↑	3.60↑	3.91↑	26.99↑	0.91↑	17.07↑	0.31↑	1.17↑	1.28↑
13.	G_2N_1	16.92↑	0.48↑	6.38↑	0.54↑	4.16↑	2.50↑	25.52↑	0.70↑	14.26↑	0.22↑	0.67	0.80↑
14.	G_2N_2	20.47↑	0.47↑	3.27	0.68↑	2.85↑	1.24	28.58↑	0.88↑	22.18↑	0.29↑	1.07↑	1.41↑
15.	G_2N_3	25.26↑	0.52↑	6.52↑	0.72↑	3.75↑	2.86↑	29.18↑	0.68↑	10.10	0.38↑	1.38↑	0.55
16.	E_2N_2	21.86↑	0.67↑	6.12↑	0.88↑	3.66↑	2.41↑	32.94↑	0.94↑	16.02↑	0.28↑	0.80↑	0.85↑
17.	C	5.87	0.26	2.60	0.28	1.34	0.57	7.34	0.38	5.74	0.12	0.52	0.32
		3.23	**0.15**	**0.82**	**0.08**	**0.56**	**0.70**	**4.03**	**0.17**	**4.52**	**0.06**	**0.20**	**0.24**

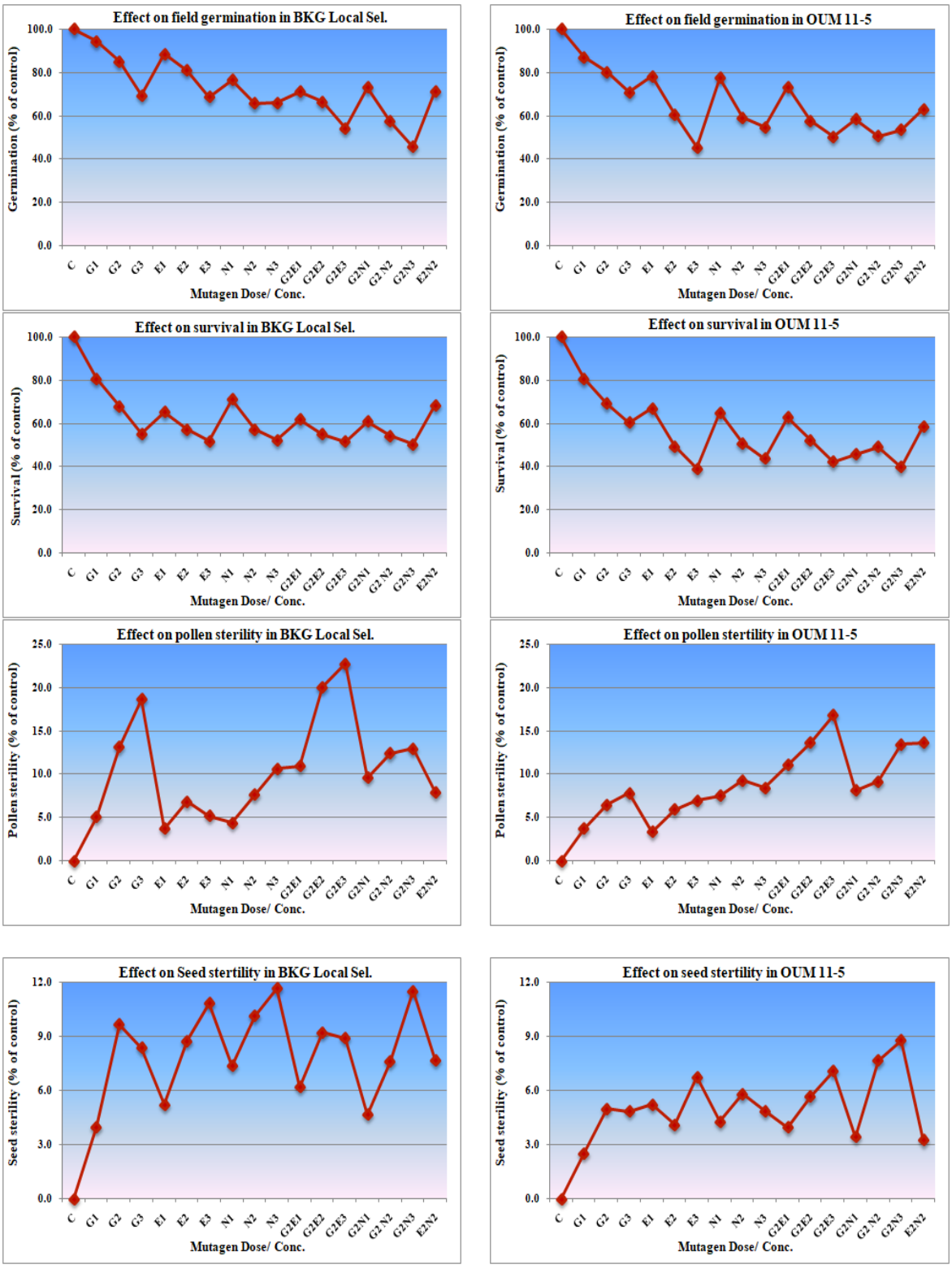
@ symbols of treatment as in Table 2 ↑ Significant increase in variance from control at 5% level

Table.5 Relationship of M_1 parameters with induced micro-mutation of M_2

Sl. No.	M_1 Parameters	M_2 population variance											
		BKG-1						OUM 11-5					
		Plant height	Clusters/ plant	Pods/ plant	Pods length	Seeds/ pod	Grain yield/ plant	Plant height	Clusters/ plant	Pods/ plant	Pods length	Seeds/ pod	Grain yield/ plant
Laboratory Experiment													
1.	Germination (%)	-0.874**	-0.558*	-0.628**	-0.651**	-0.565*	-0.724**	-0.861**	-0.723**	-0.563*	-0.767**	-0.744**	-0.518*
2.	Seedling shoot length	-0.790**	-0.540*	-0.747**	-0.693**	-0.520*	-0.720**	-0.553*	-0.584*	-0.515*	-0.743**	-0.525**	-0.483*
3.	Seedling root length	-0.763**	-0.461	-0.472	-0.596*	-0.483*	-0.594*	-0.751**	-0.639**	-0.561*	-0.739*	-0.684**	-0.516*
4.	Seedling fresh weight	-0.813**	-0.360	-0.611**	-0.583*	-0.578*	-0.578*	-0.716**	-0.566*	-0.541*	-0.825**	-0.711**	-0.504*
5.	Seedling dry weight	-0.691**	-0.348	-0.518*	-0.685**	-0.542*	-0.383	-0.705**	-0.493*	-0.529*	-0.770**	-0.687**	-0.402
Field Experiment													
1.	Germination (%)	-0.885**	-0.678**	-0.674**	-0.564*	-0.584*	-0.665**	-0.821**	-0.822**	-0.787**	-0.769**	-0.720**	-0.753**
2.	Survival (%)	-0.795**	-0.671**	-0.684**	-0.457	-0.723**	-0.573*	-0.785**	-0.753**	-0.720**	-0.778**	-0.755**	-0.675**
3.	Pollen Sterility (%)	0.537*	0.627**	0.499*	0.373	0.376	0.542*	0.591*	0.734**	0.491*	0.799**	0.514*	0.587*
4.	Seed sterility (%)	0.862**	0.661**	0.636**	0.619**	0.774**	0.656**	0.549*	0.502*	0.545*	0.800**	0.690**	0.549*

*Significant at 5% level ** Significant 1% level

Fig.1 Effect of mutagens on germination, survival, pollen sterility and seed sterility



Test of significance of the correlation coefficients indicated that all the correlation coefficients of M_1 parameters were significant at 5% level in both the genotypes. Out of total

42 negative correlation coefficients estimated from M_1 parameters with M_2 population variance of six quantitative traits, 36 and 41 correlation coefficients were significant at 5% level in BKG-1 and OUM 11-5, respectively. M_1 pollen sterility showed significant positive correlation with M_2 population variance in four traits in BKG-1 (except pod length and seeds/ pod) and in all six traits in OUM 11-5, while M_1 seed sterility showed a positive significant correlation with M_2 population variance of all the six traits in both the genotypes. The positive relationship between M_1 biological injury parameters with M_2 macro- and micro-mutations is in corroboration to the earlier findings (Blixt *et al.,* 1964; Thakur and Sethi, 1995; Singh and Mohapatra, 2004 and Mishra and Singh, 2013). Hence, M_1 biological injuries can serve as reliable parameters for early identification of effective mutagenic treated population for widening genetic variability in M_2 and later generations.

References

Blixt, I., Gelin, O., Ahnstrom, G., Eherenberg, L. and Lofgraen, R.A. 1964. Studies of induced mutations in peas, *Agri Hort. Genet*., *22*: 1-2.

Gaul, H. 1977. Mutagen effects in the first generation after seed treatment. *In*: Manual on Mutation Breeding, Tech. Rep. Ser. 119, IAEA, Vienna, 1977. pp.87-96.

Goda, T., Teramura, H., Suehiro, M., Kanamaru, K., Kawaguchi, H., Ogino, C. 2016. Natural variation in the glucose content of dilute sulfuric acid–pretreated rice straw liquid hydrolysates: implications for bioethanol production. *Bioscience, Biotechnology and Biochemistry*, 80(5): 863-869.

Khan, S. and Wani, M.R.. 2006. Induced Mutations for Yield Contributing Traits in Green Gram. *International Journal of Agriculture & Biology*, 4: 528–530.

Mishra, D. and Singh, B. 2013. Prediction of M_2 macro and micro-mutation frequency based on M_1 effect in greengram [*Vigna radiata* (L.) Wilczek]. *IOSR Journal of Agriculture and Veterinary Science*, 2(1): 01-04.

Mori, Vaishali, K, Kumar, R. and Mori K.K. and Ribadiya, K.H. 2016. EMS and gamma rays induced mutation in greengram [*Vigna radiata* (L.) Wilczek]. The Ecoscan, 10(1&2): 75-80.

Singh, B. and B.K. Mohapatra. 2004. Prediction of M_2 mutation frequency based on M_1 estimates in blackgram. *Legume Res*., 27(2): 137-139.

Sujay, Rakshit, Singh,Y.P. and Rakshit, S. 2001. Chemosensitivity studies in mungbean and urdbean. *Indian J Pulse Res.* 14 (2): 112-115.

Swaminathan MS, Siddiq EA, Savin VN and Varughese G. 1968. Studies on the enhancement of mutation frequency and identifications of mutations in plant breeding and phylogenetic significance in some cereals. Mutations in Plant Breeding II (Proc. Panel Vienna, 1967), IAEA, Vienna, p. 233-248.

Thakur, J.R. and Sethi, G.S. 1995. Mutagenic interaction of gamma rays with EMS and NaN3 in barley [*Hordeum vulgare* (L.) em. Bowden]. *Crop Research*, *9(2):* 303-308.

Wani, M.R. 2004. Effect of EMS on seed germination and pollen fertility in mungbean. *Bionotes*, 6 (2) : 56.

How to cite this article:

Digbijaya Swain, Bhabendra Baisakh, Devraj Lenka and Swapan K. Tripathy 2019. M_1 Biological Injuries: Indicators for M_2 Macro- and Micro-mutation in Mungbean [*Vigna radiata (L.)* Wilczek]. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 1685-1696.
doi: https://doi.org/10.20546/ijcmas.2019.809.191