Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 2489-2495
Received: 14-07-2019
Accepted: 18-08-2019

Digbijaya Swain
Ph.D. Scholar & Maize Breeder, AICRP on maize, Directorate of Research, OUAT, Bhubaneswar, Odisha, India

Bhabendra Baisakh
Dean, PGF-DRI, OUAT, Bubaneswar, India

Swapan K Tripathy
Professor, Department of Agricultural Biotechnology, College of Agriculure, OUAT, Bhubaneswar, India

Devraj Lenka
Professor, Department of Plant Breeding & Genetics, College of Agriculture, OUAT, Bhubaneswar, India

Corresponding Author:
Swapan K Tripathy
Professor, Department of Agricultural Biotechnology, College of Agriculure, OUAT, Bhubaneswar, India

Mutagenic effect of gamma rays, EMS, NG and their combinations for induction of Chlorophyll and macro-mutations in mungbean (*Vigna radiata* (L.) Wilczek)

Digbijaya Swain, Bhabendra Baisakh, Swapan K Tripathy and Devraj Lenka

Abstract
Seed treatment of two mungbean genotypes, BKG-1 and OUM 11-5, with gamma rays, EMS, NG and their combinations induced wide spectrum of chlorophyll mutations (albina, xantha, chlorina, viridis and sectorial) and morphological macro-mutations in M_2. Chlorina types were more frequent in both the genotypes followed by xantha and albino. Among morphological mutations, quadrifoliate, dwarf plant, lobed leaf and pentafoliate mutants induced at high frequency in both the genotypes. NG in single and combination treatments induced higher chlorophyll and morphological mutations than EMS and GR in both the genotypes. The frequency of chlorophyll, morphological and total macro-mutations showed a dose dependent increase in both single and combined treatments in case Gamma-rays and EMS in both the genotypes. While, morphological macro-mutation frequency was highest in 0.010% NG among single treatments; and 0.4% EMS + 0.010% NG in BKG-1 and 400 Gy gamma rays + 0.010% NG in OUM 11-5 among combination treatments in both the genotypes.

Keywords: Mungbean, morphological macro-mutation, chlorophyll mutation, EMS, NG, gamma-rays

Introduction
Mungbean (*Vigna radiata* (L.) Wilczek) is an important crop of India due to short duration and better adaptability to varied agro-ecological conditions throughout the year. Mungbean is the cheap source of easily digestible protein component of Indian diet. But the low average national productivity of this crop is a matter of great concern. The improvement of productivity in this crop is very slow due to narrow genetic base and erosion of a large part of genetic variation for its cultivation in marginal and sub-marginal land since long. However, mutagenesis offers an opportunity for induction of desirable traits which are either not found in the existing germplasm or have lost during the process of evolution. Induced mutagenesis has been proved to generate genetic variation and has been successfully utilized to develop a large number of mutant cultivars with high yield and other desirable characters (Kharkwal and Shu, 2009) [7].

Chlorophyll mutations are the first important biological changes in any induced mutation project. Though these mutants are not useful for plant breeding purpose, the spectrum and frequency of chlorophyll mutation are considered as indices to evaluate the genetic effect of the mutagen (Gustafsson, 1951) [6]; and such approach is *in vogue* used in several crops (Reddy *et al*. 1995) [12]. The present investigation was undertaken to study spectrum and frequency of macro-mutations (chlorophyll and morphological mutations) in an experiment designed to induce mutation in two mungbean genotypes.

Materials and Methods
Dry, uniform, genetically pure and healthy seeds of two mungbean genotypes *viz*., BKG-1 and OUM 11-5 were used for induction of mutation using Gamma-rays, Ethyl methane sulphonate (EMS), N-nitro-N-Nitrosoguanidine (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 of these two genotypes were irradiated with three doses of gamma rays (G1, G2, G3: 200 Gy, 400 Gy and 600 Gy) in the ^{60}CoGamma chamber at Bhabha Atomic and Research Centre (BARC), Mumbai. Besides, chemical mutagen treatment was administered with three different concentration of EMS (E1, E2, E3: 0.2%, 0.4%, and 0.6%) and NG (N1, N2, N3: 0.005%, 0.010% and 0.015%) aqueous solutions for six hours following a pre-soaking period of six hours. Besides, 400 Gy gamma-

ray irradiated seeds were pre-soaked in distilled water for six hours and then treated with above mentioned three concentrations of EMS(G2E1, G2E2, G2E3) and NG(G2N1, G2N2, G2N3). For combination treatment of two chemical mutagens (E2N2), distilled water pre-soaked seeds were first treated with 0.4% of EMS followed by 0.01% NG for three hours each. All the treatments were carried out at room temperature (22 ± 1 ºC) with intermittent shaking. The seeds treated with chemical mutagens were thoroughly washed under tap water to leach out residual chemicals absorbed to the treated seeds and then the seeds were dried on the blotting paper. The mutagen treated seeds from every treatment along with the parental genotypes were sown in two trials in a completely randomized block design with two replication 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 M_1 generation. The bulk seeds harvested from all the surviving M_1 plants of each treatment from two test genotypes were used along with the parents to grow M_2 generation in two separate trials in completely randomized block design with three replications. Standard agronomic package of practices were followed to raise the crop. The effects of mutagenic treatments in M_2 generation were evaluated on the basis of spectrum and frequency of macro-mutations (chlorophyll and viable morphological mutation). Observation on different types of chlorophyll mutations in each treatment were recorded daily from emergence of seedlings to 15th days after sowing, while The morphological mutations were scored throughout the life span of the plant from germination to physiological maturity of the crop. The frequencies of chlorophyll & morphological mutations were estimated on the basis of 100 M_2 plants following Gaul (1960) [4].

Results and discussion

Spectrum and frequency of chlorophyll mutants

In the present study, five distinct categories of chlorophyll mutations *viz*., albina, xantha, chlorina, viridis and sectorial chimaera were observed in M_2 generation of both the genotypes (Table 1, Table 2 and Fig. 4). Chlorophyll mutations in M_2 generation have been reported earlier in mungbean by several workers (Das and Baisakh, 2009; Kousar *et al*. 2013, Vairam *et al*. 2014; Rukesh *et al*. 2017 and Arulsevi, 2019) [2, 8, 18, 13, 1]. Chlorophyll development seems to be controlled by many genes located on several chromosomes (Goud (1967) [5]. It could be adjacent to centromere and proximal segments of chromosomes (Swaminathan, 1964 & 1965) [16, 17]. The origin of chlorophyll deficiencies is mainly due to mutation in these genes, which are responsible for synthesis of photosynthetic pigments. Among different types of chlorophyll mutations, chlorina types appeared more frequently in both the genotypes (49.5% in BKG-1 and in 51.6% in OUM 11-5). Chlorina was followed by xantha (28.8% and 26.6%, respectively in BKG-1 and OUM 11-5) and albino (11.7% and 14.8%, respectively in BKG 1 and OUM 11-5) suggesting high mutability of the genes controlling the phenotype. Appearance of higher frequency of Chlorina type was in agreement with the findings of Das and Baisakh (2009) [2] and Vairam *et al*. (2014) [18]. Sectorial chimaera and viridis types were observed in least frequency in both the genotypes. The chlorophyll mutation spectrum was wider (four types) in seven treatments of both BKG-1 (N_1, N_2, G_2E_3, G_2N_1, G_2N_2, G_2N_3 and E_2N_2) and OUM 11-5 (E_2, E_3, N_1, N_3, G_2E_3, G_2N_2 and G_2N_3).

The frequency of chlorophyll mutation per 100 M_2 plant was 0.61 (G_2) to 2.71 (G_2N_3) in BKG-1 and 0.77 (E_1) to 3.08 (G_2N_3) in OUM 11-5. The frequencies of chlorophyll mutations were in order of GN (2.29, 2.65%) > GE (1.79, 2.01%) > NG (1.58, 1.85%) > EN (1.43, 1.58%) > EMS (1.18, 1.20%) > GR (0.81, 0.93%) in both the genotypes (Figure 1 and 2) indicating that the chlorophyll mutation frequency recorded in combined treatments of gamma rays with NG and EMS was higher than the single treatments. Among the nine single mutagenic treatments, the mutation frequency was in order of N_3 (1.72%) > N_2 (1.67%) > N_1 (1.33%) > E_2 (1.26%) > E_3 (1.25%) > G_3, E_1 (1.03%) > G_1 (0.79%) > G_2 (0.61%) in BKG-1; and while, it wasN_3 (2.20%) > N_2 (1.83%) > E_3 (1.58%) > N_1 (1.52%) > E_2 (1.26%) > G_3 (1.21%) > G_2 (0.81%) > G_1 (0.78%) > E_1 (0.77%) in OUM 11-5. Among seven combined mutagenic treatments, G_2N_3 (2.71%) revealed highest mutation frequency followed by G_2N_2 (2.37%) and G_2E_2 (2.12%) in BKG-1, while in OUM 11-5 the order was G_2N_3 (3.08%) > G_2N_2 (2.89%) > G_2E_3 (2.41%) > G_2E_2 (2.39%) > G_2N_1 (1.97%) > E_2N_2 (1.58%) > G_2E_1 (1.28%).

Spectrum and frequency of morphological macro-mutations

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 BKG-1 and OUM 11-5, respectively (Table 1, Table 2 and Fig. 5). Wide spectrum of morphological mutants following mutagen treatment has been reported earlier in mungbean by several workers (Mishra, 2004; Singh and Singh, 2007; Ananda Kumar *et al.* 2009 and Mishra *et al.* 2013) [11, 14, 9, 10]. Among single mutagenic treatments of BKG-1, the mutation spectrum was wider in N_2 (12 types) followed by E_3 and N_1 (10 types), while in OUM 11-5, wider mutation spectrum was observed in G_3 and E_3 (11 types) followed by G_1, N_1 and N_2 (9 types). Among the combination treatments, the spectrum was highest in G_2E_3 in both the genotypes (15 and 13 types, respectively). Quadrifoliates, dwarf plants, lobed leaf, pentafoliates and flowering duration mutants were most common and higher spectrum in both the genotypes as compared to other mutants suggesting high mutability of the gene controlling these phenotypes.

The frequency of morphological mutations per 100 M_2 plant was 2.18 (G_1) to 10.45 (E_2N_2) in BKG-1 and was 1.96 (G_1) to 8.00 (G_2N_2) in OUM 11-5. The frequencies of morphological mutations per 100 M_2 plants were in order of EN (10.45%) > GN (7.65%) > GE (7.01%) > NG (4.96%) > EMS (4.50%) >GR (3.38%), while in OUM 11-5, the order wasGN (6.91%) > GE (6.70%) > NG (4.43%) > EN (4.34%) >EMS (3.99%) > GR (2.66%) indicating that the like chlorophyll mutation frequency morphological mutation frequency was higher in combination treatments than single treatments. Among the nine single mutagenic treatments, the frequency of morphological mutation was in order of N_2 (5.44%) > E_3 (5.42%) > G_3 (4.96%) > N_1 (4.89%) > N_3 (4.53%) > E_2 (4.40%) > E_1 (3.70%) > G_2 (3.06%) > G_1 (2.18%) in BKG-1 and in OUM 11-5, the order was N_2 (5.48%) > E_3 (4.75%) > N_1 (4.33%) > E_2 (4.21%) > N_3 (3.52%) > G_3 (3.43%) > E_1 (3.07%) > G_2 (2.63%) > G_1 (1.96%). Among the combined mutagenic treatments, the frequencies of morphological mutations per 100 M_2 plants were in order of E_2N_2 (10.45%) > G_2N_2 (9.00%) > G_2E_3 (8.65%) > G_2N_3 (7.92%) > G_2E_2

(6.60%) > G_2N_1 (6.08%) > G_2E_1 (5.86%) in BKG-1, while in OUM 11-5 the order was G_2N_2 (8.00%) > G_2E_3 (7.71%) > G_2N_3 (7.47%) > G_2E_2 (6.94%) > G_2E_1 (5.56%) > G_2N_1 (5.26%) > E_2N_2 (4.34%).

Total mutation frequency

The frequency of total mutations (both chlorophyll and morphological) per 100 M_2 plants in mutagenic treated population (Table 1 and 2) ranged from 2.98 (G_1) to 11.89 (E_2N_2) in BKG-1, while in OUM 11-5; the range was from 2.75 (G_1) to 10.89 (G_2N_2). On an average, the total mutation frequency was higher in BKG-1 (7.25%) than OUM 11-5 (6.53%). With respect to total mutation frequency, the mutagens followed the similar trend as in case of morphological mutation frequency in both the genotypes, while treatment-wise analysis indicated almost similar trend as in morphological mutation frequency with little change in positions for some treatments.

In general, the results of present investigation indicated that both chlorophyll mutation and morphological mutation frequency were higher in combination treatments than single treatments revealing the synergistic effect of mutagens in combination treatments. The frequencies of chlorophyll mutations increased with increase in dose for single as well as combined treatments. But the frequencies of morphological mutations and total macro-mutation increased with increase in dose except in case of NG for both single and combination treatments in both the genotypes. In case of NG, the highest values for morphological and total mutation frequencies were observed at medium doses of single (N_2) and combination treatments (G_2N_2). Dose dependent increase in mutation frequency was also reported in mungbean by several workers (Singh and Singh, 2007; Kumar, *et al*. 2009 and Gandhi *et al*. 2014) [14, 9, 3]. The observed maximum mutation frequency at medium doses as in case of NG in the present investigation may be referred as 'saturation effect' (Sree Ramulu, 1970) [15] which could possibly due to gradual inactivation of the repair system by NG and its combination treatments at higher doses and thereby inducing lethality. It was observed that NG in single and combination treatments induced higher chlorophyll and morphological mutations than EMS and GR in both the genotypes. On an average, the chlorophyll mutation frequency was higher in OUM 11-5 (1.69%) than BKG-1 (1.50%), while the morphological mutation frequency was higher in BKG-1 (5.75%) than OUM 11-5 (4.83%) indicating differential response of the genotypes to mutagenic treatments and more sensitivity of OUM 11-5 to chlorophyll mutation and BKG-1 to morphological mutations.

Table 1: Spectrum and frequency of macro-mutations in M_2 generation of BKG -1

Macro-mutation	Mutagenic Treatment																	C
	Single									Combination								
	G_1*	G_2	G_3	E_1	E_2	E_3	N_1	N_2	N_3	G_2E_1	G_2E_2	G_2E3	G_2N_1	G_2N_2	G_2N_3	E_2N_2	Total	
Total plants scored	504	490	484	487	477	480	450	478	464	444	424	416	444	422	442	488	7394	544
A. Chlorophyll mutation																		
1. Albina	-	-	1	-	1	1	1	1	2	1	-	1	1	1	1	1	13	-
2. Xantha	1	1	1	2	2	2	2	2	3	-	2	2	2	3	4	3	32	-
3. Chlorina	2	2	3	2	3	3	2	4	3	4	6	4	4	5	6	2	55	-
4.Viridis	1	-	-	1	-	-	1	-	-	1	-	-	1	1	1	-	7	-
5. Sectorial	-	-	-	-	-	-	-	1	-	-	1	1	-	-	-	1	4	-
Total- A	4	3	5	5	6	6	6	8	8	6	9	8	8	10	12	7	111	0
Frequency - A (%)	0.79	0.61	1.03	1.03	1.26	1.25	1.33	1.67	1.72	1.35	2.12	1.92	1.80	2.37	2.71	1.43	1.50	0.00
B. Morphological mutation																		
1. Monocotyledonary	-	1	1	1	-	-	1	1	1	-	-	2	1	1	1	1	12	-
2. Tricotyledonary	-	-	-	-	-	1	-	-	-	-	1	1	-	-	-	-	3	-
3. Tetracotyledoary	-	-	-	-	-	1	-	1	-	-	-	1	-	-	-	-	3	-
4. Unifoliate	-	-	-	-	1	-	-	-	-	-	-	1	-	1	2	-	5	-
5. Bifoliate	1	1	2	1	1	1	2	1	1	2	-	2	1	2	2	1	21	-
6. Quadrifoliate	2	5	8	4	6	6	4	5	5	6	8	10	7	11	8	5	100	-
7. Pentafoliate	-	1	-	1	1	2	1	1	2	-	1	2	2	4	2	1	21	-
8. Lobed leaf	4	3	4	4	3	5	4	4	4	6	7	4	7	6	5	2	72	-
9. Serrated leaf	-	-	1	1	-	1	1	1	-	1	-	1	-	2	3	-	12	-
10. Fasciatedstem	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
11. Hypocotyl pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	8	-
12. Early flowering	-	-	-	1	-	4	2	1	1	2	1	1	2	2	1	7	25	-
13. Late flowering	-	-	2	-	-	-	-	-	-	1	1	-	-	-	2	2	8	-
14. Tall	-	-		-	-	-	1	1	-	-	-	-	-	-	1	-	3	-
15. Dwarf	4	4	5	4	2	4	5	4	5	6	4	6	7	7	5	5	77	-
16. Short pod	-	-	-	1	3	-	-	3	-	1	2	1	-	-	-	7	18	-
17. Trailing type	-	-	-	-	1	-	1	-	1	-	-	-	-	1	1	-	5	-
18. Profuse podded	-	-	-	-	3	1	-	3	-	-	2	1	-	-	-	5	15	-
19. Small seed	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	7	9	-
20. Sterile plant	-	-	1	-	-	-	-	-	1	-	1	2	-	1	2	-	8	-
Total - B	11	15	24	18	21	26	22	26	21	26	28	36	27	38	35	51	425	0
Frequency - B (%)	2.18	3.06	4.96	3.70	4.40	5.42	4.89	5.44	4.53	5.86	6.60	8.65	6.08	9.00	7.92	10.45	5.75	0.00
Total - A+B	15	18	29	23	27	32	28	34	29	32	37	44	35	48	47	58	536	0
Frequency - A+B (%)	2.98	3.67	5.99	4.72	5.66	6.67	6.22	7.11	6.25	7.21	8.73	10.58	7.88	11.37	10.63	11.89	7.25	0.00

***G_1:** 200 Gy gamma rays; **G_2:** 400 Gy gamma rays; **G_3:** 600 Gy gamma rays; **E_1:** 0.2% EMS; **E_2:** 0.4% EMS; **E_3:** 0.6% EMS; **N_1:** 0.005% NG; **N_2:** 0.010% NG; **N_3:** 0.015% NG; **G_2E_1**: 400 Gy gamma rays + 0.2% EMS; **G_2E_2**: 400 Gy gamma rays + 0.4% EMS; **G_2E_3**: 400 Gy gamma rays + 0.6% EMS; **G_2N_1**: 400 Gy gamma rays + 0.005% NG; **G_2N_2**: 400 Gy gamma rays + 0.010% NG**;** **G_2N_3**: 400 Gy gamma rays + 0.015% NG

Table 2: Spectrum and frequency of macro-mutations in M2 generation of OUM 11-5

Macro-mutation	Mutagenic Treatment																	C	Mutagen					
	Single									Combination									Single			Combination		
	G_1@	G_2	G_3	E_1	E_2	E_3	N_1	N_2	N_3	G_2E_1	G_2E_2	G_2E3	G_2N_1	G_2N_2	G_2N_3	E_2N_2	Total		GR	EMS	NG	GE	GN	EN
A. Chlorophyll mutation																								
Total plants scored	510	495	496	522	475	505	462	438	455	468	461	415	456	450	455	507	7570	552	1501	1502	1355	1344	1361	507
1. Albina	-	-	-	-	1	1	1	1	2	1	1	2	2	2	3	2	19	-	-	2	4	4	7	2
2. Xantha	1	2	2	2	1	2	2	2	1	2	3	2	2	4	4	2	34	-	5	5	5	7	10	2
3. Chlorina	2	2	3	2	3	4	3	5	6	3	7	5	5	6	6	4	66	-	7	9	14	15	17	4
4.Viridis	1	-	-	-	-	-	-	-	1	-	-	1	-	1	-	-	4	-	1	-	1	1	1	-
5. Sectorial	-	-	1	-	1	1	1	-	-	-	-	-	-	-	1	-	5	-	1	2	1	-	1	-
Total- A	4	4	6	4	6	8	7	8	10	6	11	10	9	13	14	8	128	0	14	18	25	27	36	8
Frequency - A (%)	0.78	0.81	1.21	0.77	1.26	1.58	1.52	1.83	2.20	1.28	2.39	2.41	1.97	2.89	3.08	1.58	1.69	0.00	0.93	1.20	1.85	2.01	2.65	1.58
B. Morphological mutation																								
1. Monocotyledonary	1	-	1	1	-	1	2	-	-	1	1	-	1	1	-	1	11	-	2	2	2	2	2	1
2. Tricotyledonary	-	-	1	-	-	1	-	-	-	-	1	-	-	-	-	-	3	-	1	1	-	1	-	-
3. Tetracotyledoary	-	-	-	-	-	1	1	-	-	1	-	-	-	-	1	1	5	-	-	1	1	1	1	1
4. Unifoliate	-	-	-	-	1	1	1	-	1	-	2	1	1	1	2	-	11	-	-	2	2	3	4	-
5. Bifoliate	1	-	1	2	-	2	-	-	-	2	1	2	-	2	2	1	16	-	2	4	-	5	4	1
6. Quadrifoliate	2	4	4	6	4	4	7	4	5	5	8	6	6	6	5	2	78	-	10	14	16	19	17	2
7. Pentafoliate	1	2	-	2	2	2	1	-	2	1	2	4	2	5	7	5	38	-	3	6	3	7	14	5
8. Lobed leaf	1	1	2	-	2	-	4	2	2	4	4	5	4	4	5	2	42	-	4	2	8	13	13	2
9. Serrated leaf	-	1	1	-	-	-	1	2	1	2	2	1	2	4	2	2	21	-	2	-	4	5	8	2
10. Fasciatedstem	1	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-	4	-	2	-	-	2	-	-
11. Hypocotyl pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-
12. Early flowering	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-
13. Late flowering	-	1	1	1	2	4	-	2	-	2	4	2	2	4	2	2	29	-	2	7	2	8	8	2
14. Tall	-	-	1	2	3	2	1	5	-	1	2	1	2	2	2	1	25	-	1	7	6	4	6	1
15. Dwarf	1	2	3	-	2	4	2	1	4	4	4	5	4	6	4	4	50	-	6	6	7	13	14	4
16. Short pod	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1
17. Trailing type	-	-	1	-	-	-	-	1	-	1	-	1	-	-	-	-	4	-	1	-	1	2	-	-
18. Profuse podded	1	1	-	2	4	2	-	5	-	2	-	1	-	1	-	-	19	-	2	8	5	3	1	-
19. Small seed	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
20. Sterile plant	-	-	1	-	-	-	-	2	1	-	-	2	-	-	2	-	8	-	1	-	3	2	2	-
Total - B	10	13	17	16	20	24	20	24	16	26	32	32	24	36	34	22	366	0	40	60	60	90	94	22
Frequency - B (%)	1.96	2.63	3.43	3.07	4.21	4.75	4.33	5.48	3.52	5.56	6.94	7.71	5.26	8.00	7.47	4.34	4.83	0.00	2.66	3.99	4.43	6.70	6.91	4.34
Total - A+B	14	17	23	20	26	32	27	32	26	32	43	42	33	49	48	30	494	0	54	78	85	117	130	30
Frequency - A+B (%)	2.75	3.43	4.64	3.83	5.47	6.34	5.84	7.31	5.71	6.84	9.33	10.12	7.24	10.89	10.55	5.92	6.53	0.00	3.60	5.19	6.27	8.71	9.55	5.92

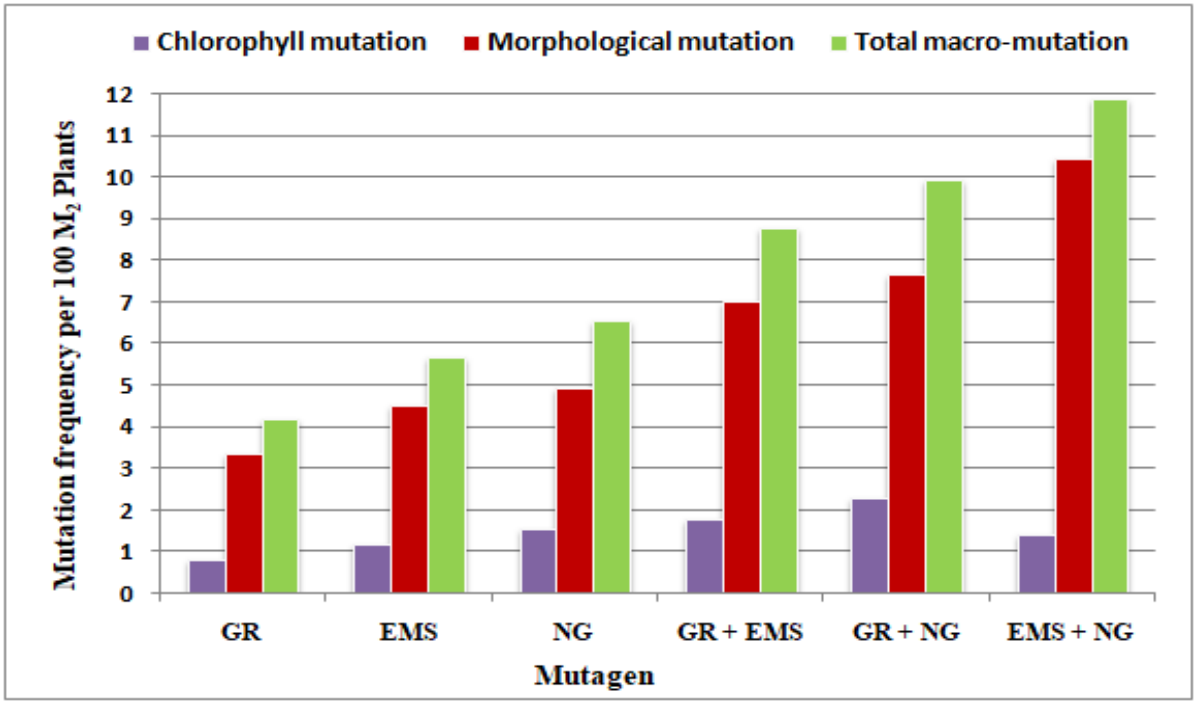

Fig 1: Macro-mutational frequency of different mutagenic treatments in BKG-1

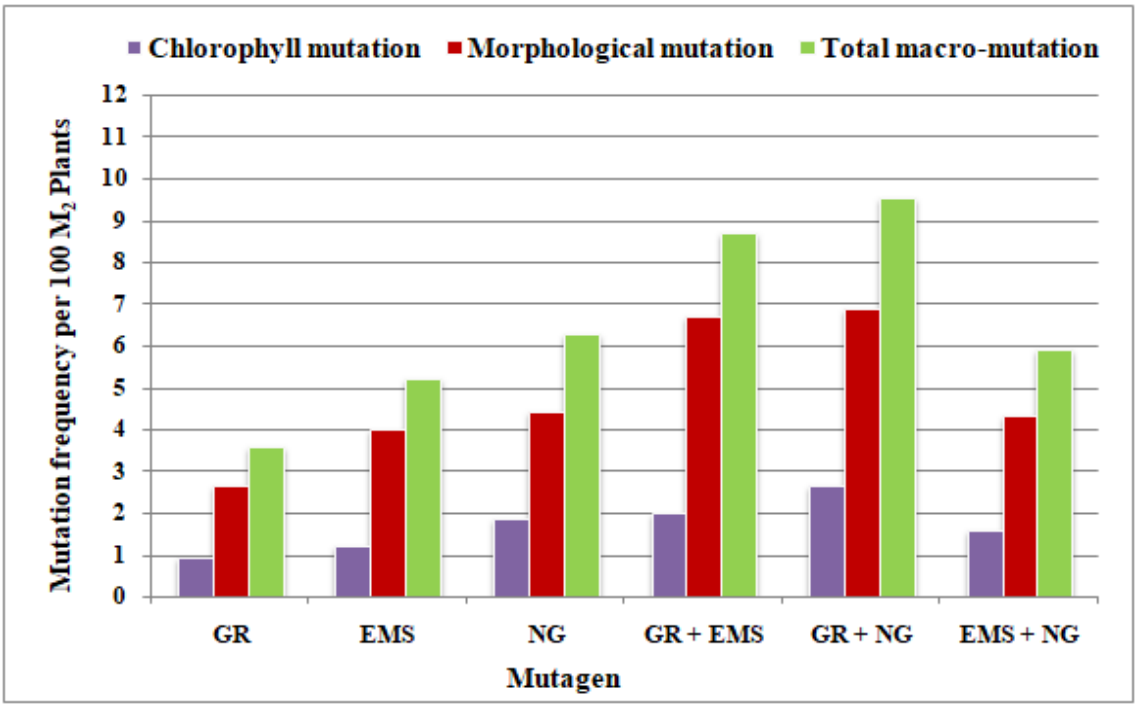

Fig 2: Macro-mutational frequency of different mutagenic treatments in OUM 11-5

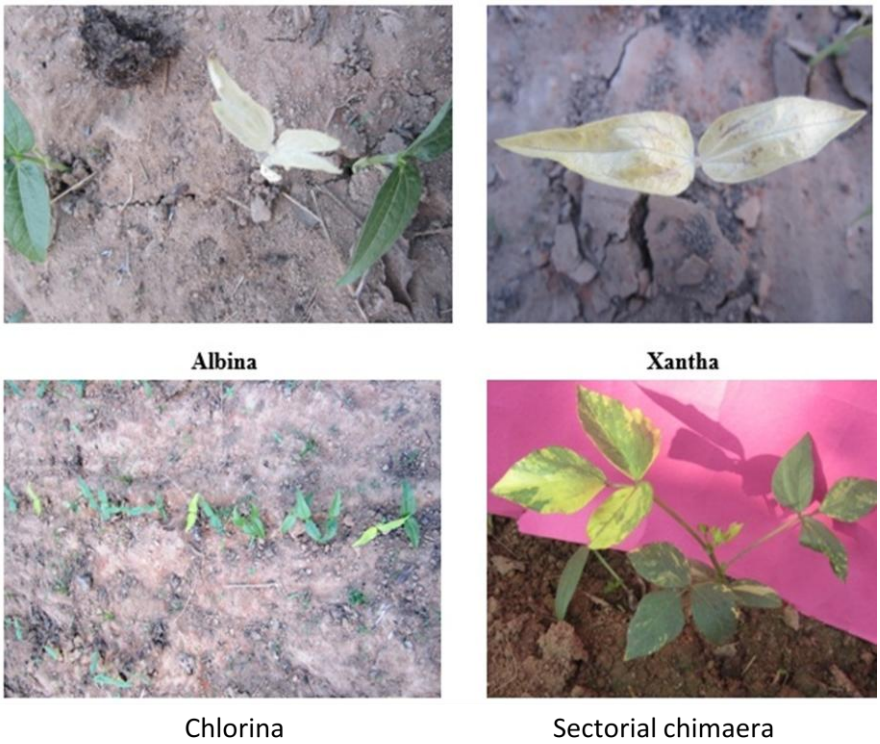

Fig 3: Chlorophyll mutations in M_2

Fig 4: Morphological Macro-mutation in M_2

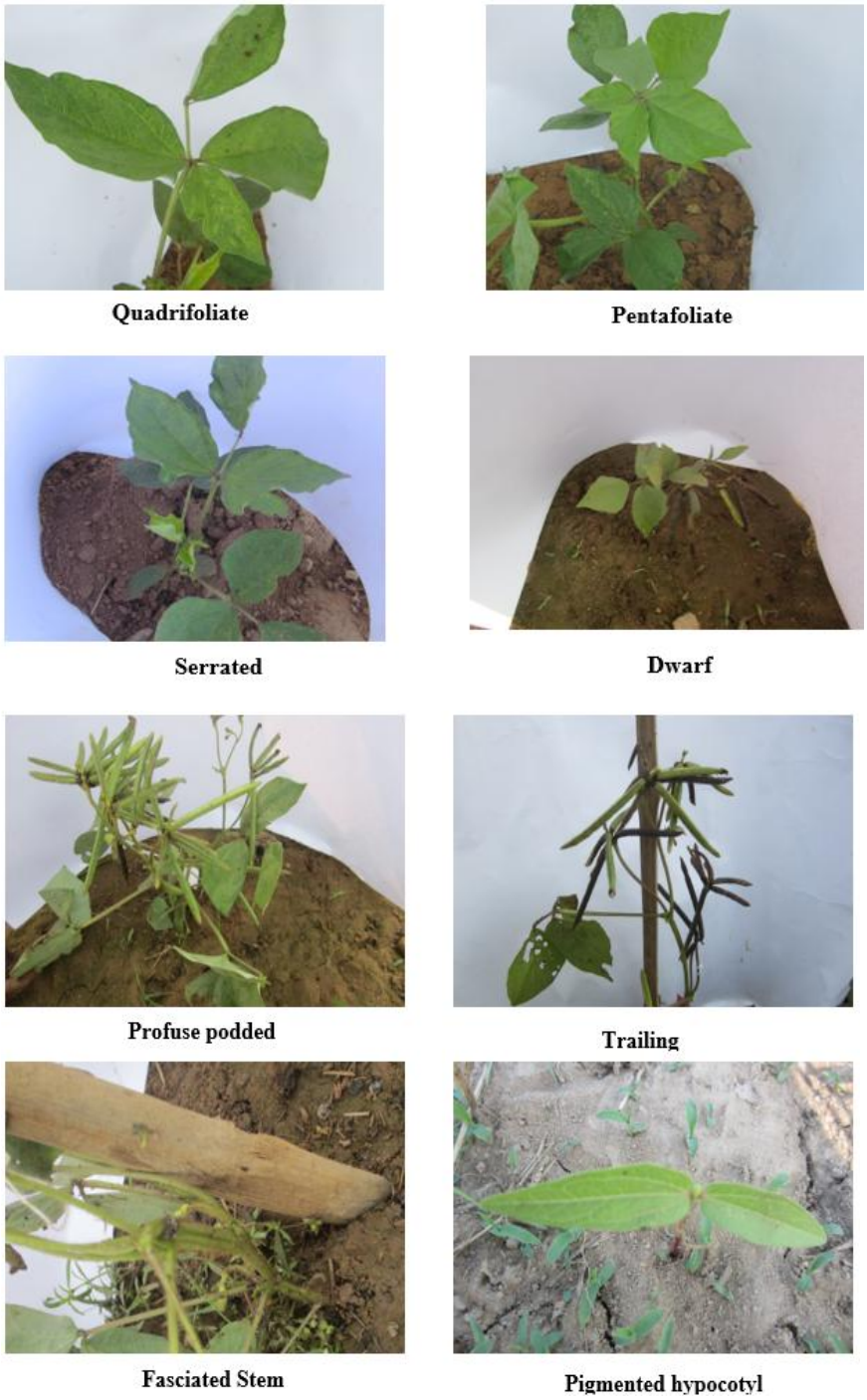

Fig 4: (Contd…..) Morphological Macro-mutation in M_2

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

References

1. Arulsevi S, Suresh S, Dhole VJ. Occurrence of chlorophyll deficient mutants in the mutated populations of greengram (*Vigna radiata* (L.) Wilczek). 2019; 10(1):303-306.
2. Das TR, Baisakh B. Effects of mutagenic treatments on chlorophyll mutation in green gram [*Vigna radiata* (L.) Wilczek]. 2009; EPlanet. 2009; 9(2):17-20.
3. Gandhi S, Umavathi ES, Mullainathan L. Studies on induced chlorophyll mutants in greengram (*Vigna radiata* (L.) Wilczek). International Journal of Advanced Research. 2014; 2(2):00-04.
4. Gaul H. Critical analysis of the methods for determining the mutation frequency after seed treatment with mutates. Genetic Agrigaria. 1960; 12(3, 4):297-318.
5. Goud JV. Induced mutation in bread wheat. Indian J Genet. 1967; **27**:40-55.
6. Gustafsson A. Induction of changes in genes and chromosomes II. Mutation, environment and evolution. Cold spring Harbour Symp, Quant. Bio. 1951; 16:263-281.
7. Kharkwal MC, Shu QY. The role of induced mutations in world food security. Q.Y. Shu (ed.). Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome, 2009, 33-38.
8. Kousar M, Suresh GB, Lavanya GR, Kumari A. Study of chlorophyll and macromutations induced by gamma rays and sodium azide in urd bean (*Vigna mungo* L. Hepper).

African Journal Agricultural Research. 2013; 8(47):5958-5961.
9. Kumar A, Parrmhansh P, Prasad R. Induced chlorophyll and morphological mutations in mungbean [*Vigna radiata* (L.) Wilczek]. Legume Res., 2009; 32(1):41-45.
10. Mishra D, Singh B, Sahu R. Gamma ray induced macro-mutations in greengram [*Vigna radiata* (L.) Wilczek]. International Journal of Agriculture and Forestry. 2013; 3(3):105-109.
11. Mishra TK. Induced genetic variability and improvement of green gram (*Vigna radiata* (L.) Wilczek).Ph. D. Thesis, OUAT, Bhubanheswar, 2004.
12. Reddy YRK, Viswanathan P, Asir R, Arumugam S. Frequency and Spectrum of Chlorophyll Mutants in Triticales with Different Rye Chromosome Constitution. J Ind. Bot. Soc., 1995; 74:335-336.
13. Rukesh AG, Rahuman MA, Latitia SC, Packiaraj D. Impact of gamma irradiation induced mutation on morphological and yield contributing traits of two genotypes of Green gram (*Vigna radiata* L.). Journal of Pharmacognosy and Phytochemistry. 2017; 6(6):1229-1234.
14. Singh AK, SinghAK. Biological influence of gamma rays and Ethyl Methane Sulphonate and their synergistic effects in mungbean. J. Food Legumes. 2007; 20(2):153-155.
15. Sree Ramulu K. Mutagenecity of radiations and chemical mutagens in sorghum. Theoretical Application of Genetics. 1970; 40:257-260.
16. Swaminathan MS. A comparison of mutation induction in diploids and polyploids. 1964, FAO/IAEA Technical Meeting on “Use of induced mutations in plant\ breeding”, 1964, 619- 641.
17. Swaminathan MS. Report of the meeting of the symposium in the use of induced mutations in plant breeding. Radiat. Bot.1965; 5:65-69.
18. Vairam N, Ibrahim SM, Vanniarajan C. Frequency and spectrum of chlorophyll mutations in greengram [*Vigna radiata* (L.) Wilczek]. Asian Journal of Bioscience. 2014; 9(2):204-207