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 M2. Chlorina types were more frequent in both the genotypes followed by xantha and albino. Among morphological mutations, quadrifoliate, dwarf plant, lobed leaf and pentalobate 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 AICRP on maize, Directorate of Agriculture, OUAT, Bhubaneswar, India. 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 °C) 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 cm² at EB-II Section, Department of Plant Breeding and Genetics, OUAT to raise M₁ generation. The bulk seeds harvested from all the surviving M₁ plants of each treatment from two test genotypes were used along with the parents to grow M₂ 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 M₂ 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 day 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 M₂ 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 chimera were observed in M₂ generation of both the genotypes (Table 1, Table 2 and Fig. 4). Chlorophyll mutations in M₂ 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, 11, 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 chimera 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 (N₁, N₂, G₁E₁, G₁N₁, G₂N₁, G₁N₅ and E₂N₅) and OUM 11-5 (E₂, E₁N₁, N₁, N₂, G₂E₂, G₅N₃) and G₂N₃) 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 N₁ (1.72%) > N₂ (1.67%) > N₃ (1.33%) > E₂ (1.26%) > E₁ (1.25%) > G₁ (1.03%) > G₂ (0.79%) > G₃ (0.61%) in BKG-1; and while, it was Nₛ₁ (2.20%) > N₇ (1.83%) > E₃ (1.58%) > N₅ (1.52%) > E₂ (1.26%) > G₁ (1.21%) > G₂ (0.81%) > G₃ (0.78%) > E₁ (0.77%) in OUM 11-5. Among seven combined mutagenic treatments, G₂N₅ (2.71%) revealed highest mutation frequency followed by G₂E₂ (2.37%) and G₃E₂ (2.12%) in BKG-1, while in OUM 11-5 the order was G₅N₁ (3.08%) > G₂N₅ (2.89%) > G₂E₂ (2.41%) > G₃E₂ (2.39%) > G₃N₁ (1.97%) > E₂N₂ (1.58%) > G₃E₁ (1.28%).

Spectrum and frequency of morphological macro-mutations

Nineteen and eighteen types of morphological macro-mutations affecting cotyledonal leaf (mono/ tri/ tetra-cotyledonal), leaf (unifoliate, bifoliate, quadrifoliate, pentafoilate, 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 (prolifer pedded) were recorded in M₂ 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 N₂ (12 types) followed by E₃ and N₁ (10 types), while in OUM 11-5, wider mutation spectrum was observed in G₃ and E₁ (11 types) followed by G₁, N₁ and N₂ (9 types). Among the combination treatments, the spectrum was highest in G₃E₂ in both the genotypes (15 and 13 types, respectively). Quadri foliates, dwarf plants, lobed leaf, pentafoilates 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 M₂ plant was 2.18 (G₁) to 10.45 (E₂N₁) in BKG-1 and was 1.96 (G₁) to 8.00 (G₃N₂) in OUM 11-5. The frequencies of morphological mutations per 100 M₂ 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 was GN (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 N₂ (5.44%) > E₁ (5.42%) > G₁ (4.96%) > N₁ (4.89%) > N₅ (4.53%) > E₂ (4.40%) > E₁ (3.70%) > G₂ (3.06%) > G₁ (2.18%) in BKG-1 and in OUM 11-5, the order was N₂ (5.48%) > E₁ (4.75%) > N₁ (4.33%) > E₂ (4.21%) > N₅ (3.52%) > G₁ (3.43%) > E₁ (3.07%) > G₂ (2.63%) > G₁ (1.96%). Among the combined mutagenic treatments, the frequencies of morphological mutations per 100 M₂ plants were in order of E₂N₁ (10.45%) > G₂N₁ (9.00%) > G₂E₂ (8.65%) > G₂N₃ (7.92%) > G₃E₁ (7.17%) > G₂N₂ (7.03%) > G₃N₁ (6.04%) > G₁E₁ (5.47%) > E₂N₂ (5.04%) > N₁E₂ (4.75%)

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(6.60%) > G2N1 (6.08%) > G2E1 (5.86%) in BKG-1, while in OUM 11-5 the order was G2N2 (8.00%) > G2E3 (7.71%) > G2N1 (7.47%) > G2E2 (6.94%) > G2E1 (5.56%) > G2N1 (5.26%) > E2N2 (4.34%).

**Total mutation frequency**

The frequency of total mutations (both chlorophyll and morphological) per 100 M2 plants in mutagenic treated population (Table 1 and 2) ranged from 2.98 (G1) to 11.89 (E2N2) in BKG-1, while in OUM 11-5; the range was from 2.75 (G1) to 10.89 (G2N2). 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 mutants 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 (N2) and combination treatments (G2N2). 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 M2 generation of BKG -1

<table>
<thead>
<tr>
<th>Macro-mutation</th>
<th>Single Mutagenic Treatment</th>
<th>Combination Mutagenic Treatment</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>Total plants scored</td>
<td>504</td>
<td>490</td>
<td>484</td>
</tr>
</tbody>
</table>

**A. Chlorophyll mutation**

1. Albina - - 1 - 1 1 1 2 1 - 1 1 1 1 1 1 1 13 -
2. Xantha 1 1 1 2 2 2 2 2 3 - 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 9 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. Monocotyledony - - - - - - - - - 1 - - - 1 - - 3 -
2. Tricotyledony - - - - - - - - - 1 - - - - - - 3 -
3. Tetracotyledony - - - - - - - - - 1 - - - - - - 3 -
4. Unifoliate - - - - - - - - - - - - - - - - - 3 -
5. Bifoliate 1 2 2 2 1 1 1 1 1 2 - 2 1 2 2 1 21 -
6. Quadrifoliate 2 5 2 2 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 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 -
12. Early flowering - - - - - - - - - - - - - - - - - 8 -
13. Late flowering - - - - - - - - - - - - - - - - - 8 -
14. Tall - - - - - - - - - - - - - - - - - 8 -
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 - - - 5 -
18. Profuse podded - - - - - 3 1 - 3 - - 2 1 - - - 5 15 -
19. Small seed - - - - - - - - - - - - - - - - - 7 -
20. Sterile plant - - - - - - - - - - - - - - - - - 8 -
Total- B 11 14 28 14 21 22 11 26 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

*G1: 200 Gy gamma rays; G2: 400 Gy gamma rays; G3: 600 Gy gamma rays; E1: 0.2% EMS; E2: 0.4% EMS; E3: 0.6% EMS; N1: 0.010% NG; N2: 0.015% NG; G2E1: 400 Gy gamma rays + 0.2% EMS; G2E2: 400 Gy gamma rays + 0.4% EMS; G2E3: 400 Gy gamma rays + 0.6% EMS; G2N1: 400 Gy gamma rays + 0.05% NG; G2N2: 400 Gy gamma rays + 0.10% NG; G2N3: 400 Gy gamma rays + 0.15% NG"
Table 2: Spectrum and frequency of macro-mutations in M2 generation of OUM 11-5

<table>
<thead>
<tr>
<th>Macro-mutation</th>
<th>Mutagenic Treatment</th>
<th>C</th>
<th>Single</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1G2 G3 E1 E2 E3 N1 N2 G2E2E3E4GNG2N3G2N3E2N2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plants scored</td>
<td>510 495 496 522 475 505 462 438 455 468 461 415 456 450 455 507 7570 552</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Albina</td>
<td>- - - - 1 1 1 1 2 - 1 1 2 2 2 3 2 19</td>
<td>- 2 4 4 7 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Xantha</td>
<td>1 2 2 2 1 2 2 2 1 2 3 2 2 4 4 2 34</td>
<td>5 5 5 7 10 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Chlorina</td>
<td>2 2 3 2 3 4 3 3 5 6 3 7 5 5 6 6 4 66</td>
<td>7 9 14 15 17 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Viridis</td>
<td>1 - - - - - 1 - - - 1 - - 4</td>
<td>- 1 - 1 1 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Sectorial</td>
<td>- - 1 1 1 1 - - - - - 1 5</td>
<td>1 2 1 1 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total - A</td>
<td>4 4 6 4 6 8 7 8 10 6 11 10 9 13 14 8 128</td>
<td>0 14 18 25 27 36 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency - A (%)</td>
<td>3.78 0.81 1.21 0.77 1.26 0.58 0.52 0.38 0.28 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</td>
<td>2.65 1.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 M2

Fig 4: Morphological Macro-mutation in M2
**Conflict of Interest Statement**
The authors declare that there is no conflict of interest.

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