Original Research Article

M1 Biological Injuries: Indicators for M2 Macro- and Micro-mutation in Mungbean [Vigna radiata (L.) Wilczek]

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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 M1 over the parents. The biological damage in M1 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 M2. Population variance in M2 increased in each mutagenic treatment of both the varieties over the respective control for six quantitative traits including seed yield. The M1 parameters e.g., germination, survival and seedling characters showed negative correlation with M2 macro-mutation frequencies and M2 population variance (micro-mutation), while pollen and seed sterility in M1 showed positive association with M2 macro-mutation frequencies and M2 population variance. Such a relationship may be useful for effective selection of mutagenic populations at even M1 generation to achieve wider genetic variability in M2 and later generations.

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

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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 M1 and its relation with M2 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 60Co 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°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 cm² at EB-II Section, Department of Plant Breeding and Genetics, OUAT to raise the M1 generation. Standard agronomic practices and recommended doses of fertilizer (20-40-20 Kg N; P2O5 and K2O/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 M1 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 M2 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 M2 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 M₁**

The analysis of variance of M₁ 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 M₁ 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 M₁ 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 11.32 cm 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 M₁ 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 M₁ 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 M₁ generation (Singh and Mohapatra, 2004). The physiological effects are generally sieved off in the M₁ generation and are not inherited, while both gene and chromosomal mutations are carried forward from M₁ to the following generations. In most
of cases meiotic abnormalities are responsible for pollen and seed sterility. Similar biological damages in M₁ 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 M₂**

Observation on different types of macro-mutations (chlorophyll and viable morphological) were recorded in M₂ population for both the genotypes. Chlorophyll mutations in each treatment of M₂ 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 M₂ 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 M₂ 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 M₂**

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 M₂ population means and variances of different treatments revealed significant differences among treatments of both the genotypes for six quantitative traits studied.

**Relationship of M₁ parameters with induced macro and micro-mutation of M₂ generation**

The effect of the M₁ parameters on induction of macro and micro-mutations in M₂ generation was ascertained from the estimates of correlation coefficient of M₁ parameters in different mutagenic treatments with chlorophyll, morphological, total macro-mutation frequency and M₂ population variance (Table 4 and 5).

In both the genotypes, all the M₁ 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 M₂ population variance in M₂, while the correlation of M₁ pollen and seed sterility showed positive correlation with M₂ frequencies in both the genotypes.
Table 1: Effect of mutagens on M₁ generation of greengram variety BKG-1 and OUM 11-5 in laboratory experiment

| Sl. No. | Treatment symbol | Treatment | BKG-1 | OUM 11-5 |
|---------|-----------------|-----------|-------|----------|
|         | Germination (%) | Germination (%) | Germination (%) | Germination (%) |
|         | Seedling shoot length (cm) | Seedling root length (cm) | Seedling fresh weight (g) | Seedling dry weight (g) | Seedling shoot length (cm) | Seedling root length (cm) | Seedling fresh weight (g) | Seedling dry weight (g) |
| 1.      | 200 Gy G₁       | 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 G₂       | 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 G₃       | 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) |
| 4.      | EMS E₁         | 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.      | EMS E₂        | 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.      | EMS E₃        | 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) |
| 7.      | NG N₁         | 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.      | NG N₂         | 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.      | NG N₃         | 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 symbol | Treatment | 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) |
| 10.     | $G_2E_1$         | 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↓ (75.3) | 2.32↓ (79.7) |
| 11.     | $G_2E_2$         | 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) |
| 12.     | $G_2E_3$         | 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) |
| 13.     | $G_2N_1$         | 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) |
| 14.     | $G_2N_2$         | 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) |
| 15.     | $G_2N_3$         | 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) |
| 16.     | $E_2N_2$         | 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) |
| 17.     | Control/ Parent  | 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) |
|         |                  |            | 7.99 (5%)    | 1.81 (0.83)  | 0.33 (0.04)  | 6.31 (1.24)  | 1.15 (0.32)  | 0.237 (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<sub>C</sub> | Mf<sub>M</sub> | Mf<sub>T</sub> | Mf<sub>C</sub> | Mf<sub>M</sub> | Mf<sub>T</sub> |
| 1.      | G<sub>1</sub>        |                          | 0.79  | 2.18     | 2.98     | 0.78  | 1.96     | 2.75     |
| 2.      | G<sub>2</sub>        |                          | 0.61  | 3.06     | 3.67     | 0.81  | 2.63     | 3.44     |
| 3.      | G<sub>3</sub>        |                          | 1.03  | 4.96     | 5.99     | 1.21  | 3.43     | 4.64     |
| 4.      | E<sub>1</sub>        |                          | 1.03  | 3.70     | 4.72     | 0.77  | 3.07     | 3.83     |
| 5.      | E<sub>2</sub>        |                          | 1.26  | 4.40     | 5.66     | 1.26  | 4.21     | 5.47     |
| 6.      | E<sub>3</sub>        |                          | 1.25  | 5.42     | 6.67     | 1.58  | 4.75     | 6.34     |
| 7.      | N<sub>1</sub>        |                          | 1.33  | 4.89     | 6.22     | 1.52  | 4.33     | 5.84     |
| 8.      | N<sub>2</sub>        |                          | 1.67  | 5.44     | 7.11     | 1.83  | 5.48     | 7.31     |
| 9.      | N<sub>3</sub>        |                          | 1.72  | 4.53     | 6.25     | 2.20  | 3.52     | 5.71     |
| 10.     | G<sub>2</sub>E<sub>1</sub> |                      | 1.35  | 5.86     | 7.21     | 1.28  | 5.56     | 6.84     |
| 11.     | G<sub>2</sub>E<sub>2</sub> |                      | 2.12  | 6.60     | 8.73     | 2.39  | 6.94     | 9.33     |
| 12.     | G<sub>2</sub>E<sub>3</sub> |                      | 1.92  | 8.65     | 10.58    | 2.41  | 7.71     | 10.12    |
| 13.     | G<sub>2</sub>N<sub>1</sub> |                      | 1.80  | 6.08     | 7.88     | 1.97  | 5.26     | 7.24     |
| 14.     | G<sub>2</sub>N<sub>2</sub> |                      | 2.37  | 9.01     | 11.37    | 2.89  | 8.00     | 10.89    |
| 15.     | G<sub>2</sub>N<sub>3</sub> |                      | 2.71  | 7.92     | 10.63    | 3.08  | 7.47     | 10.55    |
| 16.     | E<sub>2</sub>N<sub>2</sub> |                      | 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<sub>C</sub>: Chlorophyll mutation frequency  
Mf<sub>M</sub>: Morphological mutation frequency  
Mf<sub>T</sub>: Total macro-mutation frequency
Table 3 Relationship of M₁ parameters with induced macro-mutation of M₂

| Sl. No. | M₁ Parameters       | Correlation coefficient of M₁ parameters with macro-mutational frequencies | Correlation coefficient of M₁ parameters with macro-mutational frequencies |
|---------|---------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
|         |                     | BKG-1                                                                      | OUM II-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 M2 population variance for different characters

| Sl. No. | Mutagenic treatments | **BKG-1** | M2 population variance | **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.      | G1@                 | 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.      | G2                   | 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.      | G3                   | 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.      | E1                   | 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.      | E2                   | 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.      | E3                   | 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.      | N1                   | 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.      | N2                   | 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.      | N3                   | 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.     | G2E1                 | 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.     | G2E2                 | 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.     | G2E3                 | 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.     | G2N1                 | 16.92↑     | 0.48↑              | 6.38↑       | 0.54↑       | 4.16↑       | 2.50↑       | 25.52↑        | 0.70↑        | 14.26↑      | 0.22↑        | 0.97↑        | 0.80↑        |
| 14.     | G2N2                 | 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.     | G2N3                 | 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.     | E2N2                 | 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         |

@ 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 |
| 1.      | Germination (%) | -0.558**                 | -0.628**   | -0.565*       | -0.724**    | -0.861**    | -0.723**   | -0.563*       | -0.767**    | -0.563*       | -0.744**   | -0.483*     | -0.518*     |
| 2.      | Seedling shoot length | -0.540*           | -0.693**   | -0.720**       | -0.553*    | -0.584*    | -0.515*    | -0.743**       | -0.525**    | -0.483*     |
| 3.      | Seedling root length | -0.461           | -0.472     | -0.594*        | -0.751**   | -0.639**   | -0.611*    | -0.739*       | -0.684**    | -0.516*     |
| 4.      | Seedling fresh weight | -0.360          | -0.611**   | -0.583*        | -0.578*    | -0.716**   | -0.756*    | -0.825**       | -0.711**    | -0.504*     |
| 5.      | Seedling dry weight | -0.348          | -0.611**   | -0.542*        | -0.383     | -0.705**   | -0.493*    | -0.770**       | -0.687**    | -0.402*     |

**Laboratory Experiment**

| Sl. No. | $M_1$ Parameters | $M_2$ population variance | \(BKG-1\) | \(OUM\ 11-5\) |
|---------|-----------------|---------------------------|-----------|--------------|
| 1.      | Germination (%) | -0.874**                 | -0.628**   | -0.565*       | -0.724**    | -0.861**    | -0.723**   | -0.563*       | -0.767**    | -0.563*       | -0.744**   | -0.483*     | -0.518*     |
| 2.      | Survival (%)    | -0.671**                 | -0.684**   | -0.457        | -0.723**    | -0.785**   | -0.753**   | -0.720**       | -0.755**    | -0.483*     |
| 3.      | Pollen Sterility (%) | 0.537*            | 0.499*     | 0.373         | 0.376      | 0.542*    | 0.591*    | 0.734**       | 0.491*     | 0.587*     |
| 4.      | Seed sterility (%) | 0.662**         | 0.661**    | 0.619**       | 0.774**    | 0.656**   | 0.549*    | 0.502*       | 0.545*     | 0.549*     |

**Field Experiment**

| Sl. No. | $M_1$ Parameters | $M_2$ population variance | \(BKG-1\) | \(OUM\ 11-5\) |
|---------|-----------------|---------------------------|-----------|--------------|
| 1.      | Germination (%) | -0.885**                 | -0.674**   | -0.564*       | -0.584*    | -0.665**   | -0.822**   | -0.787**       | -0.769**    | -0.720**    | -0.753**   |
| 2.      | Survival (%)    | -0.671**                 | -0.684**   | -0.457        | -0.723**   | -0.785**   | -0.753**   | -0.720**       | -0.755**    | -0.675**   |
| 3.      | Pollen Sterility (%) | 0.537*            | 0.499*     | 0.373         | 0.376      | 0.542*    | 0.591*    | 0.734**       | 0.491*     | 0.587*     |
| 4.      | Seed sterility (%) | 0.662**         | 0.661**    | 0.619**       | 0.774**    | 0.656**   | 0.549*    | 0.502*       | 0.545*     | 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 M1 parameters were significant at 5% level in both the genotypes. Out of total
42 negative correlation coefficients estimated from M₁ parameters with M₂ population variance of six quantitative traits, 36 and 41 correlation coefficients were significant at 5% level in BKG-1 and OUM 11-5, respectively. M₁ pollen sterility showed significant positive correlation with M₂ population variance in four traits in BKG-1 (except pod length and seeds/ pod) and in all six traits in OUM 11-5, while M₁ seed sterility showed a positive significant correlation with M₂ population variance of all the six traits in both the genotypes. The positive relationship between M₁ biological injury parameters with M₂ 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, M₁ biological injuries can serve as reliable parameters for early identification of effective mutagenic treated population for widening genetic variability in M₂ and later generations.

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