

## EMS Induced Variability, Frequency and Spectrum of Chlorophyll Mutations in Pigeonpea (*Cajanus cajan* L.)

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**Abstract** Two genotypes of pigeonpea namely, MA 156 and MAL 13 were treated with three different concentrations of EMS (0.01M, 0.015M and 0.02M) to validate induced variability frequency and chlorophyll mutations. Germination of treated seed varied from 71% in MAL 13 (at 0.02M) to 88% in MA 156 (at 0.01M) in laboratory condition. Average root and shoot length was highest at lower dose (0.01M) and lowest at higher doses (0.02M) in both the genotypes. The highest frequency of chlorophyll mutants (3.31%) were observed at 0.015 M in MAL 13, followed by 2.73% at 0.015M in MA 156 and lowest (0.72%) 0.01M in MA 156. Mutagenic efficiency and mutagenic effectiveness were also higher at

0.015M in MAL 13. Chlorophyll mutations like, *albino*, *xantha*, *chlorina* and *viridis* were observed.

**Keywords** Pigeonpea, Seed, EMS, Chlorophyll mutation.

### Introduction

Pigeonpea [*Cajanus cajan* (L.) Mill sp.] is the second important pulse crop of India belonging to the Cajaninae sub-tribe of the economically important leguminous tribe *Phaseoleae*. It is consumed as split *dal* as well as green vegetable in many countries. It contains approximately 21% protein [1] and seeds are rich in iron, iodine and essential amino-acids like lycine, cystine and arginine. In spite of its nutritional importance, their grain production is very low. Now-a-days world's food insecurity is posing a great threat to the human civilization which can be combat by widening the genetic variability being autogamous crop. In this respect mutation breeding may be an alternative breeding method to create genetic variability for yield attributing traits to improve the yield within short time. Since last two decades, with the sole objective of developing better varieties of economically important crops, mutants were ignored which argued high yield in pigeonpea by EMS treatment [2]. Chlorophyll mutants are employed as markers for the evaluation of gene action of mutagenic

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factors in induced mutation studies [3]. Being the ultimate source of variation, this investigation was started to create EMS induced variability, frequency and spectrum of chlorophyll mutations in pigeonpea.

## Materials and Methods

The present investigation was conducted at Agricultural Research Farm, Institute of Agricultural sciences, Banaras Hindu University, Varanasi during *kharif* 2009-2011 with these two genotypes : MA 156 : It has a vigors, compact and determinate plant type, red streaks appear on the flower petal with late in flowering, produced pods in clusters having bold seeds and mature in about 260 days. MAL 13 (Malviya Chamatkar) : Resistant to wilt, Sterility Mosaic Disease and toletant to *Phytophthora* blight, spreading plant type and bear long constricted pods containing medium bold seeds and mature within 230 days.

Four hundred pure seeds each of above genotypes were presoaked in distilled water for 6 h at room temperature  $25 \pm 2^\circ\text{C}$  and dried on blotting paper for 30 minutes and then treated with Three different concentrations (0.01, 0.015 and 0.02M) in phosphate buffer of Ethyl Methane Sulfonate by submerging the seed in the freshly prepared solution for 6 h. the treated seeds were thoroughly washed for 4 h in running tap water to minimize the residual effects of chemical. simultaneously, 400 genetically pure seeds (untreated) were also presoaked in distilled water for six h and used as control. 20 seeds of each treatment were placed in petri dishes for germination test in lab condition. Moreover, a total of 8 treatments including control were immediately sown in the field following randomized block design with three reoplications during *kharif*, 2009-2010. each plot consisted of 2 meter length with spacing of 75 and 25 cm between and within rows, respectively. The recommended agronomic practices were followed to raise a good crop. Germination percentage, pollen fertility, ovule fertility and survival up to maturity were recorded in  $M_1$  generation. Pollen and ovule fertility were calculated by the following formulae :

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

$$\text{Ovule fertility (\%)} = \frac{\text{Number of under developed seed}}{\text{Total number of ovule scars}} \times 100$$

50 plants having high fertility were harvested separately from  $M_1$  generations from each treatment. Single plant progenies were grown for  $M_2$  generation during *kharif* 2010-2011 as per layout mentioned earlier. The germination percentage, frequency and spectrum of the different chlorophyll mutants were scored in  $M_2$  population. Both mutagenic effectiveness and efficiency were scored as per the procedure recommended by Konzak et al. [4].

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{time (t) } \times \text{ concentration (C)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage (S)}}$$

## Results and Discussion

The range of germination varied from 71% in MAL 13 (at 0.02M) to 88% in MA 156 (at 0.01M) as compared to control, MA 156 and MAI 13, being 94 and 92%, respectively in laboratory condition (Table 1). Average root and shoot length was longest at lowest concentration (0.01M) and shortest at higher doses (0.02M) in both the genotypes. However average reduction in root and shoot length was much higher in MAL 13 as compared to MA 156.

In field condition, the data of  $M_1$  generation on pollen fertility, ovule fertility and survival up to maturity is depicted in Table 2 and revealed that maximum germination percentage was MA 156 (84.21%) at 0.01M among all the treatments. Considering the effect of different concentrations separately, it is obvious that the reduction in germination percent (70.26 and 67.89%) was more pronounced at higher concentration of EMS (0.02M) in both the geno-

**Table 1.** Effect of different concentrations EMS on germination in lab condition.

| Treatments | Concentrations (molar) | No. of seeds sown | No. of seeds germinated | Seed germination (%) | Root length (cm) |         |         | Shoot length (cm) |         |         |
|------------|------------------------|-------------------|-------------------------|----------------------|------------------|---------|---------|-------------------|---------|---------|
|            |                        |                   |                         |                      | Minimum          | Maximum | Average | Minimum           | Maximum | Average |
| MA 156     | 0.01M                  | 20                | 17                      | 88                   | 4.2              | 10.5    | 7.6     | 7                 | 24      | 15.04   |
| MA 156     | 0.015M                 | 20                | 15                      | 78                   | 4                | 10      | 6.7     | 4                 | 17.8    | 12.87   |
| MA 156     | 0.02M                  | 20                | 14                      | 74                   | 2                | 4.5     | 2.96    | 4.5               | 7       | 5.94    |
| MAL 13     | 0.01M                  | 20                | 16                      | 83                   | 1                | 6.2     | 3.26    | 2.8               | 15      | 8.72    |
| MAL 13     | 0.015M                 | 20                | 15                      | 79                   | 1                | 6       | 3.06    | 4                 | 10      | 6.87    |
| MAL 13     | 0.02M                  | 20                | 14                      | 71                   | 1.2              | 3.2     | 1.82    | 3.8               | 8       | 6.43    |
| Control    |                        |                   |                         |                      |                  |         |         |                   |         |         |
| MA 156     | –                      | 20                | 19                      | 94                   | 4.4              | 11.5    | 8.32    | 4.6               | 18.2    | 17.16   |
| MAL 13     | –                      | 20                | 18                      | 92                   | 4                | 11.2    | 7.62    | 13                | 24.5    | 18.06   |

types. the minimum pollen fertility was observed at 0.02M concentration (75.74 % in MA 156 and 79.89% in MAL 13) while maximum at 0.01M in both the genotypes. The percentage of ovule sterility was obviously higher at higher doses of mutagen in both the genotypes as it was maximum i.e. 28.57 % in MA 156 followed by 26.31% in MAL 13 at 0.02M concentrations. Per cent plant survival at maturity data indicated that maximum 91.23% could survive in MA 156 at 0.01M concentration while minimum of 78.63% in MAL 13 at 0.02M concentration. The results of present investigation showed that there was a decrease in seed germination, plant survival, ovule

fertility and pollen fertility with increasing the doses of EMS in  $M_1$  generation. The reduction in pollen and ovule fertility with increasing doses/concentrations as obvious in this experiment are also in conformity of the result observed by Sangle and Kothekar [5], Giri [6] in *Cajanus cajan*; Sagade and Apparao [7] in *Vigna mungo*. These reductions in fertility/sterility are probably due to changes/alteration in nucleotide sequence of DNA present on the chromosome which is also corroborated by Badere and Chaudhary [8]. Moreover, a mutagen may effectively cause desirable mutations, but simultaneously undesirable effects like lethality or sterility may increase/decrease its efficiency.

**Table 2.** Germination, Pollen fertility, ovule fertility and survival up to maturity in  $M_1$  generation in field condition.

| Treatments | Seed germination (%) | Pollen fertility (%) | Ovule fertility (%) | Plant survival up to maturity (%) |
|------------|----------------------|----------------------|---------------------|-----------------------------------|
| MA 156     | 84.21                | 88.66                | 82.34               | 91.23                             |
| MA 156     | 75.00                | 77.42                | 75.02               | 87.45                             |
| MA 156     | 70.26                | 75.74                | 71.43               | 81.39                             |
| MAL 13     | 81.05                | 92.80                | 86.44               | 87.93                             |
| MAL 13     | 77.37                | 83.91                | 78.35               | 84.56                             |
| MAL 13     | 67.89                | 79.89                | 73.69               | 78.63                             |
| Control    |                      |                      |                     |                                   |
| MA 156     | 92.37                | 100                  | 100                 | 95.32                             |
| MAL 13     | 90.53                | 100                  | 100                 | 92.65                             |

### Chlorophyll mutations

The chlorophyll mutation spectrum was also studied and mutants were classified as per pattern given by Gustafsson [9] including modifications. Chlorophyll mutants are indicator of mutagenic sensitivity of the crop against various mutagenic agents and their dose effects. Different doses of EMS produced different frequency of chlorophyll mutants which varied from 0.72% to 2.73% in MA 156 and 2.36% to 3.31% in MAL 13. Among all the chlorophyll mutants *albina*, *xantha*, *chlorina* and *viridis* were abundantly evident but frequency of *viridis* was highest followed by *albina*, *xantha* and *chlorina*. This experiment is also argued that frequency of chlorophyll mutants are increasing with the dose of mutagens

**Table 3.** Frequency of chlorophyll mutations in M<sub>2</sub> generation in pigeonpea. A= *Albina*, X = *Xantha*, C = *Chlorina* and V= *Viridis*.

| Treat-ments | Seed germination (%) | No. of seedling analyzed | Frequency of chlorophyll mutant |    |   |    | Chlorophyll mutants (%) | pollen sterility (%) | Mutagenic efficiency | Mutagenic effectiveness |
|-------------|----------------------|--------------------------|---------------------------------|----|---|----|-------------------------|----------------------|----------------------|-------------------------|
|             |                      |                          | A                               | X  | C | V  |                         |                      |                      |                         |
| MA 156      | 79.5                 | 1934                     | 5                               | 1  | 1 | 6  | 0.72                    | 11.34                | 0.063                | 1.109                   |
| MA 156      | 62.9                 | 965                      | 10                              | 3  | 7 | 7  | 2.73                    | 22.58                | 0.121                | 2.809                   |
| MA 156      | 58.3                 | 646                      | 3                               | 3  | 0 | 5  | 1.66                    | 24.26                | 0.068                | 1.281                   |
| MAL 13      | 78.0                 | 1486                     | 9                               | 9  | 2 | 18 | 2.63                    | 7.20                 | 0.365                | 4.051                   |
| MAL 13      | 69.0                 | 1032                     | 9                               | 11 | 1 | 13 | 3.31                    | 16.09                | 0.206                | 3.405                   |
| MAL 13      | 73.0                 | 642                      | 2                               | 1  | 2 | 9  | 2.36                    | 16.89                | 0.146                | 1.821                   |

and recessive in nature as revealed by segregating pattern. It means, there was a dose dependent relationship in the spectrum and frequency of chlorophyll mutations either mutagens are employed singly or in combination. Sangle and Kothekar [10] also observed different types of chlorophyll chimeras viz. yellow, light green, yellowish green and dull green by treating with EMS which affected the leaflet totally totally and/or partially and maximum mutants were on the margins. the differences in the frequency of chlorophyll mutations were indicated that number of genes controlling chlorophyll development may differ in different varieties of pigeonpea [10, 11]. Girija and Dhanavel [12] also stated that EMS is more efficient than gamma rays to induce chlorophyll mutations.

#### Efficiency and effectiveness of the mutagen

The term efficiency and effectiveness was coined by Konzak et al. [4]. The efficient mutagenesis is the production of desirable change free from association with undesirable ones while the mutagenic effectiveness denotes the frequency of mutation induced by a unit dose of mutagen.

From the perusal of Table 3, it is obvious that the different concentrations of EMS showed variation in mutagenic efficiency and effectiveness. Among all the treatments higher efficiency of mutants and effectiveness of EMS was observed at 0.01M in MAL 13 and at 0.015M in MA 156. These findings indicated that lower doses of mutagens cause point mutation resulting micromutation which can be utilized extensively particularly in autogamous

crops and their genetic base can be broadened to utilize in breeding program. Sangle and Kothekar [10] have made attempts to determine the most efficient mutagenic concentration for the induction of variability for desirable traits in pigeonpea. Bhosale et al. [13] reported decreased seed germination with increasing concentration, while seedling injury, pollen sterility and lethality increase with increasing concentration/dose of the mutagens in urdbean. Results of Singh and Singh [14] also corroborate our findings as they found inverse relationship in the efficiency and concentrations of EMS, Gamma rays and SA treatments. Ariraman et al. [15] also used various concentrations of EMS and found decreased seed germination with increasing the concentration/doses as compared to control.

#### Conclusion

On the basis of above findings it is concluded that the growth trend of average shoot and root length was diminishing with increasing dose of EMS in laboratory condition. Similar pattern was also observed in field condition for germination, pollen fertility, ovule sterility and plant survival up to maturity. Both genotypes showed differential response to induce mutation at different doses of mutagens. Among all the concentrations, 0.015M was more effective and potent to induce chlorophyll mutations than others. Overall effect of different mutagen concentrations on both varieties, the *viridis* type chlorophyll mutations were most frequent and *Chlorina* type was least common. Genetic variances even change in a single gene, induces significant changes that influences not only the rate but also the spectrum of recoverable mutations. It is further suggested that all the doses/

concentration of EMS used in the investigation are effective to produce genetic variability which may be further utilize in crop improvement.

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