

Full Length Research Paper

Comparative Analysis of Gamma Rays and EMS-Induced Chlorophyll Mutations in Two Soybean Cultivars

Mudasir Hafiz Khan¹ and Sunil Dutt Tyagi²

¹Department of Agriculture, Srinagar (J&K), India.

²Department of Plant Breeding and Genetics, Kisan (P. G.), College, Simbhaoli, Ghaziabad, (U. P.), India.

Accepted 14 December, 2024

Mutagenic effectiveness and efficiency of gamma rays, EMS and combined treatments was studied in terms of M₂ (progenies) lethality and chlorophyll mutations in two cultivars of soybean (Pusa-16 and PK-1042). In general the frequencies of chlorophyll mutations were high in gamma rays and combined treatments. Four types of mutants viz., albina, xantha, chlorine and viridis were observed in the study. Gamma rays were found to be more effective to induce chlorophyll mutations in both cultivars. PK-1042 cultivar exhibited higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment.

Key words: Effectiveness, efficiency, gamma rays, EMS, soybean.

INTRODUCTION

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen, while mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility induced by the mutagen (Wagner and Foster, 1965). The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Chlorophyll mutations are used to evaluate the genetic effects of various mutagens. Gaul (1964) reported the appearance of greater number of viridis type which is attributed to the involvement of polygene in chlorophyll formation. The present study was undertaken to gather information on efficiency, effectiveness and chlorophyll mutations on the consequences of induction of physical (Gamma rays) and Chemical (EMS) mutagens in Soybean.

MATERIALS AND METHODS

Dry seeds (9 - 12% moisture) of two cultivars Pusa-16 and PK- 1042 of soybean were treated with ethyl methane sulphonate (EMS) (0.1, 0.2 and 0.3% concentration) and Gamma rays (15, 30 and 45 Kr) at

NRL, IARI, New Delhi. 100 irradiated seeds (Gamma ray) were subjected to 0.2% EMS for eight hours. The treated material along side with two controls (untreated) were immediately sown in a single unreplicated plots with 4 rows at a distance of 30 and 10 cm between rows and plants, respectively at Research Farm of Kisan (P.G.) College, Simbhaoli, UP, India. The seeds treated with chemical mutagen were washed in running water before sowing. The data was recorded from 20 randomly selected plants from each treatment.

Survival of plants was recorded at the time of maturity in the field and was expressed as percentage of control. Selfed seeds of the individual M₁ plants were harvested separately and were grown as individual M₂ families in separate lines in Modified Single Seed Bulk Design. The treated and the control material were screened for the frequency of chlorophyll mutations in the M₂ generation. Mutagenic frequency was estimated as percentage of segregating M₁ plant progenies (Gaul, 1964). The mutagenic efficiency and effectiveness were determined by the methods suggested by Konzak et al. (1965).

RESULTS

Chlorophyll mutation

The chlorophyll mutation frequencies were calculated as per cent M₁ plants (M₂ progenies) and the data is presented in Table 1. It may be noticed from the data that in cultivar PK-1042, the mutation frequency increased with increase in the dose of gamma rays, while in case of

*Corresponding author. E-mail: kmudasirhafiz@yahoo.com.

Table 1. Frequency of chlorophyll mutations in EMS and Gamma rays treated M₂ generations of soybean cultivars.

| Cultivars | Dose | Number of M ₂ seedlings | | Mutation frequency/100 M ₂ seedlings | Spectrum (relative %) of Chlorophyll mutants | | | |
|------------------------------------|-------------|------------------------------------|---------------------|---|--|--------|----------|---------|
| | | Tested | Chlorophyll mutants | | Albina | Xantha | Chlorina | Viridis |
| Control | | | | | | | | |
| PK-1042 | 0 | 600 | - | - | - | - | - | - |
| Pusa-16 | 0 | 580 | - | - | - | - | - | - |
| Gamma rays (kR) | | | | | | | | |
| PK-1042 | 15 | 500 | 9 | 1.80 | 0.00 | 0.00 | 55.56 | 44.44 |
| | 30 | 420 | 13 | 3.09 | 7.69 | 23.07 | 30.76 | 38.46 |
| | 45 | 400 | 15 | 3.75 | 13.33 | 33.34 | 20.00 | 33.33 |
| Pusa-16 | 15 | 535 | 15 | 2.80 | 6.66 | 20.00 | 33.34 | 40.00 |
| | 30 | 495 | 17 | 3.43 | 0.00 | 29.41 | 41.17 | 29.41 |
| | 45 | 480 | 13 | 2.70 | 23.07 | 0.00 | 15.38 | 61.53 |
| EMS (%) | | | | | | | | |
| PK-1042 | 0.1 | 600 | 9 | 1.50 | 0.00 | 33.33 | 22.22 | 44.45 |
| | 0.2 | 574 | 15 | 2.61 | 6.66 | 33.33 | 26.67 | 33.33 |
| | 0.3 | 525 | 12 | 2.28 | 16.67 | 16.67 | 41.67 | 25.00 |
| Pusa-16 | 0.1 | 520 | 5 | 0.96 | 0.00 | 25.00 | 50.00 | 25.00 |
| | 0.2 | 515 | 9 | 1.74 | 11.11 | 22.22 | 33.34 | 33.33 |
| | 0.3 | 485 | 16 | 3.29 | 0.00 | 31.25 | 25.00 | 43.75 |
| Combination [EMS + Gamma rays (%)] | | | | | | | | |
| PK-1042 | 15 kR + 0.2 | 485 | 11 | 2.26 | 0.00 | 36.36 | 27.27 | 36.36 |
| | 30 kR + 0.2 | 320 | 15 | 4.68 | 20.00 | 0.00 | 33.34 | 46.46 |
| | 45 kR + 0.2 | 315 | 13 | 4.12 | 7.69 | 38.46 | 23.07 | 46.15 |
| Pusa-16 | 15 kR + 0.2 | 435 | 12 | 2.75 | 16.66 | 41.66 | 25.00 | 16.67 |
| | 30 kR + 0.2 | 410 | 15 | 3.65 | 20.00 | 6.67 | 33.33 | 40.00 |
| | 45 kR + 0.2 | 385 | 19 | 4.93 | 10.52 | 26.31 | 36.84 | 26.31 |

EMS and combined treatments, the mutation frequencies exhibited addition up to the intermediate dose levels and then showed a decline with further increase in the dose of mutagen. In Pusa-16, the situation was somewhat different. An increase in the dose of gamma rays showed

increase in the mutation frequencies at intermediate level and a gradual decrease at higher doses, whereas EMS and combined treatment showed slight increase in the mutation frequency with the increase in the dose/conc. of mutagen.

Spectrum and frequency of chlorophyll

The data of frequencies and spectra of different chlorophyll mutants in the two soybean cultivars that is PK-1042 and Pusa-16 are presented in Table 1. The details for individual mutation types

are as follows: -

a) Albina: In PK-1042, out of nine mutagenic treatments the *albina* mutants were observed in six treatments. Among these treatments, the highest frequency (20.00%) of mutants was observed at 30 + 0.2% combination treatment. In other treatments, the values ranged from 0.00 to 16.67%. In Pusa-16, the *albina* mutants were observed in six treatments. The highest frequency (23.07%) was observed at 45 Kr gamma rays and 30 + 0.2% (20.00) combination treatment. Other treatments showed a range of 0.00 to 16.66%.

b) Xantha: The frequency of *xantha* chlorophyll mutants showed a slight increase in PK-1042 as compared to Pusa-16 in EMS and combined treatments. The frequencies of *xantha* mutants showed a decrease at intermediate dose levels and then increase with the increase in dose of mutagen, whereas in Pusa-16 it showed a decreasing trend. With gamma rays treatments in PK-1042, an increase in the frequency of *xantha* mutants was noticed with the increase in the doses of mutagen, while in Pusa-16 an increase upto intermediate level and thereafter a gradual decrease (0.00%) at higher dose (45 Kr) was noticed.

c) Chlorina: Dissimilar to the *xantha* mutants, the frequency of the *chlorina* mutants were high in Pusa-16 then PK-1042. In Pusa-16, the frequencies of *chlorina* mutants showed an increase with the increase in dose of mutagens. In PK-1042, on the other hand, the increase in the dose of gamma rays was associated with the decrease in the frequency of *chlorina* mutants, whereas EMS and combined treatments showed increase at the intermediate levels. In Pusa-16 as compared to other treatments, the frequencies of *chlorina* mutants were high following combined treatments. The increase in the dose was associated with the increase in the frequencies of mutants. 45 Kr gamma rays and combination treatment (45Kr + 0.2%) showed the highest frequency of *chlorina* mutants in Pusa-16 among all the treatments in both cultivars.

d) Viridis: The frequencies of the *viridis* mutants were high as compared to the other types of chlorophyll mutants. In both the cultivars, the frequencies of the *viridis* showed an increase at the intermediate level of combination treatment. Contrary to this, an increase in the dose of gamma rays was associated with the decrease in the frequency of mutants, whereas it showed a reverse trend in case of EMS treatment.

The frequencies of different chlorophyll mutant types in both the cultivars were found in the following order: - *Viridis* > *Chlorina* > *Xantha* > *Albina*.

Mutagenic effectiveness and efficiency

The effectiveness of different mutagens and that of different treatments of mutagens were calculated and the data are presented in Table 2. The mutagenic effective-

ness was as high as 1.6310 in gamma rays treatments. Whereas the highest effectiveness of EMS treatments was 1.3291 in the cultivar Pusa-16. EMS was 19 to 60 times more effective than gamma rays, while it was less effective that is 0.40 to 5 times in case of PK-1042. In both cultivars mutagenic effectiveness decreased with the increase in the dose of the mutagens except in EMS treatment of Pusa-16 where the increase (1.3291) was observed at higher concentration (0.3%). Thus, the order of mutagens based on effectiveness was: - EMS > Gamma rays.

The mutagenic efficiency was calculated on the basis of lethality and the data are presented in Table 2. The highest (0.0297) and lowest (0.092) mutagenic efficiency was exhibited by Pusa-16 (15 Kr + 0.2%) and Pk-1042 and Pusa-16 (0.3%), respectively. In general, PK-1042 exhibited higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment. However, Pusa-16 showed higher frequency in combined treatment. The mutagenic efficiency decreased with the increase in the dose of the mutagen in both cultivars the differences in number of genes controlling chlorophyll development in the two cultivars. It may also be noted that the frequencies of chlorophyll mutants were higher in gamma rays and combined treatments. This is in conformity with the previous reports of Prasad and Das (1980). In 50% of the treatments (Gamma rays, PK-1042; EMS, Pusa-16 and Combined treatment, Pusa-16) an increasing trend in the frequency of mutants with increase in the dose of mutagen was observed but the increase was not linear. The non-linearity in the frequency of mutants and dose of mutagens may to some extent, be attributed to the irregular segregation ratios of chlorophyll mutants leading to deficits of recessives in the progenies.

Albina, xantha, chlorina and viridis were the different types of chlorophyll mutants found in the present study. Xantha and Chlorina types of chlorophyll mutants in soybean were earlier reported by Geetha and Vaidyanathan (2000). In general, viridis occurred in maximum proportion than other types of chlorophyll mutants in gamma rays and combined treatments.

Since chlorophyll mutations are most conspicuous and easily detectable as they have been extensively used to find out sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of mutagens (Gaul, 1960). It may be noted from the Table 2 that relative to the EMS, gamma rays were more effective in inducing chlorophyll mutation in both the cultivars. Similar results have also been reported in Lathyrus by Nerkar (1977). In the present study, the efficiency of EMS, Gamma rays and combined treatments decreased considerably except Pusa-16 (combined treatment) with the increase in the dose of mutagens in both cultivars. The decrease in efficiency may be attributed to the failure in proportionate increase in dose of mutagens (Table 2).

Similar findings have also been reported by Dixit and Dubey (1986). It is obvious that the higher efficiency

Table 2. Effectiveness and efficiency of various EMS and Gamma rays treatments in M₂ generation in two cultivars of soybean.

| Dose | Mutation frequency/100 M ₂ seedlings | | Chlorophyll mutant frequency as (per cent) M ₂ seedlings | | Mutagenic effectiveness | | Mutagenic efficiency | |
|------------------------|---|---------|--|---------|-------------------------|---------|----------------------|---------|
| | PK-1042 | Pusa-16 | PK-1042 | Pusa-16 | PK-1042 | Pusa-16 | PK-1042 | Pusa-16 |
| Control | | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Gamma rays (kR) | | | | | | | | |
| 15 | 1.80 | 2.80 | 1.80 | 2.80 | 0.1200 | 0.1866 | 0.181 | 0.203 |
| 30 | 3.09 | 3.43 | 3.09 | 3.43 | 0.1030 | 0.1143 | 0.253 | 0.137 |
| 45 | 3.75 | 2.70 | 3.75 | 2.70 | 0.0833 | 0.0600 | 0.098 | 0.108 |
| EMS (%) | | | | | | | | |
| 0.1 | 1.50 | 0.96 | 1.50 | 0.96 | 1.8750 | 1.2000 | 0.166 | 0.168 |
| 0.2 | 2.61 | 1.74 | 2.61 | 1.74 | 1.6310 | 1.0875 | 0.169 | 0.133 |
| 0.3 | 2.28 | 3.29 | 2.28 | 3.29 | 0.9500 | 1.3291 | 0.092 | 0.092 |
| Combination | | | | | | | | |
| 15 kR + 0.2(%) | 2.26 | 2.75 | 2.26 | 2.75 | --- | --- | 0.208 | 0.297 |
| 30 kR + 0.2(%) | 4.68 | 3.65 | 4.48 | 3.65 | --- | --- | 0.172 | 0.243 |
| 45 kR + 0.2(%) | 4.12 | 4.93 | 4.12 | 4.93 | --- | --- | 0.106 | 0.232 |

at lower and intermediate doses of mutagens may be due to the fact that the biological damage (lethality and sterility) increased with the dose at a rate greater than the frequency of mutations (Konzak et al., 1965).

REFERENCES

- Dixit P, Dubey DK (1977). Mutagenic efficiency of gamma rays, NMU and their combinations in Lentil (*Lens culnaris* Medik.) var. T. 36. Indian J. Genet. Plant Breed. 46(3): 501-505.
- Gaul H (1960). Critical analysis of the methods for determining the mutation frequency after seed treatment with mutagens. Genet. Agraria. 12: 297-318.
- Gaul H (1964). Mutation in Plant Breeding. Radiation Bot. 4: 155-232.
- Geetha K, Vaidyanathan P (2000). Studies on induction of chlorophyll mutation in soybean through physical and chemical mutagens. Agricultural Science Digest 20(1): 33-35.
- Konzak CF, Nilan RA, Wagner J, Foster RJ (1965). Efficient chemical mutagenesis. In the use of Induced Mutations in Plant Breeding. Radiation Bot. (Suppl.) 5: 49-70.
- Nerkar YS (1977). Mutagenic effectiveness and efficiency of gamma rays, EMS and NMU in *Lathyrus sativus*. Indian J. Genet. Plant Breed. 37(1): 137-141.
- Prasad AB, Das AK (1980). Studies of induced chlorophyll mutations in *Lathyrus sativus* L. Cytologia 45: 335-341.