e-planet **20** (1): 1-7 (June 2022)

Mutagenic effects of gamma-rays and EMS on chromosomes and pollen sterility in greengram

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Date of receipt: 25.03.2022 Date of acceptance: 29.05.2022

ABSTRACT

To determine the potency of different doses of physical and chemical mutagens and deduce an optimum dose, cytological analysis for induced chromosomal variation is considered an accurate index in mutation breeding. Therefore, the present investigation was carried out to estimate the relative frequency and spectrum of meiotic chromosomal abnormalities at various stages of cell division using gamma-rays, Ethyl methanesulfonate (EMS), and their combination treatments in the M_1 generation of greengram (*Vigna radiata* L. Wilczek) varieties Sujata and OBGG-52. The analysis revealed a wide range of induced meiotic chromosomal abnormalities like univalents, multivalents, chromosome stickiness, laggards, bridges, and micronuclei by different mutagen doses. In general, the meiotic chromosomal abnormalities increased along with the increase in concentration in mutagens in both varieties. However, the induction of meiotic aberrations was observed to be higher in gamma rays treatments, suggesting that gamma rays could be more effective in inducing additional variability than EMS, in greengram. It was observed that the combined treatments induced meiotic abnormalities at a higher frequency as compared to individual treatments of gamma rays and EMS. Comparative estimation of induced chromosomal abnormalities suggested higher mutagenic sensitivity of var. Sujata than the var. OBGG-52 towards the single mutagenic treatments used whereas in combined treatment of moderate doses OBGG-52 expressed higher mutagenic sensitivity than Sujata. The pollen sterility observed in mutagenic treatments may be due to the induced mutation in chromosomes. A positive and significant correlation between induced chromosomal abnormality and pollen sterility was observed in both varieties.

Key words: Chromosomes, EMS, gamma-rays, greengram, induced mutation, pollen sterility

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek] known as mungbean, is an important short-duration grain legume having wider adaptability and low input requirements. It is widely grown in the sub-tropical countries of the South and South-east Asia, Australia, West Indies, South and North America, and Tropical and Subtropical Africa. The conventional approaches of plant breeding have exploited the available genetic variability which has in turn led to a narrow genetic base in this crop. Induced mutations provide a powerful means of creating new and useful variability in crop plants both in qualitative and quantitative traits (Das and Misra, 2005). Physical and chemical mutagens induce genes to mutate at rates above spontaneous baselines, thus producing a range of novel traits and broadening of genetic diversity of plants (Das and Baisakh, 2013). Physical or chemical mutagen-induced quantitative variation not only serves as an alternative source of germplasms for natural variation but is also useful in generating appropriately linked gene complexes that are responsible for the improvement in yield and

other characteristics of economic interest (Das and Prusti, 2020).

Gamma rays, one of the most commonly used physical mutagen in mutation breeding are known to influence plant growth and development by inducing cytological, genetic, biochemical, physiological, and morphological changes in cells and tissues (Das and Prusti, 2020). Gamma rays are the highly energetic ionizing radiations with a higher penetration power and thus can induce various changes at the chromosomal and molecular level and proved to be an effective physical mutagen in creating variation and effective mutation. Chemical mutagens also play important role in inducing chromosomal aberrations and mutations that are useful for crop improvement. Among the chemical mutagens used for induction of mutations in various crops, Ethyl methanesulfonate (EMS) an alkylating carcinogenic organic compound is one of the most effective, efficient, and frequently used mutagens that generate random mutations in genetic content through nucleotide substitution (Minocha and Arnason, 1962; Das and Baisakh, 2020). It is a popular mutagenic agent, that donates alkyl group i.e., ethyl group (CH_3-CH_2) to the guanine producing O_6-ethyl guanine which pair with thymine to eventually produce point mutations and induces mispairing and base changes due to chemical modification of nucleotides (Okagaki et al., 1991; Greene et al., 2003). Tarar and Dnyansagar (1980) and Zeerak (1991) found physical mutagens more effective than chemical mutagens while many other researchers reported chemical mutagens are more effective than physical mutagens (Dhanayanth and Reddy, 2000; Bhat et al., 2005; Baisakh et al., 2011).

Chromosomal rearrangements are one of the most frequently produced cases of mutation that result from the action of both physical and chemical mutagenic agents. Mutation of any of the genes disrupts meiosis, gametes sterility, and other abnormalities. Analysis of chromosomal behavior at various meiotic stages is one of the most dependable indices for estimation of the potency of any mutagen. Thus, the investigation of meiotic aberrations and their genetic consequences forms an integral part of most mutation studies. They also provide a considerable clue to assessing the sensitivity of plants to different mutagens (Zeerak, 1992). To induce genetic variability and utilize useful mutants in plant breeding programs, the identification of appropriate mutagen and its appropriate dose/ concentration is essential (Das and Baisakh, 2011). Hence a study was undertaken to assess the effect of different doses of gamma-rays and EMS on pollen sterility and meiotic behavior in the M_1 generation of greengram.

MATERIALS AND METHODS

Dry and well-filled seeds of two greengram varieties, namely Sujata and OBGG-52 were administered mutagenic treatments with three doses each of gamma rays (20, 40 and 60 kR), ethyl methane sulphonate (0.2, 0.4 and 0.6%), and combine mutagens of 40 kR gamma rays with 0.4% EMS and were coded as G1, G2, G3, E1, E2, E3 and GE2, respectively. Dry seeds were irradiated with gamma ray treatment at Bhaba Atomic Research Centre, Trombay. For treatment with EMS, the seeds were pre-soaked in distilled water for six hours, blotted dry and then treated with a freshly prepared aqueous solution of above chemical mutagen for 6 hours, with intermittent shaking. For combination treatment, seeds were first irradiated with 40 kR gamma rays and then treated with 0.4% EMS solution in the same manner as described above. After treatment, the seeds were thoroughly washed with running water to bleach out the residual chemicals and then dried on blotting paper after treatment. To grow the M_1 generation, the treated seeds were sown in RBD in two replications with spacing of 25 × 10 cm^2. Young flower buds from 50 randomly selected plants from each treatment were fixed in Carnoy's fluid (1 part glacial acetic acid: 3 parts chloroform: 6 parts ethyl alcohol), separately for 24 hours. Then these flower buds were transferred to vials containing 70% alcohol and preserved at 5° C. Chromosomal abnormalities were scored by Sqush Technique. Mean pollen sterility was determined based on acetocarmine stainability.

RESULTS AND DISCUSSION

In the present study, a broad spectrum of chromosomal aberrations was induced at various

stages of meiotic division in M_1 generation using gamma-rays, EMS alone as well as in combination in both varieties of greengram (Table 1 and 2). The spectrum of meiotic chromosomal abnormalities observed in various mutagenic treatments in both varieties included univalents, multivalents, chromosome stickiness, laggards, bridges, and micronuclei.

Table 1. Frequency and spectrum of chromosomal abnormalities induced by gamma rays, EMS and their combination in greengram var. Sujata

Treatments	Univalent (%)	Multivalent (%)	Stickiness (%)	Bridge (%)	Laggard (%)	Micro-nucleic (%)	Total chromosomal abnormality (%)	Pollen sterility (%)
G1	0.37	1.12	0.75	0.37	-	-	2.61	2.11
G2	1.22	0.81	1.63	1.22	1.63	0.81	7.32	4.56
G3	1.81	0.90	3.17	1.81	2.71	1.81	12.21	7.81
E1	-	0.72	1.44	0.72	-	0.36	3.24	2.46
E2	0.77	1.16	1.93	-	1.54	0.77	6.17	4.79
E3	2.04	1.63	2.86	1.22	2.04	1.63	11.42	7.43
G2E2	1.19	1.19	2.38	1.59	1.59	-	7.94	3.41
Sujata (C)	-	-	-	-	-	-	-	

Table 2. Frequency and spectrum of chromosomal abnormalities induced by gamma rays, EMS and their combination in greengram var. OBGG-52

Treatments	Univalent (%)	Multivalent (%)	Stickiness (%)	Bridge (%)	Laggard (%)	Micro-nucleic (%)	Total chromosomal abnormality (%)	Pollen sterility (%)
G1	-	0.72	0.72	1.08	-	-	2.52	2.14
G2	1.14	0.76	1.52	1.14	1.52	0.76	6.84	3.78
G3	1.15	0.77	2.69	1.54	1.92	1.15	9.22	5.47
E1	0.33	-	0.99	-	0.33	-	1.65	1.43
E2	1.07	0.71	1.07	0.71	0.36	-	3.92	1.77
E3	1.81	1.08	1.08	0.72	1.81	0.72	7.22	3.69
G2E2	1.50	1.13	2.26	0.75	1.50	0.75	7.89	4.23
OBGG-52 (C)	-	-	-	-	-	-	-	-

The univalents were found in all most all treated populations (except E1 in Sujata and G1 in OBGG-52) and their frequency was maximum at the higher dose of mutagen (Table 1 and 2). The occurrence of univalents indicates non-homology between certain chromosomes in the compliment. The mutagenic treatments induce structural changes in chromosomes and induced gene mutations might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents. According to Kumar and Tripathi (2004) the chemical mutagens induce univalent formation through cryptic structural changes in chromosomes, which restrict the pairing and in turn reduce the chiasma frequency. The multivalent were observed in all treated populations (except E1 in OBGG-52) and followed dose dependency in EMS treatments. The moderate dose combination treatment G2E2 produced higher multivalent in comparison to single moderate dose mutagenic treatments

(G2 or E2). Multivalents can be attributed to irregular pairing and breakage followed by translocation and inversions. (Dixit and Dubey, 1986). The occurrence of multivalent association is a common feature in the treated plants with the presence of more than two homologous chromosomes.

All the mutagenic treatments in both varieties induced stickiness of chromosomes and their frequencies were increased with increasing the dose of the mutagens in both varieties (Table 1 and 2). It was also observed that the moderate dose combination treatment (G2E2) produced higher stickiness of chromosomes in comparison to single moderate dose mutagenic treatments (G2 or E2). This stickiness of chromosomes resulted due to depolymerization of DNA (Darlington, 1942; Tarar and Dnyansagar, 1980), partial dissolution of nucleoprotein (Kaufmann, 1956), and alteration in the pattern of organization of chromosomes by Evans (1962). McGill et al. (1974) and Klasterska et al. (1976) suggested that stickiness arises due to improper folding of chromosome fibers, while Rao and Laxmi (1980) attributed it to be due to the disturbances of cytochemical balanced reactions by the mutagens. In addition, Gaulden (1987) postulated that stickiness may result from defective functioning of one or two types of specific non-histone proteins involved in chromosome organization which is necessary for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens (induced stickiness). It may also be possible that the mutagen itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to improper folding of DNA, thereby causing them to clump or stick. The stickiness of chromosomes at metaphase-I adversely affected the normal disjunctions of chromosomes at anaphase-I, which resulted in the formation of laggards and unequal separation of chromosomes at the anaphase stage.

In this study, the chromosomal bridges were observed in almost all the mutagenic treatments in both varieties (Except E2 in Sujata and E1 in OBGG-52) and their frequencies were increased with increasing the dose of the mutagens in both varieties of greengram. The chromosomal bridge formation may be attributed to the general stickiness of chromosomes at the metaphase stage or breakage and reunion of chromosomes. The chromosome bridge was useful for obtaining information on clastogenic activity. The Chromosomal bridges occur due to sister chromatid exchange followed by delayed or failure of their separation during later stages of anaphase and telophase chromosome. According to Saylor and Smith (1966), the bridge formation could be due to the failure of chiasmata in a bivalent to terminalize, and the chromosomes get stretched between the poles. Sinha and Godward (1972) suggested that paracentric-inversion may lead to the formation of chromatin bridges at anaphase I/II and telophase I/II. The bridges may be due to the stickiness of chromosomes. This stickiness interfered in the normal arrangement of chromosomes at metaphase and further led to their inability to separate, thus leading to sticky bridges. When the spindle fibers pulled the chromosomes towards the poles these bridges were broken into fragments, which either moved towards the poles or formed the laggards and micronuclei (Rees, 1955). The presence of single and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridge cycles (McClintock, 1941; Kumar and Singh, 2002).

The laggards observed, in the present study, have also been reported earlier and maybe the result of delayed terminalization, the stickiness of chromosomes, or the failure of chromosomal movement due to abnormal spindle formation, and as a result spindle fibers failed to carry the respective chromosomes to the polar region and resultantly lagging chromosome appeared (Tarar and Dnyansagar, 1980; Jayabalan and Rao, 1987). The formation of laggards may also be due to chromosomal breakage by binding to DNA in GC-rich regions (Bhat et al., 2007). In the present study, Lower doses are rarely induced laggards and the frequencies of laggards were increased with increasing the dose of gamma-rays and EMS in both the varieties. During telophase, a high frequency of micronuclei was observed at high dose treatments

of gamma-rays as well EMS in both varieties. Micronuclei might have arisen from the fragments and lagging chromosomes which failed to reach the poles and get included in the daughter nuclei (Kumar and Dubey, 1998).

The cytological study of the control plants was having normal meiosis activities in comparison to mutagen-treated populations. Cytological studies of mutagen treatment revealed that here was an increase in the frequency of meiotic chromosomal abnormality as increased the mutagen dose of gamma-rays and EMS confirmed the observations of earlier workers (Dhamayanthi and Reddy, 2000; Bhat et al., 2007). Although the types of chromosomal abnormalities were more or less common in both the varieties, the frequency of such aberrations was comparatively more in var. Sujata than the OBGG-52 indicates that it is more sensitive towards the mutagens (Table 1 and 2). Among the different doses/concentrations of mutagens, gamma-rays show more chromosomal abnormalities than the EMS except for the case of the lower dose of gamma-ray in the var. Sujata. These results support the general hypothesis that physical mutagens produce more cytological abnormalities than chemical ones (Kozgar, 2014). However, EMS was earlier found to be more effective in inducing meiotic irregularities than Gamma rays individually as well as in combination with Gamma-rays treatments (Dhamayanthi and Reddy, 2000). A dose-dependent increase in meiotic abnormalities has also been reported by Ignacimuthu and Babu (1989) in urdbean (*V. mungo*) and mung beans (*V. radiata*). Such chromosomal abnormalities may lead to the formation of nonfunctional spores.

The pollen sterility was increased with the increases in the dose/concentration of gamma rays and EMS treatments. A very high per cent of sterility was observed at high dose treatments of gamma rays and EMS in both varieties (Table 1 and 2). Gamma-ray treatments recorded the maximum pollen sterility (7.81% in Sujata and 5.47% in OBGG-52) at higher dose (60kR) whereas the minimum pollen sterility (2.11% in Sujata and 2.14% in OBGG-52) at a lower dose (20kR). In the case of EMS treatments, the maximum pollen sterility (7.43% in Sujata and 3.69% in OBGG-52) was observed at 0.6%, and the minimum (2.46% in Sujata and 1.43% in OBGG-52) at 0.2%. In Combination treatment, the pollen sterility was observed at 3.41% in Sujata and 4.23% in OBGG-52. The negative effect of mutagens on pollen fertility may be due to the cumulative effects of various meiotic aberrations that occurred due to the induction of mutations. The increased pollen sterility with increasing doses of mutagens was also reported by several investigators in greengram (Das et al., 2006; Tah, 2006; Das and Baisakh, 2020). The probable reason for increased pollen sterility might be due to more meiotic irregularities such as translocations (Das and Baisakh, 2020). Ramanna (1974) reported that any deviation in karyokinesis or cytokinesis could produce non-viable microspores. It may therefore be assumed that cytological disturbances caused as a result of

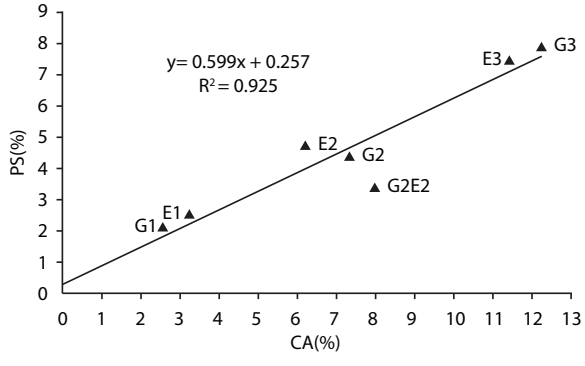

Fig. 1. Relationship between chromosomal aberrations and pollen sterility in different mutagenic treatments in greengram var. Sujata

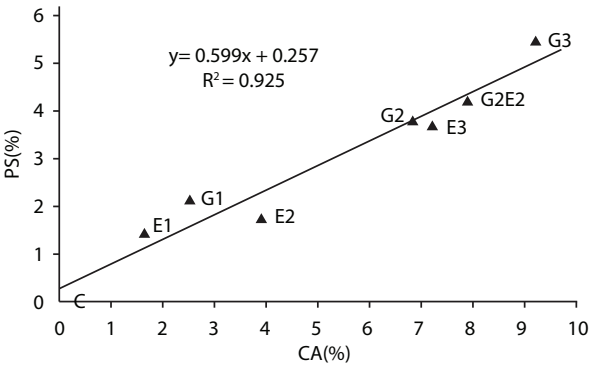

Fig. 2. Relationship between chromosomal aberrations and pollen sterility in different mutagenic treatments in greengram var. OBGG-52

physical or chemical mutagenesis were responsible for pollen sterility. Moreover, due to the mutations caused by gamma rays and EMS, the changed protein product as a result of changes in amino acid sequences might have affected the fertility of pollens. The relationship between chromosomal aberration and pollen sterility in different mutagenic treatments of both varieties are presented in Fig. 1 and 2 which suggested that induced pollen sterility may be the result of chromosomal aberrations and increases with increasing the frequency of chromosomal aberrations in both varieties of greengram. Correlation coefficient values between chromosomal abnormality and pollen sterility due to mutagenic treatments (0.962 in Sujata and 0.975 in OBGG-52) were positive and highly significant.

CONCLUSION

In the present investigation, various meiotic chromosomal variations were noticed in the mutagen treated populations of both varieties of greengram whereas, in the control population of both varieties, meiosis was normal. The percentage of chromosomal abnormalities as well as pollen sterility percentage increased with an increase in dose/concentration of gamma rays and EMS. Among the different doses/concentrations of mutagens, gamma rays show more chromosomal abnormalities than the EMS. A positive and significant correlation between chromosomal abnormality and pollen sterility was observed in this study. The relationship between chromosomal variation and pollen sterility suggested that induced pollen sterility may be due to the induced mutation in chromosomes and chromosomal aberrations. Moreover, due to such induced mutations, the changed protein product as a result of changes in amino acid sequences might have affected the morphology and fertility of pollen grains. It is concluded that both the mutagen are effective in inducing genetic variability for the improvement of greengram. Even though all mutations are not beneficial thus it is the skill of geneticist and plant breeder to select the appropriate dose, mutagen, plant characters, purposes, and methods for the betterment of crop improvement.

ACKNOWLEDGEMENT

The authors are thankful to the Vice-Chancellor, OUAT, Bhubaneswar (Odisha) and Director, IARI, New Delhi for their support and encouragement along with providing all necessary facilities during this research.

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