e-planet **21** (2): 122-130 (December 2023)

Gamma rays and maleic hydrazide induced cytogenetic effects and pollen sterility in greengram (*Vigna radiata* L. Wilczek)

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Date of receipt: 07.07.23 Date of acceptance: 27.12.23

ABSTRACT

Cytogenetic studies for induced chromosomal variations and effects are considered as an accurate index in mutation breeding for determination of the potency of different doses of gamma rays and maleic hydrazide (MH) and deducing an optimum dose. 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, Maleic hydrazide, and their combination treatments in the M1 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 of mutagens in both varieties. However, the induction of meiotic aberrations was higher in MH treatments, suggesting that MH could be more effective in inducing additional variability than gamma rays 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 MH. The comparative study of induced chromosomal abnormalities in different varieties suggested that the variety Sujata expressed higher mutagenic sensitivity 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 mutations in chromosomes. A positive and significant correlation between the induced chromosomal abnormality and the pollen sterility was observed in both varieties.

Key words: Chromosomes, gamma rays, induced mutation, maleic hydrazide, pollen sterility

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek] also known as golden gram, mungbean, mashbean, moong, etc is one of the most important pulses crops and belongs to the family *Fabaceae (Leguminosae)*. It is widely grown in the subtropical countries of South and Southeast Asia, Australia, West Indies, South and North America, and Tropical and Subtropical Africa. It is an excellent source of dietary fiber, high-quality protein, minerals, vitamins, and significant amounts of bioactive compounds, including polysaccharides, polyphenols, and peptides thus becoming a popular functional food in promoting good health. Mutation breeding is now a pillar of modern plant breeding, along with recombinant breeding and transgenic breeding. 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). Selection of efficient mutagens and their treatment doses is a prerequisite for successful mutagenesis in crop plants as mutagens are potential tools for direct improvement of qualitative and quantitative characters.

Gamma rays, one of the most used physical mutagens in mutation breeding are known to influence plant growth and development by inducing cytological, genetic, biochemical, physiological, and morphological changes in cells and tissues. Gamma rays are highly energetic ionizing radiation with a higher penetration power and thus can induce various changes at the chromosomal and molecular level and prove to be an effective physical mutagen in creating variation and effective mutation. Chemical mutagens also play a key role in inducing chromosomal aberrations and mutations that are useful for crop improvement (Das and Baisakh, 2022). Among the chemical mutagens used for induction of mutations in various crops, Maleic hydrazide ($C_4H_4N_2O_2$) is one of the most effective, efficient, and frequently used mutagens. It is a structural isomer of uracil, a pyrimidine compound of RNA. The mode of action of Maleic hydrazide (MH) is through its interference with the synthesis of uracil or by incorporating into RNA molecule replacing the uracil or it reacts with sulfhydryl groups of nucleic acids. Darlington and Mcleish (1951) were the first to report that it induces chromosome breaks. Many of these breaks induced by MH were in the heterochromatic region of the chromosome. Mcleish (1952) observed that breakage occurs during interphase leading to chromosomal break. Since heterochromatin is the site of the majority of the polygenes, MH can be effectively utilized for the induction of micromutation in quantitative traits. More recently, numerous experiments performed with various plant species have shown that MH acts as an inhibitor of the synthesis of nucleic acids and proteins (Swietlińska and Zuk, 1978).

The cytogenetic investigation is an important source of information regarding the genetic changes due to mutagens as they deal with the genetic material, the chromosomes, and more appropriately the DNA which controls the phenotype. The cytogenetic abnormalities due to any mutagen have been regarded as one dependable parameter for estimating the mutagenic potential of a mutagen which can be judged by the percentage of abnormalities it induces. The induced genetic changes occurred by the mutagens provide good scope for further improvement of greengram crop. It also provides considerable information to assess the sensitivity of plants for different mutagens and to ascertain the most effective mutagens and their treatment doses for a given crop to realize maximum results. Mutation of any of the genes disrupts meiosis, gametes sterility, and other abnormalities. Chromosomal rearrangements are one of the most frequently produced cases of mutation that result from the action of both physical and chemical mutagenic agents. Analysis of chromosomal behavior at various meiotic stages is one of the most dependable indices for the 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. To induce genetic variability and utilize useful mutants in plant breeding programs, the identification of the 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 MH on meiotic behavior and pollen sterility 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), Maleic Hydrazide (0.01, 0.02 and 0.03 %), and combine mutagens of 40 kR gamma rays with 0.02% MH

and were coded as G1, G2, G3, M1, M2, M3 and G2M2, respectively. Dry seeds were irradiated with gamma ray treatment at Bhaba Atomic Research Centre, Trombay. For treatment with MH, the seeds were pre-soaked in distilled water for six hours, blotted dry and then treated with a freshly prepared aqueous solution of the above chemical mutagen for 6 hours, with intermittent shaking. For combination treatment, seeds were first irradiated with forty kR gamma rays and then treated with 0.02% MH 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 two replications with spacing of 25×10 cm^2. Young flower buds from fifty randomly selected plants from each treatment were fixed in Carnoy's fluid (1-part glacial acetic acid: three parts chloroform: six 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, MH alone as well as in combination in both varieties of greengram (Table 1 and 2). The spectrum of meiotic chromosomal abnormalities (CA) 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, MH, and their combination in greengram var. Sujata

Treatments	Univalent (%)	Multivalent (%)	Stickiness (%)	Bridge (%)	Laggard (%)	Micro-nuclei (%)	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
M1	1.21	1.61	1.21	0.81	2.02	0.40	7.26	4.27
M2	1.69	2.12	3.39	1.27	3.81	0.85	13.13	9.03
M3	2.71	2.26	3.62	1.36	4.07	1.36	15.38	12.19
G2M2	2.06	2.06	3.29	1.65	3.29	1.23	13.58	9.70
C	-	-	-	-	-	-	-	

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

Treatments	Univalent (%)	Multivalent (%)	Stickiness (%)	Bridge (%)	Laggard (%)	Micro-nuclei (%)	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
M1	1.09	0.73	1.46	-	1.82	-	5.10	2.68
M2	1.15	1.15	2.69	0.38	3.08	0.77	9.22	7.45
M3	2.37	1.58	3.56	1.19	3.95	1.19	13.84	10.23
G2M2	1.61	1.21	2.82	0.81	3.22	0.81	10.48	8.60
C	-	-	-	-	-	-	-	-

The univalents were found in almost all treated populations (except 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 complement (Goyal et al., 2019). 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 (Das and Baisakh, 2022). Kumar and Tripathi (2004) reported that 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 and followed dose dependency in MH treatments. The moderate dose combination treatment G2M2 produced higher multivalent in comparison to single moderate dose mutagenic treatments (G2 or E2) in the variety OBGG-52 but in Sujata, the pattern observed was M2 > G2M2 > G2. 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 treated plants with the presence of more than two homologous chromosomes.

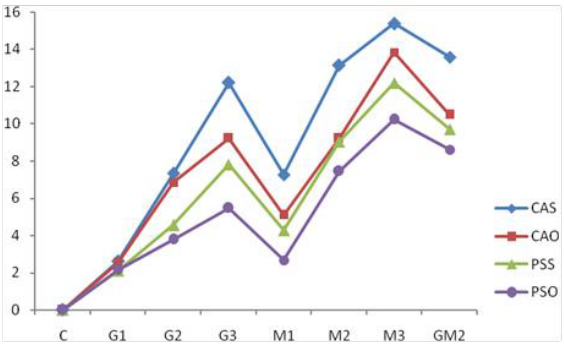
All the mutagenic treatments in both varieties induced stickiness of chromosomes and their frequencies were increased with increasing the dose of the mutagens in both varieties (Table 1 and 2). It was also observed that the moderate dose combination treatment (G2M2) produced higher stickiness of chromosomes in comparison to single moderate dose mutagenic treatments (G2 or M2). This stickiness of chromosomes resulted due to depolymerization of DNA (Darlington, 1942), partial dissolution of nucleoprotein (Kaufmann, 1956), and alteration in the pattern of organization of chromosomes (Evans, 1962). McGill et al. (1974) and Klasterska et al. (1976) suggested that stickiness arises due to improper folding of chromosome fibers, while Jayabalan and Rao (1987) reported that it is due to the disturbances of cytochemical balanced reactions in the nucleic acids by the mutagens. Gaulden (1987) postulated that stickiness may result from the 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 leads to stickiness which is caused by the mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens (induced stickiness). Gulfishan et al. (2010) explained that some kinds of gene mutations lead to incorrect coding of some non-histone proteins involved in chromosome organization and lead to chromosome clumping. 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 which causes 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 (Das and Baisakh, 2022).

The chromosomal bridges were observed in almost all the treatments in both varieties (Except M1 in OBGG-52) and their frequencies were increased with increasing the dose of both mutagens in both varieties of greengram. The chromosomal bridge formation may be attributed to the general stickiness of chromosomes at the metaphase stage or the breakage and reunion of chromosomes. The chromosome bridge was useful for obtaining information on clastogenic activity (Das and Baisakh, 2022). Chromosomal bridges occur due to sister chromatid exchange followed by delayed or failure of their separation during later stages of anaphase and telophase chromosome. Saylor and Smith (1966) reported that 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 with 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 because of breakage fusion bridge cycles (McClintock, 1941; Das and Baisakh, 2022).

In the present study, the laggards observed may be 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; Das and Baisakh, 2022). 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 rarely induced laggards in gamma rays and the frequencies of laggards were increased with increasing the dose of gamma-rays and MH in both the varieties. The moderate dose combination treatment G2M2 produced higher lagging chromosomes in comparison to single moderate dose mutagenic treatments (G2 or E2) in the variety OBGG-52 but in Sujata, it found different i.e., M2 > G2M2 > G2. Dose dependency result observed for the frequency of micronuclei in the present study. During telophase, a high frequency of micronuclei was observed at high-dose treatments of gamma rays as well as MH in both varieties. It was also found that the moderate dose combination treatment (G2M2) produced a higher frequency of micronuclei in comparison to single moderate dose mutagenic treatments (G2 or M2) 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; Das and Baisakh, 2022). The cytological study of the control plants had normal meiosis activities in comparison to mutagen-treated populations.

Cytological studies of these treatments revealed that there was an increase in the frequency of the total meiotic chromosomal abnormality as increased the mutagen dose of gamma rays and MH (Fig. 1) confirmed the observations of earlier workers (Dhamyanthi and Reddy, 2000; Bhat et al., 2007). Although the types of chromosomal abnormalities were 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 or concentrations of mutagens, MH shows more chromosomal abnormalities than gamma-rays. Such chromosomal abnormalities may lead to the formation of nonfunctional spores. 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*).



(CAS: Chromosomal abnormality in Sujata, CAO: Chromosomal abnormality in OBGG-52, PSS: Pollen sterility in Sujata, PSO: Pollen Sterility in OBGG52)

Fig 1. Effect of gamma-rays and MH treatments on chromosomal aberrations and pollen sterility in greengram var. Sujata and OBGG-52

Pollen sterility is an index of the meiotic behavior. The reasons for pollen sterility in mutagenic treatments may be due to induced gene mutation or invisible deficiencies. In the present investigation, the pollen sterility (PS) was increased with the increases in the dose/concentration of both mutagens, i.e., gamma rays and MH treatments (Fig. 1). An exceedingly high percentage of sterility was observed at high-dose treatments of gamma rays and MH in both varieties (Tables 1, 2). Gamma-ray treatments recorded the maximum pollen sterility (7.81% in Sujata and 5.47% in OBGG-52) at the 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 MH treatments, the maximum pollen sterility (12.19% in Sujata and 10.23% in OBGG-52) was observed at 0.03%, and the minimum (4.27% in Sujata and 2.68% in OBGG-52) at 0.01%. In Combination treatment, the pollen sterility was observed at 9.70% in Sujata and 8.60% 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; Das and Baisakh, 2020; Das and Baisakh, 2022). 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 due to any physical or chemical mutagenesis were responsible for pollen sterility. Moreover, due to the mutations caused by gamma rays and MH, the changed protein product because 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. 2 and 3 which suggest that induced pollen sterility may be the result of chromosomal aberrations and increases with increasing the frequency of the total chromosomal abnormalities in both varieties of greengram. Correlation coefficient values between chromosomal abnormality and pollen sterility due to mutagenic treatments (0.985 in Sujata and 0.969 in OBGG-52) were positive and highly significant. This agrees with many workers (Bhamburker and Bhalla, 1985; Das and Baisakh, 2022) who have also reported dose-dependent decrease in pollen fertility. Reduction in pollen fertility observed in mutagen treated population is attributed to the vast array of meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains (Rana and Swaminathan, 1964; Sinha and Godward, 1972). The relationship between diverse types of induced chromosomal abnormalities and pollen sterility in both varieties are presented in Fig. 4 and 5. The correlation coefficient values between diverse types of chromosomal abnormalities and pollen sterility were positive and significant in both varieties of greengram (Table 3).

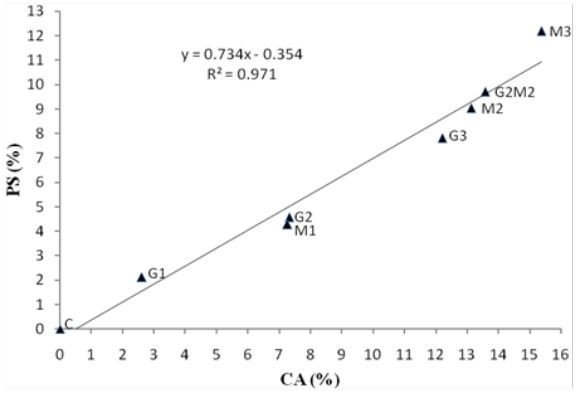


Fig. 2. Relationship between chromosomal aberrations and pollen sterility in different mutagenic treatments in greengram var. Sujata

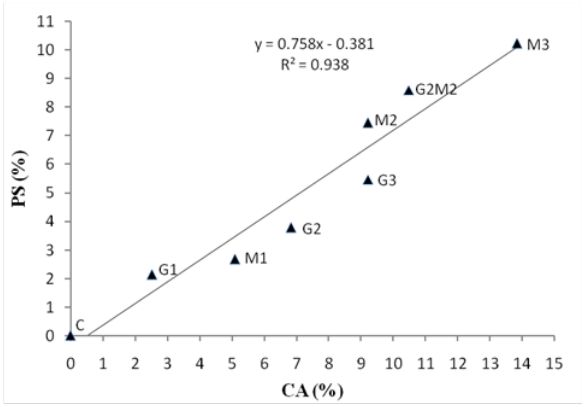
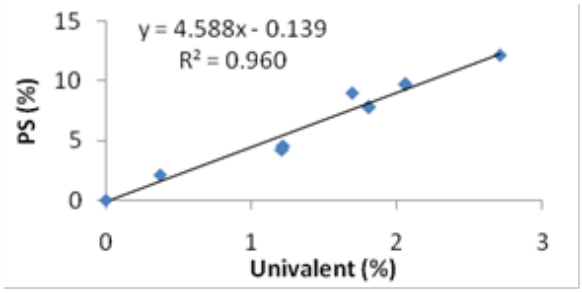
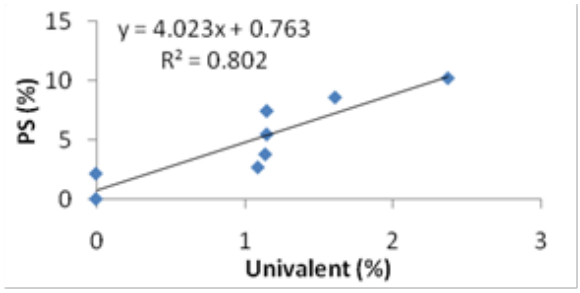
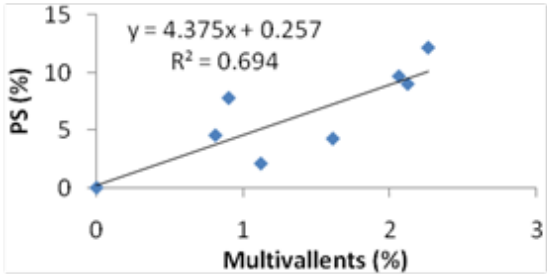
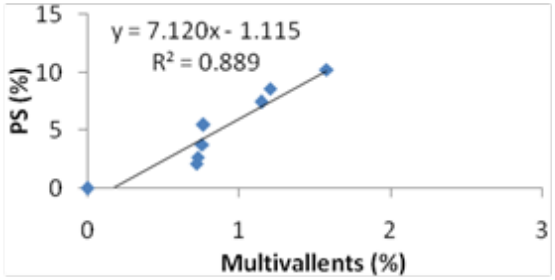


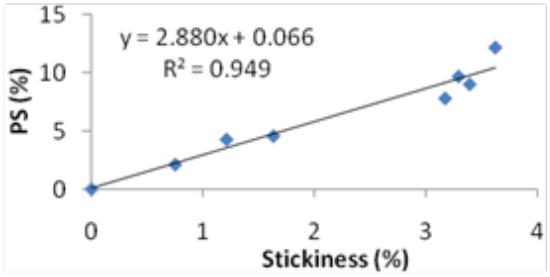
Fig. 3. Relationship between chromosomal aberrations and pollen sterility in different mutagenic treatments in greengram var. OBGG 52

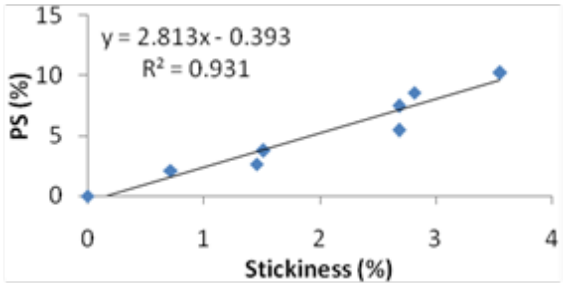

y = 4.588x - 0.139
R² = 0.960
PS (%)
Univalent (%)

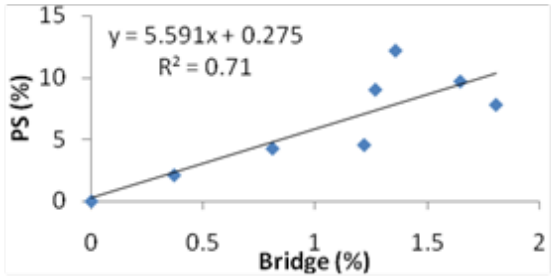

y = 4.023x + 0.763
R² = 0.802
PS (%)
Univalent (%)

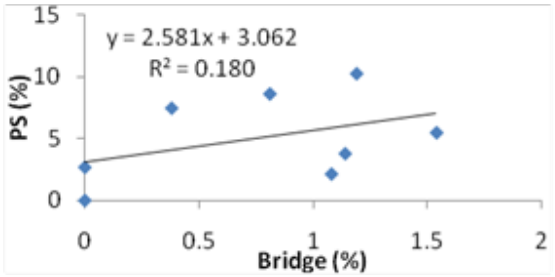

y = 4.375x + 0.257
R² = 0.694
PS (%)
Multivallents (%)

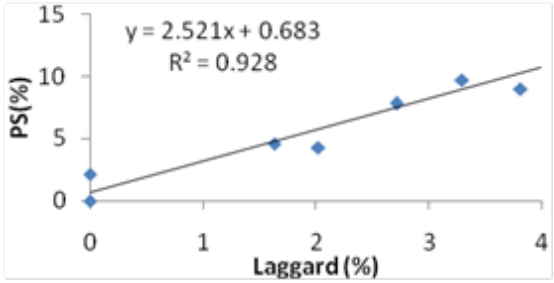

y = 7.120x - 1.115
R² = 0.889
PS (%)
Multivallents (%)

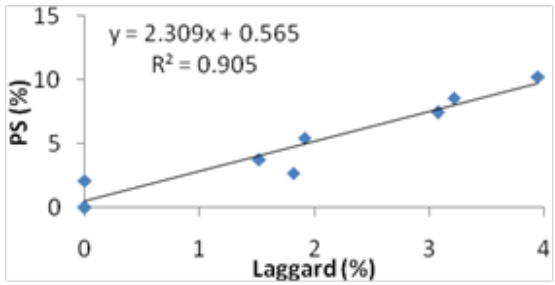

y = 2.880x + 0.066
R² = 0.949
PS (%)
Stickiness (%)


y = 2.813x - 0.393
R² = 0.931
PS (%)
Stickiness (%)


y = 5.591x + 0.275
R² = 0.71
PS (%)
Bridge (%)


y = 2.581x + 3.062
R² = 0.180
PS (%)
Bridge (%)


y = 2.521x + 0.683
R² = 0.928
PS(%)
Laggard (%)


y = 2.309x + 0.565
R² = 0.905
PS (%)
Laggard (%)

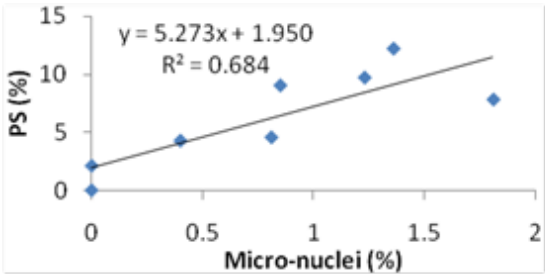


Fig. 4. Relationship between different chromosomal abnormality and pollen sterility in Sujata

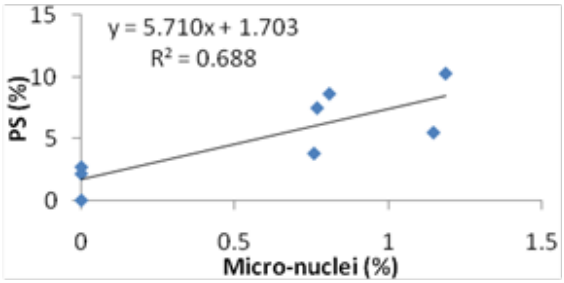


Fig. 5. Relationship between different chromosomal abnormality and pollen sterility in OBGG-52

Table 3. Correlation coefficient between different induced chromosomal abnormalities and pollen sterility.

Sl. No.	Chromosomal abnormalities	Correlation coefficient with pollen sterility	
		Sujata	OBGG-52
1	Univalent	0.980	0.896
2	Multivalent	0.833	0.943
3	Stickiness	0.974	0.965
4	Bridge	0.843	0.424
5	Laggard	0.964	0.951
6	Micro-nuclei	0.827	0.829

CONCLUSION

In the present investigation, various meiotic chromosomal variations i.e., univalent, multivalent, chromosome stickiness, laggards, bridges, and micronuclei were noticed in the gamma rays and MH-treated populations of both varieties of greengram whereas, the meiosis was normal in the control populations of both varieties. The percentage of chromosomal abnormalities as well as pollen sterility percentage increased with an increase in dose/concentration of gamma rays and MH. Based on the cytogenetic effect of different doses or concentrations of mutagens, MH shows more chromosomal abnormalities than gamma rays. A positive and significant correlation between chromosomal abnormality and pollen sterility was observed in this study. The relationship between chromosomal variations and pollen sterility suggested that induced pollen sterility may be due to the induced mutation in chromosomes and chromosomal aberrations which induce for production of a changed protein product as a result of changes in amino acid sequences and this changed protein product might have affected the morphology and fertility of pollen grains thus pollen sterility observed. It is concluded that both the mutagens are effective in inducing genetic variability for the improvement of greengram. Even though all mutations are not beneficial, it is the skill of a researcher to select the appropriate dose, mutagen, plant characters, purposes, and methods for breeding programmes to improve yield and other desirable characters in greengram.

ACKNOWLEDGEMENT

The authors are thankful to the Vice-Chancellor, OUAT, Bhubaneswar, Odisha, and Director, Indian Agricultural Research Institute, New Delhi for their support and necessary facilities during this research.

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