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BRIEF NOTE

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Cytological effects of different mutagens in soybean [*Glycine max* (L.) Merrill]

Abstract The effect of gamma rays, ethyl methane sulphonate, and their three combinations on various cytological parameters in M_1 generation was studied in two soybean cultivars, Pusa-16 and PK-1042. Combined treatments exhibited high meiotic abnormalities in Pusa-16 as compared with the individual treatments of ethyl methane sulphonate and gamma rays. In PK-1042 the highest frequency of meiotic abnormalities was exhibited by gamma rays, while in Pusa-16 the frequencies of univalents were high as compared with other meiotic abnormalities. Fragments/bridges, laggards, ring and rod bivalents were other types of meiotic abnormalities that were noticed in both the cultivars. The frequencies of irregular cells in the two cultivars were high in combined treatments followed by ethyl methane sulphonate and gamma rays. The highest percentage of pollen sterility was exhibited by the combined treatments and the lowest was exhibited by gamma rays. In Pusa-16, the increase in doses of gamma rays and ethyl methane sulphonate increased the pollen sterility percentage, while in the combined treatments, the increase was noticed up to the intermediate dose and then gradually decreased at higher doses. On the other hand, in PK-1042 the increase in ethyl methane sulphonate dose increased sterility percentage, while no definite trend was noticed in gamma rays and combined treatments.

Keywords *Glycine max*, mutagen, cytological abnormality

1 Introduction

Cytogenetic studies are important for obtaining information regarding the role and effect of various mutagens and elucidating the responses of various genotypes to a particular mutagen. The genus *Glycine*, which includes the cultivated soybean, comprises predominantly diploid ($2n = 2x = 40$) and tetraploid ($2n = 4x = 80$) species. Soybean contains $2n = 40$ small (1.42–2.82 μm), morphologically similar somatic chromosomes (Sen and Vidyabhusan, 1960) that do not show sufficiently different banding patterns to allow chromosome identification (Ladizinsky et al., 1979). Palmer (1976) has pointed out the usefulness of cytogenetic methods for the improvement of soybeans. However, information on the cytogenetics of cultivated soybean is minimal when compared with other important crops. In recent years, several important sterile male soybean mutants have been described (Graybosch and Palmer, 1988; Palmer et al., 1992), and a cytogenetic map of the 20 soybean chromosomes has been constructed for the relatively uncondensed pachytene chromosomes (Singh and Hymowitz, 1988). Hence, our research was to find out the effect of gamma (γ) rays, ethyl methane sulphonate (EMS), and their combinations on various cytological parameters in M_1 generation of *Glycine max* cv. Pusa-16 and PK-1042.

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2 Materials and methods

2.1 Materials

Seeds of two soybean varieties, Pusa-16 and PK-1042, were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

2.2 Methods

The experimental materials (constituting 20 treatments) were sown in single unreplicated plots with four rows at a distance of 30 cm between the rows and 10 cm between the plants in a row at the Research Farm of Kisan (P. G.) College, Simbhaoli. The experimental materials were divided into three treatment groups as follows.

T1 was the treatment with physical mutagen, i.e. 15, 30,

and 45 kR of gamma rays. Seed irradiation (200 seeds of each treatment) was done at the Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi.

T2 was the treatment with chemical mutagen, i.e. 0.1%, 0.2%, and 0.3% concentration of EMS. One hundred seeds of each treatment were treated with chemical mutagen for 8 hours and washed in running water before sowing.

T3 was the treatment with both physical and chemical mutagens, i.e. 15 kR γ ray + 0.2% EMS, 30 kR γ ray + 0.2% EMS, and 45 kR γ ray + 0.2% EMS. One hundred irradiated seeds in 15, 30, and 45 kR γ ray were subjected to 0.2% EMS treatment for 8 hours, respectively. The seeds treated with chemical mutagen were washed in running water before sowing.

The treated materials along with two controls (untreated) were immediately sown in single unreplicated plots with four rows at spacing of 30 cm × 10 cm at the Research Farm of Kisan (P. G.), College, Simbhaoli. The data of 20 randomly selected plants from each treatment were recorded.

2.3 Cytological studies

Cytological studies were conducted in M_1 generation. Meiotic studies were conducted on 5 randomly selected plants for each treatment. Young unopened flower buds were fixed in freshly prepared Carnoys Fluid (6 absolute alcohol: 3 chloroform: 1 acetic acid) with a few drops of saturated solution of ferric chloride added in the morning (8:00 am to 9:00 am). Squashes were made in 2% acetocarmine. For estimation of pollen sterility of the treated and control materials, the squashes of fixed flower buds were made in iodine. The pollen that stained blue-black in iodine was considered viable while the unstained was nonviable. The ratio of viable pollen to the total number in each treatment was expressed as percentage pollen viability. The procedure was repeated for all buds in a sample belonging to a particular treatment. Pollen sterility was recorded as 100-pollen viability.

The data of the following parameters of quadrivalents, bivalents, univalents at metaphase I; fragments/bridges, laggards at anaphase I; cell (%) showing other chromosomal irregularities; and pollen sterility (%) were taken at appropriate stages.

3 Results

3.1 Induced meiotic aberrations

Meiosis was studied on 10 randomly selected plants from each treatment of two varieties. The data pertaining to the spectrum of various meiotic abnormalities due to the different mutagenic treatments in two cultivars of soybean are presented in Table 1, Table 2, and Fig. 1.

In general, the combined treatments in Pusa-16 produced a high meiotic abnormality as compared with the treatments in ethyl methane sulphonate and gamma rays, while in PK-1042 the highest frequency was exhibited by gamma rays. In Pusa-16 the frequency of univalents was high as compared with that of other types of meiotic abnormities and the increase in the dose of mutagens reduced the frequency of univalents. Gamma rays produced more numbers of bridges/fragments than EMS and combined treatments in Pusa-16, with the frequency of bridges/fragments increasing at the highest dose level, while in PK-1042 the frequency of bridges/fragments decreased up to the intermediate dose level of gamma rays. Among the nine mutagenic treatments, the quadrivalents in PK-1042 were exhibited by dose levels of 15 kR, 30 kR, and 45 kR + 0.2% EMS. The frequency of irregular cells in both cultivars was high in the combined treatment and low in the gamma ray treatments. In Pusa-16 the frequencies of laggards were noticed at 30 kR of gamma rays, 15 kR + 0.2% EMS, and 45 kR + 0.2% EMS levels of the combined treatment, but in PK-1042 the frequency of laggards was exhibited by the lower doses of ethyl methane sulphonate and combined treatment.

Table 1 Cytological effects of various mutagenic treatments in M_1 generation in soybean cultivar Pusa-16

serial no.	treatment	no. of cells tested	cytological effects/%						
			bridges/fragments	laggards	I	II	IV	irregular cells	pollen sterility
1	control	44	0.00	0.00	0.00	4.54	0.00	0.00	–
2	15 kR	56	5.36	0.00	0.00	3.57	1.78	3.57	16.81
3	30 kR	32	0.00	3.12	3.12	0.00	0.00	0.00	27.63
4	45 kR	62	6.25	0.00	1.61	3.22	0.00	0.00	32.43
5	0.1% EMS	43	0.00	0.00	2.31	6.98	0.00	0.00	31.93
6	0.2% EMS	46	4.34	0.00	2.17	4.34	0.00	6.00	35.31
7	0.3% EMS	50	0.00	0.00	2.00	6.00	0.00	0.00	58.23
8	15 kR + 0.2% EMS	32	3.12	9.37	0.00	6.25	3.12	15.62	41.20
9	30 kR + 0.2% EMS	41	2.43	0.00	2.43	7.31	0.00	0.00	50.85
10	45 kR + 0.2% EMS	50	0.00	2.00	0.00	6.00	2.00	8.00	47.83

Table 2 Cytological effects of various mutagenic treatments in M_1 generation in soybean cultivar PK-1042

serial no.	treatment	no. of cells tested	cytological effects/%						
			bridges/fragments	laggards	I	II	IV	irregular cells	pollen sterility
1	control	58	0.00	0.00	0.00	5.17	0.00	0.00	–
2	15 kR	43	4.65	0.00	2.32	6.98	4.65	0.00	19.42
3	30 kR	39	2.56	0.00	5.13	0.00	5.13	0.00	47.86
4	45 kR	56	5.35	0.00	1.78	3.57	0.00	7.14	32.38
5	0.1% EMS	55	0.00	1.22	3.63	1.82	0.00	0.00	35.16
6	0.2% EMS	60	1.67	3.33	0.00	3.33	0.00	5.00	42.05
7	0.3% EMS	52	3.85	0.00	1.92	0.00	0.00	7.69	45.92
8	15 kR + 0.2% EMS	32	0.00	3.13	6.25	0.00	0.00	0.00	46.52
9	30 kR + 0.2% EMS	45	2.22	0.00	0.00	4.44	0.00	6.67	36.41
10	45 kR + 0.2% EMS	36	0.00	0.00	0.00	5.55	2.78	8.30	52.20

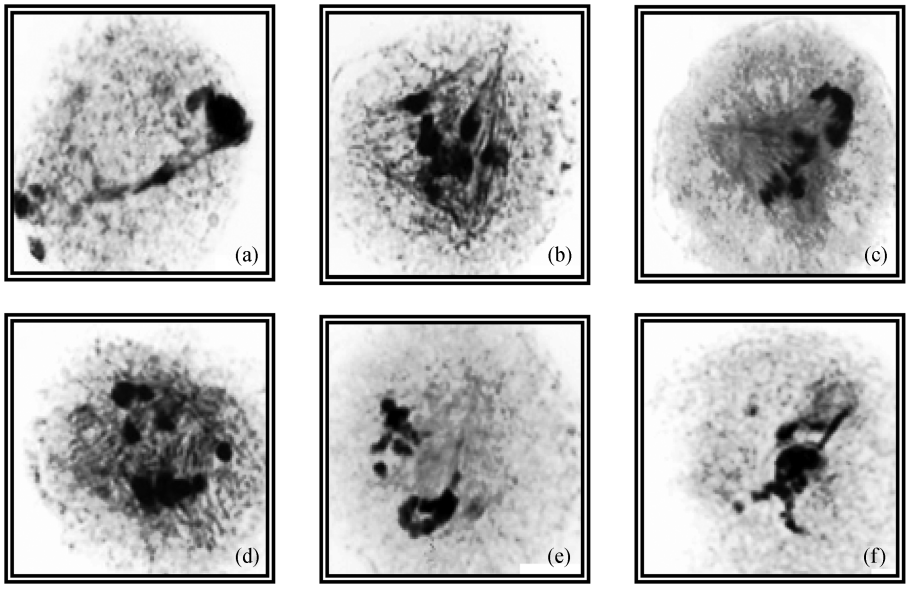

Fig. 1 Meiotic anomalies in mutagen treated soybean

Note: (a)–(f) represent telophase with thick bridge, metaphase with tripolar spindle, metaphase showing a convergent spindle, prophase I showing micronuclei, metaphase I with some linked chromosomes, and prophase involving entire chromosome complement, respectively.

3.2 Pollen sterility

The pollen sterility among the different mutagenic treatments in Pusa-16 ranged from 16.81% (15 kR) to 58.23% (0.3% EMS), while in Pk-1042 it ranged from 19.42% (15 kR) to 52.20% (45 kR + 0.2% EMS; Table 3, Fig. 2).

In both the cultivars, the combined treatments exhibited the highest percentage of pollen sterility, followed by ethyl methane sulphonate and gamma rays. In Pusa-16 the percentage of pollen sterility increased with the increase in the dose/concentration of the gamma rays and ethyl methane sulphonate, while in the combined treatments the pollen sterility increased up to the intermediate dose level followed by a decrease at the highest level of the mutagens. On the other hand, in PK-1042 the gamma rays exhibited an increase in pollen sterility at the intermediate dose level, while the combined treatments showed a reverse trend of gamma rays. In case of ethyl methane sulphonate treatment, the increase in the dose of mutagen was associated with the increase in the pollen sterility.

4 Discussion

Meiosis is a complex process that includes cytogenetic events such as chromosome pairing, formation of synaptonemal complex, recombination of chromosome segregations, and the creation of gamete meiotic products. During the present investigation various meiotic abnormalities

Table 3 Effect of mutagens on pollen sterility in two cultivars (Pusa-16 and PK-1042) of soybean

serial no.	dose	pollen sterility pollen sterility over control/%			
		Pusa-16	PK-1042	Pusa-16	PK-1042
1	control	15.76	14.42	0.00	0.00
2	15 kR	16.81	19.42	1.05	5.30
3	30 kR	27.63	47.86	11.87	33.74
4	45 kR	32.43	32.38	16.67	17.96
5	0.1% EMS	31.93	35.16	16.17	20.74
6	0.2% EMS	35.31	42.05	19.55	27.63
7	0.3% EMS	58.23	45.92	42.47	31.50
8	15 kR + 0.2% EMS	41.20	43.52	25.44	29.10
9	30 kR + 0.2% EMS	50.85	36.41	35.09	21.99
10	45 kR + 0.2% EMS	47.83	52.20	32.87	37.78

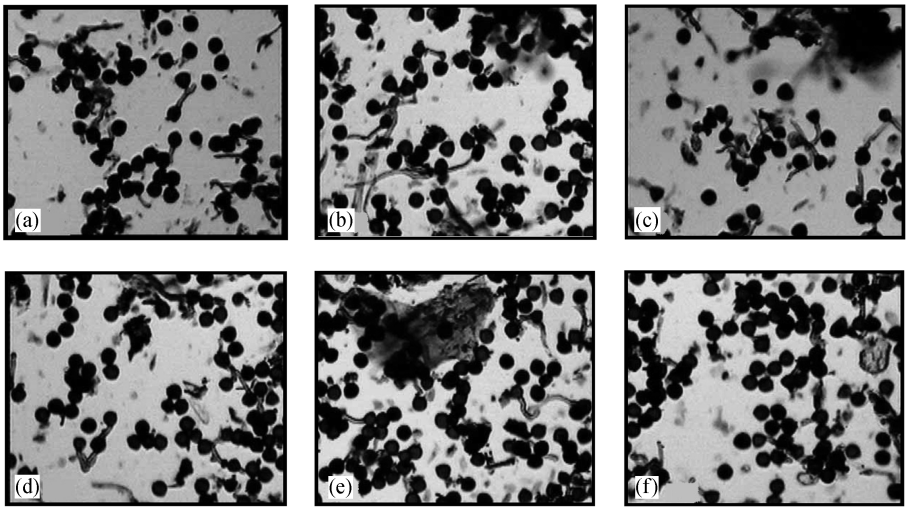

Fig. 2 Pollen sterility due to various mutagenic treatments in two cultivars of soybean
Note: (a)–(f) represent Pusa-16 control, Pusa-16 with highest sterility (0.3% EMS), Pusa-16 with lowest sterility (15 kR), PK-1042 control, PK-1042 with highest sterility (45 kR + 0.2% EMS) and PK-1042 with lowest sterility (15 kR), respectively.

such as fragments/bridges, laggards, quadrivatents, univalents, and other irregular cells were noticed. Furthermore, it was also noticed that gamma rays produced more abnormalities than EMS treatments in both the cultivars. Similar findings were also reported by Reddy and Annadurai (1992). The above findings supported the general hypothesis that physical mutagens produce more cytological abnormalities than chemical mutagens. A considerable number of abnormalities in chemical mutagen–treated populations revealed that they are capable of inducing mutations for cytological characters. The presence of univalents and bivalents of various types in the present study may be due to the reduction in chiasma frequency. This may be attributed to the mutations in the genes governing homologous chromosome pairing as also reported by Reddy et al. (1991). The occurrence of univalent, ring and rod bivalents due to the mutagenic treatments was also previously reported by Mansour (1994), Bione et al. (2002a), and Vinita et al. (2004). Bridges/fragments and laggards were also noticed in all the three groups of mutagenic treatments except laggards in EMS treatment in Pusa-16. The formation of a bridge between the two sister chromosomes may be attributed to the following: (i) the aberrant bivalent may pass intact to one pole in the first meiotic diversion and the separation of chromosomes at anaphase II would give rise to the bridge; or (ii) if one chiasma occurs in the inversion loop and another in the region between the centromere and the inversion loop, then a monocentric loop chromatid will appear at anaphase I, which gives rise to a direct-type anaphase II bridge, while sometimes bivalents lag behind and cannot be incorporated in any of the chromosome group at telophase I and in such a way laggards as well as chromosome fragments are formed. The formation of laggards and bridges/fragments have earlier been reported by several cytologists, namely, Newell and Hymowitz

(1980), Bione et al. (2000), Arslan et al. (2001), and Bione et al. (2002b).

The pollen sterility was high in EMS treatments as compared with that of the gamma ray treatments. This is in conformity with the earlier reports of Dixit and Dubey (1986). The above results are in strong contrast to the general hypothesis that physical mutagens may produce higher meiotic abnormalities than chemical mutagens. Moreover, in EMS treatments a dose-dependent increase was noticed in both cultivars. The earlier dose-dependent increase was also reported by Ignacimuthu and Babu (1992) in mungbean and Kumar and Dubey (1998) in khesari. In general, during the present study the sterility was high at intermediate or higher dose levels, which may be due to the fact that the cultivars were more sensitive to higher doses and therefore, the genic, chromosomal, and physiological disturbances were more at the higher doses which are in conformity with Ekberg (1969).

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