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nduced chlorophyll and macro-mutational spectrum and frequency n sesame cv. B 67

.C. KAR AND D. SWAIN
irectorate of Research, Orissa University of Agri. and Tech., Bhubaneswar-751 003,

Abstract: Seed treatment of sesame cv.B67, with EMS, NG, Gamma rays, Gamma rays + EMS and Gamma rays + NG induced five types of chlorophyll mutations and 17 types of viable macro-mutations in M_2. Chlorophyll mutation frequency was the highest in 900 Gy gamma rays for single mutagen treatments and in 700 Gy + 0.04% NG for combined treatments. Macro-mutational frequencies for single and combined treatments were the highest in 0.25% EMS and 700 Gy + 0.02% NG, respectively. Relative difference in mutability of gene loci for chlorophyll and macro-mutations with respect to different mutagens were clearly observed.

Key words : Sesame, EMS, NG, Gamma rays, Chlorophyll mutations, Macro-mutations.

ntroduction

Mutation breeding is a quicker method or genetic improvement in crop plants. In ndia, 71 varieties of cereals and millets, 33 f oilseeds, 49 of grain legumes, 14 of fibre rops, 10 of vegetables and 10 of cash crops, eveloped through induced mutagenesis, have een released for cultivation (Kharkwal *et al.* 001). Induced mutation is directed to improve ield and other quantitative characters, amend pecific defects in adopted varietis and to create ufficient genetic variabilities. The success in nutation breeding programme for any crop an be achieved by increasing the spectrum nd frequency in viable mutations. Therefore, ne present investigation was undertaken to study ne mutagenic response of ethyl methane sulphonate EMS), nitroso-guanidine (NG), gamma rays nd the combination treatments of gamma rays vith EMS and NG in sesame.

Materials and Methods

The materials for the present investigation vere comprised of samples of 300 dry, uniform nd well-filled seeds of sesame cv.B67 (Tilottama) or each treatment. The seeds were irradiated vith three doses of gamma rays (500 Gy, 700 Gy, 900 Gy) in Co^{60} gamma cell at the Division of Genetics, I.A.R.I., New Delhi, for gamma rays treatment. For chemical mutagenesis, seeds were presoaked in distilled water for 12 hours followed by treatment with three different concentrations of EMS (0.25%, 0.50%, 0.75%) and NG (0.01%, 0.02%, 0.04%) aqueous solutions separately for eight hours. For combined treatments, 700 Gy gamma ray irradiated dry seeds were presoaked in distilled water for 12 hours and then treated with the above mentioned three concentrations of EMS and NG for eight hours. All the chemical treatments were carriedout at room temperature (21 + 1°C) with intermittent shaking. The seeds, treated with chemical mutagens, were washed under tap water for 30 minutes to leach out the residual chemicals adsorbed to the seeds and then dried on blotting paper. Seeds of 15 treatments along with the control were sown in rows of 5 m length with a spacing of 30 x 10 cm at Central Research Station, OUAT, Bhubaneswar, during summer irrigated situation of 1999, to raise M_1 generation. Selfed seeds from M_1 plants were harvested and sown to raise M_2 generation. The effects of the mutagenic treatments were evaluated on the basis of spectrum and frequency of chlorophyll and viable macro-mutations in M_2 generations. The frequencies of mutations were estimated on the basis of 100 M_2 plants.

Results and Discussion

Chlorophyll mutations

Five types of chlorophyll mutations such as albina, xantha, viridis, chlorina and sectorial, were isolated in M_2 generation (Table 1). Xantha, viridis and chlorina types were induced by all the three mutagens in single as well as combined treatments; whereas albina types appeared

Table 1. Spectrum and frequency of chlorophyll mutations in M2 generation

Mutagens	Dose with treatment symbols	Total plants scored	Number, spectrum and frequency of chlorophyll mutants					
			Albina	Xantha	Viridis	Chlorina	Sectorial	Total
Single treatment								
Ethyl methane sulphonate (EMS)	0.25 % (E_1)	3724	0 (_)	19 (0.510)	0 (_)	2 (0.054)	0 (_)	21 (0.564)
	0.50 % (E_2)	3219	0 (_)	5 (0.155)	5 (0.155)	7 (0.217)	0 (_)	17 (0.528)
	0.75 % (E_3)	2484	0 (_)	7 (0.282)	7 (0.282)	6 (0.242)	0 (_)	20 (0.805)
	EMS pooled	9427	0 (_)	31 (0.328)	12 (0.127)	15 (0.159)	0 (_)	58 (0.615)
Nitrosoguanidine (NG)	0.01 % (N_1)	3240	2 (0.062)	9 (0.278)	3 (0.093)	5 (0.154)	0 (_)	19 (0.586)
	0.02 % (N_2)	2932	0 (_)	12 (0.409)	9 (0.307)	0 (_)	0 (_)	21 (0.716)
	0.04 % (N_3)	2503	0 (_)	10 (0.399)	4 (0.160)	4 (0.160)	0 (_)	18 (0.719)
	NG pooled	8675	2 (0.023)	31 (0.357)	16 (0.184)	9 (0.104)	0 (_)	58 (0.668)
Gamma rays (GR)	500 Gy (G_1)	3592	0 (_)	10 (0.278)	2 (0.056)	4 (0.111)	0 (_)	16 (0.445)
	700 Gy (G_2)	3454	0 (_)	8 (0.232)	8 (0.232)	6 (0.174)	0 (_)	22 (0.637)
	900 Gy (G_3)	2972	0 (_)	10 (0.336)	2 (0.067)	7 (0.236)	7 (0.236)	26 (0.875)
	GR pooled	10018	0 (_)	28 (0.279)	12 (0.120)	17 (0.170)	7 (0.070)	64 (0.639)
Combined treatment								
Gamma rays + EMS (GE)	700 Gy + 0.25% EMS (G_2E_1)	3063	0 (_)	14 (0.457)	3 (0.098)	0 (_)	0 (_)	17 (0.555)
	700 Gy + 0.50% EMS (G_2E_2)	2771	0 (_)	14 (0.505)	7 (0.253)	3 (0.108)	6 (0.217)	30 (1.083)

contd.

Table 1 : Number, spectrum and frequency of chlorophyll mutants

Mutagens	Dose with treatment symbols	Total plants scored	Albina	Xantha	Viridis	Chlorina	Sectorial	Total
	700 Gy + 0.75% EMS (G_2E_3)	2469	0 (_)	13 (0.527)	6 (0.243)	10 (0.405)	0 (_)	29 (1.174)
	GE pooled	8303	0 (_)	41 (0.494)	16 (0.193)	13 (0.157)	6 (0.072)	76 (0.915)
Gamma rays+NG (GN)	700 Gy + 0.01% NG (G_2N_1)	2879	0 (_)	11 (0.382)	2 (0.069)	2 (0.069)	8 (0.278)	23 (0.799)
	700 Gy + 0.02% NG (G_2N_2)	2637	0 (_)	12 (0.455)	7 (0.265)	4 (0.152)	3 (0.114)	26 (0.986)
	700 Gy + 0.04% NG (G_2N_3)	2358	0 (_)	16 (0.678)	7 (0.297)	9 (0.382)	2 (0.085)	34 (1.442)
	GN pooled	7874	0 (_)	39 (0.495)	16 (0.203)	15 (0.191)	13 (0.165)	83 (1.054)
	Control (C)	2093	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)

Note : Figures in parentheses indicate frequency of chlorophyll mutations per 100 M_2 seedlings.

only in the single treatment of NG (N_1). The gamma rays in single (G_3) as well as in the combined treatments (G_2E_2, G_2E_3, G_2N_1, G_2N_2 & G_2N_3) induced sectorial types. The mutational spectrum was much wider (four types) at 0.01% NG, 900 Gy gamma rays, 700 Gy + 0.50% EMS, 700 Gy + 0.01% NG, 700 Gy + 0.02% NG and 700 Gy + 0.04% NG treatments. Chlorophyll mutant xantha (50.15%) appeared more frequently followed by others>viridis (21.24%)>chlorina (20.35%)>sectorial (7.67%)> albina (0.59%).

The frequencies of chlorophyll mutations per 100 M_2 plants (Table 1) were in order of GN>GE>NG>GR>EMS. Along the nine single mutagenic treatments, the frequencies of the chlorophyll mutations per 100 M_2 plants were in order of $G_3>E_3>N_3>N_2>G_2>N_1>E_1>E_2>G_1$. Among the six combined mutagenic treatments, the frequencies of chlorophyll mutations per 100 M2 plants were in order of $G_2N_3 > G_2E_3 > G_2E_2 > G_2N_2 > G_2N_1 > G_2E_1$. In general, the frequencies of total chlorophyll mutations increased with the increase in dose for single as well as combined treatments except for 0.50% EMS, possibly due to increase in physiological damage at a faster rate with the increase in dose.

All the treatments, except 0.50% EMS, induced the highest frequencies of xantha type compared to any other types (Table 1). Among the single mutagenic treatments, NG induced the highest frequencies of xantha and viridis, while gamma rays induced highest frequency of chlorina types. Drastic chlorophyll mutations (albina, xantha, viridis) were induced more by chemical

Table 2. Spectrum and frequency of macromutations in M_2 generation

Treatments	Number, spectrum and frequency of macromutation								
	Fasciated cotyledon	Uniculm stem	Fasciated stem	Tall	Dwarf	Bushy	Whorled leaf phyllotaxy	Stipulate	Long-nar leaf
E_1	0	14	4	0	3	7	0	0	0
	(_)	(0.376)	(0.107)	(_)	(0.081)	(0.188)	(_)	(_)	(_)
E_2	0	0	0	0	4	0	0	0	0
	(_)	(_)	(_)	(_)	(0.124)	(_)	(_)	(_)	(_)
E_3	1	0	1	0	0	6	0	0	0
	(0.040)	(_)	(0.040)	(_)	(_)	(0.242)	(_)	(_)	(_)
EMS pooled	1	14	5	0	7	13	0	0	0
	(0.011)	(0.149)	(0.053)	(_)	(0.074)	(0.138)	(_)	(_)	(_)
N_1	2	0	0	8	0	0	1	0	0
	(0.062)	(_)	(_)	(0.247)	(_)	(_)	(0.031)	(_)	(_)
N_2	0	0	0	0	0	0	0	0	0
	(_)	(_)	(_)	(_)	(_)	(_)	(_)	(_)	(_)
N_3	0	8	0	0	3	0	0	0	0
	(_)	(0.320)	(_)	(_)	(0.120)	(_)	(_)	(_)	(_)
NG pooled	2	8	0	8	3	0	1	0	0
	(0.023)	(0.092)	(_)	(0.092)	(0.035)	(_)	(0.012)	(_)	(_)
G_1	0	0	0	10	0	8	0	0	0
	(_)	(_)	(_)	(0.278)	(_)	(0.223)	(_)	(_)	(_)
G_2	0	0	0	0	3	9	0	0	0
	(_)	(_)	(_)	(_)	(0.087)	(0.261)	0	0	0
G_3	0	0	0	0	0	0	5	0	0
	(_)	(_)	(_)	(_)	(_)	(_)	(0.168)	(_)	(_)
GR pooled	0	0	0	10	3	17	5	0	0
	(_)	(_)	(_)	(0.100)	(0.030)	(0.170)	(0.050)	(_)	(_)
G_1E_1	0	15	0	14	6	4	0	7	17
	(_)	(0.490)	(_)	(0.457)	(0.196)	(0.131)	(_)	(0.229)	(0.55
G_1E_2	0	10	6	6	0	0	0	0	5
	(_)	(0.361)	(0.217)	(0.217)	(_)	(_)	(_)	(_)	(0.180)
G_1E_3	0	0	0	0	4	14	0	3	7
	(_)	(_)	(_)	(_)	(0.162)	(0.567)	(_)	(0.122)	(0.284)
GE pooled	0	25	6	20	10	18	0	10	29
	(_)	(0.301)	(0.072)	(0.241)	(0.120)	(0.217)	(_)	(0.120)	(0.349)

contd

Treatments	Number, spectrum and frequency of macromutation								
	Fasciated cotyledon	Uniculm stem	Fasciated stem	Tall	Dwarf	Bushy	Whorled leaf phyllotaxy	Stipulate	Long-narrow leaf
G_2N_1	0 (_)	0 (_)	0 (_)	8 (0.278)	4 (0.139)	0 (_)	2 (0.069)	0 (_)	9 (0.313)
G_2N_2	0 (_)	14 (0.531)	4 (0.152)	0 (_)	2 (0.076)	18 (0.683)	5 (0.190	0 (_)	0 (_)
G_2N_3	0 (_)	0 (_)	0 (_)	11 (0.466)	2 (0.085)	8 (0.339)	0 (_)	0 (_)	0 (_)
GN pooled	0 (_)	14 (0.178)	4 (0.051)	19 (0.241)	8 (0.102)	26 (0.330)	7 (0.089	0 (_)	9 (0.114)
E_1	0 (_)	0 (_)	0 (_)	0 (_)	2 (0.054)	10 (0.269)	8 (0.215	0 (_)	48 (1.289)
E_2	9 (0.280)	0 (_)	0 (_)	0 (_)	0 (_)	11 (0.342)	0 (_)	0 (_)	24 (0.746)
E_3	4 (0.161)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	12 (0.483)
EMS pooled	13 (0.138)	0 (_)	0 (_)	0 (_)	2 (0.021)	21 (0.223)	8 (0.085	0 (_)	84 (0.891)
N_1	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	9 (0.278	0 (_)	20 (0.617)
N_2	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)
N_3	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	3 (0.1200	0 (_)	14 (0.559)
NG pooled	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	12 (0.138)	0 (_)	34 (0.392)
G_1	5 (0.139)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	15 (0.418)	0 (_)	38 (1.058)
G_2	0 (_)	0 (_)	0 (_)	0 (_)	10 (0.290)	0 (_)	8 (0.232)	0 (_)	30 (0.869)
G_3	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	0 (_)	11 (0.370)	0 (_)	16 (0.538)
GR pooled	5 (0.050)	0 (_)	0 (_)	0 (_)	10 (0.100)	0 (_)	34 (0.339)	0 (_)	84 (0.838)

contd...

Number, spectrum and frequency of macromutation

Treatments	Fasciated cotyledon	Uniculm stem	Fasciated stem	Tall	Dwarf	Bushy	Whorled leaf phyllotaxy	Stipulate	Long-narrow leaf
G_2E_1	3	0	0	0	0	12	0	0	78
	(0.098)	(_)	(_)	(_)	(_)	(0.392)	(_)	(_)	(0.2547)
	13	0	4	0	9	0	8	11	72
G_2E_2	(0.469)	(_)	(0.144)	(_)	(0.325)	(_)	(0.289)	(0.397)	(2.598)
	0	4	0	1	3	4	15	1	56
G_2E_3	(_)	(0.162)	(_)	(0.041)	(0.122)	(0.162)	(0.608)	(0.041)	(2.268)
	16	4	4	1	12	16	23	12	206
GE pooled	(0.193)	(0.048)	(0.048)	(0.0120	(0.145)	(0.193)	(0.277)	(0.145)	(2.481)
G_2N_1	0	0	0	0	0	13	8	0	44
	(_)	(_)	(_)	(_)	(_)	(0.452)	(0.278)	(_)	(1.528)
	0	1	3	0	12	0	0	13	72
G_2N_2	(_)	(0.038)	(0.114)	(_)	(0.455)	(_)	(_)	(0.4930	(2.730)
	0	1	9	2	0	0	5	14	52
G_2N_3	(_)	(0.042)	(0.382)	(0.085)	(_)	(_)	(0.212)	(0.594)	(2.205)
	0	2	12	2	12	13	13	27	168
GN pooled	(_)	(0.025)	(0.1520	(0.025)	(0.152)	(0.165)	(0.165)	(0.343)	(2.134)

Note : – Symbols of treatments as in Table 1.
– Figures in parentheses indicate frequency of macromutations per 100 M_2 plants.
– Number of plants scored in each treatment as mentioned in Table 1.

mutagens (EMS and NG), whereas gamma rays produced higher proportion of less drastic types (Chlorina and sectorial). The alkylating agents, such as EMS and NG, due to their specificity nature could be possibly severely affected adjacent regions of centromore and proximal segments of chromosome, where the genes controlling the chlorophyll development seem to be located (Swaminathan, 1964 & 1965), resulting higher proportion of more drastic types of chlorophyll mutations. Thus, relative differences in mutability of genes for various chlorophyll mutations by different mutagens were clearly observed.

Macro-mutations

Seventeen types of morphological macro-mutations affecting cotyledon (fascinated cotyledon), plant type (tall, dwarf, busy, uniculm), stem (fascinated stem), leaf and leaf phyllotaxy (long narrow leaf, small leaf, stipulated, whorled phyllotaxy), flower (tiny flower, coloured flower), capsule (rudimentary capsule, long-bold capsule, small-bold capsules, multi-loculed capsule) and fertility (polypetalous or gamopetalous flowers with sterile pollen grains) were recorded in M_2 (Table 2). Among single mutagenic treatments, the mutation spectrum was wider (seven types) for 0.25% EMS followed by five types to 500 Gy gamma rays and four types at 0.75% EMS, 0.01% NG and 700 Gy gamma rays. Interestingly, the spectrum of macromutations at 0.02% NG was zero. Among the combined treatment, the

pectrum was highest (ten) for G_2E_3 followed y nine for G_2E_2 and G_2N_2. In general, the pectrum for single mutagenic treatments showed decreased tendency with the increase in dose.)ccurrence of higher spectrum of macro-mutation n combined treatments than single treatments uggested the cumulative action in case of combined reatments. Out of seventeen macromutants, long old capsule mutants were most frequent and ascinated cotyledon and coloured flower mutants /ere least. Bushy, uniculm, tall and small bold apsule mutants were also quite common as ompared to other mutants.

Among the single mutagenic treatments, nacro-mutational frequency per 100 M_2 plants ras the highest at 0.25% EMS (1.29%) followed y 500 Gy gamma ray (1.05%). Among the ombined treatments, the highest macro-mutational equency was observed at 700 Gy gamma rays 0.02% NG (2.73%) followed by 700 Gy amma rays + 0.50% EMS (2.60%). In overall, ie frequencies of macromutations estimated on the basis of 100 M_2 plants were in order of GE>GN>EMS>GR>NG (Table 2). The frequencies of viable macro-mutations per 100 M_2 plants were the highest at the lowest doses of EMS, NG and gamma rays for single treatments and for combined treatments, the highest value(s) was observed at medium doses. This inverse relationship of dose with frequency could possibly due to gradual inactivation of the repair system with the increase in dose.

Relative differences in mutability of gene loci for various macromutations with respect to different mutagens were also clearly observed and these observations can be analysed from wo aspects. Firstly, the rate of mutation of ndividual gene loci was found to be highly variable for different mutagens. For example, EMS induced the highest number of small bold capsule mutants, whereas both NG and gamma rays induced the highest number of long bold capsule mutants. The results also clearly suggest hat some gene loci were affected by one mutagen nuch more than the other. For example, EMS nduced uniculm stem, bushy plant and small eaf mutants more frequently, whereas gamma rays induced more long-bold capsule, busy plant, fascinated stem, tall plant and rudimentary capsule type mutants. Secondly, some of the gene loci were affected by one mutagen, but not by the other. For example, only EMS induced uni-culm stem, bushy plant and small leaf mutants more frequently, whereas gamma rays induced more long-bold capsule, busy plant, fascinated stem, tall plant and rudimentary capsule type mutants. Secondly, some of the gene loci were affected by one mutagen, but not by the other. For example, only EMS induced fascinated stem and small bold capsule mutants. Similarly, gene loci like uniculm stem and fascinated cotyledon were affected by both EMS and NG but not by gamma rays. The gene loci, which were affected by both EMS and NG but not by gamma rays. The gene loci, which were affected by EMS and/or NG but not by gamma rays might be located in some regions on the chromosome that are more likely to yield mutations when exposed to chemical mutagens than the radiations (Auerbach and Kilbey, 1971).

The differential sensitivity of sesame genes to different mutagens indicates that the mutation process varies from one mutagen to another. The alkylating agents, such as EMS and NG, specifically react with DNA by alkylating the phosphate groups as well as the purine and pyramidine bases, but guanine is more affected leading to the formation of 7-alkyl guanine. Similarly, gamma rays result chromosomal aberrations like deletion, duplication, inversion and translocation. However, the differential sensitivyt of different genes of sesame to EMS, NG and gamma rays cannot be interpreted on the basis of action of these mutagens on a particular base (all the genes contain all the bases) or on the basis of chromosomal aberrations. Auerbach and Kilbey (1971) suggested that differetnial sensitive of genes even in prokaryotes cannot be determined by the reaction between base or base sequences and mutagens. Besides, several other factors such as genetic background, cell cycle, treatment condition, environmental factors (Auerbach, 1965), numbe of essential sites in the code message of gene (Lifschytz and Falk, 1969) etc. determine the differential response of the mutagens. Therefore, it is difficult to determne the particular cause(s) for the differential sensitivity of sesame genes to different mutagens. However, a number of such and

related investigations might help for inducing desired mutations at specific gene loci or for directed mutagenesis upto some extent.

References

Auerbach, C. (1965). Past achievements and future task of research in chemical mutagenesis, in Genetics Today, Vol.2, pp.275-284. Ed.Greets, S.I.Paragon Press, Oxford.

Auerbach, C. and Kilbey, B.J. (1971). Mutation in Eukaryotes. *Ann. Rev. Genet.* 5: 163-218.

Kharkwal, M.C., Pawar, S.E. and Pandey, R.N. (2001). Seventy-five years of research on induced mutations with special reference to crop improvement in India. In: Proceedings of NAARRI International Conference on Applications of Radioisotopes and Radiation Technology in the 21st Century. (Ed.N.Ramamoorthy, M.Ananthakrishnan and A.N.Nandakumar) December 12-14, 2001, Mumbai, India. pp.230-235.

Lifschytz, E. and Falk, R. (1969). Fine structure analysis of chromosome segment in *Drosophila melanogaster*. Analysis of ethyl methane sulphonate induced lethal. *Mutation Res.* 8: 147-155.

Swaminathan, M.S. (1964). A comparison of mutations induction in diploid and polyploids. *In*: The use of induced mutation in plant breeding. *Rad. Mut. Organ. FAO IAEA, Vienna*: 619-641.

Swaminathan, M.S. (1965). The experimental manipulation of genes. *Curr. Sci.* 34: 108-11.

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