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Research article

MUTAGENIC EFFICIENCY AND EFFECTIVENESS OF GAMMA RAYS, ETHYL METHANE SULPHONATE (EMS), NITROSOGUANIDINE (NG) AND THEIR SYNERGISTIC EFFECT FOR DIFFERENT POLYGENIC TRAITS IN BLACK GRAM (*VIGNA MUNGO* (L.) HEPPER) THROUGH INDUCED MUTAGENESIS.

Kaushik Kumar Panigrahi[1]*, Ayesha Mohanty[2], Jyotshnarani[3] and Bhabendra Baisakh[1].

[1]Department of Plant Breeding and Genetics,
[2]Department of soil sciences and Agricultural Chemistry,
[3]Department of Plant Physiology, OUAT, Bhubaneswar, India.
***E mail-*kaushikpbg@gmail.com**

ABSTRACT: The effects of mutagens in M_1 were carried out with two varieties of blackgram. Keonjhar Local (a promising local landrace of Odisha) designated as V_1 and OBG-31(a released variety) as V_2 were subjected to different degrees of mutagenic treatments to study the behaviour and effects of mutagens on M_1 population of blackgram. Three doses each of gamma rays (G1-200, G2-400 and G3-600Gy) were used as physical mutagens. Chemical mutagens like nitrosoguanidine (NG) (N1-0.005, N2-0.010 and N3-0.015%) and ethyl methane sulphonate (EMS) (E1-0.2, E2-0.4 and E3-0.6%) used singly or in combinations. Six combination treatments were G2N1- 400Gy gamma rays + 0.005% NG, G2N2- 400Gy gamma rays + 0.010% NG, G2N3- 400Gy gamma rays + 0.015% NG, G2E1- 400Gy gamma rays + 0.2% EMS, G2E2- 400Gy gamma rays + 0.4% EMS, G2E3- 400Gy gamma rays + 0.6% EMS and C- untreated check was used. Among various dose/concentration treatments, G1 (200 Gy), G3 (600 Gy), N1 (0.005 NG), N2 (0.010 NG), E1 (0.2 % EMS), G2N1 (400 Gy gamma rays + 0.005% NG) of gamma rays and combination treatments were more desirable, which resulted in low plant damage and higher genetic effects.G2E3 (400 Gy gamma rays + 0.6% EMS) did not germinate at all, neither in the laboratory nor in the field condition for both of the parents.
Key words: - Black gram, Ethyl methane sulphonate (EMS), Gamma rays, Nitrosoguanidine (NG).

INTRODUCTION

In order to improve yield and other polygenic characters, mutation breeding should be effectively utilized [5]. Mutation induction has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain specific traits [20]. Induced mutations represent the same kind of changes that occur from natural causes [10]. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement [35]. Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic [46]. Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations [18]. Therefore, an attempt has been made to study their effects in this direction.

MATERIALS AND METHODS

The material comprised of two morphologically distinct varieties of black gram (OBG-31/Mahuri) and Keonjhar Local was chosen for the study to evaluate the genetic variation on quantitative characters in M_1 generation. Dry and well filled seeds were administered with mutagenic treatments with three doses each of gamma rays (200, 400 and 600 Gray). The seeds were irradiated at Bhaba Atomic Research Centre, Trombay, Mumbai and chemical mutagen like ethyl methane sulphonate (EMS) (0.2, 0.4 and 0.6%), nitrosoguanidine (NG) (0.005, 0.010 and 0.015%) used singly or in combinations.

Six combination treatments were 400 Gy gamma rays + 0.4% EMS, 400 Gy gamma rays + 0.2% EMS, 400 Gy gamma rays + 0.6% EMS, 400 Gy gamma rays + 0.015% NG, 400 Gy gamma rays + 0.010% NG and 400 Gy gamma rays + 0.005% NG. Single mutagenic treatments were coded as G1, G2, G3, N1, N2, N3, E1, E2, E3 and combinations were coded as G2N1, G2N2, G2N3, G2E1, and G2E2and G2E3. The untreated check was denoted with C. The treatments were prefixed O and K for OBG-31 and Keonjhar Local respectively. For G1 it was denoted as OG1 for OBG-31 and KG1 for Keonjhar Local respectively.

For treatment with EMS and NG the seeds were pre-soaked in distilled water for 10 hours, blotted dry and then treated with freshly prepared aqueous solution for 6 hours, with intermittent shaking. For combination treatments, seeds were first irradiated with 400 Gy gamma rays and then treated with EMS and NG 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 ensure a uniform absorption of the mutagen, the volume of mutagen solution was maintained at 10 times proportion to that of the seed volume. The whole treatment was carried out at a room temperature of 28±1°C after washing in running water and untreated seeds were used as control. The treated seeds and control seeds were immediately sown in the field in a randomized block design (RBD) with three replications. Each treatment consists of three rows of 5 m length, in which 50 seeds per row were sown with 10 × 30 cm distance between plants and rows, respectively. Fifteen quantitative characters (5 laboratory characters + 10 Field evaluated characters) related to growth, vigor and productivity including seed yield were recorded. Laboratory evaluation was carried out by growing the plant with earthen pot and evaluated inside the laboratory, days to 50% flowering was recorded on the plot basis and for other characters the 15 randomly selected competitive plants per plot in each replication were recorded.

RESULTS AND DISCUSSION

The treatment and their code with procedure adopted are mentioned in Table 1and 2 respectively and results are presented in Table 5and 6. The analysis of variance revealed highly significant differences existed among the treatments themselves for almost all the traits studied for both the parents (Table 3 and 4). They all are highly significant for all the traits under investigations. The ANOVA revealed that mean squares due to genotypes were highly significant for all the yield attributing traits under evaluation indicating presence of sufficient amount of variability among all the mutagenic treatments. Highest germination percentage was recorded with the treatment N1 for both the varieties followed by N2 and G2 respectively. A dose related reduction in seed germination and pollen fertility by both gamma rays and EMS have shown by various workers [9, 44]. Similar results were in accordance with [14, 30, 42, 23, 17].

Table 1. Details of mutagenic treatments

Tr. No.	Treatment symbol	Mutagen	Dose/ concentration	Duration of pre-soaking in distilled water	Duration of treatment with mutagenic solution
VT1	G1	Gamma-rays	200 Gy	-	-
VT2	G2	Gamma-rays	400 Gy	-	-
VT3	G3	Gamma-rays	600 Gy	-	-
VT4	N1	NG	0.005 %	10 hours	6 hours
VT5	N2	NG	0.010 %	10 hours	6 hours
VT6	N3	NG	0.015 %	10 hours	6 hours
VT7	E1	EMS	0.2 %	10 hours	6 hours
VT8	E2	EMS	0.4 %	10 hours	6 hours
VT9	E3	EMS	0.6 %	10 hours	6 hours
VT10	G2N1	Gamma-rays 400 Gy + NG (0.005 %)		10 hours	6 hours
VT11	G2N2	Gamma-rays 400 Gy + NG (0.010%)		10 hours	6 hours
VT12	G2N3	Gamma-rays 400 Gy + NG (0.015 %)		10 hours	6 hours
VT13	G2E1	Gamma-rays 400 Gy + EMS (0.2 %)		10 hours	6 hours
VT14	G2E2	Gamma-rays 400 Gy + EMS (0.4 %)		10 hours	6 hours
VT15	G2E2	Gamma-rays 400 Gy + EMS (0.6 %)		10 hours	6 hours
VT16	C	Control (distilled water)		10 hours	-

1 kilorad (kR) = 10 Gray (Gy)

The impact of mutagens to the shoot length of the plant can be adjudged by the observation recorded in the laboratory condition. Treatment G1 and N1 resulted longer shoot growth as compared to all other treatments. All the mutants for both of the varieties V_1 and V_2 had reduced root growth for all the treatments as compared to untreated check C. Treatment G2E2 produced longest root in the mutant derived from V_1 (6.763) whereas G1 produced longest root in V_2 (4.527). Dose linked effectiveness of EMS and gamma rays were noted in chickpea in terms of germination, reduction in pollen fertility, chlorophyll mutations and seedling height (Parveen, 2006). Similar effects were also reported in peas [29], pearl millet [36], *Vigna radiata* [38, 16,], *Lens culinaris* [15, 43], *Arachis hypogea* [41, 24] *Nigella sativa* . The effect of physical and chemical mutagens and their combination treatments to demonstrate different biological parameters such as germination, survival, injury and sterility was studied by many workers, [30, 42, 23, 17]. Reduction in seedling height following treatments with gamma rays and EMS was observed in barley [32].Gupta and Yashvir [11] reported a radioprotective effect of EMS in *Abelmoschus esculantum*. The combined treatments of gamma rays and EMS showed higher germination percentage than in corresponding EMS treatments. Chaudhary [4] reported a symmetric reduction in germination in different varieties of wheat with higher doses of gamma rays. Parveen [25] reported the effect of seed treatment with different concentration of EMS on germination and growth of seedlings in chickpea. There was a proportionate decrease in germination percentage with the increasing concentrations of EMS.

Highest biomass production in fresh weight basis can be witnessed with G2N1 (3.779) for V_1 followed by C (2.379) and G2N2 (2.254). For V_2 maximum fresh weight was produced with untreated control C (2.498) followed by G2N2 (2.106) and E1 (2.004). Dry weight under mutagenic treatment was more with G2 (0.239) and G3 (0.225) for V_1 whereas minimum dry weight was recorded with G2E2 (0.043) followed by G2E1 (0.057) for V_1. Similarly for V_2 maximum dry weight was recorded with N1 (0.293) followed by N3 (0.286) and minimum dry weight was recorded with treatment E3. Kumar *et al*. [19] induced the mutation in green gram (*var.* PS16 and Sona) by different doses of gamma rays (10, 20, 30, 40 and 50kR), EMS (0.1, 0.2 and 0.3%) and their combinations (all gamma-rays treatments with 0.2% EMS) and studied the induced genetic variability for eight quantitative characters. A study on mutagenic effectiveness and efficacy of EMS and NG in mungbean was undertaken by Singh and Nalinikanta 34]. They observed higher effectiveness of NG treatments in comparison to EMS treatments. The reverse trend was observed in case of efficacy. Both effectiveness and efficacy of both chemicals decreased with increase in the mutagenic dose. The possible reason for this could be less damaging effect of lower doses of both chemicals on the genetic materials. Mutants derived from V_1 exhibited normal days to 50% flowering of 38.173 (N1) days to 44.297 (G2E1) days for all the treatments. Whereas mutants of V_2 exhibited abnormal behaviours in relation to this characters. The range of days to 50% flowering varies from 82.963 (G2N1) days to 96.147 (G2) days for all derived mutants of V_2. This might have been due to the photosensitivity of the parental genotype. Similar results were observed for days to maturity as like days to 50% flowering. Mutants of V_1 were harvest as normal at a range of 77.100 days (N1) to 88.117 days (E1). Whereas mutants of V_2 were matured much later than V_1, the range of maturity for V_2 were 118.880 (G2N1) to 136.14 (G2) days. The longest maturity duration might be due to photo sensitivity of the variety. Lower doses of gamma rays and EMS were ineffective for creation of desired variability for yield and yield components in lentil [37]. Effect of gamma rays and ethyl methane sulphonate on quantitative and qualitative traits in sunflower has been reported by Selvaraj and Jaykumar [31]. The micro mutations increase variability in yield protein content, plant height, flowering, pod production, seed weight or other yield related traits that are quantitatively inherited. In case of vegetative propagation, mutagen treatment produces chimera, which is basically the mixture of one or more genotypes and hence needs to be dissolved. These chimeras are unstable in clonal crops hence several are needed to extract true morphological mutants [1]. Plant height was found to be significantly reduced in case of mutants of V_1 as compared to V_2. For V_1 maximum plant height was observed by N1 (51.240) and N3 (71.897) for V_2. The maximum reduced plant height was observed in C (26.603) in V_1 and E1 (55.897) in V_2. Maximum number of primary branches/plant in case of V_1 was recorded with treatment G2N3 (2.297) followed by G1 (2.037) and minimum number of primary branches was recorded with G2E2 (1.163) followed by N2 (1.260) and E3 (1.273). For V_2 maximum number of primary branches /plant was recorded with G1 (2.087) followed by G2N3 (2.060) whereas minimum number of primary branches/plant was recorded with N3 (1.587). Mandell and Greenberg (1960) reported that mutagenicity of NG was more due to its structure, ratherthan to its breakdown product. Eisenstark *et al.* [7] and Baker and Tessman [2] reported that NG produces equal proportion of GC to AT and AT to GC transition.

Singer *et al.* [33] have observed that methyl group of NG reacts with purine bases and produces more of methylated guanine than methylated adenine. Cerda-Olmedo *et al.* [3] observed that NG acts specifically at the replication fork causing mutations. Dong [6] working on barley observed that NG treatments induced structural chromosome and chromatid rearrangement and the effect increased with concentration. Flora *et al.* [8] reported that NG induced more of chromosomal aberrations than EMS. NG treatments were reported to have drastic effect on germination survival, seedling growth, pollen and seed fertility in M_1 generation [12, 28, 21, 45].

Table 2. Details procedure for mutagenic treatments

Tr. No.	Treatment symbol	Mutagen	Dose/ concentration	Procedure for mutagenic treatment
VT1	G1	Gamma rays	200 Gy	Gamma irradiated by BARC
VT2	G2	Gamma rays	400 Gy	Gamma irradiated by BARC
VT3	G3	Gamma rays	600 Gy	Gamma irradiated by BARC
VT4	N1	NG 0.005 %	75 ml. of 0.015 stock solution taken and added water to make volume to225 ml.	Taken 60ml. of NG 0.005% and added 500 seeds
VT5	N2	NG 0.010 %	150 ml. of 0.015 stock solution taken and added water to make volume to225 ml.	Taken 60 ml. of NG 0.010% and added 500 seed
VT6	N3	NG 0.015 %	2gm. Of NG was added to 500 ml. of water	Taken 60ml. of 0.0015 % NG in a 100 ml beaker and added 500 seeds
VT7	E1	EMS 0.2 %	Taken 75 ml. of stock solution of 0.6% EMS and water added to make the volume 225 ml.	Taken 60 ml. of 0.2% EMS in a 100 ml beaker and added 500 seeds
VT8	E2	EMS 0.4 %	Taken 150 ml. of stock solution of 0.6% EMS and water added to make the volume 225 ml.	Taken 60ml. of 0.4% EMS in a 100 ml beaker and added 500 seeds
VT9	E3	EMS 0.6 %	3ml. of EMS + 497 ml. of water	Taken 60ml. of 0.6% EMS in a 100 ml beaker and added 500 seeds
VT10	G2N1	Gamma-rays 400 Gy + NG (0.005 %)		400Gy + 0.010% NG in a 60-40 ml. beaker and added 500 seed
VT11	G2N2	Gamma-rays 400 Gy + NG (0.010%)		400Gy + 0.010% NG in a 100 ml. beaker and added 500 seed
VT12	G2N3	Gamma-rays 400 Gy + NG (0.015 %)		400Gy + 0.015% NG (60 ml.) and added 500 seed
VT13	G2E1	Gamma-rays 400 Gy + EMS (0.2 %)		400Gy + 0.2 % EMS (60 ml.) and added 500 seed
VT14	G2E2	Gamma-rays 400 Gy + EMS (0.4 %)		400Gy + 0.4 % EMS (60 ml.) and added 500 seed
VT15	G2E3	Gamma-rays 400 Gy + EMS (0.6 %)		400Gy + 0.6 % EMS (60 ml.) and added 500 seed
VT16	C	Control (distilled water)		

BARC- Bhaba Atomic Research Centre

Table 3.Analysis of variance for different yield parameters of mutants derived from Keonjhar Local (V_1)

Treatment name	Treatment number	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Days to 50% Flowering	Days to Maturity	Plant height (cm)	Number of Primary branches/ plant	Number of clusters/ plant	Number of Pods/ plant	Number of Seeds/ pod	Pod length (cm)	100 seed weight (g)	Yield/ plant (g)
Replication	2	11.9453	0.0668	4.4272	0.2766	0.0006	2.4570	5.9063	4.8359	0.0910	0.5105	1.5361	0.0709	0.0082	0.0206	0.0129
Genotype	15	2443.8127 **	14.5189 **	168.1035 **	2.7204 **	0.0138 **	314.2790 **	1338.5778 **	477.1873 **	0.7703 **	8.1408 **	118.6565 **	8.5826 **	7.7992 **	4.3126 **	4.6623 **
Error	30	5.9853	0.0389	1.6860	0.0610	0.0004	4.3678	7.1538	12.7889	0.1044	0.5089	4.6979	0.2298	0.1067	0.1064	0.0629

Table 4. Analysis of variance for different yield parameters of mutants derived from Mahuri [OBG-31] (V_2)

Treatment name	Treatment number	Germination (%)	Root length (cm)	Shoot Length (cm)	Fresh weight (g)	Dry weight (g)	Days to 50% flowering	Days to Maturity	Plant height (cm)	Number of Primary branches/ plant	Number of clusters/ plant	Number of Pods/plant	Number of Seeds/ pod	Pod length (cm)	100 seed weight (g)	Yield/ plant (g)
Replication	2	37.5156	0.0853	9.2383	0.0198	0.0009	8.7813	2.1875	12.7344	0.0177	0.1410	5.0654	0.0233	0.0588	0.1315	0.0423
Genotype	15	2598.8503**	5.7439**	155.5517**	1.4792**	0.0223**	1500.9973**	3081.1221**	815.3028**	0.7231**	9.3549**	136.1010**	9.4165**	7.8036**	5.1577**	5.0458**
Error	30	3.9358	0.0519	0.5446	0.0192	0.0003	10.7201	26.7493	7.5033	0.0253	0.2618	4.4849	0.1954	0.1005	0.0708	0.0566

Table 5. Mean performance of all mutants derived from V_1 (Keonjhar Local)

Treatment name	Treatment number	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of Primary branches/ plant	Number of clusters/ plant	Number of Pods/ plant	Number of Seeds/ pod	Pod length (cm)	100 seed weight (g)	Yield/ plant (g)
KG1	V1T1	74.507	4.663	23.223	1.099	0.152	41.190	82.610	42.247	2.037	7.627	25.063	6.630	6.420	4.697	5.170
KG2	V1T2	86.283	5.237	20.520	0.522	0.239	39.983	80.940	43.483	1.877	5.983	24.977	6.350	6.193	4.840	4.923
KG3	V1T3	65.667	4.577	23.723	0.882	0.225	41.030	82.870	44.593	1.823	6.613	25.027	7.090	6.407	4.953	4.640
KN1	V1T4	95.937	4.343	27.790	0.428	0.159	38.173	77.100	51.240	1.557	5.483	25.517	6.603	6.277	4.873	5.137
KN2	V1T5	74.700	1.640	20.180	1.459	0.181	40.560	86.250	49.100	1.567	6.493	24.880	6.887	6.373	4.267	5.107
KN3	V1T6	86.703	2.667	20.503	1.325	0.155	40.377	83.790	49.687	1.260	6.097	25.997	6.433	6.293	4.173	4.927
KE1	V1T7	54.227	3.667	16.200	1.746	0.103	41.630	88.117	51.160	1.490	5.737	26.783	7.260	7.037	4.457	5.047
KE2	V1T8	23.880	1.100	10.113	0.415	0.098	41.163	84.013	46.220	1.410	5.717	23.437	6.883	6.693	4.567	4.903
KE3	V1T9	17.710	3.230	15.187	0.487	0.092	38.770	86.387	48.077	1.273	5.567	24.960	6.427	6.190	4.413	4.933
KG2N1	V1T10	83.410	2.337	16.530	3.779	0.207	38.407	82.910	42.720	1.587	5.723	21.173	6.270	6.177	4.933	5.223
KG2N2	V1T11	74.070	2.457	11.317	2.254	0.191	38.990	82.267	45.153	1.937	5.767	24.130	6.117	6.063	4.983	4.693
KG2N3	V1T12	44.150	1.737	9.567	1.548	0.167	40.133	79.473	43.793	2.297	5.467	22.663	6.390	5.917	4.730	4.867
KG2E1	V1T13	23.410	1.433	12.553	1.941	0.057	44.297	85.483	45.057	1.483	6.113	23.197	6.977	6.333	4.893	4.920
KG2E2	V1T14	56.033	6.763	25.207	1.453	0.043	39.687	85.627	46.493	1.163	6.220	25.433	6.030	6.003	4.660	4.923
KG2E3	V1T15	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
KC	V1T16	67.173	8.380	26.667	2.379	0.121	42.470	87.417	26.603	1.533	7.187	25.033	6.880	5.000	4.897	4.677
Mean		57.991	3.389	17.455	1.357	0.137	37.929	78.453	42.227	1.518	5.725	23.017	6.202	5.836	4.396	4.631

Table 6. Mean performance of all mutant derived from V_2 (Mahuri / OBG-31)

Treatment name	Treatment number	Germination (%)	Root length (cm)	Shoot length(cm)	Fresh weight (g)	Dry weight (g)	Days to 50% Flowering	Days to Maturity	Plant height (cm)	Number of Primary branches/ plant	Number of clusters/ plant	Number of Pods/ plant	Number of Seeds/ Pod	Pod length (cm)	100 seed weight (g)	Yield/ plant (g)
OG1	V2T1	75.810	4.527	30.097	0.946	0.205	91.127	128.027	62.077	2.087	7.960	21.990	6.777	6.463	5.130	5.375
OG2	V2T2	84.707	3.593	21.903	1.418	0.239	96.147	136.147	58.227	1.877	6.650	25.993	6.940	6.407	5.157	5.463
OG3	V2T3	56.110	3.213	21.727	1.763	0.143	92.917	131.403	65.603	1.933	6.947	28.297	7.090	6.497	5.167	4.640
ON1	V2T4	96.590	4.427	22.160	1.819	0.293	89.880	128.550	64.550	1.810	6.483	25.927	6.920	6.360	5.417	5.100
ON2	V2T5	96.397	3.450	24.240	0.878	0.191	90.277	129.783	66.657	1.753	6.827	25.400	7.320	6.660	5.267	5.310
ON3	V2T6	82.013	3.107	21.813	1.642	0.286	89.510	129.430	71.897	1.587	6.163	24.960	6.500	6.467	5.263	5.333
OE1	V2T7	62.557	3.407	13.513	2.004	0.181	85.810	130.670	55.897	1.723	6.137	26.943	7.260	6.990	4.983	5.273
OE2	V2T8	28.923	2.323	17.257	0.994	0.070	86.697	123.663	67.220	1.723	6.213	24.623	7.320	6.643	5.200	4.903
OE3	V2T9	27.707	1.410	14.363	0.270	0.033	86.913	120.107	69.567	1.773	5.900	21.900	6.790	6.190	5.150	5.157
OG2N1	V2T10	63.773	3.650	15.283	1.351	0.152	82.963	118.880	64.783	1.953	7.580	22.207	6.423	6.177	5.033	5.363
OG2N2	V2T11	46.593	1.284	11.110	2.106	0.153	89.430	120.260	64.630	2.070	6.687	29.800	6.463	6.063	4.983	4.943
OG2N3	V2T12	81.930	1.368	18.650	1.307	0.162	83.663	121.033	56.147	2.060	6.437	24.690	6.940	5.917	5.143	4.493
OG2E1	V2T13	36.960	1.800	13.120	0.639	0.083	89.610	120.493	60.960	2.057	6.313	25.660	7.310	6.333	5.740	4.910
OG2E2	V2T14	21.883	0.960	8.317	0.500	0.075	86.800	127.107	57.843	1.797	6.260	26.803	7.297	6.133	5.187	4.923
OG2E3	V2T15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
OC	V2T16	86.553	4.283	21.380	2.498	0.230	82.673	131.910	63.557	1.900	7.520	20.167	7.250	6.160	5.097	4.880
Mean		59.282	2.675	17.183	1.258	0.156	82.776	118.536	59.351	1.756	6.255	23.460	6.538	5.966	4.870	4.753

For V_1 G1 recorded maximum number of clusters/plant (7.627) followed by G3 (6.613). Lowest number of clusters/plant for V_1 was recorded with treatment G2N3 (5.467) followed by N1 (5.483). For V_2 treatment G1 topped the number of clusters/plant (7.960) followed by G2N1 (7.580). Lowest number of clusters/plant for V_2 was recorded with E2 (5.900). A study on mutagenic effectiveness and efficacy of EMS and NG in mung bean was undertaken by Singh and Nalinikanta [34]. They observed higher effectiveness of NG treatments in comparison to EMS treatments. The reverse trend was observed in case of efficacy. Both effectiveness and efficacy of both chemicals decreased with increase in mutagenic dose. The possible reason for this could be less damaging effect of lower doses of both chemicals on the genetic materials. The stimulating effect of low doses of gamma rays irradiation on plant growth may be due to the stimulation of cell division or elongation, or the alteration of metabolic processes that affect the synthesis of phytohormones or nucleic acids. In addition, high doses of gamma irradiation were reported to be harmful in several studies like that of Ramachandran and Goud [26], who reported that higher doses of gamma irradiation reduced plant height, number of leaves and branching capacity of black gram. E1 (26.783) exhibited highest pods/plant in V_1 followed by N2 (25.997). Lowest pods/plant was recorded with treatment G2N1 (21.173) followed by G2N3 (22.663). For V_2 G2N2 (29.800) yielded maximum number of pods/plant followed by G3 (28.297). Lowest pods/plant for V_2 was recorded with treatment C (20.167) followed by E3 (21.900) and G1 (21.990). E1 (7.260) witnessed highest seeds/pod in V_1 followed by G3 (7.090) whereas lowest seeds/pod recorded with treatment G2E2 (6.030) followed by G2N2 (6.117). N2 (7.320) and E2 (7.320) both recorded highest number of seeds/pod with V_2 whereas lowest seeds/pod was recorded with G2N1 (6.423) for V_2. Treatment E1 (7.037) again topped the list for pod length followed by E2 (6.693) for V_1 whereas lowest pod length for V_1 was recorded with C (5.000) followed by G2 N3 (5.917). For V_2 maximum pod length was exhibited by E1 (6.990) followed by E2 (6.643) whereas lowest pod length was recorded with G2N3 (5.917). Similar effects of gamma rays, EMS and their combination on M_1 parameters in barley was studied by Khalatkar and Bhatia [13].

Treatment G2N2 (4.983) exhibited highest 100 seed weight followed by G3 (4.953) whereas N2 (4.173) followed by E3 (4.413) exhibited lowest 100 seed weight in V_1. Treatment G2E1 (5.740) exhibited highest 100 seed weight followed by N1 (5.417), whereas G2N1 (5.033) followed by C (5.097) exhibited lowest 100 seed weight in V_2. Maximum yield/plant in V_1 was recorded with treatment G2N1 (5.223) followed by G1 (5.170). The minimum yield/plant for V_1 was recorded with G3 (4.640) followed by C (4.677). Treatment G2 (5.463) topped yield/plant for V_2 followed by G1 (5.375) and G2N1 (5.363). Lowest yield/plant for V_2 was exhibited by treatment G2N3 (4.493) followed by G3 (4.640). Though mutation breeding attempts may be made to broaden the variation spectrum to facilitate selection of lines with improved nutritional qualities, especially with respect to protein associated with high yield (Tah, 2006).As yield increment was the primary objective in most of the plant breeding programme, mutation breeding played a key role in achieving the goal of this study.

G2E3 (400 Gy gamma rays + 0.6% EMS) did not germinate at all, neither in the laboratory nor in the field condition for both of the parents. A direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS doses in *Vigna mungo* was reported by Gautam *et al*. [9].

Mutation induction has proven to be a workable, sustainable, highly efficient, environmentally acceptable, flexible, unregulated, non-hazardous and a low-cost technology in the breeder's toolbox to enhance crop improvement. In this study, the quantitative traits of the M_1 generation revealed the enhancement of the significant level of yield attributes in black gram. Among the various dose/concentration treatments, T1 (200 Gy), T3 (600 Gy), T4 (0.005 NG), T5 (0.010 NG), T7 (0.2 % EMS), T10 (400 Gy gamma rays + 0.005% NG) of gamma rays and combination treatments were more desirable, which resulted in low plant damage and higher genetic effects. As such, the maximum variation in quantitative characters may show the stable gene mutations in subsequent generation. The results indicate that black gram mutant lines are useful for crop improvement and further study is needed for the analysis of the mutants. Swaminathan *et al.* [39] in rice, barley and wheat observed that NG and NMU were more potent mutagens than EMS and qualify to group under the so called "super mutagens" class of Rapoport *et al.* [27].

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