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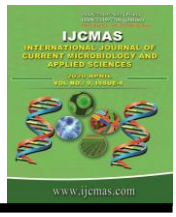

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Mutagenic Effectiveness and Efficiency of Gamma Rays, EMS and NG in Greengram (*Vigna radiata* L. Wilczek)

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A B S T R A C T

An experiment was conducted to study the effectiveness and efficiency of one physical mutagen *i.e.* gamma rays and two chemical mutagens *i.e.* ethyl methanesulfonate (EMS) and nitrosoguanidine (NG) in greengram. To study the nature and effect of mutagens in greengram, the percentage of lethality, pollen sterility, frequency of chlorophyll mutations, mutagenic effectiveness, mutagenic efficiency and mutation rates of each mutagen were estimated. The values of mutagenic effectiveness indicated that 30kR in gamma rays, 0.3% in EMS and 0.005% in NG treatment are found most effective than other doses/concentrations. NG exhibited as the most effective mutagen whereas gamma rays found as the most efficient mutagen and comparatively higher in mutation rate than EMS and NG. Among all the mutagenic treatments, the maximum efficiency observed in gamma rays 30KR treatment and the lowest efficiency observed in NG 0.015% treatment based on lethality and pollen sterility. Based on lethality and pollen sterility, the maximum mutation rate among all mutagens was observed in gamma rays treatments followed by EMS.

Introduction

Greengram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops in India. The conventional approaches of plant breeding have exploited the available genetic variability in greengram, 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 or chemical mutagen induced quantitative variation not only serves as an alternative source of germplasm for natural variation, but it is also useful in generating appropriately linked gene complexes that are responsible for the improvement in yield and other characters of economic interest. Shah *et al.*, (2008) reported that mutagens may cause

genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Gamma rays, one of the most commonly used physical mutagen in mutation breeding is known to influence plant growth and development by inducing cytological, genetic, biochemical, physiological and morphogenetic changes in cells and tissue (Gunckel and Sparrow, 1961).

Among the chemical mutagens used for induction of both macro- and micro-mutations in various crops, EMS and NG are most effective and frequently used. EMS is reported to be the most effective and powerful mutagen (Minocha and Arnason, 1962) whereas NG reported as the super mutagen (Swaminathan *et al.,* 1968). However, it is observed that only a few mutagenic treatments have been effective in inducing a high frequency of mutation while in others the frequency of induced mutation is low leading to wastage of resources. Thus early knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover the high frequency of desirable mutations (Smith, 1972; Das *et al.,* 2006).

The term "mutagenic effectiveness" is a rate of mutations produced by the mutagen concerning its dose whereas the "mutagenic efficiency" is an estimate of mutation rate in relation to the damage (Konzak *et al.,* 1965). An effective mutagen doesn't need to be an efficient one also. Both of these though are two different properties, the use of any mutagen in a plant breeding program depends on both of them.

Hence a study was undertaken to assess the effect of different doses of physical (gamma-rays) as well as chemical mutagens (EMS and NG) on the frequency of chlorophyll mutation, lethality and pollen sterility to evaluate the relative effectiveness and efficiency of mutagenic treatments and the mutation rate of different mutagens.

Materials and Methods

Dry and well-filled seeds of a greengram variety, namely Pusa Vishal administered mutagenic treatments with different doses of one physical mutagen *i.e.* gamma rays and two chemical mutagens *i.e.* Ethyl Methane sulphonate (EMS) and N-Methyl-N-Nitrosoguanidine commonly called as Nitrosoguanidine (NG).

Dry seeds were irradiated with gamma rays treatment at Bhaba Atomic Research Centre (BARC), Trombay(India). For treatment with Ethyl Methane sulphonate (EMS) and Nitrosoguanidine (NG), the seeds were pre-soaked in distilled water for six hours. After pre-soaking, the seeds were blotted dry and treated with freshly prepared chemical mutagen solutions of different concentrations i.e. EMS (0.15%, 0.30%, 0.45% and 0.60%) and NG (0.005%, 0.010%, 0.015% and 0.020%). The seeds were put in the solution for six hours at room temperature 26^0C with intermittent shaking for providing uniform treatment conditions for all the seeds.

A set of seeds was soaked in distilled water only, which used as control. After the treatment was over, the seeds were thoroughly washed in running tap water for two hours to bleach out the residual chemicals and then dried on blotting paper. The details of mutagenic treatments and the symbols used for treatments are presented in Table 1. To grow the M_1 generation, the treated seeds were sown in earthen pots (for laboratory study) and in the field in a randomized block design (RBD) with two replication with spacing of 25×10cm^2. A basal dose of 40 kg P_2O_5/ha was applied and nitrogen @ 20 kg/ha was given as top dressing at 18 days after

sowing. Observations on survival were recorded in each plot on 30th days after sowing and were calculated as the percent of control from which the lethality (%) calculated. Mean pollen sterility was determined based on acetocarmine stainability. The selfed seed of all survived plant harvested was used to grow the M_2 generation in RBD with three replications with the spacing of 25x10 cm^2. The mutagenic treated and control populations were screened daily for different types of chlorophyll mutations such as Albina, Xantha, Chlorine, striata, Viridis from 5th to 12th day after sowing. The frequency of chlorophyll mutants was calculated according to (Gaul, 1960).

The formula proposed by Konzak *et al.,* (1965) was followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness (Physical mutagen)} = \frac{Mf}{\text{Dose in kR}}$$

$$\text{Mutagenic effectiveness (Chemical mutagens)} = \frac{Mf}{c \times t}$$

$$\text{Mutagenic efficiency} = \frac{Mf}{\text{\% Lethality (L) or \% Pollen sterility(P)}}$$

Mf = Mutagenic Frequency i.e. frequency of chlorophyll mutations in M_2 generation.
kR = unit of gamma radiation.
t = duration of treatment with chemical mutagen in hours.
c = Concentration of chemical mutagens in %
L = % Lethality in M_1 generation
P= % Pollen sterility in M_1 generation

Mutation rate (MR) which provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration was calculated as follows.

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

Results and Discussion

In the present study, the biological damages like lethality and pollen sterility were recorded in M_1 generation (Table 2). Both parameters were found to increase with increasing doses of mutagens (Fig. 1). In gamma-rays treatments recorded maximum lethality 25.8% and pollen sterility 8.75% at 60kR whereas minimum lethality 2.36% and pollen sterility 0.58% at 15kR. In the case of EMS treatments, the maximum lethality (24.2%) and pollen sterility (6.58%) observed at 0.6% and a minimum lethality (1.5%) and pollen sterility (0.75%) at 0.15%. Similar trends were also found in NG *i.e.* recorded maximum lethality 49.89% and pollen sterility 9.77% at 0.02% dose whereas minimum lethality (6.53%) & pollen sterility (1.8%) recorded at 0.005%. The increased lethality and pollen sterility with increasing doses of mutagens also reported by several investigators Das *et al.,* (2006) and Tah (2006) in greengram, Bhosle and Kothekar (2010) in clusterbean. They proved that most of the higher doses of mutagens showed increased pollen sterility and lethality. The probable reason for increased pollen sterility might be meiotic irregularities such as translocations.

Since chlorophyll deficient mutants could not survive long and observed in the treated population for a variable-length period depending on the deficiency of chlorophyll. Therefore, these mutants are of no agronomic value but their frequency in different

mutagenic treatments of M_2 generation was considered to be a standard measure for estimation of effectiveness, efficiency and rate of induced mutation by different mutagens which would ultimately provide the information about the dose for inducing mutations in greengram. In this study, it was observed that an increase in dose or concentration of the mutagen did not increase the relative frequency of chlorophyll mutants; rather a random trend was observed (Table 2).

The frequent of chlorophyll mutation in different treatments of M_2 generations varied from 0.27(E1) to 1.31(N4). In general, there was no dose-dependency relationship with chlorophyll mutation. The occurrence of chlorophyll deficient mutant was noticed due to change in gene and a set of genes responsible for chlorophyll mutations (Monika and Seetharaman, 2017). The occurrence of chlorophyll mutations through induced mutagenesis had reported earlier by several researchers in greengram (Vikram *et al.,* 2014), blackgram (Goyal and Khan, 2010), in horse gram (Kulkarni and Mogle, 2013).

The mutagenic effectiveness can be explained the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency gives an idea of the proportion of mutations in relation to biological damages such as lethality, pollen sterility and chromosomal aberrations. Mutagenic effectiveness at different doses of different mutagens indicated that there is no dose relationship (Table 2). Among the different doses of gamma rays irradiated, the mutagenic effectiveness was maximum at 30kR followed by 15kR. In EMS, the mutagenic effectiveness was maximum at 0.3% followed by 0.15%. In the case of NG, the mutagenic effectiveness was maximum (25.33) at 0.005% followed by 0.01% (13.83). Hence it could be concluded that NG has higher mutagenic effectiveness compared to all other mutagens and lower doses are more effective among different doses of each mutagen. Similar results previously reported by Rao and Rao (1983); Reddi and Rao (1988); Sharma *et al.,* (2005); Khan *et al.,* (2010) and Girija and Apparao (2011). The greater effectiveness of chemical mutagens over physical mutagen has also been reported by Shah *et al.,* (2008) and Satpute and Fultambkar (2012). Swaminathan *et al.,* (1968) in a study with rice, barley and wheat observed that NG is the more potent mutagen than EMS.

Konzak *et al.,* (1965) showed that mutagenic efficiency provides the best available measure to evaluate different mutagenic treatments. It varies depending upon the criteria selected for its estimation. In the present investigation, mutagenic efficiency based on the lethality in M_1 varied from 0.022 (N3) to 0.222 (G2) and observed that there is no dose-dependent relationship i.e. it did not follow any particular (increasing or decreasing) trend in gamma rays treatments but in case of chemical mutagens, the value of efficiency decreased as there were increases in doses of mutagens i.e. the dose-dependent relationship observed.

Similar results were obtained by Gaikwad and Kothekar (2004) in lentil and Bhosle and Kothekar (2010) in clusterbean. The mutagen efficiency based on pollen sterility demonstrated that there is no dose-dependent relationship for gamma rays and NG whereas, dose-dependent relationship observed for EMS treatments i.e. the value of efficiency decreased as there were increases in doses of EMS (Table 2 and Fig. 2). It was ranged from 0.133 to 1.250 in gamma-rays treatment. In EMS treatments, the range was 0.149 to 0.360 whereas, in NG, the range was 0.078 to 0.422. Among all the twelve treatments the maximum efficiency based on pollen sterility

observed in gamma rays 30kR whereas the lowest efficiency based on pollen sterility observed in NG 0.015% treatment. Higher efficiency at lower doses of mutagen as observed in the present study might be due to that pollen sterility increased with an increase in doses at a rate faster than the frequency of mutation. Nilan and Konzak (1961) and Konzak *et al.,* (1965) opined that higher efficiency at the lower concentration of a mutagen is due to the fact that biological damage (lethality and sterility) increased within dose at a faster than the mutations.

The mutation rate was calculated by taking the mean values of efficiency for each treatment. This provides an idea of the average rate of mutation induced per mutagen. The mutation rates estimated from the value of mutagen efficiency based on lethality and pollen sterility (Table 3 and Fig. 3). Based on lethality the mutation rate varied from 0.053 (NG) to 0.117 (Gamma-rays). Similar trend also observed in case of the mutation rate based on the mutagenic efficiency calculated from pollen sterility value which varied from 0.195 (NG) to 0.535 (Gamma-rays).

A mutagen is useful only if it is effective as well as efficient. Konzak *et al.,* (1965) reported that reduced the rate of injury and the increases the mutation should be compared to the objective in any mutation program. Thus in a mutation breeding program, a high mutation rate accompanied by minimal deleterious effects is desired. But generally, the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects. In the present study, among all the mutagenic treatments, gamma-rays treatments were found to be most efficient.

Table.1 Details of mutagenic treatments

Treatment symbol	Mutagens	Dose/ concentration	Pre-soaking period (hours)	Duration of Mutagenic treatments (hours)
G1	gamma-rays	15 KR	--	--
G2	gamma-rays	30 KR	--	--
G3	gamma-rays	45 KR	--	--
G4	gamma-rays	60 KR	--	--
E1	EMS	0.15%	6	6
E2	EMS	0.30%	6	6
E3	EMS	0.45%	6	6
E4	EMS	0.60%	6	6
N1	NG	0.005%	6	6
N2	NG	0.010%	6	6
N3	NG	0.015%	6	6
N4	NG	0.020%	6	6
C	Control	--	6	--

Table.2 Effectiveness and efficiency of different mutagenic treatments on greengram

Code	Mutagenic treatment	% of lethality (L)	% of Pollen sterility (S)	Frequency of chlorophyll mutation (Mf)	Mutagenic Effectiveness (ME)	Mutagenic Efficiency based on lethality (MEFL)	Mutagenic Efficiency based on Pollen sterility (MEFS)
G1	γ-rays 15kR	2.36	0.58	0.36	0.024	0.153	0.621
G2	γ -rays 30kR	4.28	0.76	0.95	0.032	0.222	1.250
G3	γ -rays 45kR	16.17	5.57	0.74	0.016	0.046	0.133
G4	γ -rays 60kR	25.80	8.75	1.18	0.020	0.046	0.135
E1	EMS 0.15%	1.50	0.75	0.27	0.300	0.180	0.360
E2	EMS 0.30%	6.10	2.46	0.83	0.461	0.136	0.337
E3	EMS 0.45%	7.82	3.31	0.69	0.256	0.088	0.208
E4	EMS 0.60%	24.20	6.58	0.98	0.272	0.041	0.149
N1	NG 0.005%	6.53	1.8	0.76	25.333	0.116	0.422
N2	NG 0.010%	18.20	5.66	0.83	13.833	0.046	0.147
N3	NG 0.015%	31.91	8.96	0.70	7.778	0.022	0.078
N4	NG 0.020%	49.89	9.77	1.31	10.917	0.026	0.134

Table.3 Mutation rate and correlation between mutagenic effectiveness and efficiency of different mutagens in greengram

Mutagens	Mutation rate based on lethality (MRL)	Mutation rate based on pollen sterility (MRS)	Linear correlation	
			ME and MRL	ME and MRS
Gamma-rays	0.117	0.535	0.954	0.971
EMS	0.111	0.264	0.411	0.609
NG	0.053	0.195	0.980	0.988

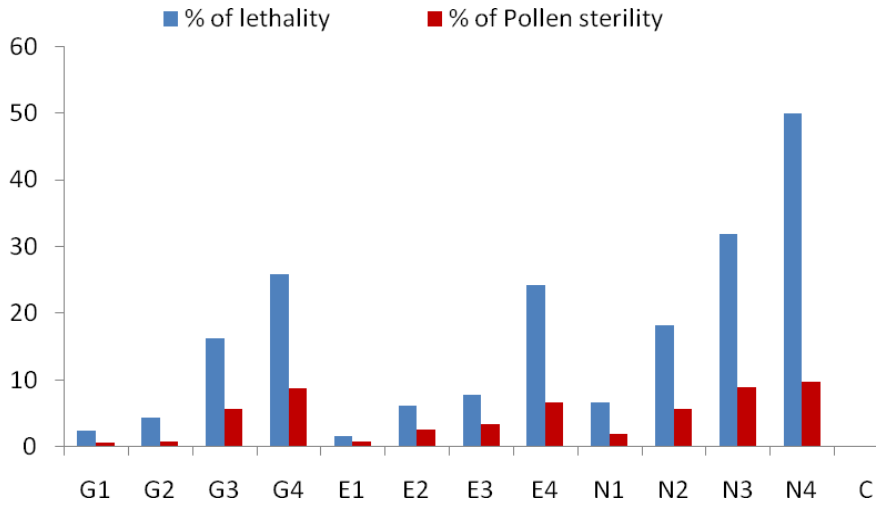

Fig.1 Effect of different mutagenic treatments on lethality and pollen sterility in greengram

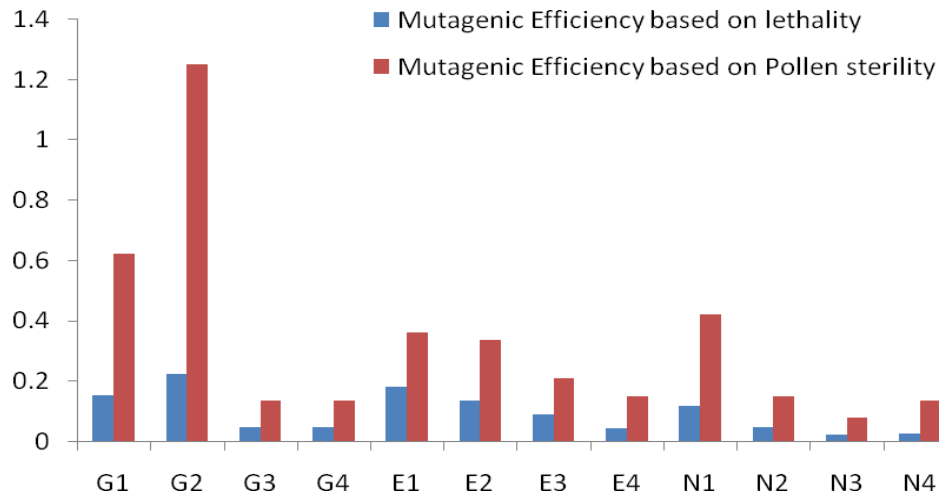

Fig.2 Mutagenic efficiency (%) of different mutagens in greengram

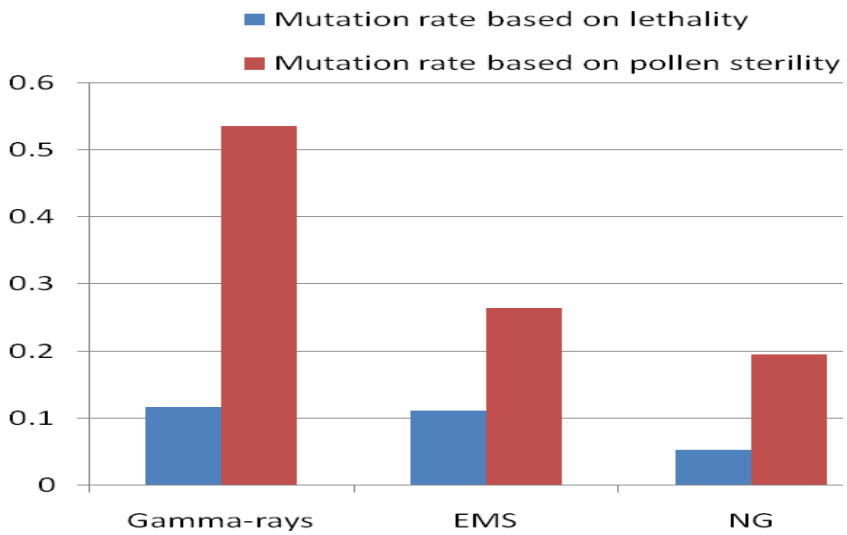

Fig.3 Mutation rates of different mutagens in green gram in terms of mutagenic efficiency for biological effects

The effectiveness and efficiency of mutagen based on leaf chlorophyll, lethality and pollen sterility in greengram are useful in identifying the genetic effect of mutagen. The study also reveals the Gamma rays, EMS and NG have a higher potential to induce significant mutations in greengram.

In this present study, it can be inferred that the lower to moderate doses of the mutagens are more effective than the higher concentrations and among mutagens for creating more useful mutations. The values of mutagenic effectiveness indicated that 30kR in gamma rays, 0.3% in EMS and 0.005% in NG treatment are found most effective than other doses/concentrations. NG has higher mutagenic effectiveness in comparison to EMS and Gamma rays. It was noted that when the mutation rate based on efficiency was considered, the maximum mutation rate (based on lethality and pollen sterility) observed in gamma-rays treatments followed by EMS and NG.

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