e-planet **18** (1): 6-12 (June 2020)

Effectiveness and efficiency of physical and chemical mutagens in greengram [*Vigna radiata* (L.) Wilczek]

T.R. DAS[1*] AND B. BAISAKH[2]

[1]*ICAR-Indian Agricultural Research Institute Regional Station, Pusa, Samastipur, 848125 Bihar, India*

[2]*Department of Plant Breeding and Genetics, OUAT, Bhubaneswar 751 003, Odisha, India*

**trdas.iari@gmail.com*

Date of receipt: 04.02.2020 Date of acceptance: 15.06.2020

ABSTRACT

A field experiment was conducted to study the effectiveness and efficiency of one physical mutagen i.e. gamma rays and three chemical mutagens i.e. ethyl methane sulfonate (EMS), nitrosoguanidine (NG), maleic hydrazide (MH) and their combinations 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 result from the study indicated that the values of mutagenic effectiveness gradually decreased with increases in dose or concentration of mutagens. NG exhibited as the most effective mutagen, whereas EMS found as the most efficient mutagen. Among combined treatments, gamma rays with NG found as more effective than other mutagenic combinations. Among all the mutagenic treatments the maximum efficiency observed in EMS 0.2% treatment (based on pollen sterility) and NG 0.01% treatment (based on lethality), whereas the lowest efficiency observed in MH 0.03% treatment basing on lethality as well as pollen sterility. Among all mutagens, the maximum mutation rate based on lethality observed in NG treatments, whereas a higher mutation rate based on pollen sterility observed for EMS treatments which can further be increased in combination with gamma rays.

Key words: Chemical mutagens, gamma rays, greengram, mutagenic effectiveness, mutagenic efficiency, mutation rate

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops in India. One of the bottlenecks in its improvement has been the lack of variability. The induction of mutation by different physical and chemical mutagens provide a powerful means of creating new and useful variability in crop plants. 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 (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 as well as chemical mutagens on the frequency of chlorophyll mutation, lethality, and pollen sterility to evaluate the

relative effectiveness of mutagenic treatments and efficiency and mutation rate in different mutagens.

MATERIALS AND METHODS

Dry and well-filled seeds of a greengram variety, namely Sujata were administered mutagenic treatments with three doses each of gamma rays (20, 40 and 60 kR), ethyl methane sulphonate (EMS; 0.2, 0.4 and 0.6%), nitroso guanidine (NG; 0.005, 0.010 and 0.015%) and maleic hydrazide (MH; 0.01, 0.02 and 0.03%) singly and combine mutagens of 40 kR gamma rays with 0.4% EMS or 0.01% NG or 0.02% MH. The twelve single mutagenic treatments of gamma rays, EMS, NG, and MH were coded as G1, G2, G3, E1, E2, E3, N1, N2, N3 and M1, M2 and M3, respectively. Three combined treatments of 40 kR gamma rays + 0.4% EMS, 40 kR gamma rays + 0.01% NG and 40 kR gamma rays + 0.02% MH were coded as GE2, GN2 and GM2, respectively. Dry seeds were irradiated with gamma ray treatment at Bhaba Atomic Research Centre, Trombay. For treatment with EMS, NG, and MH, the seeds were pre-soaked in distilled water for six hours, blotted dry and then treated with a freshly prepared aqueous solution of above chemical mutagens for 6 hours, with intermittent shaking. For combination treatments, seeds were first irradiated with 40 kR gamma rays and then treated with 0.4% EMS or 0.01 % NG or 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 (400 of each treatment) were sown in RBD in two replications with a spacing of 25 × 10 cm^2. Observations on survival were recorded in each plot at the time of maturity and were calculated as the percent of control from which the lethality percentage was calculated. Mean pollen sterility was determined based on acetocarmine stainability. The selfed seeds of all survived plants harvested were used to grow the M_2 generation in RBD with three replications with spacing of 25 × 10 cm^2. Different types of chlorophyll mutants were recorded daily from 5th to 12th day after sowing. The treated and control populations were screened for different chlorophyll mutations such as Albina, Xantha, Chlorine, Striata, Viridis. The frequency of chlorophyll mutants was calculated according to (Gaul, 1960), i.e. Number of mutants per 100 M_2 plants.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. Mutation rate (MR) which provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration was calculated as follows.

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

$$\text{Mutagenic effectiveness (Chemical mutagens)} = \frac{\text{Mutagenic Frequency (Mf)}}{\text{Conc. (c) in \% } \times \text{ duration of treatment (hrs)}}$$

$$\text{Mutagenic effectiveness (Combination)} = \frac{\text{Mutagenic Frequency (Mf)}}{\text{Dose of physical Mutagen (kR)} \times \text{Conc. of chemical mutagen (\%)} \times \text{duration (hrs)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic Frequency}}{\text{Biological damage (\% Lethality or \% Pollen sterility) in } M_1 \text{ generation}}$$

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

RESULTS AND DISCUSSION

In the present study, the biological damages like lethality and pollen sterility were recorded in M_1 generation (Table 1). Both parameters were found to increase with increasing doses of mutagens (Fig. 1). In gamma- rays treatments recorded the maximum lethality 26.7% and pollen sterility 7.81% at 60kR whereas the minimum lethality 16.5% and pollen sterility 2.11% at 20kR. In the case of EMS treatments, the maximum lethality (58.7%) observed at 0.6% and the minimum (31.5%) at 0.2%. The pollen sterility increased with increasing doses of EMS i.e. 2.46% at a low dose to 7.43. Similar trends were also found in other chemical mutagens NG and MH. In NG recorded the maximum lethality 28.0% and pollen sterility 10.51% at 0.015% dose whereas the minimum lethality 14.5% and pollen sterility 3.87% at 0.005%. In MH the lethality varies from 50.0% at 0.01% dose to 71.0% at 0.03% dose and pollen sterility from 4.27% to 12.19%. In combined treatments of gamma-ray with NG recorded the minimum lethality (35.7%), whereas gamma-ray with EMS recorded the minimum pollen sterility (3.41%). The maximum lethality (69.0%) and pollen sterility (9.7%) observed in gamma rays with MH combinations. 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, Sonone et al. (2008) in groundnut and Khan and Tyagi (2010) in soybean. 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. The lethality and pollen sterility increased in combined treatments indicating the additive or synergistic effects of mutagen.

Since chlorophyll deficient mutants could not survive long and observed in 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. The data on chlorophyll mutation frequency and effectiveness of different mutagenic treatments are presented in Table 1. The frequent of chlorophyll mutation in different treatment in M_2 generations varied from 0.88 (M3) to 2.57 (E2). In general, there was an increase in chlorophyll mutation frequency with an increase in the dose of the physical mutagen (gamma-rays) whereas in chemical mutagens there was no dose-dependency relationship. In combine treatments, maximum chlorophyll mutation (2.15) recorded in GN2 followed by GE2. The occurrence of chlorophyll mutations had reported earlier by several researchers in greengram (Vikram et al., 2014), blackgram (Thilagavathi and Mullainathan, 2009; Goyal and Khan, 2010) and in horse gram (Kulkarni and Mogle, 2013). 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).

Mutagenic effectiveness showed a decreasing trend with the increase in doses or concentration of mutagens. Among the different doses of gamma rays irradiated, the mutagenic effectiveness was the maximum (0.057) at 20kR followed by 40kR (Table 1). In EMS, the mutagenic effectiveness was the maximum (1.19) at 0.2% followed by 0.4%. In the case of NG, the mutagenic effectiveness was the maximum (58.67) at 0.005% followed by 0.01% (39.83) and in MH, the mutagenic effectiveness was the maximum (25.33) at 0.01% followed by 0.02% (18.5) treatment. 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 and Tyagi (2010) and Girija and Apparao (2011). On the contrary to the present study, Siddiq and Swaminathan (1968) found that EMS was most efficient mutagen followed by gamma rays and Nitroso-Guanidine. Among the combine treatments, the maximum effectiveness observed

in gamma-rays (40kR) + NG (0.01%) combination followed by gamma-rays (40kR)+MH (0.01%). 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 decreasing trend was observed at higher doses. 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 greater effectiveness of chemical mutagens over physical mutagen has also been reported by Shah et al. (2008) and Satpute and Fultambkar (2012).

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 0.012 (M3) to 0.122 (N1) and observed that there is no dose-dependent relationship i.e. it did not follow any particular (increasing or decreasing) trend in mutagenic treatments. 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 concentrations of dependent enhancement in the majority of the mutagenic treatments in the M_1 generation of greengram. The value of efficiency decreased as there were increases in doses of mutagens (Table 1, Fig. 2). It ranged from 0.256 to 0.536 in gamma- rays treatment. In EMS treatments, the range was 0.292 to 0.581 whereas in NG, the range was 0.155 to 0.455 and in MH it was 0.072 to 0.356. The efficiency based on pollen sterility varied from 0.151 to 0.531 in the case of combination treatments. Among all the fifteen treatments the maximum efficiency based on lethality and pollen sterility observed in NG 0.005% and EMS 0.2% treatment, respectively whereas the lowest efficiency based on lethality as well as pollen sterility observed in MH 0.03% treatment. Higher efficiency at lower doses of mutagen as observed in the present study might be due to the fact that pollen sterility increased with an increase in doses at a rate faster than the frequency of mutation. Mutagenic efficiency increased with an increased dose similar results were also noticed by Awnindra (2007) and Velu et al. (2008) in greengram and Sharma et al. (2005) in blackgram.

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 induction per mutagen. The mutation rates estimated from the value of mutagen efficiency based on lethality and pollen sterility (Table 2). Based on lethality the mutation rate varied from 0.021 (MH) to 0.100 (NG) whereas the mutation rate based on the mutagenic efficiency calculated from pollen sterility value varied from 0.151 (Gamma-rays + MH) to 0.531 (Gamma-rays + EMS). It could be noted that when the mutation rates based on efficiency were considered, the order of mutagens changes as the mutagens have varied values based on lethality and pollen sterility (Fig. 3).

A mutagen is useful only if it is effective as well as efficient. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. 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 this study, among all the mutagens EMS, NG, and Gamma-ray treatments were found to be most efficient. With respect to lethality the order of efficient mutagens could be framed as *NG > Gamma rays> Gamma rays + NG > EMS > Gamma rays + EMS > MH > Gamma rays + MH*. When the mutation rate for pollen sterility is taken into consideration, the order of mutagens could be framed as *Gamma rays + EMS > EMS > Gamma rays>NG > Gamma rays + NG > MH > Gamma rays + MH*. Many researchers have been reported that the effectiveness and efficiency of mutagens vary to a greater extent in different pulse crops like in Urdbean (Sharma et al., 2005), in Chickpea (Shah et al., 2008), in Pea (Dhulgande et al., 2011), in Clusterbean (Bhosale, 2010) and in French bean (More and Borkar, 2016).

Table 1. Effectiveness and efficiency of different mutagenic treatments on greengram variety cv. Sujata

Code	Mutagen	Percentage of lethality (L)	Percentage of pollen sterility	Frequency of chlorophyll mutation	Mutagenic effectiveness	Mutagenic efficiency	
						Based on lethality	Based on sterility
G1	Gamma-rays 20kR	16.5	2.11	1.13	0.057	0.068	0.536
G2	Gamma-rays 40 kR	22.0	4.56	1.92	0.048	0.087	0.421
G3	Gamma-rays 60 kR	26.7	7.81	2.00	0.033	0.075	0.256
E1	EMS 0.2 %	31.5	2.46	1.43	1.192	0.045	0.581
E2	EMS 0.4 %	43.7	4.79	2.57	1.071	0.059	0.537
E3	EMS 0.6 %	58.7	7.43	2.17	0.602	0.037	0.292
N1	NG 0.005 %	14.5	3.87	1.76	58.667	0.122	0.455
N2	NG 0.010 %	19.7	7.74	2.39	39.833	0.121	0.309
N3	NG 0.015 %	28.0	10.51	1.63	18.111	0.058	0.155
M1	MH 0.01 %	50.0	4.27	1.52	25.333	0.030	0.356
M2	MH 0.02 %	66.0	9.03	2.22	18.500	0.034	0.246
M3	MH 0.03 %	71.0	12.19	0.88	4.889	0.012	0.072
GE2	Gamma-rays 40 kR + EMS (0.4 %)	51.0	3.41	1.81	0.019	0.035	0.531
GN2	Gamma-rays 40 kR + NG (0.010 %)	35.7	7.97	2.15	0.896	0.060	0.270
GM2	Gamma-rays 40 kR + MH (0.02 %)	69.0	9.70	1.46	0.304	0.021	0.151

***** All mutagenic treatments found significant (at 5 % level) for lethality and pollen sterility

Table 2. Mutation rate of different mutagens in greengram

Mutagens	Mutation rate based on lethality	Mutation rate based on pollen sterility
Gamma-rays	0.077	0.404
EMS	0.047	0.470
NG	0.100	0.306
MH	0.025	0.225
Gamma rays +EMS	0.035	0.531
Gamma rays +NG	0.060	0.270
Gamma rays+ MH	0.021	0.151

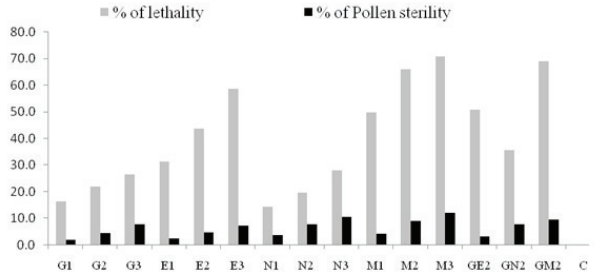

Fig. 1. Effect of different mutagenic treatments on lethality and pollen sterility in greengram

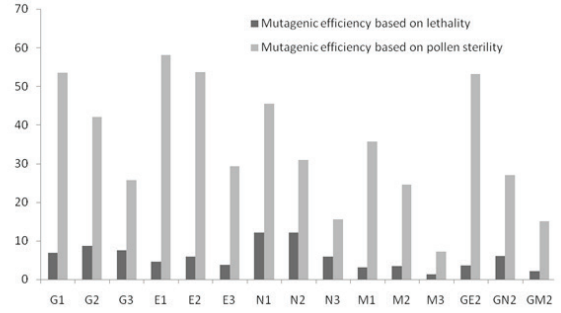

Fig. 2. Mutagenic efficiency (%) of different mutagens in greengram

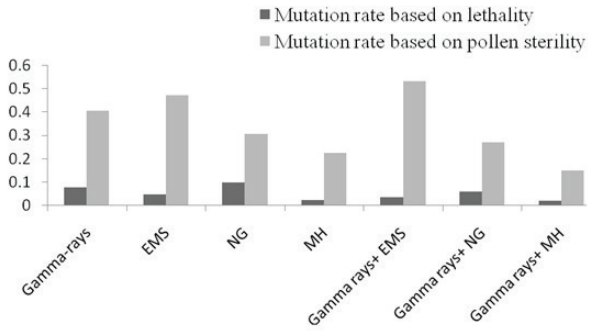

Fig. 3. Mutation rate of different mutagens in greengram

CONCLUSION

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. In the present study, it can be inferred that the values of effectiveness gradually decreased with increases in dose or concentration of mutagen. The lower to moderate concentrations of the mutagens are more effective than the higher concentrations. The study also reveals the Gamma rays, EMS, NG, and their combinations have a higher potential to induce significant mutations in greengram. It was noted that when the mutation rate based on efficiency was considered, the order of mutagen changes as according to the lethality and pollen sterility. Lethality studies indicate that NG treatments maximum mutation rate followed by gamma-rays and gamma rays + NG treatment whereas maximum mutation rate (based on pollen sterility) found in gamma rays + EMS treatment followed by EMS and Gamma rays.

ACKNOWLEDGEMENT

We express our deep sense of gratitude to Dr. D.P. Roy, Ex Vice-Chancellor, OUAT, Bhubaneswar (Odisha) for his valuable guidance, moral support and encouragement along with providing required facilities during the entire period of research.

REFERENCES

Awnindra, K.S. 2007. Mutagenic effectiveness and efficiency of gamma-rays and ethyl methane sulphonate in mungbean. *Madras Agric. J.* **94**(1-6): 7-13.

Bhosle, S.S. and Kothekar, V.S. 2010. Mutagenic efficiency and effectiveness in Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.). *J. Phytol*. **2**(6): 21-27.

Das, T.R., Misra, R.C. and Sahu, P.K. 2006. Efficiency of mutagenic treatments in expression of macro and micro mutations in M2 generation in greengram and its early predictability on basis of M1 parameters. *Environ. Ecol*. **24**(2): 283-288.

Dhulgande, G.S., Dhale, D.A., Pachkore, G.L. and Satpute, R.A. 2011. Mutagenic effectiveness and efficiency of gamma rays and Ethyl methane sulphonate in pea (*Pisum sativum* L.). *J. Exp. Sci*. **2**(3): 07-08.

Gaikwad, N.B. and Kothekar, V.S. 2004. Mutagenic effectiveness and efficiency of ethyl methane sulphonate and sodium azide in lentil (*Lens culinaris Medik*.). *Indian J. Genet*. **64**(1): 73-74.

Gaul, H. 1960. Critical analysis of the methods for determining the mutation frequency after seed treated with mutagens. *Genet. Agric*. **12**: 297-318.

Girija, S.P. and Apparao, J. 2011. Studies on effectiveness and efficiency of EMS in pigeonpea (*Cajanus cajan* L.). *Biosci. Discovery* **2**: 29-31.

Goyal, S. and Khan, S. 2010. Induced mutagenesis in Urdbean [*Vigna mungo* (L.) Hepper]: A review. *Int. J. Bot*. **6**(3) 194-206.

Khan, M., Tyagi, H. and Dutt, S. 2010. Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean *Glycine max* L. Merrill. *J. Plant Breed. Crop Sci*. **2**(3):55-58.

Konzak, C.F., Nilan, R.A., Wagner, J. and Foster, R.J. 1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding (FAO / IAEA Meeting, Rome). *Radiat. Bot*. (Suppl.) **75**: 49-70.

Kulkarni, G.B. and Mogle, U.P. 2013. Effects of mutagen on chlorophyll mutation in horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt] *Biosci. Discovery* **4** (2): 214-219.

Monika, S. and Seethraman, N. 2017. Induced chlorophyll and viable mutation in *Lablab purpureus* (L.) sweet var. *typicus* through gamma rays and Ethyl Methane Sulphonate. *Int. J. Pharm. Bio. Sci*. **8** (2): (B) 240-249.

More, A.D. and Borkar, A.T. 2016. Mutagenic effectiveness and efficiency of gamma rays and EMS in *Phaseolus vulgaris* L. *Int. J. Curr. Microbiol. App. Sci*. **5**(10): 544-554.

Nilan, R.A. and Konzak, C.F. 1961. Increasing the efficiency of mutation induction. In: *Mutation and Plant Breeding*, NAS-NRC, pp. 437-460.

Rao, G.M. and Rao, V.M. 1983. Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. *Cytologia* 48: 427-436.

Reddi, T.V.S and Rao, D.R.M. 1988. Relative effectiveness and efficiency of single and combination treatments using gamma rays and sodium azide in inducing chlorophyll mutations in rice. *Cytologia* **53**: 491-498.

Satpute, R.A. and Fultambkar, R.V. 2012. Effectiveness and efficiency of the mutagens gamma rays and EMS in soybean [*Glycine max* (L.) Merrill]. *Curr. Bot*. **3**(2): 18-20.

Shah, T.M., Mirza, J.I., Haq, M.A. and Atta, B.M. 2008. Induced genetic variability in chickpea (*Cicer arietinum* L.). II. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Pak. J. Bot*. **40**(2): 605-613.

Sharma, S.K, Sood, R. and Pandey, D.P. 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper). *Indian J. Genet*. **65**: 20-22.

Siddiq, E.A, and Swaminathan, M.S. 1968. Enhanced mutation induction and recovery caused by nitroso-guanidine in *Oryza sativa* L. *Indian J. Genet*. **28** (3): 297-300.

Sonone, N.G., Deshmukh, S.N., Patil, S.P., Rathod, S.T. and More, G.B. 2008. Comparison of mutagenic efficiency and effectiveness of EMS and gamma rays in groundnut *Arachis hypogea* L. *Crop Res.* **36**(1-2, 3): 336-390.

Tah, P. 2006. Induced macromutations in mungbean [*Vigna radiata* (L.) Wilczek]. *Int. J. Bot*. **2**(3): 219-228.

Thilagavathi, C and Mullainathan, L. 2009. Isolation of macro mutants and mutagenic effectiveness, efficiency in blackgram [*Vigna Mungo* (L.) Hepper]. *Global J. Mol. Sci.* **4**:76-79

Velu, S., Mullainathan, L., Arulbalachandran, D., Poongkuzhalir and Dhanavel, D. 2008. Spectrum of morphological mutation and effectiveness and efficiency of physical and chemical mutagens in greengram. *Int. J. Pl. Sci.* **3**(1): 1-4.

Vikram, N., Ibrahim, S.M. and Vanniarajan, C. 2014. Frequency and spectrum of chlorophyll mutations in greengram [*Vigna radiata* (L.) Wilczek] *Asian J. Bio Sci.* **9** (2): 204-207.