Electronic Journal of Plant Breeding, 6(1): 210-217 (Mar 2015)
ISSN 0975-928X

Research Note

Mutagenic efficiency and effectiveness of gamma rays and EMS and their combination in inducing chlorophyll mutations in M_2 generation of Urdbean (*Vigna mungo* (L.) Hepper)

K.S. Usharani[1]* and C.R. Ananda Kumar[1]
[1]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore - 03, Tamil Nadu, India.
*Email: usharaniagri@gmail.com

(Received: 28 Oct 2014; Accepted:26 Feb 2015)

Abstract
The usefulness of any mutagenic agent depends on its ability to induce high frequency of desirable changes as compared to undesirable ones. Hence, often it is necessary to assess the efficiency and effectiveness of mutagens for efficient and effective use. Though some studies have been carried out in blackgram, studies involving gamma rays and Ethyl Methane Sulphonate (EMS) are scanty. The present investigation was undertaken in a blackgram variety VBN 4 to assess the efficiency and effectiveness of physical and chemical mutagen *viz.*, gamma ray and EMS respectively in single and combination dose/concentration. The mutagenic efficiency was found to be highest, at lower and intermediate concentration of mutagenic treatments. Based on lethality, injury and sterility, EMS was more efficient than gamma rays and combination of both in producing chlorophyll mutants. The effectiveness of chlorophyll mutants was high in gamma rays treatment than EMS.

Keywords
Blackgram, Efficiency, effectiveness, gamma rays, EMS, chlorophyll mutations

Since chlorophyll mutations are most conspicuous and are easily detectable. They have been extensively used to find out sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of mutagen (Gustafsson, 1940). Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency is an estimate of biological effects induced such as lethality, sterility and injury (Konzak *et al.*, 1965). To obtain high efficiency, the mutagenic effect must greatly surpass the damage in the cells, such as chromosomal aberrations and toxic effects as proposed by Gaul *et al*. (1972). According to them, the effectiveness of mutagens was of theoretical importance, but did not have any immediate practical implication. Further, they reported that low biological damage of mutagens permitted the use of high dose of mutagens but for practical purpose, the aim was to obtain high efficiency.

Blackgram is a small annual plant and is commonly known in India as Urdbean. It is an important food grain legume and rich in protein. Crop improvement of urdbean through hybridization and recombination is very difficult, because of its autogamous nature (Deepalakshmi and Ananda Kumar, 2004). Due to autogamous nature, genetic variability is also lacking in blackgram. As genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central base to crop breeding. Experimentally, induced mutations provide an important source for variability. Many physical and chemical mutagens have been used for induction of useful mutations in number of crops. The practical utility of induced mutations for the improvement of quantitatively inherited characters in urdbean is well recognized.

A highly effective mutagen may not necessarily show high efficiency and vice versa. The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, pollen sterility, ovule sterility etc.,) in relation to the mutagens induced (Shah *et al.,* 2008). Selection of effective and efficient mutagen is very essential to recover high frequency of the desirable mutations in a mutation breeding studies. Hence, previous knowledge of effectiveness and efficiency of most commonly used chemical mutagens in relation to the ionization radiations in a number of genotypes is indispensable to classify the range of dose concentrations of useful mutagens in a number of breeding programme. At present no conclusive information on relative effectiveness and efficiency of different physical and chemical mutagen is available for urdbean. The present experiment/investigation was undertaken to assess the mutagenic effectiveness and efficiency of gamma rays, EMS and their combination treatments of mutagens based on frequency of chlorophyll mutations in M_2 generation in urdbean.

Seeds of VBN 4 urdbean were subjected to three different doses of gamma rays *viz.*, 40kR, 50kR and 60kR, EMS 50mM, 60mM and 70mM and combination treatments. To raise M_1 generation, a total of fifteen treatments along with the control was sown in the field at the rate of 150 seeds for each treatment at a spacing of 30 x 15cm at AC&RI, Madurai during August, 2010 in Randomized block design (RBD) with three replications. All the surviving individual plants were harvested in each treatment in M_1 generation. M_2 generation was raised on M_1 plant basis following plant to progeny method in a RBD with three replications during January, 2011. Frequency of chlorophyll mutations was calculated per 100 M_2 seedling basis. The chlorophyll mutants were classified in accordance with the system of Gustaffson (1940). Mutagenic efficiency and effectiveness were calculated on the basis of formula suggested by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness} = \frac{M \times 100}{kR \text{ or } c \times t}$$

Mutagenic efficiency = M x 100/L; M x 100/I; M x 100/S

Where,
M - Chlorophyll mutation frequency
kR - Dose of gamma radiation
c - Concentration of the chemical mutagen (mM)
L - Percentage of lethality
t - Duration of treatment with chemical mutagen (hrs)
I - Percentage of injury
S - Percentage of sterility

To evaluate the effect of combined treatments on mutation frequency the data was analyzed using the formula suggested by Sharma and Swaminathan (1969).

$$K = \frac{(a+b)}{(a) + (b)}$$

Where, a & b - two mutagens
K - hypothetical interaction coefficient

Any deviation from the unit value of K would reveal either a synergistic effect (K>1) or antagonistic effect (K<1).
Data on biological abnormalities such as lethality, injury and sterility in M_1 generation and chlorophyll mutation frequencies in M_2 generation were used to determine the mutagenic efficiency and effectiveness.

Biological abnormalities in M_1 generation: The date of plant survival (%), plant height and seed fertility (%) in M_1 generation for various mutagenic treatments in VBN 4 is given in the Table 1. Under field conditions, the percentage of seedlings survival progressively decreased with the increasing dose or concentration of mutagens. Similar results were reported by Ahmed John (1996), Kouser *et al.* (2007), Jain and Khandelwal (2008), Lal *et al.* (2009), Sagade and Apparao (2011). Reduction in plant height on 30^{th} day and maturity was reported by Kundu and Singh (1982), Chaturvedi *et al*. (1983) and Vanniarajan (1989) in blackgram, in greengram the similar results have been reported by Yogesh kumar and Mishra (1999).

In the current study, reduction in plant height was observed but dose dependent response could not be observed. The plant height at maturity decreased over the control in all the mutagenic treatments. In contrast to this, increase in plant height has been reported by Verma and Singh (1984) in greengram, Karthik (2008). The reduction in the seed fertility was gradual and the maximum was observed at highest dose or concentration of the mutagens (Ahmed John, 1996; Sagade and Apparao, 2011).

Frequency of chlorophyll mutants in M_2 generation: The mutagenic effectiveness of any given mutagen is fully explicit only from the frequency of induced mutations and induced chlorophyll mutation frequency is a very dependable index for evaluating the efficiency of mutagenic treatments, destined to generate wide array of variability. To estimate the chlorophyll mutation, periodical scoring was carried out starting from 5^{th} day to 15^{th} day after sowing. The chlorophyll mutation frequency was estimated as percentage of plants that segregated for chlorophyll deficiency on the basis of M_2 seedlings and presented in Table 2. The gamma rays induced chlorophyll mutant frequency in VBN 4 ranged from 1.60 in 50kR to 3.10 per cent in 60kR. In case of EMS treated population, the frequency of chlorophyll mutations ranged from 1.97 per cent in 60mM to 3.61 per cent in 50mM. In combination treatments, the frequency of chlorophyll mutations ranged from 0.82 per cent in 50kR+70mM to 4.97 per cent in 60kR+70mM. The greater effectiveness of combined treatments in inducing highest frequency of chlorophyll mutations has been reported by many workers (Khalatkar and Bhatia, 1975) in barley, Gautam *et al.* (1992) and Singh *et al*. (1999) in urdbean and Singh and Singh (2007) in mungbean. The frequency of chlorophyll mutations observed in the present study was very low and also fluctuating under different treatments. The findings are in confirmity with Ignacimuthu and Babu (1992).

Different types of chlorophyll mutations *viz.*, albina, chlorina, xantha and viridis were observed in the M_2 generation. Almost all the mutagenic treatments showed different degree of mutants with respective dose. Albina was white in colour, due to absence of all pigment and relatively smaller than the normal seedlings. This was leaded to the death of the plants at 10-15 days after germination. Viridis was seedlings with whitish tips of leaves, leads to lethal were observed. Xantha was straw yellow seedling with normal growth in the beginning but it was started withering after 12 - 15 days. Chlorina was light green coloured seedlings, lethal at 10 - 12 days after germination. In the present investigation, it can be represented as chlorina > xantha > viridis > albina. This is in agreement with the reports of Vanniarajan *et al.* (1993) in blackgram.

Mutagenic effectiveness: The effectiveness of both the mutagens in inducing chlorophyll mutations was calculated on the percentage basis of M_2 seedlings. The effectiveness of gamma rays in inducing chlorophyll mutations ranged from 3.2 to 5.16 per cent and the highest effectiveness was observed at 60kR (5.16 per cent). The effectiveness of EMS in inducing chlorophyll mutations ranged from 0.54 to 1.20 per cent and the highest effectiveness was observed at 50mM (1.20 per cent). The effectiveness of chlorophyll mutants was high in gamma rays treatment compare to EMS (Table 3). The high effectiveness of gamma rays was reported by Gautam *et al.* (1992) and Deepalakshmi and Ananda Kumar (2003) in blackgram and Mehraj *et al*. (1999) in greengram. There was no consistent trend between mutagen dose and effectiveness in chlorophyll mutants in this study.

Mutagenic efficiency: EMS treatments were more efficient than gamma ray in producing chlorophyll mutations (Dhulgande *et al.,* 2011). Since maximum efficiency of 43.13, 19.40 and 73.97 was obtained at 50mM based on lethality, injury and sterility basis. Based on lethality, efficiency of combination treatments in inducing chlorophyll mutations ranged from 3.04 to 43.78 per cent and the highest efficiency was observed at 60kR+70mM. Based on injury, efficiency ranged from 4.69 to 22.10 per cent and the highest efficiency was observed at 60kR+50mM. Based on sterility, efficiency ranged from 4.77 to 31.61 per cent and the highest efficiency was observed at 50kR+50mM. The efficiency of combination treatments was not high than the individual treatments except 40kR+60Mm combination. Based on interaction coefficient (K<1), all the combination treatments show less than additive effects in inducing chlorophyll mutations (Table 3). Similar results were reported by Srinivas and Veerabadhiran (2010) in lablab and Rao and Ayengar (1964) in rice.

In the present study, the mutagenic efficiency was higher, mostly at lower and intermediate doses of chlorophyll mutants than at higher doses. This is in confirmation with the findings of Ahmed John (1995) and Khan (1999) in blackgram. In contrast to this Gautam *et al.* (1992) in blackgram and Singh and Singh (2007) in greengram reported a dose dependent increase in mutagenic efficiency. The reason for the greater efficiency at lower concentrations of mutagens is relating to the fact that lethality, injury and sterility increased with the mutagen level at a much faster rate. So the lower concentration causes relatively less damage enabling the organisms to manifest the induced mutations more frequently (Shadakshari *et al*., 2001).

Solanki and Sharma (1994) considered that the higher efficiency of a mutagen indicates relatively less biological damage (*i.e.,* lethality, seedling injury, sterility etc.) in relation to mutations induced. According to Khan and Hashim (1979) and Reddi and Suneetha (1992), the effectiveness and efficiency do not necessarily increase linearly with the increasing doses, rather every dose has its own effectiveness and efficiency, independent of the other lower and higher doses.

Generally, it was inferred from the present study mutagenic effectiveness of chlorophyll mutants was high in gamma rays treatment than EMS. The efficiency was found to be highest at lower and intermediate concentration of mutagenic treatments.

Based on lethality, injury and sterility, EMS was more efficient than gamma rays and combination of both in producing chlorophyll mutants. Combination treatments in general proved to be most effective followed by chemical mutagens and gamma rays in inducing maximum frequency of chlorophyll mutations. The chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations.

References

Ahmed John, S. 1995. Effectiveness and efficiency of gamma rays in blackgram. *Madras Agric. J.,* **82**(6-8): 417-419.

Ahmed John, S. 1996. Impact of gamma irradiation on germination and survival in blackgram. *Madras Agric. J.,* **83**(9): 592-593.

Chaturvedi, S.N., Gautam, R.B. and Sharma, R.P. 1983. Mutagenic effects of gamma rays, EMS and NMU in *Vigna mungo* (L). Hepper. *Pulse Crop Newsl.,* **3**: 10-12.

Deepalakshmi, A.J. and Ananda Kumar, C.R. 2003. Efficiency and effectiveness of physical and chemical mutagens in urdbean (*Vigna mungo* (L.) Hepper). *Madras Agric. J.,* **90**(7-9): 485-489.

Deepalakshmi, A. J. and Ananda Kumar, C.R. 2004. Creation of genetic variability for different polygenic traits in blackgram (*Vigna mungo* (L.) Hepper) through induced mutagenesis. *Legume Res.,* **27**(3): 188-192.

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

Gustafsson, A. 1940. The mutation system of the chlorophyll apparatus. *Lunds Univ. Arsskr.,* **36**: 1-40.

Gaul, H., Frimmel, G., Gichmer, T. and Velonska, E. 1972. Plant improvement. Proc. Study group meeting, Buenos Aires, (1970). *IAEA*, Vienna.

Gautam, A.S., Sood, K.C. and Riccarria, A.K. 1992. Mutagenic effectiveness and efficiency of gamma rays, EMS and their synergistic effects in blackgram (*Vigna mungo* (L.) Hepper). *Cytologia,* **57**: 85-89.

Ignacimuthu, S. and Babu, C.R. 1992. Induced variation on pod and seed traits of wild and cultivated beans. *J. Nuc. Agric. Biol.,* **21**(4): 286-292.

Jain, S.K. and Khandelwal, V. 2008. Mutagenic efficiency and effectiveness of EMS and DMS in blackgram (*Vigna mungo* (L.) Hepper). *J. Arid Legumes,* **5**(2): 110-113.

Karthik, A. 2008. Induced mutagenesis in blackgram (*Vigna mungo* (L.) Hepper) M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore.

Khalatkar, A.S. and Bhatia, C.R. 1975. Synergistic effect of combined treatments of gamma radiation and ethyl methane sulphonate in barley. *Rad. Bot.,* **15**: 223-229.

Khan, I.A. and Hashim, M. 1979. Mutagenic effectiveness and efficiency of gamma rays, EMS and hydrazine hydrate in *Phaseolus aureus* Roxb. *Ind. J. Bot.,* **2**: 107-110.

Khan, M.N. 1999. Mutagenic effectiveness and efficiency of EMS, gamma rays and their combinations in blackgram. *Advan. Plant. Sci.,* **12**(1): 203-205.

Konzak, C.F., Nilan, R.A., Wagner, J. and Foster, R.J. 1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding. (Rep. FAO/IAEA Tech. Meeting, Rome, 1964), *Pergamon Press*, pp. 49-70.

Kouser, M., Suresh Babu, G. and Lavanya, G.R. 2007. Effects of mutagen on M_1 population in urdbean. *J. Food Legumes,* **20**(1): 109-110.

Kundu, S.K. and Singh, D.P. 1982. Gamma ray induced variability for quantitative characters in black gram. *Madras Agric. J.,* **69**: 644-646.

Lal, G.M., Toms, B. and Lal, S.S. 2009. Mutagenic sensitivity in early generation in blackgram. *Asian J. Agric. Sic.,* **1**: 9-11.

Mehraj, U.D., Siddiqui, B.A., Samiullah, K. and Mujeeb, U.R. 1999. Induced mutations in mungbean : Efficiency and effectiveness of chemical mutagens. *Legume Res.,* **22**: 245-248.

Rao, N.S. and Ayengar, A.R.G. 1964. Combined effect of thermal neutrons and diethly sulphate on mutation frequency and spectrum in rice. Biological Effects of Neutrons and Proton Irradiations-I. *IAEA*, Vienna. 383-391.

Reddi, T.V.V.S. and Suneetha, J. 1992. Chlorophyll deficient mutations induced in rice by alkylating agents and azide. *Cytologia,* **57**: 283-288.

Sagade, A.B. and Apparao, B.J. 2011. M_1 generation studies in urdbean (*Vigna mungo* (L.) Hepper). *Asian J. Exp. Biol. Sci.,* **2**(2): 372-375.

Shadakshari, Y.G., Chandrappa, H.M., Kulkarni, R.S. and Shashidar, A.E. 2001. Induction of beneficial mutants in rice (*Oryza sativa* L.). *Indian J. Genet.,* **61**: 274-276.

Shah, T.A., Iqbal, J., Haq, M. and Atta, B. 2008. Induced genetic variability in chickpea. *Pak. J. Bot.,* pp: 606-613**.**

Sharma, R.P. and Swaminathan, M.S. 1969. The combined effects of physical and chemical mutagens. *Radiations and Radiomimetic Substances of Mutation Breeding*. (Proc. Symp. Bombay, 1969). Dept. Atomic Energy, India, 70-78.

Singh, A.K. and Singh, A.K. 2007. Biological influence of gamma rays and Ethyl Methane Sulphonate and their synergistic effects in mungbean. *J. Food Legumes,* **20**(2): 153-155.

Singh, V.P., Singh, M. and Pal, J.P. 1999. Mutagenic effects of gamma rays and EMS on frequency and spectrum chlorophyll and macromutations in urdbean (*Vigna mungo* L.). *Indian J. Genet.,* **59**: 203-210.

Solanki, I.S. and Sharma, B. 1994. Mutagenic effectiveness and efficiency of gamma rays, Ethylene imine and N-nitroso N-ethyl urea in macrosperma lentil

(*Lens culinaris* Medik.). *Indian J. Genet.,* **54**: 72-76.

Srinivas, T.R. and Veerabadhiran, P. 2010. Efficiency and effectiveness of physical and chemical mutagens and their combination in inducing chlorophyll mutations in M_2 generation of Lablab (*Lablab purpureus* (L.) Sweet var. *Typicus*). *Electronic J. Pl. Breed.,* **1**(4): 752-757.

Vanniarajan, C. 1989. Studies on induced mutagenesis in blackgram (*Vigna mungo* (L.) Hepper). M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore.

Vanniarajan, C., Vivekanandan, P. and Ramalingam, J. 1993. Spectrum and frequency of chlorophyll and viable mutations in M_2 generation of blackgram. *Crop Improv.,* **20**(2): 215-218.

Verma, R.K. and Singh, D.B. 1984. Gamma ray induced variability in greengram. *Indian J. Agric. Sci.,* **54**(4): 277-279.

Yogesh Kumar and Mishra, V.K. 1999. Effect of gamma rays and diethyl sulphate on germination, growth, fertility and yield on greengram. *Ann. Agric. Res.,* **20**(2): 144-147.

Table 1. Estimation of plant survival (%), plant height and seed fertility (%) in M_1 generation

Treatment	Plant survival (%)		Plant height		Seed fertility (%)	
	Mean	% reduction	Mean	% reduction	Mean	% reduction
Gamma rays (kR)						
Control	86.56	0.00	18.35	0.00	92.02	0.00
40kR	76.32	11.83	15.00	18.26	85.00	7.63
50kR	65.43	24.41	16.20	11.72	82.00	10.89
60kR	54.00	37.62	13.20	28.07	76.00	17.41
Mean	70.58	18.46	15.69	14.51	83.76	8.98
SE(d)	0.90		0.27		1.23	
CD(0.05)	2.20		0.68		3.02	
EMS (mM)						
Control	86.22	0.00	18.92	0.00	91.46	0.00
50mM	79.00	8.37	15.40	18.60	87.00	4.88
60mM	74.67	13.40	14.20	24.95	81.00	11.44
70mM	66.00	23.45	13.60	28.12	74.00	19.09
Mean	76.47	11.31	15.53	17.92	83.37	8.86
SE(d)	0.95		0.27		1.21	
CD(0.05)	2.34		0.67		2.98	
Gamma rays + EMS						
Control	86.22	0.00	18.92	0.00	91.46	0.00
40kR+50mM	74.62	13.45	13.20	30.24	84.00	8.16
40kR+60mM	76.00	11.85	15.67	17.18	81.12	11.31
40kR+70mM	56.53	34.44	16.00	15.43	80.05	12.48
50kR+50mM	72.00	16.49	15.00	20.72	78.50	14.17
50kR+60mM	65.83	23.65	14.67	22.46	78.34	14.35
50kR+70mM	63.00	26.93	17.00	10.15	75.76	17.17
60kR+50mM	82.00	4.89	17.50	7.51	65.40	28.49
60kR+60mM	65.02	24.59	13.00	31.29	64.00	30.02
60kR+70mM	76.43	11.35	11.02	41.75	61.09	33.21
Mean	71.77	16.76	15.19	19.67	75.97	16.94
SE(d)	1.15		0.37		1.37	
CD(0.05)	2.43		0.77		2.87	

Table 2. Frequency of chlorophyll mutants in M_2 generation

Treatment	Number of M_2 seedlings		Mutation frequency
	Examined	Showing chlorophyll mutants	
Gamma rays (kR)			
Control	342	--	--
40kR	287	5	1.74
50kR	249	4	1.60
60kR	193	6	3.10
EMS (mM)			
Control	328	--	--
50mM	249	9	3.61
60mM	203	4	1.97
70mM	182	6	3.29
Gamma rays + EMS			
Control	328	--	--
40kR+50mM	302	6	1.98
40kR+60mM	254	9	3.54
40kR+70mM	223	3	1.34
50kR+50mM	312	14	4.48
50kR+60mM	276	7	2.53
50kR+70mM	243	2	0.82
60kR+50mM	300	5	1.66
60kR+60mM	272	4	1.47
60kR+70mM	241	12	4.97

Electronic Journal of Plant Breeding, 6(1): 210-217 (Mar 2015)
ISSN 0975-928X

Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutants - VBN 4

Treatments	% Survival reduction at 30^{th} day (lethality)	%Height reduction at 30^{th} day (injury)	%Seed fertility reduction (Sterility)	Mutation (M) per 100 M_2 seedlings	Effectiveness MX100 Cxt (or) kR	Mutagenic Efficiency			Interaction coefficient (K)
						MX100 L	MX100 I	MX100 S	
Gamma rays (kR)									
40kR	11.83	18.26	7.63	1.74	4.35	14.70	9.52	22.80	--
50kR	24.41	11.72	10.89	1.60	3.20	6.55	13.65	14.69	--
60kR	37.62	28.07	17.41	3.10	5.16	8.24	11.04	17.80	--
EMS (mM)									
50mM	8.37	18.60	4.88	3.61	1.20	43.13	19.40	73.97	--
60mM	13.40	24.95	11.44	1.97	0.54	14.70	7.89	17.22	--
70mM	23.45	28.12	19.09	3.29	0.78	14.02	11.69	17.23	--
Gamma rays + EMS									
40kR+50mM	13.45	30.24	8.16	1.98	--	14.72	6.54	24.26	0.37
40kR+60mM	11.85	17.18	11.31	3.54	--	29.87	20.60	31.29	0.95
40kR+70mM	34.44	15.43	12.48	1.34	--	3.89	8.68	10.73	0.26
50kR+50mM	16.49	20.72	14.17	4.48	--	27.16	21.62	31.61	0.85
50kR+60mM	23.65	22.46	14.35	2.53	--	10.69	11.26	17.63	0.71
50kR+70mM	26.93	10.15	17.17	0.82	--	3.04	8.07	4.77	0.16
60kR+50mM	4.89	7.51	28.49	1.66	--	33.94	22.10	5.82	0.24
60kR+60mM	24.59	31.29	30.02	1.47	--	5.97	4.69	4.89	0.28
60kR+70mM	11.35	41.75	33.21	4.97	--	43.78	11.90	14.96	0.77