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Research Paper

Mutagenic effectiveness and efficiency of gamma rays and EMS in black gram (*V.mungo* l.).

Agriculture

KEYWORDS : Mutagen, Gamma Rays, EMS, and Macro mutation.

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ABSTRACT

Studies on induced mutagenesis gamma rays and EMS were performed by sensational the healthy uniform size and dry seeds of blackgram variety TNAUCO (Bg) 6 to gamma rays 150, 200,250, 300, 350 Gy and EMS 10, 15, 20, 25,30mM. The study was to evolve efficient mutants. The LD50 values were found at 250Gy for gamma rays and 20mM for EMS under field conditions based on germination, seedling survival at 30th day, plant height on 30th day, pollen fertility, seed fertility, pods per plant, pod length, seeds per pod, 100 seed weight and single plant yield was reduced as compared to the control. In M2 generation macro mutants like dwarf mutant, spreading type, late mutant, semi sterile type, leaf mutant, basal branching and seed coat colour mutant. Gamma rays induced higher proportion of chlorophyll mutations. Single type and multiple type mutations occurred more frequently. The incidence of mutagenic efficiency and effectiveness was found to be lower at higher doses.

Introduction

Grain legume is protein rich crops and also known as poor man's meat is grown all over. In vegetarian diet, it serves as a source of dietary protein, mineral as well as considered as an essential meal to correct the malnutrition by Mahalik and Routray 2009. India agriculture black gram occupying unique position and it stands fourth in production and acreage Arulbalachandran and Mullainathan 2009. But currently the productivity of blackgram is very low down and has inactive in recent years and to lack of genetic variability. Induction of mutation by physical and chemical mutagenic treatments has been proved as an effective methodology for creating variability in both quantitative and qualitative characters in various crop plants. The potential of induced mutagens to contribute to improved growth traitsresistance in crops are considered important including disease. Hence, the present investigation aims at creation of variation through induction of mutation through chemical mutagens

Materials and methods

A study was carried out black gram variety TNAU Co (Bg) 6 through physical and chemical mutagen to encourage mutation.

Mutagenic Treatment

Physical Mutagen:

Hundred uniform size well developed seeds of black gram were taken for gamma irradiation. These sets of seeds treated with 150, 200, 250, 300, and 350 Gy of gamma rays. Irradiation was done with Cobalt (Co^{60}) was used for source of gamma rays in TNAU at Coimbatore, India.

Chemical Mutagen:

Hundred health well matured healthy seeds with 10 to 14 per cent moisture content were subjected to the mutagenic treatment. The seeds were pre-soaked in distilled water for five hours at room temperature prior to treatment. After presoaking, the excess of moisture in the seeds were removed. Then, the seeds are soaked in six hours the freshly prepared aqueous solution of EMS, which concerning three times than that of volume of seeds with corresponding concentrations of EMS *viz,* 10, 15, 20, 25 and 30 mM at room temperature (28±2 °C) with intermittent shaking. Seeds were washed thoroughly with distilled water. The control seeds pre-soaked in water. Both treated and untreated control seeds were sown in the field in randomized black design (RBD) with three replication to raised the M_1 generation. The mutant seed were grown during *summer* 2012 and *kharif* 2012.Each plant of M_1 and M_2 was rasied complete proper package of practices in single replication. Each single plant chosen from the M_1 generation. Pollen fertility and sterility in initial five immature buds and ovule sterility from the pods at the time of physiological maturity were recorded. M_1 plants having adequate seeds in different treatments were grown in plants to progeny rows to rise M_2 generation. Lethal chlorophyll mutations were identified during the first four weeks after germination, while viable chlorophyll and morphological viable mutations were observed right through the crop duration. Mutation frequency was workout as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. Mutagenic effectiveness is a measure of the frequency of mutations induced by a unite dose of mutagen. The mutagenic efficiency even though the proportion of mutation in relation to biological damage induced. Konzak *et al*. (1965) were proposed by formulae which were followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic Frequency}}{\text{Dose or (Concentration X time)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic Frequency}}{\text{Biological damage}}$$

Mutation rate (MR) was calculated by the following formula

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

This provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration.

Result and Discussion

In the current study from table 1 in which pollen sterility (%) and ovule sterility (%) are presented that increase in the pollen and ovule sterility showed direct relationship with gamma-rays and EMS, the maximum pollen and ovule sterility occurring at highest doses of gammarays and EMS. Effectiveness of gamma rays decreased with lower dose. However 15mM of EMS treatment was most effective on the basis of both M_1 plant progenies and M_2 plant basis .Whereas in case of Gamma ray, the most effective treatment was 200Gy.In general, EMS treatment were more effective than gamma rays. Gautam and Sood 1992 and Ashok kumar *et al.,* 2010 have also conducted it in Black gram. Physical and chemical mutagens encourage physiological damages (injury), gene mutations (point mutations) and chromosomal mutations (chromosomal aberrations) in

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the biological material in M_1 generation reported by Gaul, 1970 and Kousar Makeen *at al.,* 2010. The biological damage caused by the mutagens in M_1 generation could be measured based on seed germination, survival reduction (lethality), plant height reduction (injury) and seed fertility reduction (sterility) in table 2. The per cent reduction for pollen fertility ranged from 10.00 (300Gy) to 82.00(10mM). the negative relation between the doses and pollen fertility. Larik 1975 reported that the pollen fertility reduction might be due to cumulative effects of various aberrant meiotic stagesas well as physiological and genetic damages that induced probably by the breakage of chromosome through formation of an antimetabolic agent in the cell or may be due to irregular disjunction of chromosomes at anaphase. The disjunction of chromosome may result from the formation of interchanges and multivalent or orientation of chromosome at metaphase I reported Kumar and Das, 1973. The mutagenic effectiveness was found to be the highest at lower concentration with all the mutagenic treatments. EMS was found to be high effective than Gamma ray. The maximum effectiveness for chlorophyll and viable mutants was observed at 4.19 in 15mM of EMS, 4.10 in 200Gy of Gamma ray and 6.39 in 15mM and 4.90 in 200Gy (Table 2 and 3). The present study showed that the effectiveness decreased with increase in concentration of EMS and Gamma ray. This was in confirmation with the findings of Packiaraj 1988 in cowpea, Khan 1999 and Sharma *et al*., 2005 in blackgram. This may be because the biological damages increased with the increased with the increase in dose at a rate greater than the frequency of mutation Konzak *et al*., 1965. Thus, the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutation or aberrations.

Mutagenic Efficiency of chlorophyll and viable mutations was based on lethality (L) injury (I) and sterility in M_1 plant and M_2 seedling basis (table 2). The viable mutants were grouped into plant height, leaf modifications, variation in branching habit, floral mutants, pod and seed mutants and others. In the present investigation, viable macro mutations with changes in attributes like stature, duration, stem, leaf, pod, flower and seed mutants were recorded. Stature mutants namely dwarf, spreading, early, and late duration mutants were observed. Vanniarajan 1989 observed semispreading mutants in gamma ray and EMS treatment in blackgram. The lethality, injury, Sterility basis maximum efficiency was achieved by 20mM of EMS (10.77, 2.41 and 0.12). Similarly, Waghmare and Mehra 2001 in Lathyrus sativus,Vanniarajan 1989, Ahmed 1995 and Sharma *et al*., 2005 made results observations in blackgram. It can be assumed that multimutational events affect several genes and thus several enzymes or proteins, resulting in pleiotropic effect. Most of the mutants bearing multimutational events thus may be lethal in the first generation, affecting the frequency of occurrence of multimutations in M_2 and future generations.

The mutation rates were calculated using 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 mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is described. 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, the highest chlorophyll mutation rate for gamma rays (3.07) and the viable mutation rate was higher in the mutation rate was found to be higher in EMS (4.99) in terms of effectiveness (Table 4). When the mutation rates based on efficiency were compared, EMS was found to efficient as far as injury, lethality and pollen sterility in both varieties of rice are concerned. Similar observation has been recorded by Girija and Dhanvel (2009) and Sharma *et al*. (2005), Kumar and Ratnam (2010).

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Table .1: Effect of different doses of mutagens on pollen and ovule sterility

Mutagenic Treatment	Pollen Sterility %	Ovule Sterility %
Gamma ray (Gy)		
0	92.20	91.00
150	73.00	55.00
200	55.00	46.00
250	45.00	33.00
300	15.00	10.00
350	10.00	5.00
Mean	48.41	40.00
SE(d)	3.326	40.05
CD(0.05)	8.42	2.528
EMS (mM)		
10	82.00	65.00
15	69.00	53.00
20	46.00	28.00
25	21.00	11.00
30	12.00	5.00
Mean	54.02	42.18
SE(d)	1.04	0.91
CD(0.05)	2.36	2.64

Table 2. Mutagenic effectiveness and efficiency based on chlorophyll mutants

Treatments	Total No of plant studied	Total No of plant mutant	lethality	Injury	Sterility	Mutation (M) per 100 M_2 Seedlings	Mutagenic Efficiency			
							MX100 Cxt (or) kR	MX100 L	MX100 I	MX100 S
Gamma rays										
200Gy	1224	10	60.96	61.08	50.55	0.82	4.10	1.35	0.33	0.08
250Gy	1062	8	39.03	33.31	36.35	0.75	3.00	1.92	0.75	0.08
EMS										
15mM	1008	38	61.80	38.90	58.27	3.77	4.19	6.10	2.31	0.07
20mM	1024	42	39.93	33.33	30.76	2.34	1.95	5.86	3.60	0.06

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EMS										
15mM	1008	38	61.80	38.90	58.27	3.77	4.19	6.10	2.31	0.07
20mM	1024	42	39.93	33.33	30.76	2.34	1.95	5.86	3.60	0.06

Table 3. Mutagenic effectiveness and efficiency based on viable mutants

Treatments	Total No of plant studied	Total No of plant mutant	lethality	Injury	Sterility	Mutation (M) per 100 M_2 Seedlings	MX100 Cxt (or) kR	Mutagenic Efficiency		
								MX100 L	MX100 I	MX100 S
Gamma rays										
200Gy	1224	12	60.96	61.08	50.55	0.98	4.90	1.61	0.34	0.10
250Gy	1062	10	39.03	33.31	36.35	0.94	3.76	2.41	0.51	0.10
EMS										
15mM	1008	58	61.80	38.90	58.27	5.75	6.39	9.30	1.20	0.11
20mM	1024	44	39.93	33.33	30.76	4.30	3.58	10.77	2.41	0.12

Table 3. Mutagenic effectiveness and efficiency based on viable mutants

Treatments	Total No of plant studied	Total No of plant mutant	lethality	Injury	Sterility	Mutation (M) per 100 M_2 Seedlings	MX100 Cxt (or) kR	Mutagenic Efficiency		
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200Gy	1224	12	60.96	61.08	50.55	0.98	4.90	1.61	0.34	0.10
250Gy	1062	10	39.03	33.31	36.35	0.94	3.76	2.41	0.51	0.10
EMS										
15mM	1008	58	61.80	38.90	58.27	5.75	6.39	9.30	1.20	0.11
20mM	1024	44	39.93	33.33	30.76	4.30	3.58	10.77	2.41	0.12

Table 4. Mutation rates of Mutagen in terms of effectiveness and efficiency in M_2 generation

Mutagen	Mutation rate in terms of Effectiveness		Mutation rate in terms of Efficiency			Mutation rate in terms of Efficiency		
	Chlorophyll mutants	Viable	Chlorophyll mutants			Viable		
			Injury	Lethality	Sterility	Injury	Lethality	Sterility
Gamma rays (Gy)	3.55	4.33	0.54	1.63	0.08	0.42	2.01	0.10
EMS (mM)	0.3104	4.99	2.96	5.98	0.07	1.80	10.04	0.11

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REFERENCE

Awan, A.M, Konzak, C.F., Rutger, J.N. and Nilan, R.A. 1980. Mutagenic effects of sodium azide in rice. Crop Sci., 20:663-668. | Ahmed JS (1995). Effectiveness and efficiency in urdbean (Vignamungo (L.) Hepper).Indian J. Genet. 65(1): 20-22. | Ashok kumar .V, R.Usha kumari, N.Vairam and R.Amutha .2010.Effect of physical mutagen on expression of characters in arid legume pulse cowpea (Vigna unguiculata (L.) Walp.).Electronic Journal of Plant Breeding, 1(4): 908-914. | Girija, M. and Dhanvel, D. 2009. Mutagenic Effectiveness and Efficiency of Gamma rays, EMS and their combined treatments in cowpea (Vigna unguiculata L. Walp.).Global J. Mol. Sci., 4: 68-75. | Gaul, H. 1970. Mutagen effects observable in the first generation. i. Plant injury, lethality, ii. Cytological effects, iii. Sterility. Manual on mutation breeding (Tech. Pl. Series, No. 119). IAEA, Vienna: 85-89. | Gautam, A.S, Sood, K.C.1992. Mutagenic Effectiveness and Efficiency of gamma-rays, EMS and synergistic effects in Black gram. Cytologia. 57: 85-89. | Kumar, P. Raja Ramesh and Ratnam, S. 2010. Mutagenic effectiveness and efficiency in varieties of sunflower (Helianthus annuus L.) by separate and combined treatment with gamma rays and sodium azide. African J. Biotech. 9: 6517-6521. | Khan, M.N. 1999. Mutagenic effectiveness and efficiency of EMS, gamma rays and their combinations in blackgram (Vigna mungo (L.) Hepper). Advances Plant Sci., 12: 203-205. | Konzak, C.F., R.A. Nilan, J. Wanger and R.J. Foster. 1965. Efficient chemical mutagenesis. The use of induced mutation in plant breeding. Rad. Bot.,5 (suppl.): 49-70. | Kousar Makeen, Suresh B.G., G.R.Lavanya.2010.mutagenic effectiveness and efficiency of gamma rays, sodium azide and their synergistic effects in black gram(V.MUNGO L.). EJEAFChe, 9 (5):860-865. | Kumar, R.P. and K. Das. 1973. Radiation induced chromosomal interchanges in Brassica campestris L. Cytologia., 38: 587-592. | Packiaraj D (1988). Studies on induced mutagenesis of parents and hybrid in cowpea. M.Sc. (Ag.) Thesis, TNAU, Coimbatore. | Sharma,S.K.,Ritu Sood and Pandey, D.P. 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (Vigna mungo (L.) Hepper). Indian J. Genet., 65: 20-22. | Vanniarajan, C. 1989. Studies on induced mutagenesis in blackgram (Vigna mungo (L.) Hepper). M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore. | Waghmare, V.N. and R.B. Mehra. 2001. Induced chlorophyll mutants, mutagenic effectiveness and efficiency in Lathyrus sativus L. Indian J. Genet., 61:53-56.