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National Academy of Agricultural Science (NAAS) Rating : 3. 03

Mutagenic Effects in M_1 Generation of Mungbean (*Vigna radiata* (*L.*)Wilczek)

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ABSTRACT: *Mungbean is used as a pulse in the preparation of "dahl", a soup porridge eaten with a cereal or other traditional cuisines, and is a main protein source (20.8 to 31.8%) for the vegetarian diet. Mutation techniques are the best methods to enlarge the genetically conditioned variability of a species within a short time. An investigation was carried out in two mungbean genotypes CO (Gg)7 and NM 65 to find out the mutagenic effects in M_1 generation. Two mutagens viz., gamma rays and ethyl methane sulphonate are used in this study. The characters* viz., *germination percentage, root length, shoot length, plant height at vegetative and maturity phase, pollen fertility and seed fertility were studied. The results revealed that the two mutagens were sensitive for the two genotypes. It is inferred that mungbean shows high response to physical and chemical mutagens.*

Key words: *Ethyl methyl sulphonate, Gamma ray, Genotypes, Mungbean, Mutagenic effect.*

INTRODUCTION

Greengram (*Vigna radiata* (L.)Wilczek), popularly known as mungbean, is third most important pulse crop of India (Grover, 2011). It is used as a pulse in the preparation of "dahl", a soup porridge eaten with a cereal or other traditional cuisines, and is a main protein source (20.8 to 31.8%) for the vegetarian diet. A mungbean crop can increase the yield of a subsequent rice crop by up to 8% through the nitrogen it fixes in the soil and by reducing pest and disease problems (Weinberger, 2003). Due to lack of sufficient natural variability for yield and its component traits in mungbean, conventional methods of breeding have limited scope.

Mutation breeding may be an effective tool for generating variability in the existing varieties and selecting desirable early maturing lines which could be proved to be an ideal crop for summer seasons (Khan and Goyal, 2009). Induced mutation provided a modern and fruitful tool in crop plants for creating genetic variability (Khan, 1988.) Physical and chemical mutagenic agents cause genes to mutate at rates above the spontaneous base line, thus producing a range of novel traits and broadening of the genetic diversity of plants (Lagoda, 2007). The direct use of mutation by gamma rays and ethyl methane sulphonate (EMS) is a valuable approach to plant breeding, particularly when it is desired to improve one or a few characters in well-adapted variety. EMS is reported to be the most effective and powerful mutagen. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue.

Biological damage caused by mutation to germination, seedling injury, pollen sterility and survival at maturity may be considered as an indication of mutagenic effect. Hence, the present investigation in mungbean was undertaken with physical (gamma rays) and chemical (Ethyl Methane Sulphonate, EMS) mutagens at different concentrations to assess their sensitivity. It is helpful in determining the effect and mechanism of action of the mutagen for further mutation breeding programme.

MATERIALS AND METHODS

The experiment materials used in the present study consisted of Co (Gg)7 and NM 65 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The investigation envisaged studying the differential sensitivity of the

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mungbean by subjecting them to different doses of the two mutagens *viz.*, gamma rays and ethyl methane sulphonate. Gamma irradiation was done using cobalt 60 sources in the Gamma chamber, installed at Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The chemical mutagen, ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) with molecular weight 124.16, from the sigma chemical company, USA was used for treating the seeds. For the assessment of LD_{50} dose three hundred seeds of uniform size were used for (Gamma rays – 300, 400, 500 and 600 Gy and EMS – 10, 20 and 30 mM) each treatment. In respect of EMS treatment, the seeds were presoaked in distilled water for six hours. Appropriate quantities of EMS were dissolved in distilled water to have the concentrations envisaged in the program. The treatment was performed at room temperature 22 +2 °C early morning hours with intermediate shaking during the treatment period of 6 hours. After the chemical treatment, the seeds were washed thoroughly with running tap water for half an hour to remove the residues of the chemical, if any and the excess moisture in seed coat was removed by using folds of blotting paper.

About 500 seeds of each treatment were sown in the experiment field along with control at the spacing of 30 x 10 cm in randomized block design with two replication to rise M_1 generation during Kharif season of 2013 at the research farm of Agricultural College and Research Institute, TNAU, Madurai. The germination percentage, survival were recorded during 3rd and 7th days after sowing. The mean values of germination percentage, shoot length and root length were worked out for each dose of a mutagenic treatment and expressed as per cent over control and per cent reduction. Based on 50 per cent reduction in germination, the LD_{50} was fixed for both physical and chemical mutagens. Survival of plants at maturity and morphological changes in different treatments for each cultivar were recorded in the field. The pollen sterility was observed at flowering stage on 30 plants per treatment selected randomly. Seed fertility percentage of well developed seeds to the total weight of seeds was worked out for each plant and the mean seed fertility was arrived.

RESULTS AND DISCUSSION

Effect of Mutagenic Treatments in M_1 Generation

EMS and gamma rays are potent mutagens well known for their action in inducing point mutations, enzyme inhibitions and chromosomal aberrations. The observed data on the effect of gamma rays and EMS treatments on seed germination are presented in significant reduction in germination percentage was observed in all the treatments. The reduction percentage obtained for different characters for both the genotypes are presented in the Table 1 and Table 2. The fifty percent reduction occurred between two treatments, 400 Gy and 500 Gy in CO (Gg) 7 and NM 65 for gamma rays and between 20 mM to 30 mM in CO (Gg) 7 and NM 65 for EMS. The gradual reduction in germination over control was found to be directly proportional to the increase in dose levels as indicated by highly significant negative correlation. Similar reduction in germination per cent over control was noticed by Satpute and Fultambkar, 2012 in Soyabean.

The influence on shoot and root growth has been related to many factors which may be attributed to chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanism and chromosomal damage cum mitotic inhibition (Riley, 1954). The shoot length per cent reduction ranged from 16.07 (300 Gy) to 53.02 (600 Gy) in CO (Gg) 7 and from 8.25 (300 Gy) to 60.15 (600 Gy) in NM 65 for gamma rays. In EMS, the range was between 0.57 (10 mM) to 17.91 (30 mM) in CO (Gg) 7 and 6.40 (10 mM) to 24.01 (30 mM) in NM 65. In gamma ray and EMS treatment, the trend observed was regular with continuous reduction for mean shoot length, when the dose level increased. An inverse relationship was exhibited between the mutagenic dosages and the rate of shoot and root development.

Observation on the survival of plants on 30th day is presented in the dose differences were found to be highly significant. The reduction in percentage of survival ranged from 23.44 (300 Gy) to 57.45 (600 Gy) in CO (Gg) 7 and 21.98 (300 Gy) to 57.63 (600 Gy) in NM 65 for gamma rays and it varied from 32.48 (10 mM) to 52.55 (30 mM) in CO (Gg) 7 and 29.06 (10mM) to 51.17 (30 mM) in NM 65 for EMS. These results indicated that survival rate was gradually reduced with increase in doses. Similar reduction in survival was reported earlier by Tambe (2009) in soybean and Satpute (2009) in lentil.

The data regarding pollen fertility was analyzed after arc sine inverse transformation. Both the mutagenic treatments revealed the existence of significant difference between the means of pollen fertility in both the genotypes. Reduction of pollen fertility occurs due to inactivation of certain genes, thereby upsets genetic and physiological equilibrium,

Table 1
Reduction Percentage of Different Characters in M_1 generation – Gamma rays

Characters	*Control*		*300 Gy*		*400 Gy*		*500 Gy*		*600 Gy*	
	CO(Gg) 7	*NM 65*	*CO(Gg) 7*	*NM 65*	*CO(Gg) 7*	*NM 65*	*CO(Gg) 7*	*NM 65*	*CO(Gg) 7*	*NM 65*
Germination percentage	-	-	27.60	26.93	50.87	49.55	52.62	53.34	55.49	55.69
Shoot length	-	-	16.07	8.25	22.11	25.40	46.14	40.92	53.02	60.15
Root length	-	-	2.90	6.27	17.40	29.22	39.50	39.31	47.11	61.22
Plant height on 30th day	-	-	5.47	6.27	9.63	11.42	17.87	15.50	23.62	26.52
Plant survival on 30th day	-	-	23.44	21.98	36.29	36.42	41.80	42.19	57.45	57.63
Pollen fertility	-	-	13.60	11.34	33.25	22.36	42.95	33.68	48.63	44.72
Plant height at maturity	-	-	4.26	6.09	16.18	12.63	21.22	24.58	29.42	30.99
Seed fertility	-	-	8.06	8.32	20.9	23.27	29.23	30.58	34.42	33.56

Table 2
Reduction Percentage of Different Characters in M_1 generation – EMS

Characters	*Control*		*10 mM*		*20 mM*		*30 mM*	
	CO (Gg) 7	*NM 65*	*CO (Gg) 7*	*NM 65*	*CO (Gg) 7*	*NM 65*	*CO (Gg) 7*	*NM 65*
Germination percentage	-	-	29.23	28.57	50.12	50.36	53.07	52.59
Shoot length	-	-	0.57	6.40	9.52	19.61	17.91	24.01
Root length	-	-	6.16	11.66	12.32	19.83	38.41	42.96
Plant height on 30th day	-	-	8.26	5.62	12.57	12.47	18.30	28.27
Plant survival on 30th day	-	-	32.48	29.06	41.38	41.59	52.55	51.17
Pollen fertility	-	-	14.71	13.54	39.80	33.61	48.01	42.80
Plant height at maturity	-	-	3.87	7.90	14.69	16.24	25.97	22.64
Seed fertility	-	-	10.21	6.54	25.62	19.74	35.50	36.11

physiological disturbance, chromosome structural changes and point mutations (Rana and Swaminathan, 1964; Gaul 1977). In CO (Gg) 7, the percent reduction for pollen fertility ranged from 13.60 (300 Gy) to 48.63 (600 Gy) and from 11.34 (300 Gy) to 44.72 (600 Gy) in NM 65 for gamma rays. In EMS, the per cent reduction for pollen fertility ranged from 14.71(10 mM) to 48.01 (30 mM) in CO (Gg) 7 and from 13.54 (10 mM) to 42.80 (30 mM) in NM 65. Balai and Ram Krishna (2009) also observed steady reduction in germination, survival and pollen viability in mungbean with increasing doses of chemical mutagens in mungbean.

The per cent reduction in plant height on 30th day ranged varied from 5.47 (300 Gy) to 23.62 (600 Gy) in CO (Gg) 7 and from 6.27 (300 Gy) to 26.52 (600 Gy) in NM 65 for gamma rays. In case of EMS treatment, the mean plant height was found to be decreased from 10mM (8.26) to 30mM (18.30) in CO (Gg) 7 and from 10mM (5.62) to 30mM (28.27) in NM 65 and it showed declining trend with increasing doses of EMS. In CO (Gg) 7, the percent reduction in plant height at maturity ranged from 4.26 (300 Gy) to 29.42 (600 Gy) in CO (Gg) 7 and from 6.09 (300 Gy) to 30.99 (600 Gy) for gamma rays. In EMS treatments, plant height at maturity was lesser than that of the control. The per cent reduction in plant height was between 3.87 (10mM) to 25.97 (30mM) in CO (Gg) 7 and from 7.90 (10mM) to 22.64 (30 Mm) due to EMS treatments. Khan and Wani (2006) also reported the reduction in plant height in greengram.

The per cent reduction of seed fertility ranged from 8.06 (300 Gy) to 34.42 (600 Gy) in CO (Gg) 7 and 8.32 (300 Gy) to 33.56 (600 Gy) in NM 65 for gamma rays. In EMS treated population the seed fertility ranged from 10.21 (10mM) to 35.50 (30mM) in CO (Gg) 7 and from 6.54 (10 mM) to 36.11 (30 mM) in NM 65 than per cent over control. The per cent of reduction in pollen ferility and seed fertility showed increasing trend with increasing doses of mutagens. Manju *et al.* (1983) recorded similar findings in horse gram.

CONCLUSION

From the present investigation, it is referred that both the genotypes might be considered as sensitive to both the mutagens used. Reduction in the characters occurred with increase in dose or concentration of mutagens. Genetic variation created through mutation in mungbean could be further studied and screened in M_2 generation.

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