Plant Archives Vol. 14 No. 1, 2014 pp. 499-501 *ISSN 0972-5210*

EFFECT OF MUTAGENESIS ON GERMINATION, SURVIVAL, POLLEN AND SEED STERILITY IN M_1 GENERATION OF BLACK GRAM [*VIGNA MUNGO* (L.) HEPPER]

B. Ramya* and G. Nallathambi

Department of Plant Genetic Resources, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore - 003 (Tamil Nadu), India.

Abstract

M_1 generation of Black gram [*Vigna mungo* (L.) Hepper] was raised by treating the seeds of TNAU Co (Bg) 6 with varied concentration of chemical mutagen (EMS) and physical mutagen (Gamma rays). A dose dependant decrease was noticed in most of the characters in M_1 generation. The results indicated that the reduction in germination percent over control was noticed in all mutagenic treatments while increased pollen sterility was associated with corresponding increases in dose / concentration of mutagens.

Key words : Black gram, EMS, gamma rays, germination, survival, pollen sterility.

Introduction

Black gram [*Vigna mungo* (L.) Hepper] is an important pulse crop of our country. It belongs to the family leguminosae and subfamily papilionaceae. The chromosome number of this crop is 2n = 2x = 22 (Bhatnagar *et al*., 1974). It is a highly self-pollinated crop with cleistogamous nature. Creation of variability through pollination and artificial hybridization is very difficult as the flowers are cleistogamous and very delicate to handle. Even if hybridization is carried out the seed set is less than 5 per cent. Also, this crop lacks proper male sterility system commercially to be utilized for hybridization. Hence, the present investigation aims at creation of variation through induction of mutation through chemical mutagens and physical mutagens.

Materials and Methods

Seeds of black gram variety TNAUCo (Bg) 6 dry healthy and uniform size were collected from department of pulses. This investigation envisaged studying the different mutagens gamma and EMS. Gamma irradiation was done using cobalt 60 (^{60}Co) sources in the gamma chamber, installed at Tamilnadu Agricultural University, Coimbatore. The chemical mutagen Ethyl methane sulphonate ($CH_3SO_2O\ C_2H_5$) with molecular weight 124.16 from sigma chemicals.

For assessment of LD_{50} doses seed were treated gamma rays at 150, 200, 250, 300, 350Gy doses and EMS at 10, 15, 20, 25, 30mM concentrations. Five hundred seeds were pre soaked for six hours in water initially then the seeds were immersed for six hours in the requisite concentration of mutagen EMS with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of mutagen solution was maintained at proportion of ten times to that of the seed volume. The whole treatment was carried out at a room temperature of 28±2°C for four hours after washed in running water, untreated seeds were used as control. The treated seeds of gamma rays, EMS and control seeds were immediately sown in the field in a randomized block design (RBD) with three replications raised M_1 generation during summer 2010. Each treatment consists of three rows of 5m length/ replication, in which 50 seeds per row were sown with 10 × 30 cm distance between plants and rows, respectively.

Germination percentage : The number of seed emergence of the radical was counted and mean was expressed as percentage.

Plant survival : The number of plant reaching maturity in the field was noted and expressed as percentage.

***Author for correspondence :** E-mail:balramagri@gmail.com

Table 1 : Effect of different mutagens in M_1 generation of black gram variety TNAUCO (Bg) 6.

Treatment	Seed germination 7th day (%)	Survival %			Fertility %	
		15th day	21st day	30th day	Pollen	Seed
Gamma ray (Gy)						
0	98.5	95.00	91.00	90.00	92.20	91.00
150	76.80	74.50	70.50	70.00	73.00	55.00
200	63.40	62.25	56.25	55.00	55.00	46.00
250	43.50	41.00	36.80	36.00	45.00	33.00
300	30.00	27.30	22.00	21.00	15.00	10.00
350	28.50	24.40	21.40	20.00	10.00	5.00
Mean	56.6	5.22	1.72	0.50	48.40	40.00
EMS (mM)						
10	79.00	76.40	72.50	68.60	82.00	65.00
15	69.30	66.30	61.50	60.00	69.00	53.00
20	41.30	40.00	38.40	38.00	46.00	28.00
25	28.20	26.30	23.20	23.00	21.00	11.00
30	12.50	9.50	7.50	7.00	12.00	5.00
Mean	54.80	74.50	49.00	47.80	53.70	42.20
SE (d)	4.13	4.00	3.74	3.63	3.77	3.31
CD (0.05)	8.68	8.40	7.85	7.64	7.93	6.96

Pollen sterility : The pollen grains were collected 10 randomly selected plants with clean glass slides by dusting anthers of single flower that were about to dehisce and stained with acetocaramine : glycerine (1:1) mixture. Well filled and fully stained pollens were counted as fertile, while the unstained and shrunken ones as sterile.

Seed fertility : Number of well filled seeds per pod was counted in five pods for each plant separately in each treatment in each replication. The percentage of well-developed seeds was worked out for each plant and mean seed fertility was arrived at.

Results and Discussion

Germination percentage

The date of germination percentage in M_1 generation for various mutagenic treatments in TNAU Co (Big) 6 is given in the table 1. In the present study, under field conditions, there was inhibition in germination percentage in the entire EMS and gamma ray doses in the comparison to the control. The similar result were reported by Sharma *et al*. (2005), Vanniarajan (1989) and Sarkar *et al*. (1996). The lowest germination 12.50 was recorded in 30mM EMS concentration in which may be due to physiological and acute chromosomal damage Singh *et al*. (1997). Delay in the one set of mitosis Yadav (1987) and chromosomal aberration induced enzyme activity such as catalyse, lipase and hormonal activity results in reduced germination (Ananthaswamy *et al*., 1971). Reduction in germination over control ranged from 98.5 to 28.50 of gamma radiation and ranged from 79.00 to 12.50 of EMS the findings are close agreement with the earlier reports of Vanniarajan (1989), Sarkar *et al*. (1996).

Survival percentage

Survival (at flowering) due to different mutagenic treatment in TNAU Co (Bg) 6 ranged from 7.00 (30mM) to 70.00 (100 Gy) (table 1). The decrease in survival percentage was associated with increases in the dose / concentration of the mutagens in both the cultivars. These findings are close agreement with the earlier reports of Ignaimuthu and Babu (1988) and Vanniarajan (1989), Sharma *et al*. (2005) in gamma rays. In EMS reduction in survival could be attributed to chromosome caused reduction in fertility or physiological distributes by mutagenic effects (Sato and Gaul, 1967).

Pollen and seed fertility

The effect of mutagen was more prominent in terms of pollen and seed sterility, which is an increase as dose increases in both the mutagens blackgram. The maximum sterility was observed in 82.00% 10 mM of EMS and 10.00% in 350Gy gamma ray. The increasing pollen sterility has been mainly attributed to chromosomal interchange, chromosomal aberration, gene mutation (Gautam *et al*., 1992), cytoplasmic factors (Malinoveskii *et al*., 1973). In most cases, meiotic abnormalities are

responsible for pollen sterility (Muthusamy and Jayabalan, 2002) in cotton and Khan and Wani (2005) in chickpea. In the present findings, the increase in pollen sterility because of mutagenesis is in accordance with the findings in Ignacimuthu and Babu (1989) wild and cultivated urd and mungbean. The gradually increase percentage of pollen sterility with increase dose/ concentration was in conformity with the earlier reports in Bhaskaran (1978), Chaturvedi *et al*. (1983) and Ahmed John (1996), Singh and Mohapatra (2004) in blackgram, Dixit and Dube (1988) in lentil, Kulkarni (2011) horsegram and Sangle *et al*. (2011) in pigeonpea.

Conclusion

From present study, it can be concluded that both mutagens showed an inhibitory effect on germination, survival and pollen and seed sterility percentage. The concentration / dose used in present study will be effective in induction of wide range of mutants.

Acknowledgments

The authors would like to acknowledge BRNS for funding the research work. We gratefully thank the Professor & Head, Department of P.G.R., Department of Millets and the Director, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University (Tamil Nadu), India for their help in the research work. Sincere thanks also to the advisory committee members Dr. J. R. Kannan Bapu, Dr. S. Ganesh Ram, Dr. N. Kumaravadivel and Dr. P. Govindaraju for their suggestions and technical support.

References

Ahmed John, S. (1991). Mutation studies in blackgram (*Vigna mungo* (L.) Hepper), *Ph.D. Thesis*, Bharathidasan University, Triruchirapalli.

Ananthswami, H. N., V. K. Vikil and A. Sreenivasan. (1971). Biochemical and physiological changes in gamma irradiated wheat during germination. *Rad. Bot.*, **11** : 1-2.

Bhaskaran, M. (1978). Studies on induced mutagenesis in blackgram. *M.Sc. (Ag.) Thesis*, Tamil Nadu Agric. Univ., Coimbatore.

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

Dixit, P. and D. K. Dube (1988). Mutagenic efficiency of gamma rays, NMU and their combination in lentil (*Lens culinaris* Medik.) var. T-36. *Indian J. Genet*., **43(3)** : 501-506.

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

Ignacimuthu, S. and C. R. Babu (1989). Induced chromosomal and pollen sterility in wild and cultivated urd and mungbean. *Cytologia*., **51(1)** : 159-167.

Khan, S. and M. R. Wani (2005). Genetic vriability and correlation studies in chickpea mutants. *J. Cytol. Genet.*, **6** : 155-160.

Malinoveskii, B. N., N. M. Zoz and A. I. Kitaev (1973). Induction of cytoplasmic male sterility in sorgum by chemical mutgens. *Genetica*., **9** : 19-27.

Muthusamy, A. and N. Jayabalan (2002). Effect of mutagens on pollen fertility of cotton (*Gossypium hirsutum* L.). *Indian J. Genet.*, **62(2)** : 187.

Sangle Sunil, M., Swapnil E. Mahamune, Sopan N. Kharat and V. S. Kothekar (2011). Effect of mutagenisis on germination and pollen starility in pegionpea. *Bioscience Discovery*, **2(1)** : 127-130.

Sarkar, K. K., S. Sharma and M. Hussian (1996). Induced genetic variability in mungbean in M_2 generation. *Environ. Biol*., **14(4)** : 815-817.

Sato, M. and H. Gaul (1967). Effect of EMS on the fertility of barley. *Rad. Bot*., **7** : 7-15.

Sharma, S. K., Ritu Seed and D. P. Pandey (2005). Studies on mutagen sensitivity, effectiveness and efficiency in urdbean [*Vigna mungo* (L.) Hepper]. *Indian J. Genet*., **65(1)** : 20-22.

Singh, G., P. K. Sareen and R. P. Saharan (1997). Mutation studies is mungbean (*Vigna radiate* L. Wilczek). *J. Nuclear Agric. Biol.*, **26(4)** : 227-231.

Singh, B. and B. K. Mohapatra (2004). Prediction of M_2 mutation frequency based on M_1 estimates in blackgram. *Legume Res*., **27(2)** : 137-139.

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

Yadav, R. D. S. (1987). Effect of mutagens on mitotic index, seedling vigor and chlorophyll mutation in mungbean (*Vigna radiate* L. Wilczek). *J. Nuclear Agric. Biol.*, **16(1)** : 13-17.