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INFLUENCE OF GAMMA RAYS ON GERMINATION, SURVIVAL AND POLLEN STERILITY IN BLACK GRAM (*Vigna mungo* L.) MUTANTS

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ABSTRACT
Induced mutation in plant improvement has been used in several crops to generate new sources of genetic variations. The M_1 generation of black gram was raised by treating the seeds of variety ADT 3 and CO 6 with varied doses of gamma rays (200 Gy, 300 Gy and 400 Gy) for studying seed germination, survival of plants and pollen sterility. The two varieties of black gram showed differential response towards mutagenic treatments to the above parameters. Seed germination and survival of plants decreased with increasing dose of the mutagen in both cultivars of black gram. The results showed that gamma irradiation had major influence on black gram varieties and two varieties also responded well.

INTRODUCTION

Black gram (*Vigna mungo* L.Hepper) is the important food legume and rich in protein. In spite of its importance, the crop however has very limited genetic variability (Arvind kumar *et al.*,2007). Therefore, development of new plant types for different situation is required. Mutation techniques are the best methods to enlarge the genetic variability of a species within a short time (Micke 1988). Biological damage caused by mutation for germination, pollen sterility and survival at maturity may be considered as an indication of mutagenic effect (Gaul 1964)

Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding of plants is useful to improve the character if the character is not located in a plant germplasm of a species, and also for generating variability in the existing varieties (Khan and Goyal , 2009).Induced mutation using physical and chemical mutagens is one method to create genetic variation resulting to new varieties with better characteristic features (Wongpiyasatid *et al.,* 2000).

Ionization radiations still remain most suitable agents for inducing genetic variability (Tah 2006). Application of radiation has been most frequently used for induction of mutation resulting in direct development of 89% mutant varieties (Velmurugan *et al.,* 2010). Gamma irradiation is one of the main physical mutagens for mutation studies in plants. It had diverse effect on traits of plants and this depended on plant species or varieties and the dose of irradiation (Saxesena *et al.,* 2014). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (wi *et al.,* 2005).

Gamma irradiation has provided number of useful mutants and still shows an elevated potential for improving vegetatively propagated plants. A great majority of mutant varieties (64%) were developed by the use of gamma rays (Ahloowalia *et al.,* 2004).There are different types of ionizing radiation *viz*., X-rays, gamma rays, protons, neutrons, alpha and beta particles. However, gamma rays are widely employed for mutation studies as they have shorter wave length and therefore, possess more energy per photon than X- rays and penetrate deep into the tissue (Khin, 2006 and Zhu *et al*.,2006).

Hence, the present study was undertaken to study the response of two black gram varieties for seed germination, survival and pollen sterility to the gamma ray exposure.

MATERIALS AND METHODS

Plant material

The experimental plant material (seed) of two black gram cultivars i.e. ADT 3 and CO 6 were collected from *Tamil Nadu Rice Research* Institute*, Aduthurai* and from the Department of Pulses Tamil Nadu Agricultural University, Coimbatore respectively. The trials were laid out in randomized block

design with two replications at Agricultural College and Research Institute (ACRI), TNAU, Madurai, during Rabi 2016.

Mutagen treatment

Well filled and healthy 500 seeds of ADT 3 and CO 6 with the moisture content of 12 per cent were packed in butter paper covers and placed in an irradiation chamber located in vertical drawer inside the lead flask in gamma chamber. The seeds for each treatment were irradiated in the gamma chamber at 200,300 and 400 Gy doses of gamma rays. Seed material was exposed to gamma irradiation from the Cobalt 60 gamma source for appropriate time for each dose based on the half-life of the source in the gamma chamber installed at the Bhabha Atomic Research Centre. Mumbai

The non irradiated dry seeds were taken as the control. After the completion of the treatment the treated seeds were sown in the field along with their respective control to raise the M_1 generation in a randomized block design with two replications. All the treatments including the control were raised adopting a spacing of 30 cm in between rows and 10 cm in between plants. Number of seeds germinated on 7th day was counted and the germination percent was calculated. Survival percentage at 45 DAS crops was recorded and the survival percent was calculated. Pollen fertility was observed during flowering period.

Germination percentage

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

Pollen sterility

Pollen sterility was determined from five randomly selected plants of each treatment along with control. The anthers from the fresh flowers were removed and burst at one end. The pollen mass was smeared on a slide followed by addition of a few drops of 1% potassium iodide (the stain was prepared by dissolving 1 gm of iodine and 2 gm of potassium iodide in 100 ml of distilled water). Fully stained pollen grains were considered as fertile while empty, partially stained and shriveled ones were classified as sterile and round pollen grains were recorded as fertile and those mixed with both the types were classified as partially fertile. The pollen fertility percentage of the pollen grains was calculated based on the formula given below. The values were expressed as percentage.

$$\text{Pollen fertility percentage} = \frac{\text{Total number of well stained pollen}}{\text{Total number of stained and unstained pollen}} \times 100$$

Survival of plants at maturity

Survival of plants in each treatment and their respective controls were recorded in the field at the time of maturity. The values were expressed as percentage.

RESULTS AND DISCUSSION

Evaluation of the effects of mutagen in M_1 generation is a common procedure in any mutation breeding experiments. Physical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal mutations (chromosomal aberrations) in the biological material of M_1 generation(Gaul The biological damages caused by the mutagens in M_1 generation could be measured based on seed

Table 1: Effect of gamma rays on germination percentage of ADT 3 and CO 6 in M_1 generation

Mutagen (Dose) ADT 3	Germination percentage		
	Transformed Mean (%)	% over control	% reduction
Control	81.87	100	-
200 Gy	57.91	70.74	29.26
300 Gy	41.10	50.20	49.80
400 Gy	34.85	42.57	57.43
CO 6			
Control	79.73	100	-
200 Gy	54.71	68.62	31.38
300 Gy	39.08	49.02	50.98
400 Gy	33.98	42.61	57.39

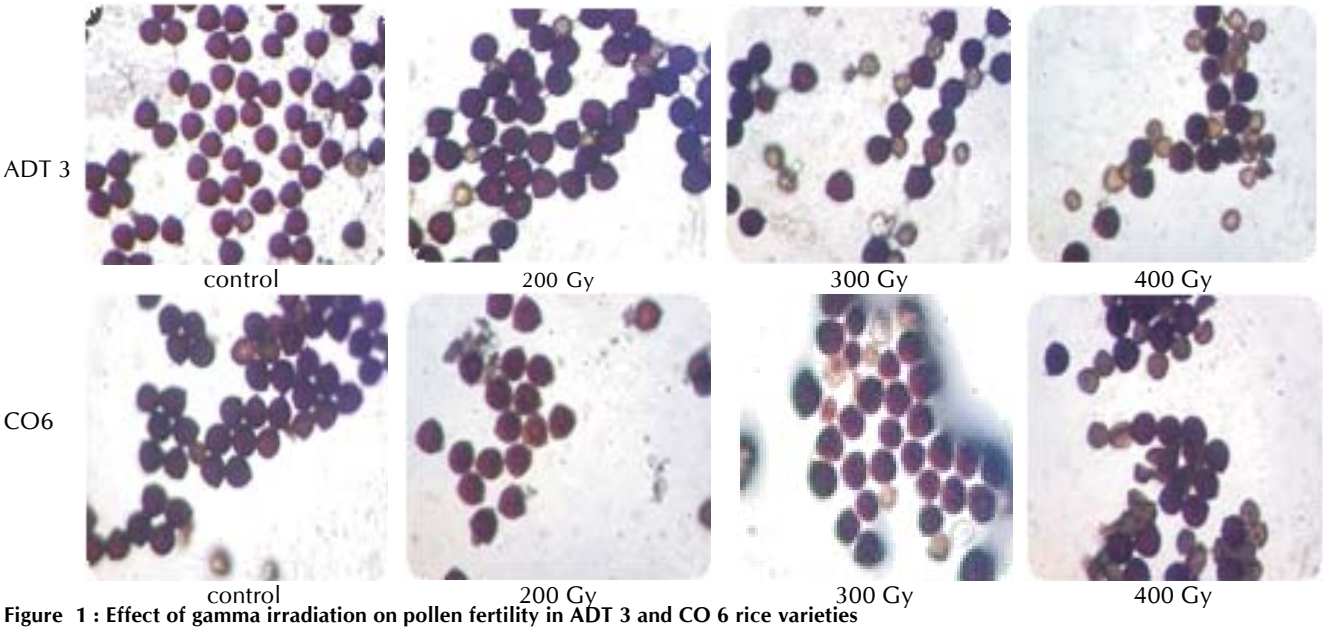

Figure 1 : Effect of gamma irradiation on pollen fertility in ADT 3 and CO 6 rice varieties

Table 2: Effect of gamma rays on plant survival of ADT 3 and CO 6 in M_1 generation

Mutagen (Dose)	Survival percentage Transformed Mean (%)	% over control	% reduction
ADT 3			
Control	75.37	100.00	-
200 Gy	58.05	77.01	22.99
300 Gy	38.96	51.69	48.31
400 Gy	30.82	40.89	59.11
CO 6			
Control	74.04	100.00	-
200 Gy	59.01	79.70	20.30
300 Gy	37.73	50.96	49.04
400 Gy	28.82	38.93	61.07

Table3: Effect of gamma rays on pollen sterility of ADT 3 and CO 6 in M_1 generation

Mutagen (Dose) ADT 3	Pollen fertility Transformed Mean (%)	% over control	% reduction
Control	81.49	100.00	-
200 Gy	62.58	76.79	23.21
300 Gy	42.63	52.31	47.69
400 Gy	35.09	43.06	56.94
CO 6			
Control	79.36	100.00	-
200 Gy	53.32	67.18	32.82
300 Gy	39.22	49.41	50.59
400 Gy	31.04	39.11	60.89

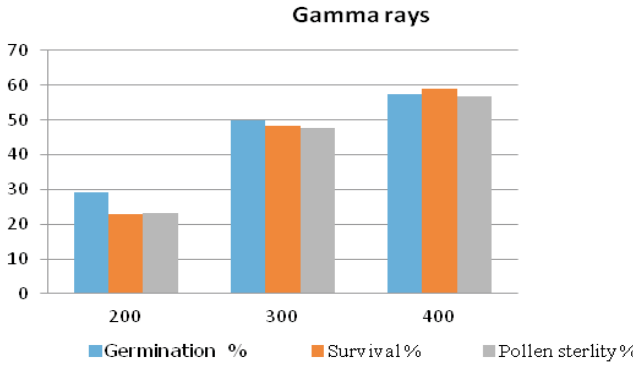

Figure 2: Effect of different doses of gamma rays on germination, seedling survival (30 DAS), and pollen fertility in ADT 3 (percent reduction over control)

germination, survival reduction (lethality) and fertility reduction (sterility). Gaul (1970) reported that the damage to the biological material as reflected in the above parameters might be considered as an indication of the mutagenic effects. As similar to aforesaid findings, the following different M_1 damages (effects) have been studied in the present investigation of black gram.

Effect of mutagens on germination

The data on germination percentage in M_1 generation for various mutagenic treatments in ADT 3 and CO 6 are given in Table 1. In both the varieties, in comparison to the control, the percent germination was low in all treatments. The maximum reduction was observed at higher concentrations of the mutagens and the LD_{50} value (50% reduction of seed germination) was observed in 309.75 Gy of gamma rays for

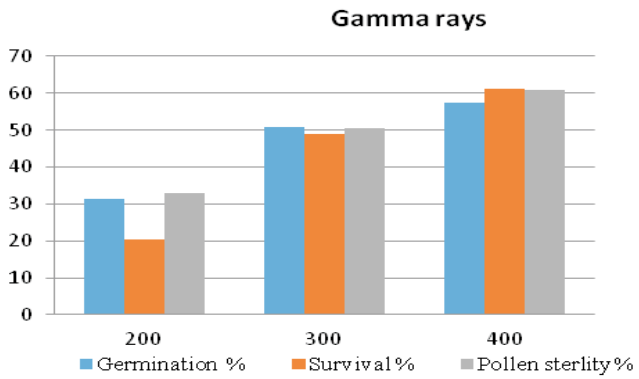

Figure 3: Effect of different doses of gamma rays on germination, seedling survival (30 DAS), and pollen fertility in CO6 (percent reduction over control)

ADT 3 and in 301.06 Gy of gamma rays for CO 6 variety. In case of ADT 3, reduction in germination ranged from 29.26 percent (200 Gy) to 57.43 percent (400 Gy). Similarly, in M_1 generation of CO 6, germination percentage ranged from percent 31.38 percent (200 Gy) to 57.39 percent (400 Gy). Similar inhibitory effects on seed germination observed by Kumari *et al.*, 2013, Padavai and Dhanavel (2004) , Singh and Kole (2005) and Mundhe and Borse (2012).

In accordance with the above findings, in the present investigation, at higher dosage of mutagens, the seed germination got delayed and the seedlings were shorter which subsequently died in a short period. This might be due to the effect of mutagens by which, affected seedlings after the cotyledonary emergence remained alive only for a particular period of time. The mutagenic sensitivity of a biological material can be attributed to the level of differentiation and development of embryo at the time of treatment and also to the extent of damage to the growth processes like rate of cell division, cell elongation, various stages of hormone and biosynthetic pathways observed by Scholz and Lehman (1962).

The reduction in germination per cent due to treatment may be attributed to a drop in the auxin level or chromosomal aberrations as reported by Reed (1959). In the present study, the characteristic feature of effect of irradiation in dicots seems to be that, the affected seedlings, after the emergence of cotyledonary leaves, remain alive in the critical stage for a considerably long time (Dubinin, 1964). During this phase there is some type of repair or unaffected cells after the primary shoot dies or the so called intra-somatic or diplontic selection (Gaul, 1958) takes place and unwanted cells and other disturbances detrimental to the plant are eliminated, alternatively, the seedlings are not able to overcome the radiation damage and hence they die without putting forth any side shoots. The seedling mortality was reported to be due to the assimilation mechanism (Quastler and Baer, 1950) inhibition of auxin synthesis (Skoog, 1935), chromosomal damage and inhibition of mitosis (Gunkel and Sparrow, 1961).

Survival percentage on 30th day

Seedling injury is widely used as an index of determining biological effects of various physical mutagens in M_1 generation. In the present study, the survival of the seedling was reduced with increase in dosage of gamma rays. All the treatments resulted in retardation in the height of seedlings. The maximum seedling injury was 59.11 % in ADT 3 and

61.07 % CO 6 with 400 Gy gamma rays treatment as compared to control (Table no 2) . Also, the increase in survival had an inverse relationship with in dose of mutagens.in the present results are in agreement with the result obtained by Ignaimuthu and Babu (1988) and Vanniarajan (1989).

The differential sensitivity of genotypes may be attributed to their metabolic processes affected in differential manner, either by mutagen uptake or degradation and sites of action in the embryo (Ahmed John, 1996). Sparrow *et al*. (1961) assigned the reduced seedling growth by radiations to inhibition of cell division and extra chromosomal damage. Conger and Stevenson (1969) correlated increased seedling injury with chromosomal damage.

Pollen sterility

In the present investigation, the pollen sterility percentage was gradually decreased with increasing dose of gamma rays when compared to control (Table 3). Pollen sterility in M_1 generation is the first sign of genetic effectiveness of the treatments. The maximum reduction was observed at higher concentrations of the mutagens. Pollen fertility may be influenced by many environmental factors as shown by the control. In M_1 generation of ADT 3, pollen sterility percentage ranged from 23.21 per cent (200 Gy) to 56.94 per cent (400 Gy) and 32.82 per cent (200 Gy) to 60.89 per cent (400 Gy) for CO 6. The gradually increased percentage of pollen sterility with increased dose/ concentration was in conformity with the earlier reports in mungbean (Ignacimuthu and Babu 1989).

The negative effect of mutagens on pollen fertility percentage in mutagenic treated plants may be due to meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains (Khan *et al.,*2005). The sterility of the pollen may be due to physiological and genetic changes or may be due to meiotic aberrations.The increasing pollen sterility has been mainly attributed to chromosomal interchange, chromosomal aberration, gene mutation (Gautam *et al*., 1992) and cytoplasmic factors. In most cases, meiotic abnormalities are responsible for pollen sterility (Muthusamy and Jayabalan, 2002) in cotton and(Khan and Wani , 2005) in chickpea. According to (knozak *et al.,* 1961) induction of the pollen sterility is due to chromosomal irregularities caused by the mutagen.

REFERENCES

Ahloowalia B. S., M. Maluszynski, K. Nichterlein. 2004. Induced mutations - A new paradigm in plant breeding. *Euphytica*. **135:** 187-204.

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

Arvind kumar, Mishra, M. N. and Kharkwal, M. C..2007..Induced mutagenesis in blackgram (*Vigno mungo* (L.)Hepper).*Indian J.Genet.***67 (1):** 41-46.

Conger, A. D. and Stevenson, H. Q. 1969. A correlation of seedling height and chromosomal damage in irradiated barley seeds. Rad. Bot. **9(1):** 1-14.

Dubinin, N. P. 1964. Problems of radiation genetics. Oliver and boyod, London, p. 445.

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

Gaul, H. 1970. Mutagen effects observable in the first generation. I. Plant injury and lethality. II. Cytological effects. III. Sterility Manual on mutation breeding. (Tech.Rep. Series No.119) IAEA, Vienna,pp. 85-99.

Gaul , H.1964. Mutation in plant breeding. *Radiation Botany*. **4(3) :**155 - 232

Gaul, H. 1958. Present aspects of induced mutation in Plant Breeding. Euphytica. **7:** 275-289.

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

Khan.s, S. Goyal. 2009. Mutation genetic studies in mungbean for selection of early maturing mutants.*Thai J. Agricultural Science*. **42(2) :** 109-113.

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

Khin, T. 2006. Rice mutation breeding for varietals improvement in Myanmar. Pl. Mut. Rep. **1(1):** 34-36.

Kumari, K., Dhatt, K. K. and Kapoor, M. 2013. Induced mutagenesis in Chrysanthemum morifolium variety 'Otome Pink' through gamma irradiation. *The Bioscan*. **8(4):** 1489-1492..

Micke, A.1988. Genetic improvement of grain legumes using induced mutation. In Proc.FAO / IEAE workshop on improvement of grain legume production using induced mutation , 1-5 july , 1986 pullman , Washington (U.S.A) IAEA, Vienna, 1985 ,pp. 1-51

Mundhe and Borse, R. D.2012. Studies of Mutagenic Sensitivity in Soybean (Glycine max L.). *J. Phytol. Res*. **25(2):** 239-240.

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

Quastler, H. and Baer, M. 1950. Inhibition of plant growth by irradiation. III. Successive radiation effects homologous responses. *Biol. Abstr*. **24:** 30984.

Reed, J. 1959. Radiation on biology of Vicia faba in relation to the general problem. Oxford Backwell scientific Publications.

Saxesena, R. R., Vidyakar, V., Manish, K., Vishwakarma, Punam, S., Meena, Y. and Lal, G. M. 2014. Genetic variability and heritability analysis for some quantitative traits in field pea (Pisum Sativum L.). *The Bioscan*. (Supplement on Genetics and Plant Breeding). **9(2):** 895- 898.

Scholz, F. and Lehman, C. O. 1962. Die Gatensle bener Mutantender saat gerstle in Bezihnug Zur Formrumarnningfeltigkeit der Art. Hordeum vulgare. L.S. IV. Kulturpflanse, **10:**312-314.

Singh R. and Kole C. R. 2005. Effect of mutagenic treatment with EMS on germination and some seedling parameters in mungbean. *Crop. Res*. **30(2):** 236-240.

Skoog, F. 1935. The effects of X-irradiation of auxin and plant growth. *J. Cell. Com. Physiol*.**7:** 227-250.

Sparrow, A. H., Cuany, R. L., Miksche, J. P. and Schairer, L. A. 1961. Some factors affecting the response of plants to acute and chronic radiation exposure. *Radiation botany*. **1:** 10-34.

Tah P. R. 2006,Radio sensitivity of rice genotypes to gamma radiations based on seedling traits and physiological indices. Asian Journal of Plant Science. **5:** 61- 70.

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

Velmurugan M., K. Rajamani, P. Paramaguru, R. Gnanam, J. R. Kannan Bapu, C. Harisudan,P. Hemalatha . 2010. *In vitro* mutation in horticultural crops – A review. Agricultural review. **31(1) :** 63- 67.

Wi . S. G., B.Y. Chung, J. H. Kim, M. H. Baek, D. H.Yang, J.W Lee, J. S.Kim.2005. Ultrastructural changes of cell organelles in *Arabidopsis* stem after gamma irradiation. *J. Plant Biol*. **48(2):** 195-200

Wongpiyasatid.A, S. Chotechuen, P. Hormchan, 2000. Radiation-induced changes in respiration of corn, wheat, sorghum and radish seeds during initial stages of germination in relation to subsequent seedling growth. *Kasetsart J. (Nat. Sci.)*.**34:** 443-449.

Zhu, X. D., Chen, H. Q. and Shan, J. X. 2006. Nuclear techniques for rice improvement and mutant inductionin China National Rice Research Institute. *Plant Mut. Rep*. **1:** 25-28.