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The effect of mutagens on M1 population of black gram (*Vigno mungo* L. Hepper)

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This study was carried out with black gram (urdbean) variety TNAUCo(Bg)6 to determine the effects of gamma rays (150, 200, 250, 300 and 350 Gy) and ethyl methane sulphonate (10, 15, 20, 25, 30 mM). Data collected were on seed germination and survival, pollen and seed fertility, plant height, number of primary branches, number of clusters per plant, number of pods per plant, pod length, number of seeds per plant, hundred seed weight and yield per plant in M_1 generation. Thereafter, progressive reduction in germination and survival percentage, pollen and seed fertility and yield related traits were observed in the mutagenic treatments. Deleterious effects were more pronounced in higher doses, indicating almost a linear relationship. LD_{50} values of 41.30 and 43.50% were observed in 20 mM of ethyl methane sulphonate and 250 Gy of gamma rays, respectively. The increasing doses of gamma rays and ethyl methane sulphonate decreased in phenotypic and yield related parameters. The reduction in quantity and yield traits has been attributed to the physiological disturbance or chromosomal damage of the cells of the plant caused by the mutagens. Ethyl methane sulphonate was observed to be more effective than gamma rays as it generated more number of mutants, which later caused higher physical injury.

Key words: *Vigna mungo*, variety TNAUCo(Bg)6, gamma rays, ethyl methane sulphonate (EMS).

INTRODUCTION

Black gram (*Vigna mungo* (L.) Hepper) is a rich source of protein (20.8 to 30.5%); its total carbohydrates range from 56.5 to 63.7%. It is also a good source of phosphoric acid and calcium. It contains a wide variety of nutrients and is popular for its fermenting action and thus it is largely used in making fermented foods. It is an important pulse crop occupying a unique position in Indian agriculture. It covers an area of about 3.24 million hectares and produces 1.46 million tonnes. Its productivity is only 526 kg per ha. In Tamil Nadu, black gram covers an area of about 3.41 lakh hectares with production of 1.21 lakh tonnes and 355 kg per ha.

Jayamani et al. (2012) and Indian Institute of Pulses Research (2011) report that about 70% of the total area is in the central and southern parts of the country, and this contributes about more than 77% of the total production. But the national productivity of black gram is around 500 kg per ha due to restricted cultivation in the marginal lands, lack of genetic variability and the absence of suitable ideotypes for different cropping systems. These cause poor harvest index and susceptibility to pests and diseases (Pawar, 2001; Banu, 2005). There is paucity of research on this species compared to cereals and other legumes.

In order to improve yield and other polygenic characters, mutation breeding should be effectively utilized (Deepalakshmi and Anandakumar, 2004). Mutation induction has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain specific traits (Kurobane et al., 1979).

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Induced mutations represent the same kind of changes that occur from natural causes (Govindan (2000). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid, 2000). Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs et al., 2002). Therefore, an attempt has been made to study their effects in this direction.

MATERIALS AND METHODS

Dry, healthy and uniform sized seeds of black gram variety TNAUCo(Bg)6 were treated with gamma rays at 150, 200, 250, 300 and 350 Gy doses and ethyl methane sulphonate at 10, 15, 20, 25 and 30 mM concentrations. Five hundred (500) seeds were pre-soaked for 6 h in water initially (Malarkodi, 2008). Then, the seeds were immersed for 6 h in the requisite concentration of mutagen ethyl methane sulphonate with intermittent shaking. To ensure a uniform absorption of the mutagen, the volume of mutagen solution was maintained at 10 times proportion to that of the seed volume. The whole treatment was carried out at a room temperature of 28±1°C for 4 h after washing in running water and untreated seeds were used as control. The treated seeds of gamma rays, ethyl methane sulphonate and control seeds were immediately sown in the field in a randomized block design (RBD) with three replications. Each treatment consists of three rows of 5 m length, in which 50 seeds per row were sown with 10 × 30 cm distance between plants and rows, respectively. Data were recorded on 11 quantitative characters and further statistically analyzed. Mean values for the 11 quantitative traits in different treatments and percentage over control are presented.

RESULTS AND DISCUSSION

Germination percentage was significantly reduced in all the gamma ray and ethyl methane sulphonate treatments presented in Table 1. The 50% reduction of germination was recorded at 250 Gy of gamma rays (43.50%) and 20 mM of ethyl methane sulphonate (41.30%). It indicated that germination percentage was reduced under the influence of mutagenic treatment with increasing doses per concentrations. Similar results were reported in red gram by Jayanthi (1986), in winged bean by Veeresh et al. (1995) and in blackgram by Thilagavathi and Mullainathan (2011). The significant survival reduction was observed in the higher dose / concentration of gamma rays 350 Gy (19.99) and ethyl methane sulphonate (30 mM, 6.58). This might have been due to the effect of mutagens on meristematic tissues of the seed. Morphological variations, especially leaf abnormalities are the indicators of effective mutagen treatment. In different treatments, morphological variations like trifoliate, tetrafoliate, pentafoliate, hexafoliata and fused leafs were observed in the present investigation. Plant height was also found to be significantly reduced in higher doses of physical and chemical mutagenic treatments. The maximum plant height reduction was observed in 350 Gy of gamma rays (3.00 cm) and 30 mM (2.00 cm) (Figure 1). Pollen fertility and seed fertility percentage among all the mutagenic treatments showed gradual decrease with respect to the increase in concentrations. In the current findings, the increase in pollen sterility as a consequence of mutagenesis is in accordance with the findings of Ignacimuthu and Babu (1989) on wild and cultivated Urd and mungbeans. In most cases, meiotic abnormalities are responsible for pollen sterility (Mathusamy and Jayabalan, 2002) in cotton and chickpea (Khan and Wani, 2005). In addition to chromosomal aberrations, some genetic and physiological changes might have caused pollen steiility.The number of primary branches per plant was also significantly affected by 350 Gy of gamma rays (1.08) and 30 mM of ethyl methane sulphonate (1.13) treatments (Figure 1). Number of pods per plant and pod length also reduced in increasing doses in Table 2. The number of cluster per plant was also significantly affected in 350 Gy of gamma rays (3.00) and 30 mM of ethyl methane sulphonate (2.00) treatments (Figure 1). Similar results were reported in the quantitative parameters such as number of primary branches per plant, number of cluster per plant, number of pods per plant, pod length, number of seeds per pod and plant yield per ha; they all decreased in gamma rays and ethyl methane sulphonate treatment than in control in M_1 generation of *Vigna mungo* (Thilagavathi and Mullainathan, 2011) and *Vigna unguiculata* (Mensah and Akomeah, 1992; Rizwana Banu, 2005).

However, plant height at 60^{th} day, number of seed per plant and 100 seed weight per plant in different treatments indicated a significant reduction in the higher doses of physical and chemical treatment. Percentage reduction in seed weight was maximum (5.00 g) in 150 Gy of gamma rays and 10 mM of ethyl methane sulphonate treatment (4.30 g). A maximum seed yield of 10.00 g per plant was observed in control. There was significant reduction in pollen fertility, seed fertility, hundred seed weight and seed yield was non-significant in all the treatments. In the present study, reduction in seed germination and germination percentage was concentration/dose dependent and linear. Reduction of germination in mutagenic treatments is due to delay or inhibition of physiological and biological processes necessary for seed germination; they include enzyme activity (Kurobane et al., 2002). The treatments showing maximum variation in quantitative characters may show stable gene mutations in subsequent generations.

Conclusion

All the quantitative and yield traits were proportionately

Table 1. Impact of gamma ray and ethyl methane sulphonate treatment on growth characters in black gram.

Treatment	Germination percentage	Survival percentage			Plant height at 30th Day	Pollen fertility percentage	Seed fertility percentage	Number of primary branches per plant
		15th Day	21th Day	28th Day				
Gamma ray (Gy)								
Control	98.50	95.00	91.04	90.22	18.00	92.20	91.00	2.67
150	76.80	74.50	70.50	70.00	13.00	73.00	55.00	2.07
200	63.41	62.25	56.25	55.00	11.00	55.00	46.00	1.68
250	43.50	41.00	38.29	35.22	6.00	45.24	33.08	1.53
300	30.00	27.30	22.34	20.95	4.00	15.00	10.25	1.29
350	28.50	24.40	21.40	19.99	3.00	10.00	5.00	1.08
Mean	56.79	54.08	49.97	48.56	9.17	48.41	40.05	1.72
Sed	2.79	4.95	1.23	1.24	0.63	3.326	2.528	0.11
CV (%)	6.01	11.2	3.12	3.12	8.43	8.42	7.73	7.93
Ethyl methane sulphonate (mM)								
Control	98.50	95.00	91.04	90.22	18.00	92.20	91.00	2.67
10	79.00	70.99	69.83	68.75	8.00	82.91	65.04	2.17
15	69.30	66.30	61.50	55.76	7.00	69.01	53.02	1.83
20	41.30	40.00	38.40	36.02	6.00	46.00	28.00	1.36
25	28.20	26.30	23.47	23.07	3.00	21.00	11.00	1.33
30	12.50	9.50	7.50	6.58	2.00	13.00	5.00	1.13
Mean	54.80	51.35	48.62	46.73	7.34	54.02	42.18	1.74
Sed	2.35	4.45	3.09	2.41	0.52	1.04	0.91	0.17
CV (%)	5.26	10.62	7.79	6.32	8.64	2.36	2.64	12.26

Table 2. Impact of gamma rays and ethyl methane sulphonate treatment on pod and yield characters in black gram.

Treatment	Number of pods per plant	Pod length cm	Number of cluster per plant	Plant height at 60th Day	Number of seeds per pod	Hundred seed weight (g)	Seed yield per plant g
Gamma ray (Gy)							
Control	39.67	5.80	12.67	33.60	6.00	6.00	10.00
150	23.67	5.00	12.00	31.37	4.33	5.00	9.35
200	20.67	3.20	9.00	27.80	3.33	4.80	7.50
250	17.00	2.80	6.00	31.37	3.67	3.20	5.70
300	13.00	2.20	4.00	27.80	2.34	2.40	3.40
350	9.00	1.00	3.00	14.48	1.67	1.00	2.00
Mean	20.50	3.33	7.78	27.74	3.56	3.73	6.33
Sed	1.02	0.27	1.02	1.93	0.35	0.42	0.24
CV (%)	6.11	10.24	6.11	8.54	12.22	13.75	4.62
Ethyl methane sulphonate (mM)							
Control	39.67	5.80	12.67	33.60	6.00	6.00	10.00
10	17.67	5.00	10.00	28.00	4.00	4.30	8.20
15	13.00	3.80	8.33	26.87	3.67	3.10	6.00
20	18.00	3.00	4.67	23.60	3.00	2.50	4.75
25	9.33	2.40	3.00	11.67	2.67	1.50	2.90
30	8.00	1.30	2.00	9.90	2.00	0.80	2.00
Mean	17.61	3.55	6.78	22.27	3.56	3.03	5.64
Sed	0.89	0.40	0.57	0.89	0.91	0.35	0.35
CV (%)	6.19	13.88	10.32	4.88	2.64	14.30	14.30

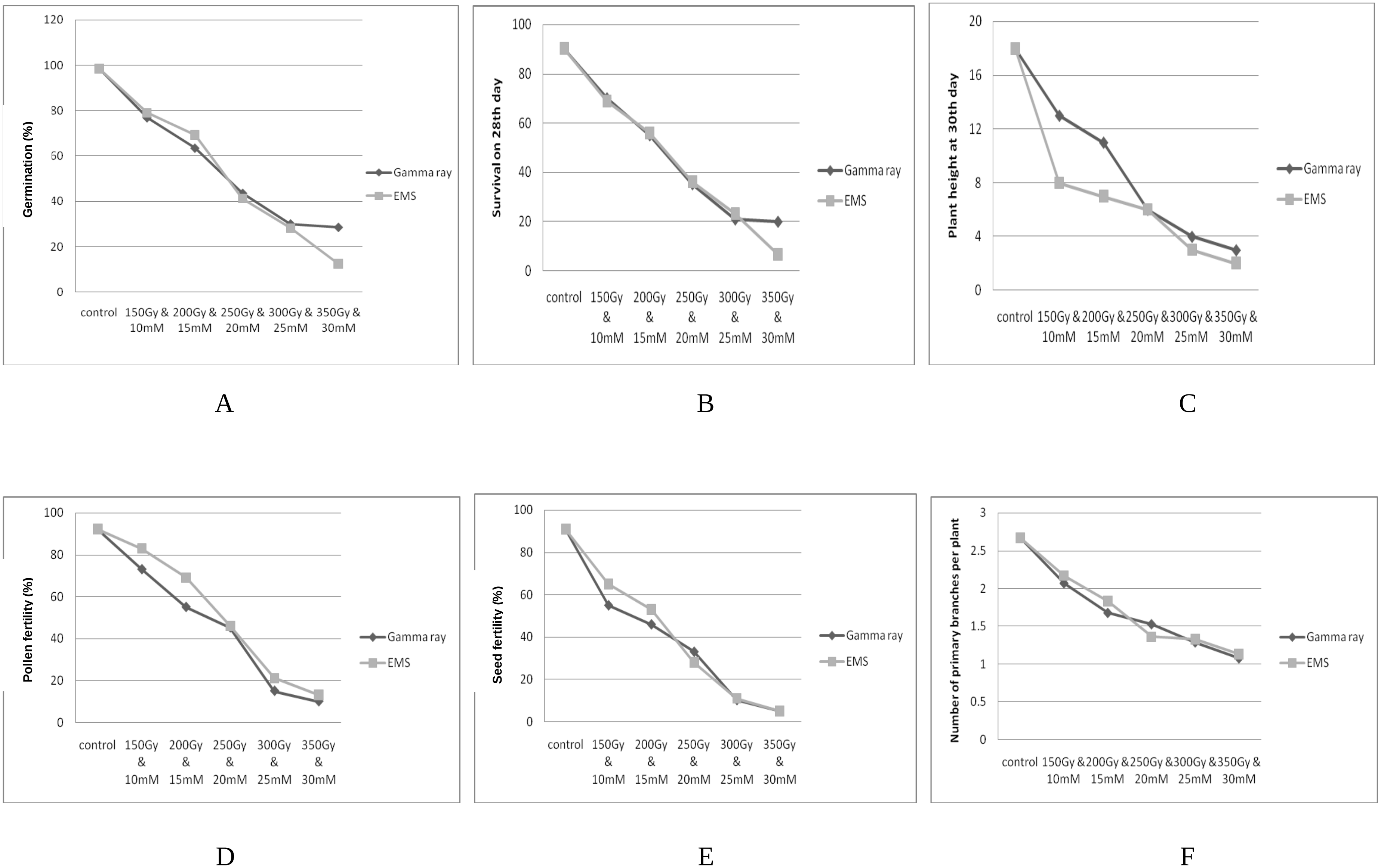

Figure 1. Comparison effect of gamma rays and EMS mutagenesis on (A) germination percentage, (B) Survival percentage (C)plant height, (D)pollen fertility (E) Seed fertility percentage (F) Number of primary branches per plant (G) Number of pods per plant (H)pod length (I) Number of cluster per plant (J) Number of seed per pod (K)Hundred seed weight, (L) seed yield per plant

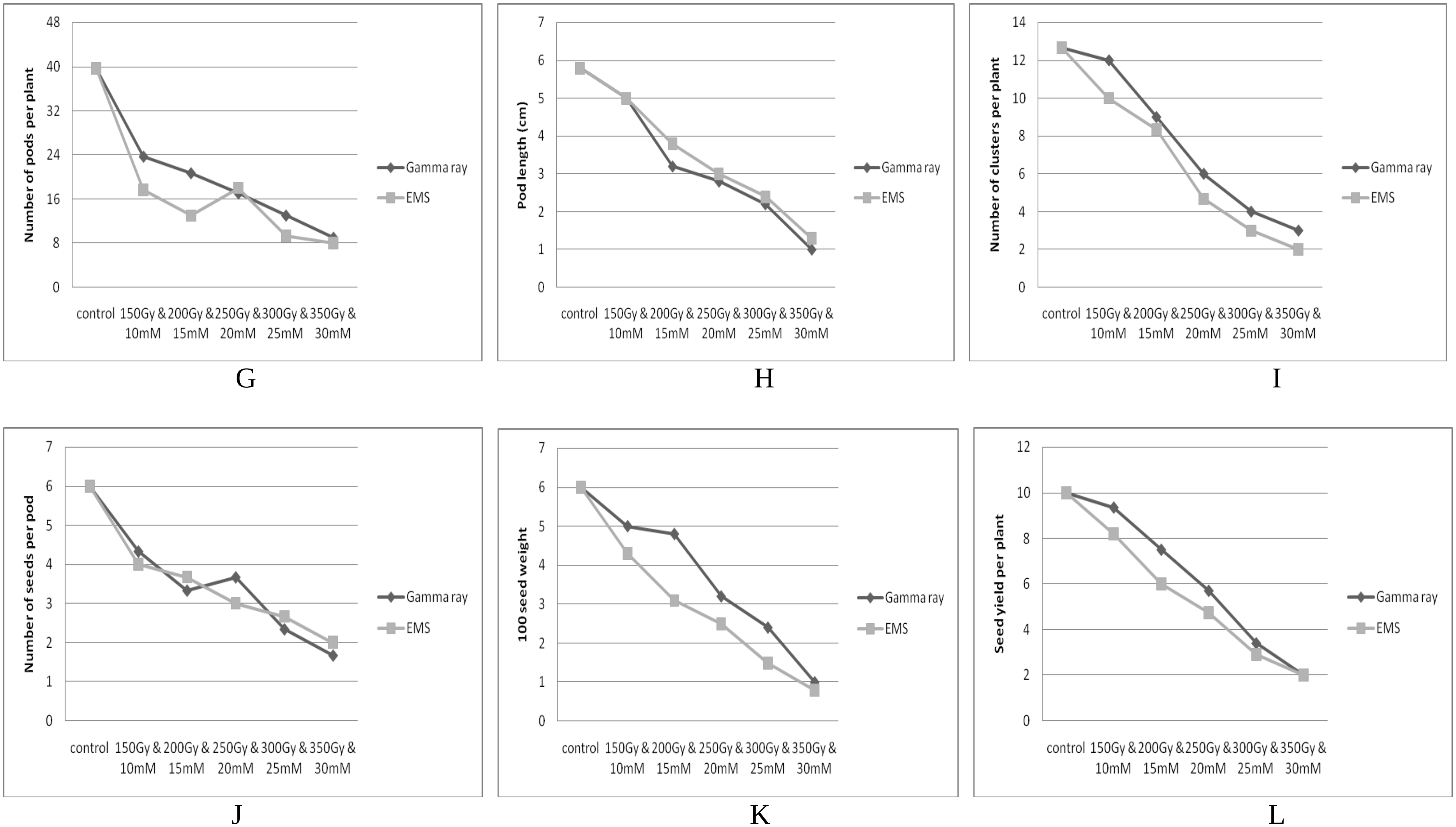

Figure 1. Contd.

decreased with increased dose/ concentrations of gamma rays and ethyl methane sulphonate. The decrease in quantitative characters has been attributed to the physiological disturbance or chromosomal damage of the cells of the plant caused by the mutagens. Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. Chemical mutagens usually cause point mutation, but the loss of a chromosome segment or deletion can also occur.

Studies show that the most important parameters for inducing physical and chemical mutagen growth and yield characters were reduced based on dose per concentration and duration of treatment. In the present study, it was observed that the gamma ray irradiation and ethyl methane sulphonate affect Urdbean. We observed morphological changes such as stunted plants, reduction of the plant height and yield parameters.

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