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Effect of mutagenesis on germination, plant survival, pollen sterility and seed sterility in m_1 generation of cluster bean (*Cyamopsis tetragonaloba* L.) variety MDU 1

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Abstract
The present study was carried out to study the impact of mutagens on biological parameters such as seed germination, shoot and root length and to determine the lethal dose (LD50) of gamma ray, electron beam and combined effect of gamma ray + EMS on cluster bean variety MDU 1. Cluster bean seeds were treated with different doses (100, 200, 300, 400, 500, 600,700 Gy) of gamma rays, electron beam and (100+20mM EMS, 200+20mM EMS, 300+20mM EMS, 400+20mM EMS, 500+20mM EMS, 600+20mM EMS,700+20mM EMS Gy) of Gamma ray with EMS combined treatment. Radiation induced morphological changes such as reduced germination, reduced in shoot and root length was observed compared to control. In cluster bean variety MDU 1. LD_{50} was noticed at 406 Gy, 302 Gy and 202 Gy for gamma rays, electron beam and combined treatment respectively. Decrease in the germination percent with increase in dose was observed in the present study. Optimal mutagenic doses were calculated based on germination percentages which were considered useful in developing cluster bean mutagenized population to create genetic variability for various qualitative and quantitative traits.

Keywords: Cluster bean, germination percentage, plant survival, pollen sterility and seed sterility

Introduction
The Cluster bean (*Cyamopsis tetragonaloba* L. Taub.) (2n=14) belongs to family Fabaceae. The importance of legume has been highly appreciated as a source of guar gum. Unlike the seeds of other legumes, cluster bean has a large endosperm containing significant amount of hydrocolloid *i.e.*, galactomannen gum. The pods grown in clusters giving the common name cluster bean. It is native to the Indian sub-continent. It is an erect, annual legume, extremely drought resistant and thrives in semi-arid regions where few plants thrive.

The availability of genetic diversity conserved in the germplasm determines the success of any crop improvement program. Further, exploitation of available diversity through hybridization is cumbersome due to small flower size and very poor seed setting in manually hybridized buds. Therefore, mutation breeding may be preferred choice in such situations for creating variability and isolating desirable mutants for specific purpose. Though, mutability of cluster bean has amply been demonstrated through various studies systematic work still lacks (Stafford, 1989; Mittal *et al.*, 1968; Vig 1965; Mahla *et al.*, 2010; Arora and Pahuja, 2008; Velu *et al.*, 2012) [21, 12, 26, 10, 2, 25]. The successful use of induced mutation depends on efficiency to create desirable changes with least undesirable effects (Harten, 1998 and Pathak, 2015) [7, 15]. Thus need to generate, basic information for proper application of mutatgenesis in cluster bean improvement. Therfore a study to create useful genetic diversity in cluster bean has been conducted using different mutagens.

Material and Methods
The seeds of cluster bean variety MDU 1 for the induction of mutation treatment were obtained from Department of Horticulture, Agricultural College and Research Institute, Madurai, India. Cluster bean seeds were packed in butter paper covers and placed in the gamma chamber, it 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 Bhabha Atomic Research Centre, Mumbai. Non-irradiated dried seeds were taken as control. Seeds of MDU 1 were treated at seven different doses (100, 200, 300, 400, 500, 600 and 700 Gy). For electron beam irradiation, seeds of variety MDU 1 were treated at seven different doses (100, 200, 300, 400, 500, 600 and 700 Gy) from electron accelerator facility at

Electron Beam Centre, Bhabha Atomic Research Centre, Mumbai, India. For combined treatment, cluster bean were exposed with gamma ray and then same seeds were treated with 20mM EMS at seven different doses (100+20mM EMS, 200+20mM EMS, 300+20mM EMS, 400+20mM EMS, 500+20mM EMS, 600+20mM EMS,700+20mM EMS Gy) of Gamma ray with EMS combined treatment. The treated seeds with control were sown in germination trays, Department of Horticulture, Agricultural College and Research Institute, Madurai for working out the LD_{50} and probit analysis was done using the germination percent. After mutagenic treatment, seeds were sown immediately in the field along with control (untreated seeds). All M_1 seeds were grown with a spacing of 60 cm between rows and 45 cm between plants with single seed per hole. Recommended dose of fertilizers, plant protection measures and the general cultural practices were uniformly followed for all the treatments. Probit analysis was carried out to determine the lethal dose (LD_{50}) of gamma rays and electron beam and combined treatment (gamma ray with EMS) in cluster bean variety MDU 1.

LD_{50} fixation and raising M_1 in field condition

The seeds of cluster bean variety MDU 1 were treated with gamma irradiation as 150 seeds per treatment. Then the seeds were sown in protrays along with control. The sown seeds were observed for its germination percentage. Based on these data the lethal dose 50 (LD_{50}) has been calculated calculated using probit analysis. All treatments were raised as M_1 generation. In this generation the Lethality, Injury and Sterility factors (LIS) were recorded and the seeds from individual plants were collected.

Optimization of LD_{50} Dosage

Well filled, undamaged and uniform sized seeds were handpicked from the seed lot. Initially to fix the LD_{50} value, the random sample of 150 seeds were treated with different doses of gamma ray and electron beam (100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy and 700 Gy) and (100+20mM EMS, 200+20mM EMS, 300+20mM EMS, 400+20mM EMS, 500+20mM EMS, 600+20mM EMS,700+20mM EMS Gy) of Gamma ray with EMS for combined treatment. Treated seeds were grown in portrays.

Determination of LD 50 value

Through the preliminary experiments, LD_{50} value was determined based on the germination percentage of the treated seeds using probit analysis. The sprouted seeds were evaluated at 7^{th} days to 15^{th} day after sowing in all the treatments and the germination and survival percentage were worked out. Based on the probit analysis the LD_{50} dose for Gamma ray found 400 Gy, electron beam found 300 Gy and 200 Gy were found in combined treatment and based on this for M_1 generation were selected for field experiments.

Seed germination

Germination counts were recorded starting from 5th day of sowing and continued up to 15th day and expressed as percentage of germinated seedling to the total seeds sown.

Shoot length

The length of the shoot from the cotyledonary node to the tip of the shoot was measured on ten randomly selected seedlings on the seventh day and expressed in centimetre.

Root length

The root length from the cotyledonary node to the tip of the primary root was measured on ten randomly selected seedlings and expressed in centimetre.

Seedling survival

Observation on survival was made on 50th day after sowing. Plants above the cotyledonary leaves are considered as surviving. It was expressed as percentage of surviving plants to the total number of seedlings emerged.

Pollen fertility

Pollen grains were collected on 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

Ten clusters were collected randomly in each mutants, number of filled walls and total number of walls were counted individually and the mean was worked out and expressed in percentage.

Result and discussion

Mutation induction is a random process determined by probability, use of appropriate mutagen and the dose plays a critical role in induction of mutation. It has been clearly shown in a number of plant species that the effect induced varies with the varying mutagens and with variation in mutagen dose (Goyal and Khan, 2010) [5]. LD_{50} is the great importance to know the sensitivity of different genotypes to the critical dose of mutagens causing 50 per cent mortality. A gradual reduction in the germination was noticed corresponding to an increase in the dosage of gamma rays, electron beam and combined treatment (gamma ray +EMS) for the cluster bean variety MDU 1. Probit analysis was done using the seed germination values in the variety MDU 1 and LD_{50} dose was determined. LD_{50} was noticed at 406 Gy, 302 Gy and 202 Gy for gamma rays, electron beam and combined treatment respectively (Table 1). Decrease in germination per cent with the increase of treatment doses was observed in this study. It indicated that germination percentage was reduced under the influence of mutagenic treatment with increasing doses. Similar results were reported in black gram (Thilagavathi and Mullainathan, 2011) [23]. Radio-sensitivity varied with cluster bean genotype and was associated with seed testa texture, thickness and seed weight. As the present variety considered in this experiment showed LD_{50} almost in the similar range and whereby it may be concluded that variety MDU 1 was sensitive to gamma rays, electron beam and combined treatment (gamma ray+ EMS gamma rays) irradiation.

Table 1: Probit Analysis for fixing the LD50 value in cluster bean cv. MDU 1

Mutagen	Treatment	Mortality percentage	Corrected Mortality / Lethality (%)	Log 10 dose	Probit value	LD_{50} Value
Control	-	02.00	00.00	-	-	-
Gamma ray	100Gy	24.66	23.47	2.00	4.31	
	200Gy	28.00	26.54	2.30	4.42	
	300Gy	44.00	39.14	2.48	4.85	
	400Gy	48.66	47.96	2.60	4.97	406
	500Gy	54.66	54.09	2.70	5.12	
	600Gy	58.66	58.17	2.78	5.22	
	700Gy	64.66	64.29	2.85	5.38	
Electron beam	100Gy	20.66	19.39	2.00	4.18	
	200Gy	46.00	44.90	2.30	4.90	
	300Gy	50.66	50.00	2.48	5.02	302
	400Gy	57.33	57.15	2.60	5.18	
	500Gy	62.66	62.25	2.70	5.32	
	600Gy	66.00	65.31	2.78	5.41	
	700Gy	68.00	67.35	2.85	5.47	
Gamma + EMS	100Gy+20mM	37.33	36.74	2.00	4.68	
	200Gy+20mM	48.66	47.96	2.30	4.97	202
	300Gy+20mM	57.33	57.15	2.48	5.18	
	400Gy+20mM	62.00	61.23	2.60	5.31	
	500Gy+20mM	67.33	67.35	2.70	5.45	
	600Gy+20mM	71.33	71.43	2.78	5.56	
	700Gy+20mM	77.33	77.56	2.85	5.75	

Seed germination

The effect of gamma radiation, electron beam and combined treatment (gamma ray + EMS) on seed germination at field was reduced with increasing dose of gamma rays, electron beam and combinations (gamma ray + EMS) (Table 2). The seed germination was decreased from 76.53 % to 35.71% for gamma rays, 80.61 % to 32.65% for electron beam and 63.26% to 22.44 % for combined treatment (gamma ray + EMS) in cluster bean variety MDU 1. Per cent over the control. This shows significant influence of mutagen on germination. Similar observations were reported previously in blackgram (Khan and Wani, 2006; Sagade and Apparao, 2011) [9, 18].

Table 2: Effect of Gamma ray, Electron beam and Combination of Gamma ray + EMS on seed germination percentage

Mutagen	Concentration / Dose	Germination percentage	Percentage over control	Percentage reduction over control
Control	-	98	100	00.00
Gamma ray	100Gy	75	76.53	23.47
	200Gy	72	73.46	26.54
	300Gy	56	60.86	39.14
	400Gy	51	52.04	47.96
	500Gy	45	45.91	54.09
	600Gy	41	41.83	58.17
	700Gy	35	35.71	64.29
Electron beam	100Gy	79	80.61	19.39
	200Gy	54	55.10	44.90
	300Gy	49	50.00	50.00
	400Gy	42	42.85	57.15
	500Gy	37	37.75	62.25
	600Gy	34	34.69	65.31
	700Gy	32	32.65	67.35
Gamma + EMS	100Gy+20mM	62	63.26	36.74
	200Gy+20mM	51	52.04	47.96
	300Gy+20mM	42	42.85	57.15
	400Gy+20mM	38	38.77	61.23
	500Gy+20mM	32	32.65	67.35
	600Gy+20mM	28	28.57	71.43
	700Gy+20mM	22	22.44	77.56

Shoot length and root length

In cluster bean variety MDU 1, all the treatments were showed significant differences in shoot and root length reduction per cent over control for all mutagenic treatments (Table 3). The higher per cent reduction in root length 35.71% and shoot length 32.78% was observed in gamma ray (700Gy). In electron beam treatments, higher dose (700 Gy) showed decreased root (20.40% and shoot length (29.50 % reduction per cent over control. The higher per cent reduction in root length (31.63% and shoot length (39.34 was recorded in higher dose of combined treatment of Gamma ray + EMS (700 Gy+20 Mm EMS). Seedling injury is broadly used as an index of determining biological effects of various physical and chemical mutagens in M1 generation (Swaminathan *et al*., 1966). In the present study, there was a pronounced dose dependent inhibitory effect of gamma rays, electron beam and combined treatment (gamma ray+ EMS gamma rays on the shoot length and root length in seedlings over the control were

observed. Similar reduction in shoot and root length was reported in mungbean (Kamini and Akhaury, 1988) [8] and black gram (Surender *et al*., 2014) [22] and Lentil (Ram narayan ahirwar *et al*., 2014) [16]. Mutagens can cause physiological damages mainly manifested as growth retardation and death is generally not restricted in M_1 generation (Mak *et al*., 1986) [11]. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Gunckel and Sparrow, 1961) [6]. In earlier studies, electron beam radiation showed less physiological damage in M_1 generation and wide mutation frequency in M_2 generation (Rui *et al*., 1995) [7]. In the present study, there is not much difference in the reduction in germination percent, shoot and root length of cluster bean var. MDU 1 caused by gamma rays, electron beam and combined treatment.

Table 3: Effect of Gamma ray, Electron beam and Combination of Gamma ray + EMS on injury percentage of cluster bean cv. MDU 1

Mutagen	Treatment	Root length (cm)	Percent reduction over control	Shoot length (cm)	Percent reduction over control
Control	-	9.8		12.2	
Gamma ray	100Gy	9.7	01.02	11.3	07.37
	200Gy	9.2	06.12	10.9	10.65
	300Gy	8.9	09.18	10.4	14.75
	400Gy	8.1	17.34	10.2	16.39
	500Gy	7.7	21.42	9.9	18.85
	600Gy	7.5	23.46	8.4	31.14
	700Gy	6.3	35.71	8.2	32.78
Electron beam	100Gy	9.5	03.06	10.7	12.29
	200Gy	9.4	04.08	10.5	13.93
	300Gy	8.9	09.18	9.6	21.31
	400Gy	8.7	11.22	9.4	22.95
	500Gy	8.6	12.24	9.1	25.40
	600Gy	8.4	14.28	8.8	27.86
	700Gy	7.8	20.40	8.6	29.50
Gamma + EMS	100Gy+20mM	9.4	04.08	9.8	19.67
	200Gy+20mM	9.1	07.14	9.4	22.95
	300Gy+20mM	8.3	15.30	8.7	28.68
	400Gy+20mM	7.9	19.38	8.4	31.14
	500Gy+20mM	7.4	24.48	8.1	33.60
	600Gy+20mM	6.9	29.59	7.9	35.24
	700Gy+20mM	6.7	31.63	7.4	39.34

Plant survival percentage

Plant survival percentage in gamma ray treatment, the survival per cent over control due to different mutagenic treatment in var. MDU 1 ranged from 76.04% in 500 Gy to 84.48% in 300 Gy. In electron beam ray treatment, the survival per cent over control ranged from 80.78% in 400 Gy to 92.61% in 200 Gy and combined treatment ranges from 49.10 % in 300 Gy+20mM EMS to 70.10 % in 100 Gy+20Mm EMS (Table 4). The decrease in survival percentage was associated with increases in the dose per concentration of the mutagens. These findings were close agreement with the earlier reports of Nawale *et al.,* 2006 [14], Ugorji *et al.,* 2012 [24], Dhanavel and Girija, 2009 [47].

Table 4: Effect of Gamma ray, Electron beam and Combination of Gamma ray + EMS on quantitative traits of M1 generation in cluster bean cv. MDU 1

Effect of Gamma ray on quantitative traits of M_1 generation in cluster bean cv. MDU 1							
Character	**Control**	**300Gy**	**Reduction over control %**	**400Gy**	**Reduction over control %**	**500Gy**	**Reduction over control %**
Germination Percentage (%)	98	56	42.85	51	47.95	45	54.08
Plant height at vegetative stage on 30th day (cm)	26.5	20.04	24.37	19.16	27.69	18.36	30.71
Plant height at harvesting stage (cm)	112	80.18	28.41	78.01	30.34	76.28	31.89
Seed fertility (%)	100	97.36	2.64	94.28	5.72	93.93	6.07
Pollen fertility (%)	98.86	93.38	5.48	92.72	6.21	90.62	8.33
Effect of Electron beam on quantitative traits of M_1 generation in cluster bean cv. MDU 1							
Character	**Control**	**200Gy**	**Reduction over control %**	**300Gy**	**Reduction over control %**	**400Gy**	**Reduction over control %**
Germination Percentage (%)	98	54	44.89	49	50	42	57.14
Plant height at vegetative stage on 30th day (cm)	26.5	17.26	34.86	15.23	42.52	14.46	45.43
Plant height at harvesting stage (cm)	112	81.75	27.00	73.53	34.34	68.64	38.71
Seed fertility (%)	100	97.22	2.78	94.11	5.89	93.54	6.46

Pollen fertility (%)	98.86	94.62	4.28	89.41	9.55	87.50	11.49

Effect of Combination of Gamma ray + EMS on quantitative traits of M_1 generation in cluster bean cv. MDU 1

Character	Control	100Gy+20 Mm	Reduction over control %	200Gy+20M m	Reduction over control %	300Gy+20M m	Reduction over control %
Germination Percentage (%)	98	62	36.73	51.23	47.42	42	57.14
Plant height at vegetative stage on 30th day (cm)	26.5	17.56	33.73	16.58	37.43	15.82	40.30
Plant height at harvesting stage (cm)	112	73.33	34.52	71.67	36.00	64.11	42.75
Seed fertility (%)	100	93.33	6.67	91.42	8.58	88.28	11.72
Pollen fertility (%)	98.86	78.27	20.82	68.09	31.12	65.90	33.34

Pollen sterility and seed sterility percentage

The effect of mutagen was more prominent in terms of pollen sterility and seed sterility percentage reduction over control increased as dose increases the dose of mutagen. The maximum seed sterility 6.07% and pollen sterility 8.33% was observed at 500 Gy gamma ray, maximum seed sterility 6.46% and pollen sterility 11.49% observed in 400 Gy of electron beam in cluster bean var. MDU 1. In combined treatment recorded maximum seed sterility 11.72% and pollen sterility 33.34% at 300 Gy +20mM of gamma +EMS was recorded in cluster bean var. MDU 1 (Table 4). Similar findings were reported by Shinde *et al*., (2010) [20] in cluster bean, Bind *et al*., (2014) [3] in cow pea, Monica *et al*., (2015) [13] in garden bean, Vanniarajan *et al.*, (1993) in black gram and Ahmed John (1996) [1] in black gram. The reason for the pollen sterility might be attributed to cryptic deletions and specific gene mutations (Sato *et al*., 1967) [19].

Conclusion

From the present investigation, it was concluded that biological parameters such as seed germination, shoot and root length and to determine the lethal dose (LD50) of gamma ray, electron beam and combined effect of gamma ray + EMS on cluster bean variety MDU 1. Decrease in the germination percent with increase in dose was observed in the present study. Optimal mutagenic doses were calculated based on germination percentages which were considered useful in developing cluster bean mutagenized population to create genetic variability for various qualitative and quantitative traits.

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