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Comparative Studies on Mutagenic Effectiveness and Efficiency in Horsegram [*Macrotyloma uniflorum* (Lam) Verdc.]

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ABSTRACT

Background: Two horsegram varieties *viz*., PAIYUR 2 and CRIDA1-18R were mutated with gamma rays (G), electron beam (EB), G+EB and G+EMS (ethyl methane sulphonate) to determine the mutagenic potency in breeding programme.

Methods: Uniform seeds treated with different mutagenic doses were raised in randomized block design which constituted M_1 generation. Each plant was harvested individually and forwarded to M_2 generation following plant to progeny row method.

Result: A dose dependant decline was observed for seed germination, plant survival, root length, shoot length, plant height, pollen fertility and seed fertility in M_1 population. Wide spectrum of chlorophyll mutants was induced in M_2 generation with maximum frequency at EB followed by combination treatments (G+EMS and G+EB) in both varieties. The mutagenic effectiveness ranged from 0.14 per cent to 1.45 per cent in PAIYUR 2 and 0.15 per cent to 1.71 per cent in CRIDA1-18R. The high mutation rate for effectiveness was exhibited by G and G+EB. With reference to sterility, EB was found to be efficient mutagen in both varieties whereas varied efficiency was noted for lethality (EB - PAIYUR 2; G+EMS - CRIDA1-18R) and injury (G - PAIYUR 2; G+EMS - CRIDA1-18R).

Key words: Chlorophyll mutants, Effectiveness, Efficiency, Horsegram, Induced Mutation.

INTRODUCTION

Horsegram [*Macrotyloma uniflorum* (Lam) Verdc.] is a nutritious legume being cultivated in diverse agro climatic zones of India. It has been quoted as potential food crop of tropics by US National Academy of Sciences owing to its enriched nutrient source coupled with drought tolerance potential (NAS, 1978). Horsegram, since time immemorial finds a part in Indian ayurvedic medicine which cures a variety of health issues starting from common cold to kidney stones (Prasad and Singh, 2015). The seeds provide cheap source of protein satisfying the nutritional requirements of developing nations (Aditya *et al*., 2019; Geetha *et al*., 2011). The crop is used as fodder source because of its enriched protein content in comparison with other legumes (Fuller and Murphy, 2018). In addition, it also helps in improvement of soil fertility by fixation of atmospheric nitrogen (Cullis and Kunert, 2017). Despite of its multi advantages, horsegram cultivation is not widely encouraged in ideal agronomic environments. The current scenario of plant type possesses many undesirable traits such as indeterminate, twining growth habit, photosensitivity and asynchronous maturity (Chahota *et al.,* 2013). More scientific efforts through plant breeding and biotechnology are to be initiated and intensified for bringing improvement in horsegram in order to meet out future nutritional security (Mabhaudhi *et al*., 2017).

Genetic variability is an essential prerequisite for effective selection. Horsegram is an autogamous crop with limited variability (Wani and Anis, 2001) which makes conventional breeding less feasible. Induced mutagenesis throws relatively high frequency of desirable mutants in comparison with spontaneous mutation. It offers scope for creation of variability in self pollinated crops and thereby useful in evolving agronomic superior varieties with short period of time (Bolbhat and Dhumal, 2009). For any induced mutation breeding programme, selection of mutagen with increased efficiency and effectiveness is needed for recovery of high frequency of desirable mutants (Solanki and Sharma, 1994). Mutagenic effect on biological material varies to a greater extent with several group of mutagen. Therefore, the present investigation was carried out to identify the effective doses of various mutagens for acquiring considerable frequency of mutation induction in horsegram.

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MATERIALS AND METHODS

Two popular horsegram cultivars of Southern India *viz.,* PAIYUR 2 and CRIDA1-18R were utilized for mutagenic studies. The seeds of PAIYUR 2 were procured from Tamil Nadu Agricultural University (TNAU), India and CRIDA1-18R from Central Research Institute for Dryland Agriculture, India. The well filled seeds were uniformly dried to a moisture content of 10-12 per cent. The seeds were irradiated with 100 Gy, 200 Gy, 300 Gy and 400 Gy doses of gamma rays (G), electron beam (EB) and its combinations. The irradiation treatments were imposed at Bhabha Atomic Research Center (BARC), Trombay, Mumbai, India. High frequency of mutations was reported at lower doses of EMS (0.2% and 0.3%) in horsegram (Bolbhat *et al*., 2012). Hence, combination treatments involving four doses of G with 0.3 per cent EMS were chosen to explore the combined effect of physical and chemical mutagens in horsegram. A part of gamma irradiated seeds were soaked in distilled water for 10 hours and the pre-soaked seeds were treated with 0.3 per cent of EMS prepared in sodium phosphate buffer maintaining pH of 7.0 for 4 hours with intermittent shaking.

A total of 34 treatment combinations including control (untreated seeds) constituted M_1 generation. Four replications with 25 seeds each were sown in roll towel method to study the effect of mutagenesis on seedling traits *viz*., seed germination (%), root length (cm), shoot length (cm) and seedling height (cm). A total of 500 seeds from each treatment were sown in a randomized block design with two replications at Department of Pulses, TNAU during *rabi* 2018. Observations on survival (%), plant height (cm) on 30^{th} day and maturity, pollen fertility (%) and seed fertility (%) were recorded. Seeds of individual M_1 plants were harvested separately and forwarded to M_2 generation following plant to progeny row method. Chlorophyll mutants were observed in M_2 population periodically from seedling stage to maturity as suggested by Bolbhat *et al*. (2012). The chlorophyll mutants were classified according to the pattern developed by Gustaffson (1940) and Blixt (1972). Parameters recorded in M_1 and M_2 generation were utilized to determine the effectiveness and efficiency of mutagenic doses (Konzak *et al*., 1965).

Mutagenic effectiveness

Physical mutagen

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic frequency (Mf)}}{\text{Dose of mutagen (Gy)}}$$

Chemical mutagen

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (Mf)}}{\text{Concentration of mutagen (\%)} \times \text{Time (Hours)}}$$

Combinations

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic frequency (Mf)}}{\text{Dose of mutagen (Gy)} \times \text{Con. of mutagen (\%)} \times \text{Time (Hours)}}$$

Mutagenic efficiency:

$$\text{Efficiency} = \frac{\text{Mutation frequency (Mf)}}{\text{Percent Injury (I)/Percent Lethality (L)/Percent Sterility(S)}}$$

Mutation frequency was estimated as percentage of M_1 plant progenies segregating for chlorophyll mutants (Gaul, 1964). The percent injury, lethality and sterility represent the percent reduction of plant height at 30^{th} day, plant survival and seed fertility respectively in M_1 generation.

RESULTS AND DISCUSSION

Several classes of physical and chemical mutagens differ in their efficiency in inducing mutations (Wani 2009) either directly or indirectly affecting cellular components. The mutagenic effect of any ionizing radiation depends upon the amount of energy lost to biological material of its path which is defined as Linear Energy Transfer (LET). Among ionizing radiations, G and EB possess low LET value of around 0.2 $KeV\mu m^{-1}$ (Magori *et al*., 2010). Earlier attempts on G led to release of superior mutants but the mutagenic effect of EB remains unexplored in horsegram. Studies on EB would also produce desirable mutations as it exhibit comparatively high relative biological effectiveness (RBE) due to increased absorption dose rate than G and other radiation methods (Zhu *et al*., 2008). Chemical mutagens have also contributed equally in development of mutants as that of ionizing radiations (Ganapathy *et al*., 2008). Point mutations *i.e*., base pair changes are often induced by chemical mutagens which results in altered gene product due to change in amino acid composition (Shu *et al*., 2012).

The degree of any mutagenic effect can be quantitatively determined by plant injuries in M_1 generation (Table 1). Drastic reduction in seed germination was observed in both varieties with increased dosage rate. The decline may be the result of altered metabolic activities due to enzymatic disturbances involved in seed germination (Kulkarni, 2011). A similar decline in survival percent was recorded at higher mutagenic doses (Kulkarni and Mogle 2013). Seedlings are highly sensitive to mutagenic effect and provide an easy means to measure injury. Traits *viz*., shoot and root length gradually declined with increased mutagenic dose in all treatments. The maximum reduction was recorded in combination treatment of G+EMS (400 Gy+0.3%). The inhibitory effect had occurred due to interrupted mitotic division leading to reduced formation of cells contributing seedling growth. Of the two seedling traits studied, the mutagenic treatments exerted greater effect on root length rather than shoot length. Girija *et al*. (2013) reported high percent reduction of root length in cowpea due to cytological changes (anaphasic bridges, laggards, stickiness) occurring in root cell on treatment of seeds with higher mutagenic doses. Vegetative traits *viz*., plant height at 30^{th} day and maturity was reduced with increased mutagenic dose in all treatments and the highest reduction was observed at 400

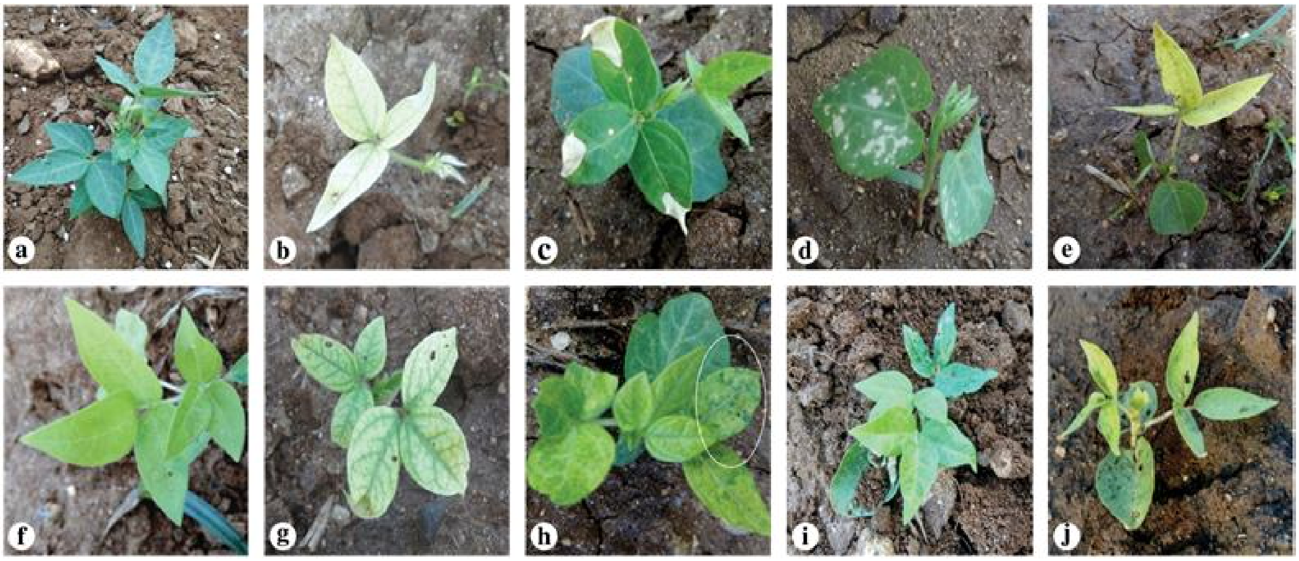
Table 1: Effect of mutagens and its combination on mean performance of traits in M_1 generation.

	Seedling traits				Vegetative and reproductive traits			
Mutagen	Seed germination (%)	Plant survival at 30 DAS (%)	Shoot length (cm)	Root length (cm)	Plant height at 30 DAS (cm)	Plant height at maturity (cm)	Pollen fertility (%)	Seed fertility (%)
Variety : PAIYUR 2								
Control	92.50	82.81	10.43	17.84	17.35	71.76	93.48	94.89
G:100 Gy	71.62	42.64	9.91	11.64	16.82	69.95	82.49	87.28
G:200 Gy	43.11	34.31	7.78	10.47	12.18	58.24	72.89	85.16
G:300 Gy	31.25	28.21	6.88	10.25	10.59	56.08	47.77	83.22
G:400 Gy	20.17	16.24	6.87	10.23	9.24	52.31	37.34	82.81
EB:100 Gy	44.58	34.25	10.36	12.38	14.25	62.68	82.80	89.81
EB:200 Gy	33.76	29.24	9.74	11.52	11.19	56.23	71.95	85.10
EB:300 Gy	23.27	21.14	8.33	11.82	9.89	49.55	32.78	80.67
EB:400 Gy	20.15	15.28	6.33	10.55	9.21	48.84	32.16	80.15
G+EMS:100 Gy+0.3%	68.76	41.08	8.46	10.19	16.04	61.90	79.74	85.77
G+EMS:200 Gy+0.3%	41.45	34.41	7.02	9.31	11.99	57.57	67.27	84.37
G+EMS:300 Gy+0.3%	29.68	21.74	6.50	8.79	9.55	54.21	40.23	79.62
G+EMS:400 Gy+0.3%	18.99	16.10	4.82	6.68	9.22	49.75	26.21	76.29
G+EB:100 Gy	37.96	33.81	9.49	11.55	12.27	58.54	71.74	83.89
G+EB:200 Gy	25.68	21.62	7.99	11.97	10.86	51.47	58.86	82.01
G+EB:300 Gy	21.72	18.01	7.63	11.28	9.21	43.31	30.19	78.32
G+EB:400 Gy	18.95	14.92	6.00	8.21	8.64	42.78	25.11	74.15
Variety : CRIDA1-18R								
Control	90.90	80.55	10.69	17.64	16.12	62.67	90.21	90.43
G:100 Gy	68.34	40.25	7.38	10.38	14.28	47.21	82.16	85.66
G:200 Gy	39.71	32.94	6.79	10.33	10.08	43.68	70.87	80.16
G:300 Gy	28.32	23.21	7.01	9.94	9.52	36.47	42.45	75.46
G:400 Gy	19.84	15.42	6.49	9.73	8.52	34.50	32.11	73.14
EB:100 Gy	41.78	31.25	8.71	12.10	11.49	46.30	68.78	80.51
EB:200 Gy	29.14	25.72	7.74	12.90	9.98	38.53	56.04	80.26
EB:300 Gy	22.78	18.64	7.71	12.42	8.49	34.25	50.10	78.32
EB:400 Gy	20.09	14.98	6.35	9.05	8.22	32.15	20.13	71.86
G+EMS:100 Gy+0.3%	65.24	38.64	7.21	7.98	13.35	37.80	74.03	80.90
G+EMS:200 Gy+0.3%	36.92	31.44	6.48	9.41	10.01	32.50	63.71	75.71
G+EMS:300 Gy+0.3%	26.24	21.09	5.79	7.32	9.47	31.30	38.65	73.70
G+EMS:400 Gy+0.3%	18.17	15.21	5.15	5.92	8.34	29.03	26.87	73.02
G+EB:100 Gy	37.76	30.03	7.79	11.21	10.25	33.16	54.29	79.81
G+EB:200 Gy	26.72	21.10	7.26	10.45	8.96	30.72	47.65	73.21
G+EB:300 Gy	22.03	17.65	6.25	9.40	7.58	28.65	40.10	71.24
G+EB:400 Gy	15.15	14.01	6.76	8.79	7.21	26.27	19.35	65.24

G- Gamma ray, EB- Electron beam, EMS - Ethyl methane sulphonate.

Gy of G+EB combination in both the cultivars. Reduction in plant height can be attributed to slow rate of cell division, altered amylase and peroxidase activity (Cherry and Lessman, 1967). An inverse relationship existed between pollen fertility level and dosage rate. Pollen sterility results due to aberrant genetic and physiological damages which are induced by chromosomal breaks and point mutations (Rana and Swaminathan, 1964). Similar decline in trend was registered for seed fertility with increased mutagenic dosage (Ramya *et al*., 2014).

Gaul (1964) insisted the use of chlorophyll mutation as markers for exploring the gene action of mutagenic factors. Wide spectrum of chlorophyll deficient mutants was observed in M_2 population which includes: *albina, albina-green, striata, chlorina, xantha, viridis, xanthaviridis, maculata* and *tigrina* (Fig 1). Average frequency of total chlorophyll mutants was varying in magnitude from 0.73% to 1.31% in PAIYUR 2 and 0.94% to 1.18% in CRIDA1-18R (Table 2). Among the chlorophyll mutants, *chlorina* recorded the highest frequency followed by *striata, viridis, xantha* and *albina* in both varieties (Fig 2a). The maximum occurrence of *chlorina* was noticed in combination of G+EMS treatment



a. Control; **b.** *Albina* - No recovery observed due to complete absence of chlorophyll or carotenoid pigment (survived up to 10 - 12days); **c.** *Albinagreen* - White and green colour appearance of mutant leaves. No recovery observed; **d.** *Maculata* - Mutant seedlings with whitish dots on green leaves; **e.** *Xantha* - Yellow coloured leaves with absence of chlorophyll pigment (survived up to 10 - 12 days from sowing); **f.** *Chlorina* - Mutants were light or pale green in colour and few survived up to maturity with normal reproductive phase; **g.** *Striata* - Mutant seedlings characterized by striped leaves. Few seedlings survived up to maturity; **h.** *Tigrina* - Mutant leaves were characterized by presence of green patches; **i.** *Viridis* - Mutants were characterized by light or viridine green colour with recovery of normal green at later stage. Survived up to maturity with normal reproductive phase; **j.** *Xanthaviridis* - Initially yellow later changed to normal green.

Fig 1: Spectrum of chlorophyll mutations recorded in M_2 generation.

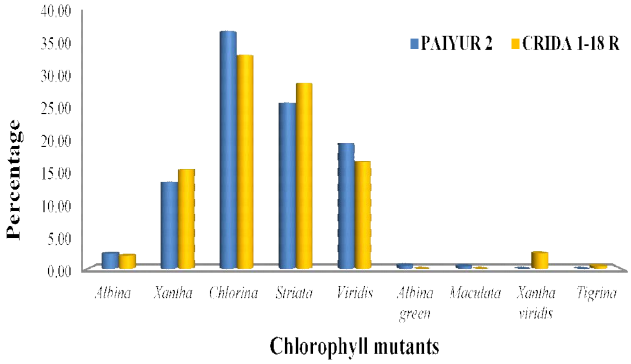


Fig 2a: Relative percentage of chlorophyll mutants in M_2 generation.

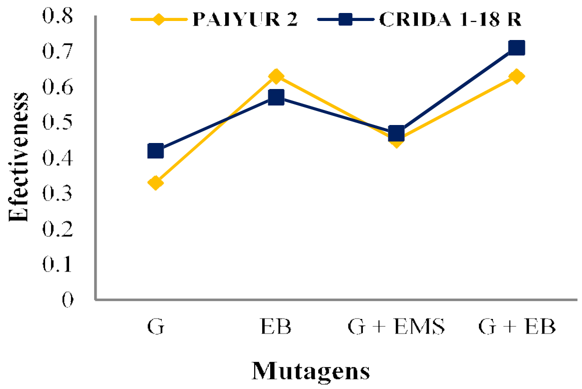


Fig 3a: Average mutagenic effectiveness in M_2 generation of horsegram.

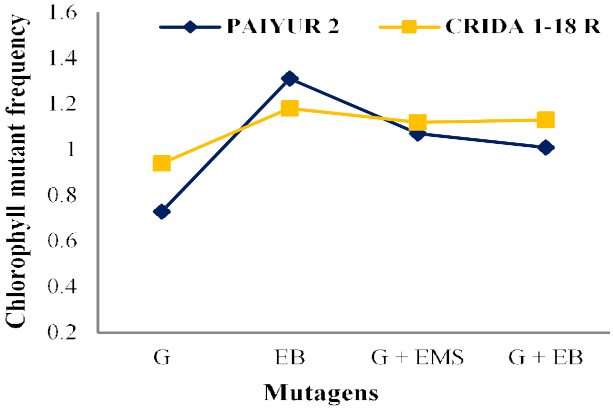


Fig 2b: Frequency of total chlorophyll mutants in M_2 generation.

(44.91% - PAIYUR 2 and 37.55% - CRIDA1-18R). A steady decline in chlorophyll mutants was observed at higher doses in all mutagenic treatments except G in this study. The decrease in mutation frequency at higher doses can be an event of chromosomal aberrations or saturation (Mehraj *et al.*, 1999). EB and its combination with G induced rare mutant types *viz*., *albina-green* and *maculata* in PAIYUR 2. A chlorophyll deficient mutant *xanthaviridis* was observed at all mutagenic treatments except G+EB combination whereas *tigrina* (rare type) was observed only at one combination dose (G+EMS:300 Gy+0.3%) in CRIDA1-18R cultivar. Among the four mutagenic treatments, EB induced maximum frequency of chlorophyll mutants followed by combinations in both varieties (Fig 2b). The improved efficiency of EB

Table 2: Frequency and spectrum of chlorophyll mutants in M_2 generation of horsegram.

Mutagen	No. of M_2 plants studied	No. of chlorophyll mutants	Frequency of chlorophyll mutants (%)	Relative percentage of chlorophyll mutants						
				Albina	*Xantha*	*Chlorina*	*Striata*	*Viridis*	*Albina -green*	*Maculata*
Variety : PAIYUR 2										
Control	1300									
G:100 Gy	1940	10	0.51	0.00	10.00	20.00	20.00	50.00	0.00	0.00
G:200 Gy	1924	11	0.57	0.00	18.18	45.45	18.18	18.18	0.00	0.00
G:300 Gy	1740	14	0.80	7.14	14.29	35.71	42.86	0.00	0.00	0.00
G:400 Gy	1723	18	1.04	0.00	16.67	27.78	22.22	33.33	0.00	0.00
Average		13.25	0.73	1.79	14.79	32.24	25.82	25.38	0.00	0.00
EB:100 Gy	1692	15	0.88	0.00	20.00	46.67	33.33	0.00	0.00	0.00
EB:200 Gy	1756	29	1.65	3.45	13.79	41.38	34.48	0.00	0.00	6.90
EB:300 Gy	1751	28	1.60	7.14	0.00	32.14	25.00	35.71	0.00	0.00
EB:400 Gy	1696	19	1.12	0.00	15.79	36.84	5.26	42.10	0.00	0.00
Average		22.75	1.31	2.65	12.40	39.26	24.52	19.45	0.00	1.73
G + EMS:100 Gy + 0.3%	1740	18	1.03	0.00	16.67	50.00	33.33	0.00	0.00	0.00
G + EMS:200 Gy + 0.3%	1732	13	0.75	7.69	7.69	46.15	38.46	0.00	0.00	0.00
G + EMS:300 Gy + 0.3%	1745	23	1.31	0.00	21.74	43.48	26.09	8.70	0.00	0.00
G + EMS:400 Gy + 0.3%	1701	20	1.18	0.00	0.00	40.00	30.00	30.00	0.00	0.00
Average		18.50	1.07	1.92	11.53	44.91	31.97	9.68	0.00	0.00
G + EB:100 Gy	1716	25	1.45	8.00	16.00	48.00	24.00	0.00	4.00	0.00
G + EB:200 Gy	1419	20	1.41	5.00	20.00	0.00	35.00	35.00	5.00	0.00
G + EB:300 Gy	1298	08	0.62	0.00	12.50	25.00	0.00	62.50	0.00	0.00
G + EB:400 Gy	1250	07	0.56	0.00	14.29	57.14	28.57	0.00	0.00	0.00
Average		15.00	1.01	3.25	15.70	32.54	21.89	24.38	2.25	0.00
Variety : CRIDA1-18R				***Albina***	***Xantha***	***Chlorina***	***Striata***	***Viridis***	***Xantha -viridis***	***Tigrina***
Control	1300									
G:100 Gy	1932	11	0.57	0.00	9.09	0.00	45.45	36.36	9.09	0.00
G:200 Gy	1920	16	0.83	0.00	6.25	43.75	43.75	6.25	0.00	0.00
G:300 Gy	1824	18	0.99	0.00	16.67	33.33	33.33	0.00	16.67	0.00
G:400 Gy	1832	25	1.36	4.00	16.00	4.00	36.00	40.00	0.00	0.00
Average		17.50	0.94	1.00	12.00	20.27	39.63	20.65	6.44	0.00
EB:100 Gy	1347	12	0.89	0.00	0.00	50.00	41.67	0.00	8.33	0.00
EB:200 Gy	1312	19	1.44	10.53	15.79	42.11	31.58	0.00	0.00	0.00
EB:300 Gy	1310	13	0.99	0.00	30.77	7.69	15.38	46.15	0.00	0.00
EB:400 Gy	1295	18	1.39	0.00	16.67	33.33	27.78	22.22	0.00	0.00
Average		15.50	1.18	2.63	15.81	33.28	29.10	17.09	2.08	0.00
G + EMS:100 Gy + 0.3%	1724	18	1.04	5.56	5.56	38.89	0.00	44.44	5.56	0.00
G + EMS:200 Gy + 0.3%	1708	14	0.82	0.00	21.43	50.00	21.43	0.00	0.00	7.14
G + EMS:300 Gy + 0.3%	1482	20	1.35	5.00	25.00	35.00	30.00	5.00	0.00	0.00
G + EMS:400 Gy + 0.3%	1481	19	1.28	0.00	10.53	26.32	31.58	31.58	0.00	0.00
Average		17.75	1.12	2.64	15.63	37.55	20.75	20.26	1.39	1.79
G + EB:100 Gy	691	12	1.74	8.33	0.00	41.67	33.33	16.67	0.00	0.00
G + EB:200 Gy	709	10	1.41	0.00	30.00	40.00	30.00	0.00	0.00	0.00
G + EB:300 Gy	656	05	0.76	0.00	20.00	40.00	20.00	20.00	0.00	0.00
G + EB:400 Gy	650	04	0.61	0.00	25.00	50.00	25.00	0.00	0.00	0.00
Average		7.75	1.13	2.08	18.75	42.92	27.08	9.17	0.00	0.00

G-Gamma ray, EB- Electron beam, EMS - Ethyl methane sulphonate

Table 3: Effectiveness and efficiency of mutagens and its combination in horsegram.

Mutagenic dose	Chlorophyll mutation frequency (%)	Mutagenic effectiveness (%)	Lethality (%)	Injury (%)	Sterility (%)	Efficiency (%)		
						Mf / L	Mf / I	Mf / S
Variety : PAIYUR 2								
G:100 Gy	0.51	0.51	37.77	3.05	10.18	1.35	16.72	5.01
G:200 Gy	0.57	0.29	45.26	29.80	12.48	1.26	1.91	4.57
G:300 Gy	0.80	0.27	51.02	38.96	14.45	1.57	2.05	5.54
G:400 Gy	1.04	0.26	63.72	46.74	14.86	1.63	2.23	7.00
Average	0.73	0.33	49.44	29.64	12.99	1.45	5.73	5.53
EB:100 Gy	0.88	0.88	45.32	17.87	7.21	1.94	4.92	12.21
EB:200 Gy	1.65	0.82	50.04	35.50	12.53	3.30	4.65	13.17
EB:300 Gy	1.60	0.53	58.22	43.00	16.92	2.75	3.72	9.46
EB:400 Gy	1.12	0.28	64.88	46.92	17.42	1.73	2.39	6.43
Average	1.31	0.63	54.62	35.82	13.52	2.43	3.92	10.32
G +EMS:100Gy + 0.3%	1.03	0.86	39.14	7.55	11.83	2.63	13.64	8.71
G +EMS:200Gy + 0.3%	0.75	0.31	45.17	30.89	13.30	1.66	2.43	5.64
G +EMS:300Gy + 0.3%	1.31	0.36	57.58	44.96	17.90	2.28	2.91	7.32
G +EMS:400Gy + 0.3%	1.18	0.25	63.88	46.86	20.90	1.85	2.52	5.65
Average	1.07	0.45	51.44	32.57	15.98	2.11	5.38	6.83
G + EB:100 Gy	1.45	1.45	45.73	29.28	13.78	3.17	4.95	10.52
G + EB:200 Gy	1.41	0.71	57.70	37.41	15.65	2.44	3.77	9.01
G + EB:300 Gy	0.62	0.21	61.67	46.92	19.09	1.01	1.32	3.25
G + EB:400 Gy	0.56	0.14	65.32	50.20	22.74	0.86	1.12	2.46
Average	1.01	0.63	57.61	40.95	17.82	1.87	2.79	6.31
Variety : CRIDA1-18R								
G:100 Gy	0.57	0.57	38.30	11.41	5.88	1.49	5.00	9.69
G:200 Gy	0.83	0.42	45.12	37.47	11.71	1.84	2.22	7.09
G:300 Gy	0.99	0.33	54.88	40.94	16.21	1.80	2.42	6.11
G:400 Gy	1.36	0.34	63.78	47.15	18.32	2.13	2.88	7.42
Average	0.94	0.42	50.52	34.24	13.03	1.82	3.13	7.58
EB:100 Gy	0.89	0.89	46.75	28.72	11.36	1.90	3.10	7.83
EB:200 Gy	1.44	0.72	52.26	38.09	11.61	2.76	3.78	12.40
EB:300 Gy	0.99	0.33	59.92	47.33	13.52	1.65	2.09	7.32
EB:400 Gy	1.39	0.35	64.33	49.01	19.48	2.16	2.84	7.14
Average	1.18	0.57	55.82	40.79	13.99	2.12	2.95	8.67
G +EMS:100Gy + 0.3%	1.04	0.87	39.79	17.18	10.96	2.61	6.05	9.49
G +EMS:200Gy + 0.3%	0.82	0.34	46.56	37.90	15.99	1.76	2.16	5.13
G +EMS:300Gy + 0.3%	1.35	0.38	57.18	41.25	17.82	2.36	3.27	7.58
G +EMS:400Gy + 0.3%	1.28	0.27	64.05	48.26	18.44	2.00	2.65	6.94
Average	1.12	0.47	51.90	36.15	15.80	2.18	3.53	7.29
G + EB:100 Gy	1.74	1.74	47.94	36.41	12.06	3.63	4.78	14.43
G + EB:200 Gy	1.41	0.71	57.15	44.42	18.27	2.47	3.17	7.72
G + EB:300 Gy	0.76	0.25	61.08	52.98	20.02	1.24	1.43	3.80
G + EB:400 Gy	0.61	0.15	65.56	55.27	25.16	0.93	1.10	2.42
Average	1.13	0.71	57.93	47.27	18.88	2.07	2.62	7.09

G - Gamma ray, EB - Electron beam, EMS - Ethyl methane sulphonate, Mf - Mutation frequency; L - Lethality; I - Injury; S - Sterility.

may be attributed to high absorbed dose rate which can deliver high density free radicals in a shorter time period leading to increased double stranded DNA breaks resulting in high mutagenic effects (Zhu *et al*., 2008).

Knowledge on relative biological effectiveness and efficiency of a mutagen is an essential pre-requisite to determine the recovery of high frequency of desirable mutants (Smith 1972). Mutagenic effectiveness refers to the rate of mutations induced per unit dose of a mutagen (dose or time × concentration), whereas mutagenic efficiency depicts the proportion of mutations as against undesirable biological damages *viz*., lethality, seedling injury and sterility. The effectiveness ranged from 0.14% (G+EB: 400 Gy) to 1.45% (G+EB:100 Gy) in PAIYUR 2 and 0.15% (G+EB:400

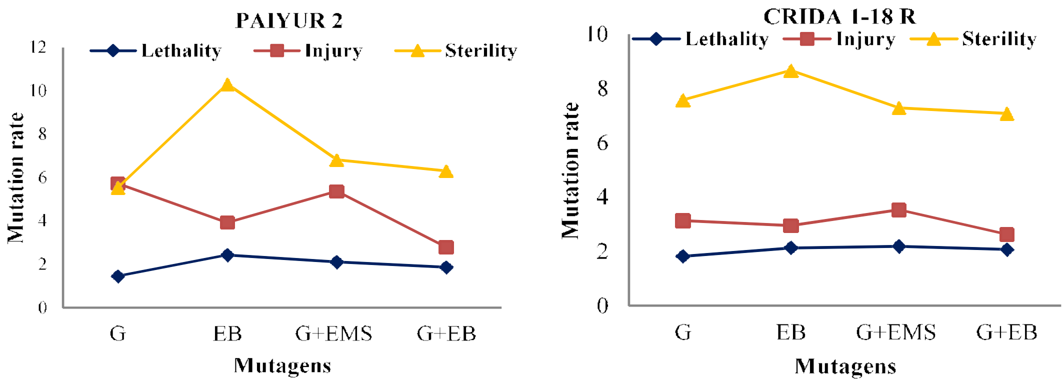


Fig 3b: Mutation rate in relation to biological damage (Lethality, Sterility and Injury)

Gy) to 1.74% (G+EB:100 Gy) in CRIDA1-18R (Table 3). Maximum effectiveness was noticed at the lowest concentration of mutagenic dose in single and combination treatments (Shirsat *et al*., 2010). The lowest dose *viz*., 100 Gy of G and G+EB can be employed in future mutation breeding programme for obtaining high frequency of desirable mutants. On an average, EB and its combination were found to be the most effective mutagen in both varieties (Fig 3a). Similar results were reported by Joshi-Saha *et al*. (2015) in chickpea. A sharp decline in effectiveness was observed at higher doses in all mutagenic treatments studied. The order of mutation rate for effectiveness was: EB (0.63%) and G+EB (0.63%) > G+EMS (0.45%) > G (0.33%) in PAIYUR 2 and G+EB (0.71%) > EB (0.57%) > G+EMS (0.47%) > G (0.42%) in CRIDA1-18R.

The percentage of lethality, injury and sterility increased steadily with high doses of mutagen while the efficiency was found to be declining with higher doses. Similar results were observed by Kavithamni *et al*. (2008) in soybean. The efficiency with relation to lethality decreased at higher mutagenic doses in all treatments except G in both varieties. Mutagenic doses *viz*., EB:200 Gy (3.30%) in PAIYUR 2 and G+EB:100 Gy (3.63%) in CRIDA1-18R scored maximum value for efficiency in terms of lethality. The highest efficiency was noticed in G:100 Gy (16.72%) in PAIYUR 2 and G+EMS:100 Gy+0.3% (3.53%) in CRIDA1-18R with reference to injury whereas, EB:200 Gy (13.17%) and G+EB:100 Gy (14.43%) showed improved efficiency for sterility in PAIYUR 2 and CRIDA1-18R respectively. Higher efficiency at lower and moderate doses of mutagenic agent is due to relatively low biological damage, which increases at faster rate on higher doses than the mutation induced (Konzak *et al*., 1965). EB recorded high mutation rate for sterility in both varieties. With respect to lethality and injury, varied mutagenic efficiency was registered between varieties. EB and G exhibited high mutation rate for lethality and sterility in PAIYUR 2 whereas G+EMS was found to efficient in CRIDA1-18 R (Fig 3b). The order of mutation rate for efficiency is as follows:

Lethality	PAIYUR 2	EB > G+EMS > G+EB > G
	CRIDA1-18R	G+ EMS > EB > G+EB > G
Injury	PAIYUR 2	G > G+EMS > EB > G+EB
	CRIDA1-18R	G+EMS > G > EB > G+EB
Sterility	PAIYUR 2	EB > G+EMS > G+EB > G
	CRIDA1-18R	EB > G > G+EMS > G+EB

In this study, the mutagenic treatments exhibiting high mutation rate for effectiveness and efficiency were considered in order to obtain high frequency of mutation changes with less undesirable effects. Electron beam was found to be effective as well as efficient (sterility and lethality - PAIYUR 2 and Sterility - CRIDA1-18R) in both varieties, hence it can be employed in future mutation breeding programme to induce variability in horsegram.

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