International Journal of Life science & Pharma Research

EFFECT OF MUTAGENS ON SEED GERMINATION, PLANT SURVIVAL AND QUANTITATIVE CHARACTERS OF HORSEGRAM (*Macrotyloma uniflorum* (LAM.) VERDC)

BOLBHAT SADASHIV N. BHOGE VIKRAM D. AND DHUMAL KONDDIRAM N.*

Dada Patil Mahavidyalaya Karjat, Dist- Ahmednagar-414402 (M.S.) India
Department of Botany, University of Pune, Pune-411007 (M.S.) India*

ABSTRACT

Seeds of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) cv. Dapoli Kulthi-1 and cv. Rajapur-5 were treated with various doses/ concentrations of gamma radiation (100 to 400Gy), EMS (0.2 to 0.5%) and their combinations. The physiological effects on seed germination (7 days) and plant survival at 45 days after sowing were investigated. Gradual reduction in seed germination and plant survival was recorded with increase in dose / concentration of mutagens. Lower doses of gamma rays 100Gy (89.3%), EMS 0.2% (86.2%) and in combination 100Gy + 0.2% (85.6%) showed highest germination percentage in cv. Dapoli Kulthi- 1. In cv. Rajapur- 5 highest germination percentage was recorded in 100Gy (87.3%), EMS- 0.2% (85.2%) and in combination 200Gy + 0.2%EMS (82.17%). The survival percent in both cultivars of horsegram was decreased as the dose of gamma radiation increased. Significant reduction in survival percentage was observed at the higher doses (400Gy) of gamma radiation, EMS (0.5%) and in their combination (400 + 0.5%EMS).Various micromutations with respect to quantitative characters were scored in M_1 population. For both cultivars almost all the mutagenic treatments (except very few) caused decrease in plant height, primary branches per plant, days required for first flowering, number of pods per plant, pod length, number of seeds per pod and seed yield per plant during M_1.

Key words : Horsegram, germination, mutagens, micromutations

INTRODUCTION

Amongst pulses horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) is highly neglected in India and hence require more emphasis on its improvement as it has nutritional, medicinal and fodder value. Horsegram is drought tolerant and having good nitrogen fixing ability, but receives a low priority in cropping system, soil types etc.(Bolbhat and Dhumal, 2010). It is grown in *kharif* and *rabbi* seasons, as main crop, or as a mixed crop. It is cultivated in areas with annual rainfall 300-600 mm, but does not tolerate flooding or water logging. The favourable average temperature is 18 to 27ºC, and adapted to a wide range of well-drained soils. The whole seeds of horsegram generally used as cattle feed. The seed, sprout and whole meal can be used by large populations in rural areas (Kanaka, 2012). The use of dry seeds of horsegram as human food is limited due to its poor cooking quality, presence of high level of enzyme inhibitors and heamagglutinin activities (Ray, 1969). The seeds are rich in tannins and polyphenols as compared to the other legumes (Kadam and Salunkhe, 1985). Antinutrients like phytates, tannins and oxalic acid reduce the availability of iron. Induced mutagenesis is useful and effective method in crop improvement. Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time (Manjaya and

Nandanwar, 2007).There fore the present investigation was undertaken study the effect of gamma rays, EMS and their combination in inducing genetic variability in quantitative and qualitative characters in M_1 generation of horsegram and results are discussed.

MATERIALS AND METHODS

The authentic seeds of horsegram (*Macrotyloma uniflorum* (Lam.)Verdc) cv. Dapoli Kulthi-1 and Rajapur- 5 received from Department of Botany, Dr. B.S.K.K.Vidyapeeth, Dapoli, Dist- Ratnagiri, (M.S., India) were dried to reduce moisture content up to 10-12 %. These seeds were irradiated with100, 200, 300 and 400Gy of gamma rays from ^{60}Co source at Bhabha Automic Research Center (BARC), Trombay, Mumbai, (MS). Few irradiated and few fresh seeds were soaked in water for 10 hours. Wet seeds were treated with different concentrations of EMS (Sigma chemical Co. Ltd. USA) such as 0.2, 0.3, 0.4 and 0.5% for four hours. Treated seeds washed thoroughly with tap water for two hours and then used for raising M_1 generation. A total of 25 treatment combinations in M_1 generation including untreated dry seeds used as control. A total of 75 seeds from each treatment was used for seed germination in field. Three replications with 25 seeds / replication sown in field were used for recording seed germination percentage on 7th day. Treated and control seeds were sown in a field at a spacing of 30 x 15 cm in randomized block design Effect of gamma radiation, EMS and their combinations on seed germination and survival of plants are presented in Table 1. Both the varieties were more sensitive to mutagens. The data presented in table 1 reveal that the seed germination in field was reduced with increasing dose / concentrations of gamma rays, EMS and their combinations. Lower doses of gamma rays (100Gy- 89.3%), EMS (0.2% - 86.2%) and in combination (100Gy + 0.2% 85.6%) showed highest germination percentage in cv. Dapoli Kulthi- 1. In cv. Rajapur- 5 highest germination percentage was recorded in 100Gy (87.3%), EMS-0.2% (85.2%) and in combination 200Gy + 0.2% EMS (82.17%). The results were in conformation with Datir et al. (2007) and Dalvi (1990) in horsegram, Potdukhe and Narkhede (2002) in pigeon pea. In both cultivers higher dose / concentrations were inhibitory while lower had stimulative effect on seed germination. Similar trend was recorded by Bolbhat and Dhumal

replicated thrice. 1000 seeds for each treatment were used to raise M_1 generation and all the M_1 plants were harvested individually. Randomly 20 plants from each treatment of M_1 populations were observed for mutations at regular intervals from seedling stage to maturity of the crop. Mean values of each parameter such as germination percentage (7th Day), survival percentage at 45 DAS and micromutations with respect to quantitative characters at maturity of both cultivars were recorded in the table.

STATISTICAL ANALYSIS

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.3.5).

RESULTS AND DISCUSSION

Seed germination

(2009) in horsegram. The physiological damage in terms of reduction in germination and survival percentage revealed that gamma radiation was more deleterious to the cv. Rajapur- 5 as compared to cv. Dapoli Kulthi- 1 (Datir et al., 2007). The inhibitory effect on seed germination was directly proportional to the dose/ conc. of gamma radiation, EMS and their combinations (Table-1). Inhibitory effects on seed germination by the above mentioned mutagens were reported earlier by Kulkarni (1978), Rudraswami (1983) and Dalvi (1990) in horsegram, Decrease in percent seed germination with increasing concentrations of EMS was reported by Senapati et al., (2008) Girija and Dhanvel (2009), Auti (2005) and Barshile (2006) in different legumes. Decrease in percent seed germination in horsegram caused by gamma radiation, EMS, and their combinations might be due to their effects on genetical and cytological processes coupled with the changes induced in metabolic processes. The

decrease in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds (Sjodin, 1962). Sinha and Godward (1972) opinioned that the reduction in percentage of seed germination and survival was due to the disturbances caused at the physiological level coupled with chromosomal damage. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens (Kulkarni, 2011). Gamma radiation, EMS, and their combinations are potent mutagens, well known for their action causing point mutations, enzyme inhibitions and chromosomal aberrations (Auti, 2005). The observed reduction in seed germination in horsegram as a result of treatments of these mutagens might be due to point mutations or the injuries caused to the genetic material. This may eventually lead to decrease the rate of respiration and energy production, which finally caused decrease in seed germination.

Table 1. ***Effect of mutagens on percent seed germination and plant survival in M_1 generation of horsegram***

Treatments	% germination		% survival (45 DAS)	
	Dapoli Kulthi- 1	Rajapur- 5	Dapoli Kulthi- 1	Rajapur- 5
Control	91.1±3.64	90.5±3.62	80.5±3.22	79.2±3.17
100Gy	89.3±4.47	87.3±4.36	78.1±3.91	75.6±3.78
200	81.7±5.72	79.7±5.58	69.5±4.87	68.3±4.78
300	73.4±2.20	69.5±2.08	62.3±1.87	59.8±1.79
400	59.6±3.58	57.8±3.47	53.8±3.23	51.5±3.09
0.2%EMS	86.2±5.17	85.2±5.11	74.6±4.48	72.5±4.35
0.3%	81.4±3.26	78.5±3.14	68.3±2.73	67.3±2.69
0.4%	76.7±5.37	73.8±5.17	64.2±4.49	62.7±4.39
0.5%	64.3±1.93	61.3±1.84	56.5±1.70	52.6±1.58
100 + 0.2%EMS	85.6±4.28	81.4±4.07	72.2±3.61	70.3±3.52
100 + 0.3%	80.3±2.41	76.7±2.30	66.5±2.00	62.5±1.88
100 + 0.4%	71.7±4.30	66.3±3.98	60.3±3.62	59.8±3.59
100 + 0.5%	58.9±4.12	55.9±3.91	51.7±3.62	47.2±3.30
200 + 0.2%	83.2±3.33	82.1±3.28	73.2±2.93	68.1±2.72
200 + 0.3%	78.5±3.93	75.3±3.77	65.9±3.30	60.3±3.02
200 + 0.4%	64.3±4.50	61.7±4.32	57.3±4.01	58.2±4.07
200 + 0.5%	49.8±1.49	48.3±1.45	41.2±1.24	45.7±1.37
300 + 0.2%	82.5±4.13	80.3±4.02	72.7±3.64	67.3±3.37
300 + 0.3%	75.1±4.51	72.1±4.33	67.3±4.04	57.9±3.47
300 + 0.4%	60.3±2.41	58.9±2.36	53.1±2.12	50.4±2.02
300 + 0.5%	48.2±1.45	47.1±1.41	40.8±1.22	42.6±1.28
400 + 0.2%	80.9±5.66	78.7±5.51	71.3±4.99	66.2±4.63
400 + 0.3%	73.6±4.42	73.4±4.40	64.5±3.87	57.4±3.44
400 + 0.4%	54.3±2.72	53.8±2.69	55.6±2.78	51.3±2.57
400 + 0.5%	45.1±1.80	44.6±1.78	38.4±1.54	38.1±1.52
SEM±	3.14	3.04	2.73	2.6
F-value	36.98	37.89	35.16	32.32
P-value	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	6.15	5.96	5.35	5.10

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Plant survival

The survival percent in both cultivars of horsegram was decreased as the dose of gamma radiation increased (Table-1). Significant reduction in survival percentage was observed at the higher doses (400Gy) of gamma radiation, EMS (0.5%) and in their combination (400 + 0.5%EMS). Amongst all the treatments, combination treatments were having more adverse impact on survival of plants. Decrease in survival

percent due to mutagenic treatments was reported by Kulkarni (1978), Rudraswami (1983) and Dalvi (1990) in horsegram, Auti (2005), Barshile (2006), Dhanavel et al., (2008) and Girija and Dhanvel (2009), Kavithamni et al., (2008) and Potdukhe and Narkhede (2002) in various pulse crops.

Quantitative characters (Micromutations)

Data on effect of mutagens on quantitative characters in M_1 generation was recorded for plant height, primary branches per plant, number of days required for 1st flowering, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight and total seed yield per plant (Table-2 and 3).

Plant height

All the mutagens were effective for inducing variability in plant height of horsegram cv. Dapoli Kulthi-1and Rajapur-5 in M_1 generation (Table-2 and 3). Gamma radiation treatments such as 100, 200 and 400Gy caused significant reduction in plant height, except 300Gy. The lower concentration treatments of EMS (0.2, 0.3 and 0.4%) had stimulatory effect on plant height over control. However the higher concentration treatment (0.5%) was responsible for reduction of plant height in both cultivars. The combination treatments of GR and EMS had also shown –ve influence on the plant height except 100Gy + 0.2%EMS in both cultivars.

Number of primary branches per plant

All doses of GR (except 200Gy) had +ve impact on this parameter. However the higher doses had shown –ve influence as compared to control. Similar trend was noted for EMS treatments except 0.4 and 0.5%EMS. All the combination treatments had –ve impact on number of branches per plant. But 300Gy + 0.2%EMS (cv. Dapoli Kulthi-1) and 100 + 0.2%EMS (cv. Rajapur- 5) (Table- 2 and 3) showed +ve impact.

Days required for first flowering

Both the mutagens gamma radiation and EMS single and in combination treatments succeeded for inducing the variability in number of days required for first flowering in M_1 generation (Table- 2 and 3). In Dapoli Kulthi- 1 almost all the treatments of GR caused slight delay in first flowering over control (49.21DAS). Same trend was repeated with EMS and combination of GR and EMS, except 400Gy + 0.4%EMS (30.17 DAS) and 400Gy + 0.5%EMS (33.46 DAS). While in cv. Rajapur- 5 all mutagens caused earlyness except 300Gy and 0.5%EMS.

Number of pods per plant

Gamma radiation and EMS single and in combination treatments were succeeded in inducing the variability in number of pods per plant in M_1 generation of both cultivars. The data recorded in Table- 2 and 3 revealed that the treatments used had +ve as well as –ve influence on number of pods per plant. In M_1 generation of cv. Dapoli Kulthi- 1 maximum number of pods per plant (140) was noted in 300Gy than control (110.47). Minimum number of pods per plant (74.00) was recorded at 200Gy. In EMS maximum number of pods per plant (156) was noted at 0.2% EMS and minimum (83.20) at 0.4%EMS over control (110.47). All the combination treatments reduced the number of pods per plant except 200Gy + 0.4%EMS (124.00).

In cv. Rajapur- 5 maximum number of pods per plant (88.25, 91.10, 90.39 and 93.05) was noted in 300 and 400Gy, 0.2, 0.3 and 0.4%EMS.While other treatments caused –ve influence.

Pod length

In both cultivars mean values for this parameter showed negative influence (Table- 2 and 3) of all the mutagens used. Gamma radiation and EMS single and in combination, all treatments showed reduction in pod length with few exceptions as compared to control.

Number of seeds per pod

In both cultivars there was no significant change in number of seeds per pod, almost all the treatments were on par with control (Table- 2 and3)

1000- Seed weight

Results recorded on 1000-seed weight (Table- 2 and 3) indicated that there was stimulatory as well as inhibitory effect on the mean values for this parameter. In Dapoli Kulthi- 1 mean values of 1000-seed weight in gamma radiation (25.90g) at 100Gy, EMS (28.82g) at 0.2% and in combination

treatments (26.01g) at 100Gy + 0.2%EMS, exerted maximum increase in 1000 seed weight over control (24.91g). While in Rajapur- 5 100Gy (22.13g), 0.2%EMS (22.62g) and 100Gy + 0.4%EMS (23.20g), exerted maximum increase in 1000 seed weight.

Seed yield per plant

Data on seed yield per plant in M_1 progeny showed (Table- 2 and 3) improvement or reduction in seed yield per plant over control. Dapoli Kulthi- 1 showed significant improvement in seed yield per plant over control (15.53g) was obtained in treatments like 0.2%EMS (24.67g) followed by 400Gy (22.09g) and 300Gy (21.34g). However treatments like 200Gy, 100Gy+0.4%EMS and 200Gy+0.5%EMS had caused highest reduction in seed yield per plant (11.12g, 10.16g and 10.05g). While cv. Rajapur- 5 showed maximum increase in treatments such as 0.2 (13.24g), 0.3 (12.98g) and 0.4%EMS (12.76g). Almost all reports on induced mutation studies in different crop plants have reveled physical damage in M_1 generation, there by inducing changes in quantitative characters. All the mutagenic treatments except few brought about decrease in the plant height, number of primary branches per plant, days required for first flowering, number of pods per plant, pod length, number of seeds per pod and seed yield per plant (Table- 2 and 3) in horsegram cv. Dapoli Kulthi-1 and cv. Rajapur- 5 during M_1 generation. Earlier reports in blackgram, soybean, and grasspea were in confirmatory with present findings (Misra, 1992, Rakshit and Singh, 2001, Rybinski, 2003, Patil et al., 2004, Sharma et al., 2005 and Senapati et al., 2008). The genes responsible for diverse types of traits, which are distributed throughout the genome, might have been affected by the mutagens all resulted in to different types of micromutations (Senapati et al., (2008), Bolbhat (2011) and Dhumal and Bolbhat (2012).

Table 2. ***Micromutations in M_1 generation of horsegram cv. Dapoli Kulthi-1.***

Treatments	Plant height (cm)	Primary branches/ plant	1st flowering	Number of pods/ plant	Pod length (cm)	Number of grains/ pod	1000 seed weight (gm)	Yield/ plant (gm)
Control	52.80±7.39	9.87±1.38	49.21±6.89	110.47±15.47	6.12±0.86	6.85±0.96	24.91±3.49	15.53±2.17
100Gy	50.41±4.03	12.40±0.99	51.34±4.11	97.00±7.76	6.08±0.49	6.80±0.54	25.90±2.07	16.27±1.30
200	49.20±5.41	9.40±1.03	49.17±5.41	74.00±8.14	5.74±0.63	6.33±0.70	25.10±2.76	11.12±1.22
300	54.20±7.05	11.20±1.46	51.05±6.64	140.00±18.20	5.86±0.76	7.00±0.91	24.78±3.22	21.34±2.77
400	47.15±6.60	10.60±1.48	49.82±6.97	139.80±19.57	6.10±0.85	7.40±1.04	24.23±3.39	22.09±3.09
0.2 %EMS	56.80±3.98	11.60±0.81	48.22±3.38	156.80±10.98	6.12±0.43	7.40±0.52	28.82±2.02	24.67±1.73
0.3 %	53.12±4.25	11.00±0.88	50.36±4.03	108.00±8.64	5.90±0.47	6.00±0.48	24.85±1.99	15.72±1.26
0.4 %	54.60±8.19	7.60±1.14	50.42±7.56	83.20±12.48	5.48±0.82	6.60±0.99	26.17±3.93	13.43±2.01
0.5 %	47.40±6.64	9.80±1.37	50.64±7.09	125.40±17.56	5.50±0.77	6.40±0.90	25.21±3.53	16.21±2.27
100Gy + 0.2 %EMS	53.40±4.81	8.00±0.72	51.24±4.61	105.60±9.50	6.14±0.55	7.20±0.65	26.01±2.34	17.86±1.61
100Gy + 0.3 %	45.20±6.78	8.80±1.32	48.35±7.25	94.60±14.19	5.88±0.88	7.00±1.05	24.65±3.70	14.37±2.16
100Gy + 0.4 %	45.29±5.89	8.20±1.07	50.15±6.52	67.20±8.74	5.62±0.73	7.00±0.91	24.07±3.13	10.16±1.32
100Gy + 0.5 %	43.05±6.03	7.80±1.09	49.11±6.88	86.20±12.07	6.10±0.85	7.00±0.98	23.12±3.24	12.09±1.69
200Gy + 0.2 %	48.25±3.38	8.00±0.56	51.27±3.59	91.80±6.43	5.88±0.41	7.00±0.49	24.83±1.74	13.30±0.93
200Gy + 0.3 %	46.65±4.20	8.40±0.76	51.57±4.64	98.20±8.84	5.92±0.53	5.96±0.54	23.90±2.15	14.07±1.27
200Gy + 0.4 %	44.34±4.88	8.60±0.95	49.71±5.47	124.00±13.64	6.04±0.66	7.00±0.77	23.75±2.61	17.12±1.88
200Gy + 0.5 %	42.16±2.53	9.80±0.59	51.75±3.11	66.80±4.01	5.92±0.36	7.00±0.42	23.85±1.43	10.05±0.60
300Gy + 0.2 %	46.80±6.08	10.00±1.30	51.29±6.67	120.00±15.60	6.00±0.78	7.00±0.91	25.30±3.29	18.34±2.38
300Gy + 0.3 %	45.81±4.12	9.80±0.88	49.46±4.45	92.40±8.32	5.92±0.53	7.00±0.63	25.14±2.26	14.12±1.27
300Gy + 0.4 %	44.60±4.01	9.60±0.86	52.82±4.75	84.80±7.63	5.88±0.53	6.60±0.59	24.05±2.16	10.49±0.94
300Gy + 0.5 %	43.69±3.06	9.40±0.66	51.73±3.62	123.00±8.61	6.22±0.44	7.00±0.49	24.87±1.74	18.35±1.28
400Gy + 0.2 %	47.40±5.69	9.60±1.15	50.33±6.04	80.60±9.67	6.00±0.72	6.80±0.82	25.63±3.08	12.47±1.50
400Gy + 0.3 %	44.60±3.12	9.00±0.63	51.75±3.62	59.80±4.19	5.56±0.39	7.00±0.49	24.72±1.73	10.35±0.72
400Gy + 0.4 %	41.20±2.47	8.20±0.49	30.17±1.81	111.00±6.66	6.08±0.36	7.40±0.44	25.95±1.56	19.11±1.15

400Gy + 0.5 %	42.30±4.65	8.33±0.92	33.46±3.68	108.00±11.88	6.17±0.68	6.67±0.73	24.79±2.73	15.22±1.67
SEM±	4.28	0.83	4.40	9.40	0.53	0.61	2.21	1.40
F-value	2.09	4.57	2.92	13.73	0.32	0.77	0.50	15.39
P-value	<0.001	<0.001	<0.001	<0.001	1.00	0.75	0.96	<0.001
$LSD_{0.05}$	8.39	1.63	8.62	18.42	1.04	1.20	4.33	2.74

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Table 3. *Micromutations in M_1 generation of horsegram cv. Rajapur- 5*

Treatments	Plant height (cm)	Primary branches/ plant	1st flowering	Number of pods/ plant	Pod length (cm)	Number of grains/ pod	1000 seed weight (gm)	Yield/ plant (gm)
Control	42.65±5.97	10.13±1.42	46.35±6.49	85.21±11.93	5.71±0.80	5.31±0.74	21.67±3.03	12.47±1.75
100Gy	42.22±3.38	11.90±0.95	45.11±3.61	83.13±6.65	5.49±0.44	5.45±0.44	22.13±1.77	12.59±1.01
200	41.09±4.52	10.07±1.11	46.78±5.15	75.36±8.29	5.11±0.56	5.29±0.58	21.65±2.38	12.11±1.33
300	45.31±5.89	12.19±1.58	44.63±5.80	88.25±11.47	5.63±0.73	5.35±0.70	20.15±2.62	12.53±1.63
400	40.51±5.67	11.33±1.59	45.14±6.32	86.79±12.15	5.69±0.80	5.11±0.72	20.41±2.86	12.18±1.71
0.2%EMS	44.63±3.12	13.17±0.92	43.92±3.07	91.10±6.38	5.43±0.38	5.65±0.40	22.62±1.58	13.24±0.93
0.3	43.13±3.45	12.41±0.99	46.98±3.76	90.39±7.23	5.21±0.42	5.69±0.46	21.71±1.74	12.98±1.04
0.4	45.37±6.81	13.26±1.99	45.28±6.79	93.05±13.96	5.53±0.83	5.28±0.79	21.29±3.19	12.76±1.91
0.5	38.80±5.43	9.72±1.36	45.11±6.32	68.14±9.54	5.67±0.79	5.09±0.71	21.13±2.96	10.22±1.43
100 + 0.2% EMS	43.22±3.89	11.29±1.02	47.18±4.25	71.43±6.43	5.70±0.51	5.17±0.47	21.75±1.96	11.19±1.01
100 + 0.3%	40.75±6.11	9.20±1.38	46.42±6.96	65.18±9.78	5.63±0.84	5.21±0.78	21.49±3.22	10.12±1.52
100 + 0.4%	39.19±5.09	9.11±1.18	45.19±5.87	68.62±8.92	5.75±0.75	5.98±0.78	23.20±3.02	10.52±1.37
100 + 0.5%	36.48±5.11	8.89±1.24	44.53±6.23	59.77±8.37	5.62±0.79	5.69±0.80	23.11±3.24	10.46±1.46
200 + 0.2%	40.31±2.82	9.26±0.65	43.89±3.07	60.11±4.21	5.31±0.37	5.23±0.37	21.65±1.52	9.29±0.65
200 + 0.3%	41.12±3.70	9.11±0.82	45.12±4.06	57.93±5.21	5.46±0.49	5.39±0.49	21.24±1.91	9.23±0.83
200 + 0.4%	36.53±4.02	8.13±0.89	44.26±4.87	55.81±6.14	5.58±0.61	5.63±0.62	20.98±2.31	9.05±1.00
200 + 0.5%	35.21±2.11	8.45±0.51	45.65±2.74	56.17±3.37	5.39±0.32	5.30±0.32	20.67±1.24	8.79±0.53
300 + 0.2%	38.82±5.05	9.25±1.20	44.74±5.82	62.24±8.09	5.60±0.73	5.35±0.70	21.12±2.75	9.12±1.19
300 + 0.3%	38.21±3.44	8.72±0.78	44.33±3.99	54.46±4.90	5.75±0.52	4.91±0.44	22.19±2.00	8.15±0.73
300 + 0.4%	33.48±3.01	7.47±0.67	45.69±4.11	51.17±4.61	5.80±0.52	5.54±0.50	22.27±2.00	8.11±0.73
300 + 0.5%	30.17±2.11	7.05±0.49	47.13±3.30	49.63±3.47	5.35±0.37	5.41±0.38	21.09±1.48	7.79±0.55
400 + 0.2%	36.64±4.40	8.19±0.98	46.71±5.61	50.95±6.11	5.11±0.61	5.20±0.62	20.62±2.47	7.51±0.90
400 + 0.3%	34.19±2.39	8.27±0.58	45.19±3.16	49.76±3.48	5.38±0.38	5.28±0.37	20.96±1.47	7.47±0.52
400 + 0.4%	30.79±1.85	7.21±0.43	44.34±2.66	46.12±2.77	5.65±0.34	5.11±0.31	21.12±1.27	7.58±0.45
400 + 0.5%	30.07±3.31	8.38±0.92	46.43±5.11	48.98±5.39	5.51±0.61	5.69±0.63	21.45±2.36	7.85±0.86
SEM±	3.54	0.89	4.05	6.33	0.49	0.48	1.91	0.95
F-value	3.31	8.33	0.13	12.08	0.31	0.52	0.32	8.95
P-value	<0.001	<0.001	1.00	<0.001	1.00	0.96	1.00	<0.001
$LSD_{0.05}$	6.94	1.74	7.94	12.41	0.96	0.94	3.74	1.86

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

CONCLUSION

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens but lower doses/ concs. of gamma rays as well as EMS had shown stimulatory effect in both cultivars. The survival rate was highly reduced with increasing dose/concs. of mutagens. Almost all the mutagenic treatments (except very few) caused decrease in plant height, primary branches per plant, days required for first flowering, number of pods per plant, pod length, number of seeds per pod and seed yield per plant during M_1.

REFERENCES

1. Auti SG. Mutational Studies in mung (*Vigna radiata* (L.) Wilczek). Ph.D. Thesis. 2005; University of Pune, Pune (MS), India.
2. Barshile JD. Studies on effect of mutagenesis employing EMS, SA and GR in Chickpea (*Cicer arietinum* L.). Ph.D. Thesis. 2006; University of Pune, Pune (MS), India.
3. Bolbhat SN and Dhumal KN. Desirable mutants for pod and maturity characteristics in M_2 generation of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). Res. on Crops. 2010; 11 (2): 437-440.
4. Bolbhat SN and Dhumal KN. Induced macromutations in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). Legume Res. 2009; 32 (4): 278-281.
5. Bolbhat SN. Studies on induced mutations in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). Ph. D. Thesis. 2011; University of Pune, Pune (MS) India.
6. Dalvi VV. Gamma rays induced mutagenesis in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). M.Sc. dissertation. 1990; Dr. B. S. K. K. Vidyapeeth, Dapoli (MS), India.
7. Datir SS, Dhumal KN and Pandey RN. Gamma radiation and EMS induced variation in seed germination and plant survival in horsegram (*Macrotyloma uniflorum* (Lam.)Verdc). J. Arid Legumes. 2007; 4 (1): 15-17.
8. Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M and Thilagavathi C. Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). African J. of Biotechnology. 2008; 7 (22): 4116-4117.
9. Dhumal KN and Bolbhat SN. Gamma Radiation. 1st ed. Rijeca (Croatia): In Tech Publisher. c2012. Chapter 10, Induction of genetic variability with gamma radiation and its applications in improvement of horsegram; p. 207-228.
10. Girija M and Dhanavel D. Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments in cowpea (*Vigna unguiculata* (L.) Walp). Global J. Mol Sci. 2009; 4 (2): 68-75.
11. Kadam SS and Salunkhe DK. Nutritional composition, processing, and utilization of horsegram and moth bean. CRC Rev. Food Sci. Nutri. 1985; 22: 1–26.
12. Kanaka KD. Variability and divergence in horsegram (*Dolichos uniflorus*). J. Arid Land. 2012; 4 (1): 71-76.
13. Kavithamni D, Kalamani A, Vannirajan C and Uma D. Mutagenic effectiveness and efficiency of gamma rays and EMS in Soybean (Glycine max (L.) Merrill). Agric. J. 2008; 95 (7-12): 448-451.
14. Kulkarni GB. Effect of mutagen on pollen fertility and other parameters in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). Bio. Sci. discovery. 2011; 2 (1): 146-150.
15. Kulkarni RN. Mutagenic effects of gamma-rays and EMS in horsegram. Genet. agri. 1978; 62-71.
16. Manjaya JG and Nandanwar RS. Genetic improvement of soybean variety JS 80-21 through induced mutations. Plant Mutation Reports. 2007; 1 (3):36-40.
17. Misra RC. Induced mutation and its implications in improvement of blackgram. Ph.D. Thesis. 1992. Orissa Univ. of. Agric. and Technol., Bhubaneswar, (Orissa), India.
18. Patil A, Taware SP and Raut VM. Induced variation in quantitative traits due to physical (g-rays), chemical (EMS) and combined mutagen treatment in soybean (*Glycine max*

(L.) Merrill). Soyb. Genet. Newsl. 2004; 31: 1-6.
19. Potdukhe NR and Narkhede MN Induced mutation in pigeonpea (*Cajanus cajan* (L.) Millsp.). J. Nuclear Agric. Biol. 2002; 31 (1): 41-46.
20. Rakshit S, Singh VP. Chemo sensitivity studies in mungbean and urdbean. Indian J. Pulses Res. 2001; 14 (2): 112-115.
21. Ray P K. Toxic factor(s) in row horsegram (*Dolichos biflorus*). J. food Sci. 1969; 6: 207-211.
22. Rudraswami P. Induced mutagenesis in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) using gamma-rays and EMS. Mysore J. of Agri. Sci. 1983; 21 (1): 95.
23. Rybinski W. Mutagenesis as a tool for improvement of traits in grasspea (*Lathyrus sativus* L.). Lathyrism Newsletter. 2003; 3: 27-31.
24. Senapati N, Misra RC and Muduli KC. Induced macromutations in blackgram *Vigna mungo* (L.) Hepper. Legume Res. 2008; 31 (4): 243-248.
25. Sharma SK, Sood R, and Pandey DP. Studies on mutagen sensitivity effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper), Indian J. Genet. 2005; 65 (1): 20-22.
26. Sinha SS and Godward N. Radiation studies in *Lens culanaris*. Induction of mutations and type of mutants. J. Cytol. Genet. 1972; 8: 131-136.
27. Sjodin J. Some observations in X_1 and X_2 of *Vicia faba* (L.) after treatment with different mutagens. Hereditas. 1962; 48: 565-586.