

EMS AND GAMMA RAYS INDUCED MUTATION IN GREENGRAM [VIGNA RADIATA (L.) R. WILCZEK]

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INTRODUCTION

Greengram (*Vigna radiata* (L.) Wilczek) ($2n = 2x = 22$) is a self-pollinated legume originated in South Asia. It is an economically important crop in India, Pakistan, Thailand, Vietnam, Myanmar, and China with the combined planted area of over 5 million ha. The crop is considered rather wild as it still gives low seed yield (< 1 t/ha), with uneven maturity. This opens an ample room for mung bean breeders to improve the crop.

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). Mutation has been successfully employed in breeding of several food crop varieties, ornamentals and export crops (Mohamad *et al.*, 2005). Gamma rays are the most energetic form of electromagnetic radiation, can be useful for the alteration of physiological characters. These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf *et al.*, 2003). Ethyl Methane Sulphonate (EMS) is mutagenic and carcinogenic organic compound, it produces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation and it is reported to be the most effective and powerful mutagen (Hajara, 1979) and typically produces only point mutations (Okagaki *et al.*, 1991). The potentiality of ionizing radiations and chemical mutagens is different and their ability to induce mutations varies from genotype to genotype in rice (Singh and Sharma, 2013).

Keeping the above facts in view, the present investigation in green gram was undertaken to induce genetic variability following EMS (chemical mutagen) and gamma (Physical mutagen) irradiation on seed quality parameters for the improvement in green gram.

MATERIALS AND METHODS

Laboratory study was conducted at the Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh, Gujarat, India, to induce genetic variability in green gram, for which two varieties of green gram (GM 4 and Meha) treated with seven different doses of gamma rays (100, 200, 400, 600, 800, 1000 and 1200 Gy) and ethyl methane sulphonate (0.03, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 %) following Factorial Completely Randomized Design repeated three times. Healthy dry seeds of both the varieties subjected to the gamma ray irradiation treatment at Bhabha Atomic Research Station, Trombay, Mumbai. Observations were recorded on germination per cent (ISTA, 1993), plumule length (cm), radical length (cm), speed of germination (Maguire, 1962) and seed vigour index (length) (AbdulBaki and Anderson, 1973) and analyzed following Factorial

ABSTRACT

In the present study, two varieties of green gram (GM 4 and Meha) was treated with seven different doses each of gamma rays (100, 200, 400, 600, 800, 1000 and 1200 Gy) and ethyl methane sulphonate (0.03, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 %) with the objectives to induce genetic variability in mungbean. The M1 generation was raised in the laboratory. The observations were recorded on germination per cent, plumule length, radical length, speed of germination and seed vigour index (length) and analyzed following Factorial CRD. The results showed that in M1 generation (laboratory), both main and interaction effects were found significant. Germination percentage, plumule and radical length, speed of germination and seed vigour index (length) were decreased with increased doses of mutagenic concentrations in both the varieties. However, overall from the overall results, it can be concluded that gamma rays was found to be more effective in inducing (reducing) seed vigour parameters. GM 4 was found to be more resistant against mutagenic treatments as compared to Meha for majority of seed vigour parameters.

KEY WORDS

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Gamma rays
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CRD as per the method suggested by Steel and Torrie (1980). In the analysis, control (zero gamma rays, zero EMS) was taken once (three repetition) in the experiment for both the varieties. However, for analysis (FCRD) purpose, same varietal controls were used as EMS control and gamma rays control for the respective varieties.

RESULTS AND DISCUSSION

Germination per cent

Both main and interaction effects, were found significant for germination per cent (Table 1). Mean values of main effects and interaction effects of germination percentage in M₁ generation with different doses of mutagen in green gram is presented in Table 2. Germination per cent varied significantly for varieties, mutagens and concentrations. Between varieties, germination per cent was significantly low in Meha (60.86%) as compared to GM 4 (76.93%). Effect of EMS mutagen (74.57%) on germination was significantly low as compared to the gamma rays mutagen (63.22%). Germination per cent was maximum affected at highest mutagen concentration (score 7), which was significantly differed from rest of the concentrations, including control. Moreover, there was gradual decrease in germination as the concentration of mutagen increased. Interactions among varieties and mutagens on seed germination per cent were found significant. GM 4 germination per cent decreased with EMS as compared to gamma rays. The same pattern was followed in Meha. GM 4 with EMS showed significantly low (59.60%) germination as compared to other combination. Interactions among varieties and concentrations on seed germination per cent were found significant. Germination per cent gradually decreased in both varieties with gradual increased in score of concentration. Minimum germination was found in Meha with score 7 (49.71%), which was at par with Meha with score 6 (52.10 %). Interactions among mutagens and concentrations on seed germination per cent were found significant. Germination per cent gradually decreased with increased concentration/doses in both the mutagens. Significantly low germination per cent was found in gamma rays with 1200 Gy, which was at par with 1000 Gy and were significantly differed from rest of the combinations. Interaction effect of varieties x mutagens x concentrations on seed germination per cent was found significant. Germination gradually decreased with increase in concentration in both the varieties and same pattern was observed in both the mutagens. Significantly higher germination was found in control of GM-4 (97.81%) which was at par with 0.03 % EMS GM 4 (96.00%), 0.05 % EMS GM 4 (92.19%) and Meha (76.33%). However, significantly lower germination was found in Meha (48.95%) in 1200 Gy gamma rays which was at par with 1200 Gy gamma rays GM-4 (49.05%), 1000 Gy gamma rays GM-4 (54.38%), 1000 Gy gamma rays Meha (51.52%), 800 Gy gamma rays Meha (54.29%).

The perusal of the results suggested that as the dose of mutagen was increased, the germination percent was reduced with impact more conspicuous at higher doses as compared to lower doses of gamma rays and EMS. The impact of germination could be attributed to damage in the seeds at the time of cell division in the meristemic activity during the process

Table 1: Analysis of variance for different characters in M₁ generation with different doses of mutagen in mungbean

Sources of variation	d.f.	Germination per cent			Plumule length (cm)			Radical length (cm)			Speed of germination			Seed vigour index (length)		
		MS	SEm	LSD	MS	SEm	LSD	MS	SEm	LSD	MS	SEm	LSD	MS	SEm	LSD
Varieties	1	6196.73**	0.52	1.49	2114.53**	0.75	2.14	4.63	0.26	-	17876.07**	1.08	3.06	176306505.78**	177.46	501.38
Mutagens	1	3094.01**	0.52	1.49	72630.25**	0.75	2.14	202.51**	0.26	0.74	10284.03**	1.08	3.06	1482667675.59**	177.46	501.38
Concentrations	7	1236.70**	1.05	2.98	2938.94**	1.51	4.18	30.16**	0.52	1.48	3417.95**	2.17	6.13	215594487.42**	354.93	1002.76
Varieties x mutagens	1	1901.80**	0.74	2.11	959.19**	1.07	3.02	27.25**	0.37	1.05	8660.54**	1.53	4.34	60748959.82**	250.97	709.06
Varieties x Concentrations	7	39.95**	1.49	4.12	132.36**	2.14	6.05	2.14**	0.74	2.09	300.68**	3.07	8.68	6592070.89**	501.94	1418.11
Mutagens x Concentrations	7	70.78**	1.49	4.12	1822.11**	2.14	6.05	10.83**	0.74	2.09	231.86**	3.07	8.68	38908025.27**	501.94	1418.11
Varieties x mutagens x Concentrations	7	42.90**	2.11	5.97	357.50**	3.03	8.56	9.13**	1.05	2.96	193.26**	4.34	12.27	7030889.72**	709.86	2005.51
Error	64	13.40	-	-	27.54	-	-	4.63	0.26	-	56.66	-	-	1511707.35	-	-
C.V %		5.31			5.87			9.99			6.62			9.14		

*, ** = Significant at P = 0.05 and 0.01 respectively.

Table 2: Mean values of main effects and interaction effects of different characters in M₁ generation with different doses of mutagen in mungbean

Main effects and Interaction effects		Germination per cent	Plumule length (cm)	Radical length (cm)	Speed of germination	Seed vigour Index (length)	
Varieties	GM 4	76.93	8.46	9.71	127.30	14803.12	
	Meha	60.86	9.40	9.94	100.00	12092.75	
Mutagens	Gamma rays	63.22	6.18	7.97	103.30	9518.00	
	EMS	74.57	11.68	11.68	124.00	17377.88	
Concentrations(Score)	0 (Control)	87.07	12.28	12.62	145.41	21626.98	
	1	75.85	9.82	11.66	123.85	16446.39	
	2	73.88	9.40	10.84	122.03	15277.33	
	3	68.77	8.03	9.61	111.42	12391.77	
	4	67.90	8.43	9.58	112.28	12562.01	
	5	63.46	8.17	9.11	103.96	11185.64	
	6	59.36	7.84	7.95	98.55	9666.82	
	7	54.88	7.48	7.27	91.69	8426.57	
Varieties X Mutagens	GM 4 X gamma rays	66.80	6.03	8.06	107.45	10077.69	
	GM 4 X EMS	59.60	6.33	7.88	99.15	8958.30	
	Meha X gamma rays	87.06	10.90	11.37	147.14	19528.56	
	Meha X EMS	62.09	12.47	12.00	100.86	15227.21	
Varieties X Concentrations	GM 4 X 0	97.81	11.52	12.87	167.04	23851.53	
	GM 4 X 1	86.05	9.29	12.62	142.36	19065.13	
	GM 4 X 2	81.19	8.69	10.28	134.33	16149.56	
	GM 4 X 3	77.69	7.81	8.89	128.43	13507.64	
	GM 4 X 4	74.93	8.54	9.76	123.13	14249.42	
	GM 4 X 5	71.12	7.32	8.42	116.28	11746.54	
	GM 4 X 6	66.62	7.23	7.82	108.75	10622.87	
	GM 4 X 7	60.05	7.28	7.06	98.03	9232.30	
	Meha X 0	76.33	13.03	12.37	123.78	19402.43	
	Meha X 1	65.64	10.35	10.71	105.34	13827.64	
	Meha X 2	66.57	10.11	11.39	109.73	14405.11	
	Meha X 3	59.86	8.26	10.32	94.41	11275.90	
	Meha X 4	60.88	8.31	9.40	101.44	10874.60	
	Meha X 5	55.81	9.01	9.80	91.63	10624.73	
	Meha X 6	52.10	8.45	8.08	88.35	8710.78	
	Meha X 7	49.71	7.69	7.47	85.35	7620.85	
	Mutagens X Concentrations	Gamma rays X 0	87.07	12.28	12.62	145.41	21626.98
		Gamma rays X 100 Gy	70.93	7.86	11.68	113.71	13865.90
		Gamma rays X 200 Gy	67.14	5.90	8.83	109.40	9812.19
		Gamma rays X 400 Gy	61.64	5.01	7.49	97.55	7681.85
		Gamma rays X 600 Gy	61.05	5.45	8.04	100.00	8249.91
Gamma rays X 800 Gy		55.98	4.72	6.33	91.30	6199.49	
Gamma rays X 1000 Gy		52.95	4.25	4.84	87.12	4832.71	
Gamma rays X 1200 Gy		49.00	3.97	3.94	81.90	3874.94	
EMS X 0		87.07	12.28	12.62	145.41	21626.98	
EMS X 0.03%		80.76	11.78	11.64	133.99	19026.87	
EMS X 0.05%		80.62	12.90	12.85	134.66	20742.48	
EMS X 0.10%		75.90	11.06	11.72	125.29	17101.69	
EMS X 0.15%		74.76	11.40	11.12	124.56	16874.11	
EMS X 0.20%		70.95	11.61	11.88	116.62	16171.79	
EMS X 0.25%		65.76	11.43	11.06	109.99	14500.94	
EMS X 0.30%		60.76	10.99	10.59	101.49	12978.21	

of germination. The similar results have reported by Singh and Chaturvedi, 1980; Khan and Goyal, 2009 and Thanga, 2015 in *Vigna radiata*; Solanki and Sharma, 1994 in lentil; Sagade and Apparao, 2011, Dhasarathan *et al.*, 2014 and Ramya *et al.*, 2014 in *Vigna mungo*; Ariraman *et al.*, 2014 in *Cajanus cajan*; Umavathi and Mullainathan, 2014 in *Cicer arietinum*; Dhulgande *et al.*, 2015 in *Pisum sativum*; and Aparna *et al.*, 2013 in *Arachis hypogaea*.

Plumule length (cm)

Both main and interaction effects, were found significant for plumule length (Table 1). Mean values of main effects and

interaction effects of plumule length in M₁ generation with different doses of mutagen in green gram is presented in Table 2. Plumule length varied significantly for varieties, mutagens and concentrations. Between varieties, plumule length was high in Meha (9.40 cm) as compared to GM 4 (8.4 cm). Effect of EMS mutagen (11.68 cm) on plumule length was high as compared to the gamma rays mutagen (6.18 cm). There was significant difference between (12.78 cm) and highest mutagenic treatment (7.48 cm) concentration. Interactions among varieties and mutagens on plumule length were found significant. In both the varieties, EMS treatment had more

Table 2: Contd.....

	SourcesMain effects and Interaction effects		Germination per cent		Plumule length (cm)		Radical length (cm)		Speed of germination		Seed vigour index (length)	
			GM 4	Meha	GM 4	Meha	GM 4	Meha	GM 4	Meha	GM 4	Meha
Varieties X Mutagens X Concentrations	Gamma rays	Control	97.81	76.33	11.52	13.03	12.87	12.37	167.04	123.78	23851.53	19402.43
		100 Gy	76.10	65.76	7.45	8.26	12.28	11.08	120.92	106.49	15009.40	12722.41
		200 Gy	70.19	64.10	4.74	7.06	7.45	10.20	111.97	106.84	8556.82	11067.55
		400 Gy	66.05	57.24	4.97	5.04	7.04	7.94	105.61	89.50	7933.43	7430.27
		600 Gy	63.19	58.90	5.50	5.41	8.28	7.81	100.53	99.48	8710.32	7789.50
		800 Gy	57.67	54.29	4.83	4.62	6.81	5.86	91.25	91.34	6709.52	5689.46
		1000 Gy	54.38	51.52	4.78	3.73	5.45	4.23	85.06	89.18	5563.39	4102.03
	EMS	Control	97.81	76.33	11.52	13.03	12.87	12.37	167.04	123.78	23851.53	19402.43
		0.03 %	96.00	65.52	11.13	12.43	12.96	10.33	163.79	104.18	23120.87	14932.88
		0.05 %	92.19	69.05	12.64	13.15	13.11	12.58	156.70	112.62	23742.30	17742.67
		0.10 %	89.33	62.48	10.66	11.47	10.74	12.70	151.26	99.32	19081.85	15121.52
		0.15 %	86.67	62.86	11.58	11.22	11.24	10.99	145.72	103.40	19788.51	13959.70
		0.20 %	84.57	57.33	9.82	13.40	10.03	13.73	141.31	91.92	16783.57	15560.00
		0.25 %	78.86	52.67	9.69	13.17	10.18	11.93	132.45	87.52	15682.35	13319.52
		0.30 %	71.05	50.48	10.12	11.87	9.82	11.37	118.87	84.10	14177.47	11778.95

Note: 0 to 7 score indicates gradual increased in mutagen concentration (gamma rays dose and EMS concentration).

plumule length than gamma rays. GM 4 with gamma rays shows significantly low (6.02 cm) plumule length as compared to other combinations. Interactions among varieties and concentrations on plumule length were found significant. Gradual increased in concentration, plumule length did not reduce gradually in both the varieties. Significantly high plumule length was found in control Meha (13.03 cm), which was at par with GM 4 control (11.52 cm), GM 4 score 1 (9.29 cm), Meha score 1 (10.35 cm), Meha score 2 (10.11 cm) and Meha score 5 (9.01 cm). Significantly low plumule length was observed in score 6 GM 4 (7.23 cm), which was at par with Meha score 7 (7.68 cm), GM-4 score 3 (7.81 cm), GM 4 score 5 (7.32 cm) and GM 4 score 7 (7.28 cm). Interactions among mutagens and concentrations on plumule length were found significant. Significantly high plumule length was found in 0.05 % EMS (12.90 cm), which was at par with control (12.28 cm), 0.03 % EMS (11.78 cm), 0.10 % EMS (11.72 cm), 0.15 % EMS (11.06 cm), 0.20 % EMS (11.40 cm), 0.25 % EMS (11.43 cm), 0.30 % EMS (10.99 cm) and 100 Gy gamma rays (7.86 cm). Significantly low plumule length was observed in 1200 Gy gamma rays (3.97 cm), which was at par with 200 Gy gamma rays (5.90 cm), 400 Gy gamma rays (5.01 cm), 600 Gy gamma rays (5.45 cm), 800 Gy gamma rays (4.72 cm) and 1000 Gy gamma rays (4.25 cm). Interaction effect of varieties x mutagens x concentrations on plumule length was found significant. Majority of treatments were at par with control.

Shoot length has been extensively used to measure the mutagenic effect. A quantitative assessment of injury in M1 plants through these traits can be used as an indicator to compare the effect of different mutagen. The reduction in plumule length recorded in laboratory study has been attributed to changes in levels of auxin and absorbic acid and to physiological and biochemical disturbance or chromosomal aberrations, changes in enzymatic activity, and impaired mitosis in the meristemic zone of growing seedlings. It might be due to decrease in respiratory quotient in the seedlings obtained from treated seeds. The similar results for plumule length are also reported earlier by Sujay and Singh, 2001 and

Thanga, 2015 in *Vigna radiata*; Rajiv Kumar *et al.*, 2008 in *Cyamopsis tetragonoloba*; Patel, 2008 in *Macrotyloma uniflorum*; Ariraman *et al.*, 2014 in *Cajanus cajan*; Umavathi and Mullainathan, 2014 in *Cicer arietinum*; Dhulgande *et al.*, 2015 in *Pisum sativum*; and Aparna *et al.*, 2013 in *Arachis hypogaea*

Radical length (cm)

Both main and interaction effects, were found significant for radical length (Table 1). Mean values of main effects and interaction effects of radical length in M₁ generation with different doses of mutagen in green gram is presented in Table 2. Radical length varied significantly for mutagens and concentrations. Effect of EMS mutagen (11.68 cm) on radical length was significantly high as compared to the gamma rays mutagen (7.97 cm). However, gradual increased in concentration radical length did not reduce gradually. However, there was significant difference at lowest (12.61 cm) and highest (7.26 cm) concentration. Interactions among varieties and mutagens on radical length were found significant. In GM 4 radical length increased with more gamma rays as compared to EMS. GM 4 with EMS showed significantly low (7.8 cm) radical length as compared to other combination. Interactions among varieties and concentrations on radical length were found significant. Gradual increased in concentrations radical length did not reduce gradually in both the varieties. Significantly high radical length was found in control in GM 4 control (12.87 cm), which was at par with Meha control (12.37 cm), GM 4 score 1 (12.62 cm) and Meha score 2 (11.39 cm) and significantly low radical length was observed in GM 4 score 7 (7.06 cm), which was at par with Meha score 7 (7.47 cm), GM 4 score 3 (8.89 cm), GM 4 score 5 (8.42 cm), GM 4 score 6 (7.82 cm) and Meha score 6 (8.08 cm). Interactions among mutagens and concentrations on radical length were found significant. Significantly high radical length was found in control of gamma rays (12.61 cm), which was at par with 100 Gy gamma rays (11.68 cm) which was at par with all the EMS treatments. Significantly low radical length was observed in 1200 Gy gamma rays (3.94 cm), which was

at par with 1000 Gy gamma rays (4.84 cm). Interaction effect of varieties x mutagens x concentrations on radical length was found significant. Significantly high radical length in GM 4 was found in control (12.86 cm), which was at par with 100 Gy (12.28 cm) and majority of EMS treatments. Significantly high radical length in GM 4 was found in 1200 Gy gamma rays (4.30 cm) which was at par with 800 Gy gamma rays (6.81 cm) and 1000 Gy gamma rays (5.45 cm). Significantly high radical length in Meha was observed in control (12.37 cm), which was at par with 100 Gy gamma rays (11.08 cm) and majority of EMS treatment. Significantly high radical length in GM 4 was observed in 1200 Gy gamma rays (3.57 cm), which was at par with 800 Gy gamma rays (5.86 cm) and 1000 Gy gamma rays (4.23 cm).

Radical length has been extensively used to measure the mutagenic effect. A quantitative assessment of injury in M1 plants through these traits can be used as an indicator to compare the effect of different mutagen. In the present study, all the treated materials exhibited significantly lower radical length than their respective control. The reduction in radical length was palpably dose/concentration dependent with more reduction in radical length with increased in dose of mutagen concentration. The reduction in radical length recorded in laboratory study has been attributed to change in levels of auxin and absorbic acid and to physiological and biochemical disturbance or chromosomal aberrations, changes in enzymatic activity, and impaired mitosis in the meristemic zone of growing seedlings. It might be due to decrease in respiratory quotient in the seedlings obtained from treated seeds. The similar results for radical length are also reported earlier by Sujay and Singh, 2001 and Thanga, 2015 in *Vigna radiata*; Rajiv Kumar et al., 2008 in *Cyamopsis tetragonoloba*; Patel, 2008 in *Macrotyloma uniflorum*; Ariraman et al., 2014 in *Cajanus cajan*; Umavathi and Mullainathan, 2014 in *Cicer arietinum*; Dhulgande et al., 2015 in *Pisum sativum*; and Aparna et al., 2013 in *Arachis hypogaea*

Speed of germination

Both main and interaction effects, were found significant for speed of germination (Table 1). Mean values of main effects and interaction effects of speed of germination in M₁ generation with different doses of mutagen in green gram is presented in Table 2. Speed of germination varied significantly for varieties, mutagens and concentrations. Between varieties, speed of germination was significantly low in Meha (100.00) as compared to GM 4 (127.30). Effect of EMS mutagen (124.00) on speed of germination was significantly high as compared to the gamma rays mutagen (103.30). Speed of germination was maximum affected at highest mutagen concentration (score 7) which was significantly differed from rest of the concentrations, including control. Speed of germination was negatively associated with concentration of mutagen. Interactions among varieties and mutagen on speed of germination were found significant. Speed of germination was significantly reduced by EMS as compared to gamma rays in case of both the varieties GM 4 and Meha. Significantly high speed of germination was found in Meha with gamma rays mutagen (147.14) interaction and lowest was found in GM 4 with EMS mutagen (99.15) interaction. Interactions among varieties and concentrations on speed of germination were

found significant. There was gradual reduction in speed of germination with increasing dose of mutagens for GM 4 variety. Significantly high speed of germination was found in control of GM 4 (167.04) whereas, significantly low speed of germination was observed in score 7 Meha (85.35) which was at par with Meha score 6 (88.35) and Meha score 5 (91.63). Interactions among mutagen and concentrations on speed of germination were found significant. Speed of germination decreased with increased concentration in both the mutagens. Significantly high speed of germination was found in control (145.41). Significantly low speed of germination was observed in 1200 Gy gamma rays (81.90) which was at par with 1000 Gy gamma rays (87.12). Interaction effect of varieties x mutagens x concentrations on speed of germination was found significant. Significantly high speed of germination was found in GM 4 control (163.73) which was at par with 0.03 % EMS GM 4 (163.79) and 0.05 % EMS GM 4 (156.70) and significantly low speed of germination was found in GM 4, 1200 Gy gamma rays (77.19) which was at par with 1000 Gy gamma rays (85.06). Significantly high speed of germination was found in control (123.78) and significantly low was observed in 0.30 % EMS Meha (84.10) which was at par with 1000 Gy Meha (89.18), 800 Gy gamma rays Meha (91.34), 0.25 % EMS Meha (87.52) and 0.20 % EMS Meha (91.92).

Speed of germination means numbers of germinated seeds were counted every day from the first day to seven day. Speed of germination decreased with increased dose of the mutagenic concentration this type of result obtained because of decrease in germination per cent with increased dose of mutagenic concentration. The impact of germination could be attributed to damage in the seeds at the time of cell division in the meristemic activity during the process of germination. The results are in accordance with the results of Aparna et al., 2013 in *Arachis hypogaea*.

Seed vigour index (length)

Both main and interaction effects, were found significant for seed vigour index (length) (Table 1). Mean values of main effects and interaction effects of seed vigour index (length) in M₁ generation with different doses of mutagen in green gram is presented in Table 2. Seed vigour index (length) varied significantly for varieties, mutagens and concentrations. Between varieties, seed vigour index (length) was significantly low in Meha (12092.75) as compared to GM 4 (14803.12). Effect of EMS mutagen (17377.88) on seed vigour index (length) was significantly high as compared to the gamma rays mutagen (9518.00). Seed vigour index (length) gradually decrease as the concentration gradually increases. Seed vigour index (length) was maximum affected at highest mutagen concentration (score 7) which was significantly differed from rest of the concentrations, including control. Seed vigour index (length) is negatively associated with concentration of mutagen. Interactions among varieties and mutagens on seed vigour index (length) were found significant. Seed vigour index (length) more affected by EMS as compared to gamma rays in case of both the varieties GM 4 and Meha. Highest seed vigour index (length) was found in Meha with gamma rays mutagen (19528.56) interaction and lowest seed vigour index (length) was found in GM 4 with EMS mutagen (8958.30) interaction.

Interactions among varieties and concentrations on seed vigour index (length) were found significant. Gradual increased in concentration seed vigour index (length) reduced gradually in both the varieties. Significantly high seed vigour index (length) was found in control concentration in GM 4 (23851.53) and significantly low seed vigour index (length) was observed in score 7 of Meha (7620.85). Interactions among mutagens and concentrations on seed vigour index (length) were found significant. Seed vigour index (length) decreased with increased concentration in both the mutagens. Gradual increased in concentration seed vigour index (length) did not reduce gradually in both the mutagens. Significantly high seed vigour index (length) was found in control (21626.98) and significantly low seed vigour index (length) was observed in 1200 Gy gamma rays (3874.94) which was at par with 1000 Gy gamma rays (4832.71) Interaction effect of varieties x mutagens x concentrations on seed vigour index (length) was found significant. Seed vigour index (length) did not gradual decreased with gradually increased score of concentration in both varieties and same pattern was observed in both the mutagens. Significantly high seed vigour index (length) was found in control GM 4 (23851.53), which was at par with 0.03 % EMS GM 4 (14932.88) and 0.05 % EMS (17742.67) and in case of Meha control (19402.43). Significantly low seed vigour index (length) was found in 1200 Gy gamma rays, GM 4 (4287.12) which was at par with 1000 Gy gamma rays GM 4 (5563.39) and in Meha 1200 Gy (3462.75) which was at par with 1000 Gy gamma rays Meha (4102.03).

Seed vigour index (length) were obtained by multiplication of germination per cent with seedlings length means the seed vigour index (length) is depend upon two factors germination per cent and seedling length. Both germination percentage and seedling length decreased with increased dose of mutagen. Therefore, seed vigour index (length) so it is decreased with increased dose of mutagenic concentration. The results are in accordance with the results of Aparna *et al.*, 2013 in *Arachis hypogaea*; and Dhulgande *et al.*, 2015 in *Pisum sativum*.

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