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DL Sundesha
Assistant Professor, College of Horticulture, S. D. Agricultural University, Jagudan, Gujarat, India

MP Patel
Research Scientist, Pulse Research Station, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India

HS Bhadauria
Associate Professor, Department of Genetics and Plant Breeding, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India

Shreya
Assistant Professor, College of Horticulture, S. D. Agricultural University, Jagudan, Gujarat, India

Corresponding Author:
DL Sundesha
Assistant Professor, College of Horticulture, S. D. Agricultural University, Jagudan, Gujarat, India

Effect of EMS on seed germination and seedling vigour in mungbean [*Vigna radiata* (L.)]

DL Sundesha, MP Patel, HS Bhadauria and Shreya

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Abstract
The pure, healthy and dry seeds of four mungbean varieties *i.e.,* Meha, K 851, GM 3 and GM 4 were treated with different concentrations of Ethyl Methane Sulphonate (0.00 M, 0.02 M, 0.03 M and 0.04 M) for study the effect on seed germination and seedling vigour such as germination per cent, shoot length (cm), root length (cm), fresh weight (g) and dry weight (g). For LD_{50} recorded near to the 0.04 M EMS as per survival per cent in the all four mungbean varieties. The present results clearly indicated that different concentrations of Ethyl Methane Sulphonate can be effectively utilized to create variability for different quantitative characters.

Keywords: Mungbean, ethyl methane sulphonate (EMS), LD_{50}, seedling vigour

Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] is the most important pulse crop in India. In the traditional vegetarian diet of Indian population, pulses occupy second place next to cereal and is the main source of protein, ranking after chickpea and pigeonpea. Mungbean, an important seed legume, is a short duration (60 to 75 days) crop and plays vital role in meeting the quantitative and qualitative requirement of food and protein throughout the world. Mungbean provide 24 per cent protein and the seeds are considered to be easily digestible. It also provides nutritional fodder to the cattle and improves the soil fertility through atmospheric nitrogen fixation with the help of *Rhizobium* species (Sundesha *et al*., 2019) [10]. Plant breeders always look for large and diverse gene pool of variability as it is a prerequisite for success in any breeding programme. In mungbean, natural variability is limited and hybridization is little difficult due to cleistogamous and small flower structure. Therefore, induced mutation technique was followed to create genetic variability by artificial means.

Material and Methods
The true pure seeds of mungbean variety Meha, K 851, GM 3 and GM 4 were procured from the pulse research station, SDAU, Sardarkrushinagar. Preparation of EMS solution in phosphate buffer at pH 7.0 in different concentration. Pure, healthy and mature seeds of individual varieties were pre-soaked in distilled water for two hours before they were treated with chemical mutagens. Pre-soaked seeds were treated for four hours at room temperature with freshly prepared solution of chemical mutagens with 0.00 M (control), 0.02 M, 0.03 M and 0.04 M. After treating seeds, quick wash with tap water. For the treatment of control seed, seeds were treated with distilled water. All the seeds along with control were sown immediately after mutagenic treatments. In laboratory condition, 25 seeds were sown treatment wise in the cement pipe structure with proper plant as well as row spacing during *summer*-2014. Germination of seeds were carefully examined everyday and the emergence of cotyledonary leaf was taken as the induction of germination, germinated seeds of each treatment were counted on eighth day after sowing and germination percentage was calculated, shoot and length was measured in centimeter after fifteen days of sowing, fresh weight (g) was recorded after fifteenth day of sowing, shoot and root length were measured in centimeter and fresh weight (g) was recorded after these seedlings were put in oven at constant 50ºC temperature for 48 hours and weight was recorded as dry weight (g) and total number of seedlings survived were counted after fifteen day of sowing and plant survival percentage was also calculated.

Result and Discussion

In the present study, the highest mean germination percent (88, 96, 96 and 92) and survival per cent (88, 96, 96 and 92) was recorded in control treatment, whereas lowest mean germination per cent (56, 56, 56 and 56) and survival per cent (44, 52, 44 and 52) was observed at 0.04 M treatment in four varieties Meha, K 851, GM 3 and GM 4, respectively (Table 1). Mean germination per cent and survival per cent were reduced in all four varieties with increase in the concentration of EMS. The perusal of the results suggested that as the concentration of EMS was increased when, the germination per cent and survival per cent were reduced. The impact on germination could be attributed to damage in the seeds at the time of cell division in the meristematic region during the process of germination (Balai and Krishna, 2009; Kumar *et al.*, 2010 and Sagade and Apparao, 2011) [1, 4, 6]. There were little genotypic differences among the varietal studies for LD_{50} doses as exhibited by seed germination. The LD_{50} value of germination was higher than the highest concentration of EMS treatment which is suggested that concentration of EMS should be increased from 0.04 M EMS for attain the LD_{50}. The LD_{50} value of survival percentage was recorded in cultivar Meha at 0.04 M. whereas, in other remaining three cultivars were recorded near to the 0.04 M. A variation was evinced for LD_{50} values in all the four varieties of mungbean. Similar results were also reported by Singh and Singh (2013) [9]. Cherry and Hageman (1961) [2] opined that impairment of mitosis or virtual elimination of cell division in the meristematic zone during germination in treated seeds with higher concentration led to seed lethality. Sato and Gaul (1967) [7] reported that seedling injury led to slow growth culminating in early mortality. Higher degree of damage incited by treating to chromosomal materials also results in inhibition of growth hormones. All these factors cumulatively may be attributed as the reason for reduction of plant survival. Maximum shoot length (6.90, 6.71, 7.06 and 7.05 cm), root length (7.40, 7.35, 7.54 and 7.41 cm), fresh weight (1.05, 1.09, 1.15 and 1.13 g), dry weight (0.52, 0.55, 0.62 and 0.59 g) were recorded in control treatment, whereas minimum shoot length (3.88, 3.81, 3.99 and 4.02 cm), root length (4.12, 4.10, 4.32 and 4.23 cm), fresh weight (0.95, 0.95, 1.01 and 0.98 g), dry weight (0.45, 0.46, 0.51 and 0.48 g) were recorded at 0.04 M EMS treatment in four varieties Meha, K 851, GM 3 and GM 4, respectively (Table 2). Mean shoot and root length as well as mean fresh and dry weight were reduced in all four varieties with increase in concentration of ethyl methane sulphonate. Highest mean shoot and root length as well as fresh and dry weight were observed at control treatment in variety GM 3, while lowest values in these traits were recorded at 0.04 M EMS treatment in K 851. The results also suggested a differential response to different concentration of EMS treatments. The reduction in shoot length recorded in the flat studies has been attributed to changes in the levels of auxin and ascorbic acid and to physiological and bio-chemical disturbances (Gunkel and Sparrow, 1954 and Singh, 1974) [3, 8] or chromosomal aberrations changes in enzymatic activity and impaired mitosis in the meristematic zone of growing seedlings (Cherry and Hageman, 1961) [2]. It might be due to decrease in respiratory quotient in the seedlings obtained from treated seeds (Woodstock and Justice, 1967) [11]. Such chromosomal aberrations caused due to induction of mutation have also been reported by Nandanwar and Patil (2000) [5].

Table 1: Per cent seed germination and Per cent seed survival in mungbean cultivars under different treatments in laboratory conditions

Varieties	Treatments (Conc. of EMS)	Number of seeds sown	Per cent seed germination			Per cent seed survival		
			Number of seeds germinated	Mean germination in per cent	Reduction over control per cent	Number of seeds survival	Mean survival in per cent	Reduction over control per cent
Meha	Control	25	22	88	-	22	88	-
	0.02 M	25	18	72	18.18	17	68	22.73
	0.03 M	25	15	60	31.82	12	48	45.45
	0.04 M	25	14	56	36.36	11	44	50.00
K 851	Control	25	24	96	-	24	96	-
	0.02 M	25	18	72	25.00	16	64	33.33
	0.03 M	25	18	72	25.00	17	68	29.17
	0.04 M	25	14	56	41.67	13	52	45.83
GM 3	Control	25	24	96	-	24	96	-
	0.02 M	25	21	84	12.50	20	80	16.67
	0.03 M	25	17	68	29.17	14	56	41.67
	0.04 M	25	14	56	41.67	11	44	54.17
GM 4	Control	25	23	92	-	23	92	-
	0.02 M	25	21	84	8.70	19	76	17.39
	0.03 M	25	17	68	26.09	16	64	30.43
	0.04 M	25	14	56	39.13	13	52	43.48

Table 2: Per cent reduction in shoot length (cm) and Root length (cm) and Fresh and Dry weight (g) in mungbean cultivars under different treatments in laboratory conditions

Varieties	Treatments (Conc. of EMS)	Shoot length (cm)		Root length (cm)		Fresh weight (g)		Dry weight (g)	
		Mean (cm)	Per cent reduction over control	Mean (cm)	Per cent reduction over control	Mean (g)	Per cent reduction over control	Mean (g)	Per cent reduction over control
Meha	Control	6.90	-	7.40	-	1.05	-	0.52	-
	0.02 M	4.55	34.06	4.85	34.46	1.00	04.32	0.48	07.61
	0.03 M	4.25	38.41	4.46	39.73	0.98	07.05	0.47	09.43
	0.04 M	3.88	43.77	4.12	44.32	0.95	09.69	0.45	13.84
K 851	Control	6.71	-	7.35	-	1.09	-	0.55	-
	0.02 M	4.45	33.68	4.77	35.07	1.01	06.89	0.49	10.48

	0.03 M	4.12	38.61	4.44	39.56	0.98	09.75	0.48	12.87
	0.04 M	3.81	43.21	4.10	44.27	0.95	12.63	0.46	16.72
GM 3	Control	7.06	-	7.54	-	1.15	-	0.62	-
	0.02 M	4.71	33.26	5.00	33.62	1.07	06.96	0.54	12.44
	0.03 M	4.29	39.28	4.64	38.40	1.04	09.57	0.54	13.57
	0.04 M	3.99	43.45	4.32	42.74	1.01	12.17	0.51	17.94
GM 4	Control	7.05	-	7.41	-	1.13	-	0.59	-
	0.02 M	4.61	34.65	4.86	34.37	1.04	08.37	0.51	13.10
	0.03 M	4.35	38.36	4.52	39.02	1.01	10.92	0.50	14.53
	0.04 M	4.02	42.92	4.23	42.90	0.98	13.56	0.48	19.45

Conclusion

In this research, seed germination per cent, seed survival per cent, shoot & root length as well as fresh and dry weight were reduced with increasing concentration of EMS. The results clearly indicated that different concentration of EMS mutagen can be effectively utilized to create variability for various quantitative traits in all four varieties of mungbean.

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