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RESEARCH ARTICLE

Chemical mutagens (Ethyl methane sulphonate and sodium azide) mediated morphological characters and biochemical variations in green gram (*Vigna radiata* L.)

■ **RICHA SHARMA AND K. SHARATH KUMAR**

SUMMARY

Green gram (*Vigna radiata* L.) of the family Leguminosae is an important legume crops in the semi-arid tropics and study was carried out to improve crop yield of two varieties of green gram *i.e* Markiv and Smart to determine the effects of ethyl methane sulphonate and sodium azide (10, 20, 30, 40 mM). The LD_{50} value was observed in 40mM of EMS and 30mM of sodium azide. For inducing mutation various concentration *viz.*, 10, 20, 30 and 40mM @ four hours were applied to 100 seed sample of each concentration and one respective control. The LD_{50} value was observed in 40 mM of EMS and 30 mM of sodium azide. The morphological and yield characters showed significant increment in seed germination, plant height, number of leaves, number of branches per plant, 50 per cent of flowering, number of nodules per plants, number of pods per plant, numbers of clusters per plant, number of grains per pod, 100 seed weight at lower concentrations. At higher concentration of EMS and sodium azide phenotypic, biochemical and yield characters spontaneously decreased. Present investigation concluded the lower concentration of EMS (upto 30mM) and sodium azide (upto 20 mM) performs positively and improved growth and yield parameters studied.

Key Words : Biochemical, Chemical mutagens, Ethyl methane sulphonate, Morphological, Mutation,Sodium azide

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Green gram (*Vigna radiata* L.) of the family Fabaceae is food legume crops in the semi-arid tropics covering Asia, Africa, Southern Europe and Central and Southern America. It is one of the important pulse crops in India and reported to be cultivated since ancient times (Ministry of agriculture, GoI, 2003). More than 70 per cent of world's green gram production comes from India and accounts for about 10

to 12 per cent of total pulse production in the country (Anonymous, 2014). There are still gaps in production and demand due to increasing population and limited agriculture land resources. Solution of such can be come only with harvesting more from the same fields. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Chemical mutagens come up with promising prospect to induce mutation and has resulting in increase crop yield (Deepalakshmi and Anandakumar, 2004). Chemical mutagenesis is considered as an effective means in improving the yield and quality traits of crop plants (Srivastava *et al.*, 2011). Mutation induction has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain specific traits (Kurobane *et al.,* 1979). Induced mutations represent the same kind of changes that occur from natural causes (Govindan, 2000). Among numerous alkylating chemical mutagens such as ethyl methane sulphonate (EMS), sodium azide, hydrazine hydrate etc. sodium azide and EMS had been and is still used as potent mutagens in variety of crop improvement programmes. EMS is a common, powerful, and one of the most effective chemical mutagen, especially recommended when mutation is introduced to the seed materials, since the application and the monitoring of the outcome of mutations are relatively easy. In plants, EMS usually causes point mutations, on the other hand, loss of a chromosome segment or deletion can also occur in lesser extent (Okagaki *et al.*, 1991). The mutant plants formed by the application of sodium azide are able to withstand a range of unfavorable conditions and have enhanced yields, improved stress tolerance, longer shelf-life and reduced agronomic input in comparison to normal plant (Ahloowalia and Maluszynski, 2001). Its application on plant is easy and inexpensive and creates mutation to improve their traits. Sodium azide applied to soil demonstrated broad spectrum activity against weeds, nematodes and soil borne phytopathogenic fungi (Robertson and Kabana, 2000). Therefore, an attempt had been made to study the efficacy of different concentration of these two chemical on vegetative, reproductive aspects along with yield attributes for improving productivity in green gram.

MATERIAL AND METHODS

Dry, healthy and uniform sized seeds of green gram varieties were treated with ethyl methane sulphonate (EMS) and sodium azide (SA) at 10, 20, 30 and 40 mM concentrations. Seeds were pre- soaked for 6 h in water initially than immersed for 4 h in the requisite concentration of mutagens with intermittent shaking to ensure a uniform absorption of the mutagen. The whole treatment was carried out at a room temperature of 28±1°C as per the guidlines (Malarkodi, 2008). Seeds were sown in the field after thoroughly washing for 6 times. Treatments adopted were (T_0- Control, T_1- 10 mM EMS, T_2- 20 mM EMS, T_3- 30 mM EMS, T_4- 40 mM EMS, T_5- 10 mM SA, T_6- 20 mM SA, T_7- 30 mM SA and T_8- 40 mM SA). Seeds were sown with 30 × 10 cm distance between rows and plants, respectively and supplied with recommended dose of NPK. Parameters observed were seed germination, plant height, number of leaves, number of branches per plant, number of flowers, number of nodules per plants, number of pods per plant, numbers of cluster per plant, number of grains per pod, 100 seed weight, chlorophyll a and b, total chlorophyll and caroteinod and further analyzed statistically at CD 0.05 per cent.

RESULTS AND DISCUSSION

Chemical mutagens were always proven an effective tool for inducing mutation to improve genetic makeup of economic crops (Adamu and Aliyu, 2007). Green gram is highly nutritive and relatively rich in dietary supplements thus, improving the yield through application of EMS and SA was studied at different concentrations. Observations on different morphological and biochemical aspects are provided in Table 1 to 4. Germination percentage was significantly reduced in all ethyl methane sulphonate and sodium azide treatments. The 50 per cent reduction of germination was recorded at 40 mM of ethyl methane sulphonate (48.33%) and 30mM of sodium azide (51.66%). It indicated that germination percentage was reduced under the influence of mutagenic treatment with increasing doses per concentrations. Similar results were reported in red gram Jayanthi (1986), in winged bean Veeresh *et al.* (1995) and in blackgram (Thilagavathi and Mullainathan, 2011). However, there are evidence of EMS treatments accelerated seed germination under several abiotic stresses conditions such as extreme temperature, water, salt, metal, ozone, gas (SO_2 and NOx) that generate cellular reactive oxygen species (ROS) or oxidative stress which alters DNA indirectly reported by Mittler (2002) and Patra *et al.* (2005).

Morphological characters shows deviation from the reductive growth pattern during the study. It suggests positive output for lower concentration of both the mutagens *viz.*, maximum plant height (60.00 cm), primary leaves per plant (33), primary branches per plant (9) was recorded in 20mM of EMS treatment, studies on green gram (Thilagavathi and Mullainathan, 2011) and *Vigna unguiculata* (Deepalakshmi and Anandakumar, 2004) confirms the findings at lower concentration and further increase in concentration reduces the counts. Root nodule formation is also an important aspect for good growth of leguminous plants. They are responsible for nitrogen fixation thus, increase the vegetative growth. For T_2 treatment number of root nodules (30) increased compared to control (24). Studies on horse gram reports increase in number of root nodules by 22 0.5 per cent EMS at over 16 for control (Bolbhat *et al.*, 2012), similar results were obtained in green gram (Gnanamurthy *et al*.,2012). Maximum number of flowers per plant (14) over control (8.00), 50 percentage flowers (44 days) over control (51 days), was recorded in T_2 treatment. Mutagens induced increase in no. of flower is reported in chickpea (Girish *et al.*, 2015).

Yield characters always found influenced by mutagens positively as well as neatively. Study suggests positive output for lower concentration of both the mutagens *viz.*, maximum number of pods per plant (25.00), number of seeds per pod (12.00), number of

Table 1 : Effect of different concentrations of chemical mutagens on morphological characters in green gram varieties at 30 days after sowing

Treatments	Plant height (cm)		Number of leaves/plant		Number of branches/plant		Number of flowers/plant		Number of root nudules/plant	
	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart
T_0	18.50	19.16	20.0	18.0	6	5	3.0	3.0	14.00	15.00
T_1	20.90	20.10	21.0	19.0	6	5	4.0	3.0	17.66	15.00
T_2	20.16	19.63	26.0	24.0	6	6	5.0	4.0	20.00	17.00
T_3	18.60	19.40	13.0	11.0	4	4	3.0	3.0	12.00	12.00
T_4	18.80	18.50	8.0	8.0	3	3	2.0	1.0	10.00	10.00
T_5	19.70	19.73	18.0	18.0	5	5	3.0	3.0	12.00	12.00
T_6	20.30	20.60	21.0	21.0	6	7	4.0	4.0	15.00	14.00
T_7	19.00	18.20	11.6	12.0	4	4	3.0	2.0	10.00	10.00
T_8	18.40	18.00	8.0	8.0	3	2	1.0	0.0	9.00	8.00
F-test	S	S	S	S	S	S	S	S	S	S
S.E.±	0.089	0.190	0.141	0.300	0.092	0.195	0.087	0.184	0.136	0.290
C.D. (P=0.05)	0.256	0.545	0.407	0.863	0.264	0.560	0.249	0.529	0.392	0.832

Table 2 : Effect of different concentrations of chemical mutagens on morphological characters in green gram varieties at 45 days after sowing

Treatments	Plant height (cm)		Number of leaves/plant		Number of branches/plant		Number of flowers/plant		Number of root nudules/plant	
	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart
T_0	52.70	47.30	28.0	26.0	8	6	8.0	9.0	24.00	25.00
T_1	59.50	50.50	31.0	28.0	8	7	11.0	9.0	27.18	25.00
T_2	60.00	55.26	32.0	33.0	9	9	14.0	11.0	30.00	27.00
T_3	45.10	39.06	27.0	22.0	5	3	6.0	6.0	22.00	22.00
T_4	37.70	32.26	22.0	21.0	4	4	4.0	4.0	20.00	20.00
T_5	50.50	49.50	29.7	30.0	8	7	6.0	6.0	22.00	22.00
T_6	55.30	55.80	32.0	31.0	9	9	9.0	8.0	25.00	24.00
T_7	39.20	39.20	22.0	22.0	4	4	4.0	4.0	20.00	20.00
T_8	32.20	30.30	21.00	20.00	4	3	3.0	3.0	19.00	19.00
F-test	S	S	S	S	S	S	S	S	S	S
S.E.±	0.086	0.183	0.137	0.291	0.109	0.232	0.096	0.204	0.127	0.270
C.D. (P=0.05)	0.248	0.525	0.394	0.837	0.314	0.667	0.276	0.586	0.366	0.777

clusters per plant (8.00), hundred seeds weight (4.79 g) was recorded in 20mM of EMS treatment, studies on green gram (Thilagavathi and Mullainathan, 2011) and *Vigna unguiculata* (Mensah and Akomeah, 1992 and Rizwana *et al.*, 2005) supports the findings. However, in a study on horse gram (Senapati *et al*., 2008) there was significant reduction in seed weight and seeds per plant in all the treatments of EMS. The treatments showing maximum variation in quantitative characters may show stable gene mutations in subsequent generations (Ramya *et al.*, 2014). Although maximum studies suggested that quantitative parameters such as, number of cluster per plant, number of pods per plant, number of seeds per pod and plant yield per ha etc were increased when treated with lower concentrations of ethyl methane sulphonate and sodium azide (Khan and Goyal, 2009; Raziuddin *et al.*, 2007; Dhanavel *et al.* 2008; Azad *et al.*, 2012 Chowdhury *et al.*, 2012; Adamu and Aliyu, 2007 and Lavanya *et al.*, 2011).

Different biochemical characters (Chl a, Chl b, total chl and carotenoid content) were estimated during the study. They were found affected negatively and followed the reductive pattern with an increase in concentration of mutagens. Different studies conducted on mutagenic effect suggest that there were development of albino and xantha mutants when treated with EMS (Girija and Dhanavel, 2009 and Rafiq *et al.*,

Table 3 : Effect of different concentrations of chemical mutagens on seed germination percentage and yield characters in green gram varieties

Treatments	Seed germination (%) at 15 DAS		Number of pods/ plant		Number of cluster/plant		Number of seeds/pod		Hundred seed weight	
	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart
T_0	91.66	86.66	19.00	18.00	6.30	4.66	10.33	9.10	3.67	3.40
T_1	83.33	80.00	22.00	20.30	7.30	6.30	9.33	8.30	4.25	2.97
T_2	73.33	71.66	24.96	21.60	8.00	7.00	12.00	10.00	4.79	3.39
T_3	63.33	58.33	17.00	14.00	5.30	4.30	6.66	6.00	3.51	3.10
T_4	51.66	48.33	14.00	11.30	4.30	4.00	5.32	5.66	2.83	3.32
T_5	46.66	76.66	21.20	20.00	6.30	6.30	9.33	9.02	3.92	3.97
T_6	66.66	66.66	22.60	22.30	7.30	7.00	12.00	11.00	4.63	4.29
T_7	53.33	51.66	15.00	15.00	5.00	4.30	6.10	6.00	2.43	2.34
T_8	48.33	43.33	13.00	12.00	4.00	4.30	5.30	5.00	2.13	2.10
F-test	S	S	S	S	S	S	S	S	S	S
S.E.±	0.062	0.131	0.124	0.264	0.090	0.191	0.095	0.202	0.045	0.096
C.D.(P=0.05)	0.178	0.377	0.357	0.758	0.259	0.549	0.274	0.581	0.131	0.277

Table 4 : Effect of different concentrations of chemical mutagens on chlorophyll (Chl a, Chl b and total chl) and carotenoid content (mg/g FW) among green gram varieties

Treatments	Chlorophyll a content (mg/g FW)		Chlorophyll b content (mg/g FW)		Total chlorophyll content (mg/g FW)		carotenoid content (mg/g FW)	
	Markiv	Smart	Markiv	Smart	Markiv	Smart	Markiv	Smart
T_0	1.46	1.44	0.65	0.60	2.03	1.96	2.08	1.99
T_1	1.20	1.18	0.54	0.45	1.90	1.52	1.65	1.41
T_2	1.14	1.10	0.49	0.41	1.81	1.37	1.77	1.67
T_3	1.02	0.97	0.43	0.36	1.62	1.22	1.49	1.56
T_4	0.93	0.92	0.38	0.32	1.36	1.21	1.43	1.49
T_5	1.22	1.15	0.47	0.44	1.68	1.49	1.60	1.53
T_6	1.12	1.07	0.42	0.39	1.54	1.39	1.83	1.86
T_7	1.01	0.96	0.37	0.35	1.38	1.23	1.46	1.62
T_8	0.91	0.92	0.33	0.31	1.26	1.20	1.59	1.45
F-test	S	S	S	S	S	S	S	S
S.E.±	0.011	0.024	0.006	0.012	0.034	0.073	0.019	0.041
C.D. (P=0.05)	0.033	0.070	0.017	0.035	0.098	0.209	0.040	0.117

2011) which supports the decrease in chlorophyll content with increasing doses. Although this contradict with having high yields in T_2 treatment when compared with control but can be explained on the basis of increase in number of leaf and branches that compensates the deficit gap. Studies show that the most important parameters for inducing chemical mutagen for growth and yield characters were based on concentration and duration of treatment.

Conclusion :

All the quantitative and yield traits examined during the study showed negative as well as positive effect of mutagens on it. At lower concentration (EMS 30 mM and SA 20 mM) observation moves in positively and enhances crop productivity but further increase in concentration proportionately show decrements due to physiological disturbance or chromosomal damage of the cells of the plant caused by the mutagens present investigation exhibited that the seed treated with lower concentration of EMS (upto 30 mM) and sodium azide (upto 20 mM) for duration of 4 hours performed positively and improved growth and yield parameters studied.

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