UDC 575
https://doi.org/10.2298/GENSR1801199R
Original scientific paper

EFFECT OF MUTAGEN- ETHYL METHANE SULPHONATE ON YIELD INCREASING PARAMETERS OF FRENCH BEAN (*Phaseolus vulgaris* L.)

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Ramandeep, T.S. Dhillon, R.K. Dhall, B.S. Gill (2018): *Effect of mutagen-ethyl methane sulphonate on yield increasing parameters of French bean (Phaseolus vulgaris L.).*- Genetika, Vol 50, No.1, 199-207.

The present investigation was undertaken at the Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, INDIA during spring season of 2015 and 2016 with objective to induce genetic variability for yield and quality traits in cultivars 'Contender' and 'Arka Komal' through chemical mutagen-ethyl methane sulphonate (EMS) at 0.15, 0.25 and 0.35%. Seed treatment with all the EMS concentration drastically reduced its germination but the germination per cent decreased with increase in concentration of EMS. The per cent seed germination was maximum (90%) in control 'Contender' whereas it was 74%, 49% and 42% in EMS at 0.15%, 0.25% and 0.35%, respectively. Similar trend of results for germination percentage were obtained in cultivar 'Arka Komal' after treatment with different concentrations of EMS, however germination percentage of 'Arka Komal' was lower than of 'Contender'. The germination percentage trends of EMS treated French bean cultivars were comparable under field and laboratory conditions but somewhat less under field due to less favourable conditions. All the plants harvested in M_1 generation in both cultivars were sown in plant to progeny fashion along with parents. Several desirable plants were selected in M_2 generation for early flowering, number of pods per plant, pod length and bean common mosaic virus (BCMV) resistance in both the cultivars at different concentrations of EMS. Selection was also performed for straight pods in case of genotype 'Contender'. The maximum frequency of desirable mutants was observed in lower EMS concentration (0.15%). The 0.15% EMS increased number of pods per plant which may be directly related to yield per plant and it will ultimately increase the total yield. Therefore, EMS concentration at 0.15% can be effectively used for inducing maximum variability in French bean. The mutant population developed in M_2 generation can be further used to check its performance in next generations.

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Key words: Bean common mosaic virus, French bean, Mutation, Pod shape, Variability, Yield

INTRODUCTION

French bean is one of important winter season vegetable crops and grown for its tender pods and dry beans. It is also known as kidney bean, haricot bean, snap bean, navy bean, string bean and garden bean. It is known for rich source of protein, vitamins and minerals particularly calcium, phosphorus and iron and thus is highly nutritious. Beans, the 'meat of the poor', contribute essential protein to the undernourished people. The 100 g of green pods contain 1.7 g protein, 4.5 g carbohydrates, 221 IU vitamin A, 11 mg vitamin C and 50 mg calcium (GOPALKRISHNAN, 2007). French bean can be used as carminative, diuretic and emollient. It is also used in treatment of diabetes, diarrhea, dysentery and kidney problems (BROUGHTON *et al.*, 2003).

India ranks first in area and production of French bean among Asian countries and occupies an area of 217.00 thousand hectare with an annual production of 2135.35 thousand metric tonnes (MT) with average productivity of 9.83 t/ha. In India, it is mostly gown in West Bengal, Andhra Pradesh, Jharkhand, Himachal Pradesh and Jammu and Kashmir. Punjab occupies an area of 1.2 thousand hectare with annual production of 1.9 thousand MT and average productivity of 1.6 t/ha (ANONYMOUS, 2015).

Mutation can play a significant role in improvement of French bean because of high response towards mutagen. The bean mutation breeding program has been started in 17 countries for obtaining disease resistance, high protein content and high yield (TULMENNNETO, 1990). In Michigan (North America), 40% cultivated area has been covered by white seeded bean mutant variety. In India, very limited work has been done for the improvement of French bean through mutation breeding. In India, only one variety 'Pusa Parvati' in French bean has been released through mutation breeding (KHARKWAL and SHU, 2009) and this mutant was developed by mutation from wax podded accession "EC 1906" followed by selection. Therefore, present investigation has been undertaken to create genetic variability in French bean through mutation breeding. Moreover, popular varieties of French bean *viz.* 'Contender' and 'Arka Komal' are highly susceptible to diseases especially bean common mosaic virus (BCMV) under Punjab conditions. Besides, 'Contender' has curved pods which are undesirable character for marketing. Keeping this in view, the present investigation was conducted to induce genetic variation in French bean through chemical mutagen- ethyl methane sulphonate.

MATERIALS AND METHODS

Plant Material and Experimental Procedure

The seeds of French bean varieties 'Contender' and 'Arka Komal' were treated with chemical mutagen ethyl methane sulphonate (EMS) at the different concentrations to know the lethal dose 50 (LD_{50}). For each treatment 100 seed of each variety was used for determination of lethal dose. Lethal dose-50 (LD_{50}) is that dose at which seed germination of 50% is remained. In this experiment LD_{50} dose was observed at 0.25% concentration of EMS. After the determination of LD_{50} (0.25%) one dose below LD_{50} (0.15%) and one dose above LD_{50} (0.35%) was used for experimental purposes. For inducing mutation through chemical mutagen EMS, seeds were first pre-soaked for four hours in distilled water, afterwards these seeds were treated with EMS for 6 hours with different concentration and finally treated seeds were post-soaked for 4 hours in distilled water.

The EMS treated 350 seeds of each genotype were sown in the field along with untreated (control) seeds. All the surviving M_1 mutant plants were harvested separately at the maturity. The harvested seeds were sown in the next season to raise M_2 generation. The respective control and treatment progenies were observed several times for different mutations throughout the crop duration.

Germination percentage

About 100 seeds of both the cultivar treated with 0.15, 0.25 and 0.35% EMS along with control were placed germinate on moist blotting papers in petri dishes using distilled water. A control set of untreated seeds was also placed for comparison. The seed germination percentage was observed 10 days after sowing. The per cent seed germination was calculated as follows.

$$\text{Per cent seed germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Germination percentage using field data

About 350 seeds of both varieties were treated with 0.15, 0.25 and 0.35% EMS were sown in the field along with the control. The data for germination per cent is taken 15 days after sowing in the field. The per cent seed germination was calculated as follows.

$$\text{Per cent seed germination} = \frac{\text{Number of seeds germinated in field}}{\text{Total number of seeds sown}} \times 100$$

Identification of mutants for desirable traits

M_2 seeds of individual M_1 harvested plants were space planted in plant to progeny rows. The seeds of parent will be sown after every 10 test rows for comparison. Within each varieties selection was performed for early germination, early flowering, number of pods and for bean common mosaic virus resistance. In case of 'Contender', selection also performed for straight pods characteristics. Several desirable plants were selected in M_2 generation and their mean performance was compared with parents' plants using Fisher's t- test of significance.

Observations recorded in M_2 generation

Individual M_2 plant along with parent plant were observed for traits *viz.* days to germination, days to flowering, number of pods per plants, pod shape and bean common mosaic virus resistant (in natural epiphytic conditions). Percent disease incidence for bean common mosaic virus was calculated by using the following formula.

$$\text{Bean common mosaic virus incidence} = \frac{\text{Number of plants infected}}{\text{Number of plants observed}} \times 100$$

The genotypes were later grouped into different categories based on 0-5 scale from immune to highly susceptible (DIWAKAR and MALI, 1976).

Category	Description	Scale
Immune	No plants showing symptoms	0
Resistant	1-5 per cent of plants showing symptoms	1
Moderately Resistant	5-15 per cent of plants showing symptoms	2
Moderately Susceptible	15-25 per cent of plants showing symptoms	3
Susceptible	25-50 per cent of plants showing symptoms	4
Highly Susceptible	More than 50 per cent of plants showing symptoms	5

Frequency (%) of selected mutant plant in M_2 generation

In M_2 generation 1150 plants for 0.15% EMS concentration, 780 plants for 0.25% EMS concentration and 0.35% EMS concentrations was observed in 'Contender' genotype. Similarly in 'Arka Komal' genotype 1110, 730 and 610 plants were observed for above mention EMS concentration. The frequency of selected mutants was determined using following formula.

$$\text{Mutant frequency (\%)} = \frac{\text{Number of mutants selected}}{\text{Number of plants observed}} \times 100$$

RESULTS AND DISCUSSION

Germination percentage in M_1 generation

The data on per cent seed germination under laboratory and field conditions of EMS treated French bean varieties *viz.* 'Contender' and 'Arka Komal' were presented in Table 1. The results of germination percentage under laboratory and field conditions indicated that the per cent seed germination in all the concentrations of EMS (0.15, 0.25 and 0.35%) was drastically reduced as compared to control. Within the treatment, the germination per cent is decreased with the increase in dose of EMS i.e. there was an inhibitory effect of increasing mutagen dose on the germination of seeds. The germination data obtained from laboratory experiment showed that seed germination in genotype 'Contender' was 90% (Table 1), whereas in three treatments of EMS @ 0.15%, 0.25% and 0.35%, it was 74%, 49% and 42%, respectively. The germination percentage was reduced under field conditions due to less favourable conditions for germination as compared to laboratory conditions. The untreated seed of genotype 'Contender' had 88% germination, whereas EMS treated seed had 70, 46 and 39% germination @ 0.15, 0.25 and 0.35% EMS concentrations under field conditions (Table 1). DHANAVEL *et al.*, (2008) studied the mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments in cowpea. They used EMS concentrations at 5, 10, 15, 20 and 25 mM and obtained 76.33, 61.66, 52.33, 41.00 and 32.33% germination, respectively in M_1 generation. They reported that EMS was more effective and efficient in causing mutations as compared to gamma rays and the combined treatments. ARIRAMAN *et al.*, (2014) studied the effects of different concentration of ethyl methane sulphonate (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM) on per cent seed germination of pigeon pea. The seed germination percentage was decreased with the increased concentration of mutagen when compared with control. DATIR *et al.*, 2007; KAVITHAMANI *et al.*,

2008; KUMAR and MISHRA, 2004 were also observed lower seed germination percentage at higher EMS concentrations in different crops.

Table 1. Effects of ethyl methane sulphonate (EMS) treatment on per cent seed germination in M_1 generation of French bean

Variety	Treatments	Seed germination under laboratory conditions (%)	Seed germination (%) under field conditions
Contender	Control	90.0	88.0
	EMS (0.15%)	74.0	70.0
	EMS (0.25%)	49.0	46.0
	EMS (0.35%)	42.0	39.0
Arka Komal	Control	85.0	81.0
	EMS (0.15%)	70.0	66.8
	EMS (0.25%)	46.0	45.1
	EMS (0.35%)	41.0	37.7

In case of 'Arka Komal' results were similar to 'Contender' in M_1 generation. However, lower germination percentage was observed in this genotype under laboratory (85%) as well as under field conditions (81%). This lower germination of 'Arka Komal' in M_1 generation may be due to the difference between germination percentages of both the varieties. The germination percentage of 'Arka Komal' after treatment with EMS @ 0.15, 0.25 and 0.35% concentration was 70, 46 and 41%, respectively which were lower than the control (85%). Similar pattern of results for per cent seed germination were observed under field conditions i.e. 81% in control and 66.8, 45.1 and 37.7% in EMS treatment at 0.15, 0.25 and 0.35% respectively (Table 1).

Mean performance of mutant plant in M_2 generation

All the plants harvested in M_1generation were again sown along with parent (control) in plant to progeny fashion. In M_2 generation, several desirable mutant plants were identified for early flowering, number of pods per plant, straight pod and bean common mosaic virus resistance while no mutant plant was observed for fewer days to germination and pod width. The mean performance of mutant plant for early flowering was 35.40 days at 0.15% EMS, 35.63 days at 0.25% EMS and 36.00 days at 0.35% EMS. The number of pods per plant was found to be 40.79, 37.40 and 37.62 in mutant plants of EMS concentration at 0.15, 0.25 and 0.35%, respectively. The mean pod length was highest (16.84 cm) in 0.15% EMS followed by 16.65 cm at 0.35% EMS and 16.63 cm at 0.25% EMS concentration (Table 2). The mean performance of observed mutant plants for flowering, number of pods per plant and pod length was significantly different from control.

The early flowering, number of pods per plants and pod length mutants were also observed in 'Arka Komal' in M_2 generation. The mean performance of mutant plants for early flowering was 39.59 days at 0.15% EMS, 39.55 days at 0.25% EMS and 39.63 days at 0.35% EMS, which were significantly different from control (46.80 days). The number of pods per mutant plant was significantly different from control (20.70 pod/plant) with mean performance of 32.78 pods/plant at 0.25% EMS, 32.33 pods/plant at 0.15% EMS and 31.83 pods/plant at 0.35% EMS. The pod length mutant had 13.92 cm pod length at 0.25% EMS followed by 13.90

cm at 0.15% EMS and 13.91 cm at 0.35% EMS. The average performance of control for flowering (46.80 days), numbers of pods per plant (20.70) and pod length (12.71) was found to be significantly different from mutant plants mean performance in different EMS concentration (Table 2). Similar results of mutation induced by EMS treatment was observed by SADASHIV and KONDIRAM (2012) for plant height, days to first flowering, number of pods per plant, pod length and yield per plant.

Table 2. Mean performance of French bean mutants and control in M_2 generation

Variety	EMS (%)	Early flowering (days)	Number of pods per plant	Pod length (cm)
Contender	0.15	35.40*	40.79*	16.84*
	0.25	35.63*	37.60*	16.63*
	0.35	36.00*	37.62*	16.65*
	Control	42.80	24.60	15.49
Arka Komal	0.15	39.59*	32.33*	13.90*
	0.25	39.55*	32.78*	13.92*
	0.35	39.63*	31.83*	13.91*
	Control	46.8	20.7	12.71

*Significant difference at 5%

Frequency of selected mutant plants in M_2 generation

The frequency of selected mutant plants was observed in M_2 generation for different traits *viz.* early flowering, number of pods per plant, pod length, straight pods, and bean common mosaic virus resistance. The best EMS concentration of a trait was selected based on frequency of selected mutant plants in M_2 generation. The maximum number of mutant plants for early flowering, number of pods per plant, pod length and bean common mosaic virus resistance were observed in lower EMS concentration (0.15%), whereas maximum straight pod mutant was observed in 0.25% EMS concentrations (Table 3). At 0.15% EMS concentration, the maximum frequency of mutant plant was observed for bean common mosaic virus resistant (1.73%) followed by number of pods per plant (1.65%), pod length (1.57%), early flowering (1.30%) and straight pod (1.04%).The least frequency of selected mutants was observed in 0.35% EMS concentration for early flowering (0.94%), number of pods per plant (1.25%), pod length (1.09%), straight pod (0.62%) and bean common mosaic virus resistance (0.62%).

The similar results were observed for frequency of mutants in 'Arka Komal' in M_2 generation. The maximum number of mutant plants for early flowering (1.59%), number of pods per plant (1.62%), pod length (1.44%) and bean common mosaic virus resistance (1.90%) were observed in lower EMS concentration (0.15%). The least frequency of selected mutants was observed in 0.35% EMS concentration for early flowering (1.31%), number of pods per plant (1.14%), pod length (1.15%) and bean common mosaic virus resistance (0.54%). DHANAVEL *et al.,* (2008) also reported that the lower concentration of EMS (0.15%) was more effective for inducing mutation as compare to higher doses and found viable mutation frequency in the range 0.86 to 3.26%. MANGAIYARKARASI *et al.,* (2014) observed that lower dose of EMS

(0.3%) induce maximum mutagens and mutation frequency of 0.51-2.72%. Similar results were also reported by KULKARNI and MOGLE (2013) in horse gram.

Table 3. Frequency (%) of selected mutant of French bean in M_2 generation (Individual plant basis)

Variety	EMS (%)	No. of plants observed	Days to flowering	Number of pods per plant	Pod length	Straight pods	BCMV* resistance
Contender	0.15	1150	1.30	1.65	1.57	1.04	1.73
	0.25	780	1.02	1.28	1.41	1.15	1.41
	0.35	640	0.94	1.25	1.09	0.62	0.62
Arka Komal	0.15	1110	1.59	1.62	1.44	-	1.90
	0.25	730	1.50	1.23	1.36	-	1.29
	0.35	610	1.31	1.14	1.15	-	0.54

* Bean common mosaic virus

The above findings clearly revealed that mutant plants for important traits can be selected by using lower concentration of EMS (0.15%). The single plants selection for early flowering, number of pods per plant, pod length and bean common mosaic virus resistance should be conducted in M_2 generation of selected mutant plants.

CONCLUSIONS

The seed germination of French bean cultivars 'Contender' and 'Arka Komal' decreases with increase in concentration of EMS from 0.15 to 0.35%. Several desirable mutants were selected in M_2 generation for early flowering, number of pods per plant, pod length and bean common mosaic virus resistance in both the cultivars at different concentrations of EMS but maximum frequency of desirable mutants was observed in EMS at 0.15%. The 0.15% EMS increased number of pods per plant which may be directly related to yield per plant and it will ultimately increase the total yield. The mutant population developed in M_2 generation can be further used to check its performance in next generations.

Received, May 08th, 2017
Accepted November 18th, 2017

REFERENCES

AHLOOWALIA, B.S., M. MALUSZYNSKI, K. NICHTERLEIN (2004): Global impact of mutation-derived varieties. Euphytica, *135*: 187–204.

ANONYMOUS (2015): Area and production of French bean. http://www.indiastat.com.

ARIRAMAN, M., S. GNANAMURTHY, D. DHANAVEL, T. BHARATHI, S. MURUGAN (2014): Mutagenic effect on seed germination, seedling growth and seedling survival of pigeon pea. Int. Letters Nat. Sci., *21*: 41-49.

BROUGHTON, W.J., G. HERNANDEZ, M. BLAIR, S. BEEBE, P. GEPS, J. VANDDRLAYDEN (2003): Beans (*Phaseolus* spp.) model food legumes. Plant Soil, *252*: 55-128.

DATIR, S.S., K.N. DHUMAL, R.N. PANDEY (2007): Gamma radiation and EMS induced variation in seed germination and plant survival in horse gram. J. Arid Legumes, *4*: 15-17.

DHANAVEL, D., P. PAVADAI, L. MULLAINATHAN, D. MOHANA, G. RAJU, M. GIRIJA, C. THILAGAVATHI (2008): Effectiveness and efficiency of chemical mutagens in cowpea. African J. Biotech., *7*: 4116-4117.

DIWAKAR, M.P., and V. R. MALI (1976): Cowpea Mosaic Virus, a new record for Marathwada. J Maharashtra Agric University *1*: 274-277.

GOPALKRISHNAN, P. (2007): *Vegetable crops.* pp. 176-177. New India Publisher, New Delhi, India.

KAVITHAMANI, D., A. KALAMANI, C. VANNIARAJAN, D. UMA (2008): Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean. Madras Agric. J., *95*: 448-451.

KHARKWAL, M.C. and Q.Y. SHU (2009): The Role of Induced Mutations in World Food Security. In: Induced Plant Mutations in the Genomics Era; Shu, Q.Y., Ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, pp. 33–38.

KULKARNI, G.B. and U.P. MOGLE (2013): Effects of mutagen on chlorophyll mutation in horse gram. Biosci. Discovery, *4*: 214-219.

KUMAR, A. and M.N. MISHRA (2004): Effect of gamma-rays, EMS and NMU on germination, seedling vigour, pollen viability and plant survival in M_1 and M_2 generation of okra (*Abelmoschus esculentus* (L.) Moench). Adv. Plant Sci., *17*: 295-297.

MANGAIYARKARASI, R., M. GIRIJA, S. GNANAMURTHY (2014): Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus.* Int. J. Curr. Microbiol. App. Sci., *3*: 881-889.

SADASHIV, B. and D. KONDIRAM (2012): Effect of mutagens on quantitative characters in M_2 and M_3 generation of horse gram. Int. J. Scientific Res., *2*: 1-7.

TULMENNNETO, A. (1990): Genetic improvement of beans *(Phaseolus vulgaris)* through mutation breeding. In: *Genetic improvement of Pulse crops. Pp* 296-327. Premier publishing house, Hyderabad.

EFEKAT MUTAGEN- ETHIL METAN SULFONATA NA PARAMETRE POVEĆANJA PRINOSA KOD FRANCUSKOG PASULJA (*Phaseolus vulgaris* L.)

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Izvod

Istraživanje je obavljeno na istraživačkoj farmi za povrće, Deparmana za povrtarstvo, Pendžab Univerziteta, Ludhiana, Indija, tokom prolećne sezone 2015 i 2016, sa ciljem da izazove genetičku varijabilnost za prinos i osobine kvaliteta kod sorata 'Contender' i 'Arka Komal' primenom hemijskog mutagena etil metan sulfonata (EMS) sa 0.15, 0.25 i 0.35%. Seme tretirano svim koncentracijama EMS imalo je drastično manju klijavost, a % klijavosti je opadao sa porastom koncentracije EMS. Najveću klijavost (90%) imalo je seme sorte 'Contender' u kontroli, dok je klijavost bila 74%, 49% i 42% u EMS-u pri koncentracijama od 0.15%, 0.25% i 0.35%. Sličan trend je dobijen i kod sorte 'Arka Komal' posle tretiranja različitim koncentracijama EMS-a, ali je % klijavosti bio niži nego kod sorte 'Contender'. Trend procenta klijavosti kod sorti pasulja tretiranih EMS-om bio je sličan u poljskim i laboratorijskim uslovima, ali je bio nešto niži u polju, zbog manje povoljnih spoljašnjih uslova. Sve biljke sakupljene u M1 generaciji obe sorte su bile posejane zajedno sa roditeljima. Nekoliko biljaka odabrano je u M_2 generaciji prema osobinama: rano cvetanje, broj mahuna po biljci, dužina mahuna i otpornost na mozaični virus pasulja (BCMV) kod obe sorte pri različitim koncentracijama EMS-a. Selekcija je urađena i na osnovu uspravnih mahuna kod genotipa 'Contender'. Maksimalna frekvencija poželjnih mutanata je utvrđena pri nižim koncentracijama EMS (15%). Ta koncentracija je utivcala na povećanje broja mahuna po biljci, što je direktno povezano sa prinosom po biljci i svakako dovodi do povećanja ukupnog prinosa. Zbog toga se EMS koncentracija od 0.15% može efikasno koristiti za indukciju maksimalne varijabilnosti kod Francuskog pasulja.

Primljeno 08.V.2017.
Odobreno 18. XI. 2017.