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EMS Induced Mutational Variability in *Vigna radiata* and *Vigna mungo*

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ABSTRACT

Ethylmethane sulphonate (EMS) has been long considered as a potential chemical mutagen for inducing genetic variability in crop plants. This study tries to elucidate out the effect of different doses (0.1 to 0.4%) of EMS on the two species of genus *Vigna* thereinafter *V. radiata* and *V. mungo* in M_1 and M_2 generations for isolation of putative mutants and their respective comparative studies. Selection of mutants is based upon the variation in genetic parameters for yield and certain yield contributing characters. The comparative studies on the estimation of total seed protein content and nitrate reductase (EC 1.7.99.4) activity, NRA, in both generations of these two species have also been studied in order to reveal the potentiality of mutants to be isolated for raising M_3 generation. In the present study, a better variant doses of, EMS were found to be very effective in generating mutation for higher yield in both species and there was a linear correlation of the total seed protein content to NRA and the total plant yield. The accuracy of the selected mutants in M_2 generation was satisfactorily evaluated and would help in isolating wide range of mutant pools accurately to larger extent in shorter duration.

Key words: Mutagen, *Vigna*, heritability, mutant, protein

INTRODUCTION

Induced mutation breeding, a much heralded short cut technique, is a powerful approach for determining the biological functions of genes in an organism, with altered phenotypes and physiological response. It is preferably, due to less cross pollination rate, suited for self-fertilizing species (Gottschalk, 1973) as none or less natural variability is produced due to conventional methods.

Genetic variation among genotypes and relationship between major yield contributing traits/characters is of vital importance to breeding programmes that aim to produce important cultivars (Tah, 2006). Various approaches have been developed for induced mutagenesis involving chemical and physical methods. Chemical mutagenic agents like ethylmethane sulphonate (EMS) produce a range of novel traits and broadening of genetic diversity of plants (Lagoda, 2007). With development of new techniques such as targeting induces local lesions in genomes (TILLING), EMS mutagenesis can be used for both forward and reverse genetic studies (Kim *et al*., 2004).

Among the various pulses, mungbean (*Vigna radiata* L. Wilczek) and urdbean (*Vigna mungo* L. Hepper), the self-fertilized crops, plays an important role in Indian agriculture and has been grown under various agro-ecological conditions. Despite this, progress in production and

productivity in mungbean and urdbean has remained far from satisfactory and is unable to cope with the demand of growing populations. This is because the breeding methodology applied on pulse crops in the past has been purely conventional. Since, the conventional techniques employed in the improvement of mungbean and urdbean have not keep pace with the demands of expanding population, the importance of development of high yielding mutant varieties has great relevance (Khan and Goyal, 2009). One of the chief advantages of mutation breeding applied to these two important pulse crops, mungbean and urdbean, is that it can give rise to many different mutant alleles with different degree of trait modifications (Chopra, 2005).

The present work aimed to understand the mutagen induced variability in two species of genus *Vigna* viz., mungbean and urdbean - self pollinated legume crops which are cultivated in different countries of the world including India for their higher nutritive values. The induced variability (genotypic and phenotypic) in M_2 generation was estimated with regards to quantitative traits, particularly yield and yield contributing traits. The induced mutagenesis for seed protein content and nitrate reductase activity in M_1 and M_2 generations were also estimated for possible isolation and evaluation of high yielding mutant lines in subsequent generations and to determine its effects at molecular level through protein and DNA profiling.

MATERIALS AND METHODS

Mutagenic treatment: Seeds of mungbean var. PDM-11 were obtained from the Indian Agriculture Research Institute, New Delhi and urdbean var. T-9 were procured from the Government Seed Store, Aligarh, India in the month of March, 2008. For each treatment three hundred healthy seeds of both species, presoaked in distilled water for 9 h, were treated with different doses (0.1, 0.2, 0.3 and 0.4%) of freshly prepared aqueous solution of EMS (Sissco Research Laboratories Pvt Ltd., Mumbai, India) in phosphate buffer (pH 7.5) for 6 h. Three hundred seeds soaked in distilled water for 15 h were used as controls.

Raising M_1 and M_2 generations: For raising M_1 generation, 1200 seeds treated with different doses of EMS and 300 seeds as controls were sown at the Agricultural field of the Aligarh Muslim University, Aligarh in 2008 kharif season (April-July) in a Complete Randomized Block Design (CRBD). Three hundred seeds harvested separately and randomly from healthy individual of M_1 plants from each treatment, as well as from control, were sown as M_2 progenies in kharif season of 2009. The spacing was maintained at 30 cm (plant to plant in a row) and 60 cm (between the rows) in the field. Three replications were maintained in each treatment.

Data were collected on certain quantitative characters viz., pods/plant, 100 seed weight (g) and total plant yield (g) in M_1 and M_2 generations. Total seed protein content (%) and nitrate reductase activity, NRA (μmol NO_2 g^{-1} (f.w.)/h) in leaf tissues at flowering stage were also studied in both generations. Total seed protein content and NRA were estimated by the methods outlined by Lowry *et al.* (1951) and Srivasttava (1975) respectively. The optical density of the samples was taken from spectrophotometer (Elico Pvt. Ltd., Hyderabad, India).

Data analysis: Data were analyzed using SPSS software (Version 16.0, SPSS Inc. Chicago, Illinois 606606) and means were compared using Duncans Multiple Range Significance test at 5% level of significance. All means are presented with±Standard Error (SE). Co-efficient of variation (CV) in M_1, Phenotypic Coefficient of Variation (PCV) and genetic variability parameters

(Genotypic Coefficient of Variation (GCV), heritability in broad sense (h^2) and Genetic Advance (GA)) in M_2 were estimated for each parameter by the formulae suggested by Singh and Chaudhary (1985).

RESULTS

Application of EMS for the induction of mutation significantly altered the mean values of yield and yield contributing characters studied in M_2 generation whereas no significant change in mean values for same characters were observed in M_1 generation in both species of genus *Vigna*. However, mean values of total seed protein content changed significantly in both generations of mungbean and urdbean (Table 1). Coefficient of variation was maximum for 100 seed weight

Table 1: Comparative estimates of mean of yield and its components and total protein conten in mungbean and urdbean in M_1 and M_2 generations

	Pods per plant		100 seed weight (g)	
Treatments	M_1	M_2	M_1	M_2
Mungbean Var. PDM-11				
Control	47.13±0.55^a	47.93±0.42^d	3.67±0.05^a	3.61±0.15^b
0.1% EMS	48.20±2.08^a	50.27±0.10^c	3.96±0.07^a	3.51±0.17^b
0.2% EMS	48.60±2.00^a	58.26±0.57^a	3.91±0.10^a	4.04±0.03^a
0.3% EMS	48.10±2.00^a	55.16±0.23^b	3.88±0.10^a	3.87±0.02^a
0.4% EMS	46.90±1.62^a	45.16±0.04^e	3.52±0.08^a	3.54±0.05^b
SD	0.73	5.32	0.19	0.23
Urdbean Var. T-9				
Control	34.73±0.55^a	35.80±0.65^c	3.44±0.11^a	3.30±0.02^b
0.1% EMS	33.20±0.29^a	46.20±1.57^a	3.67±0.28^a	3.37±0.01^a
0.2% EMS	29.86±0.87^a	44.73±1.49^{ab}	3.64±0.12^a	3.34±0.01^a
0.3% EMS	32.46±0.97^a	42.46±0.66^b	3.57±0.12^a	3.28±0.01^{bc}
0.4% EMS	32.93±0.79^a	31.63±0.64^d	3.40±0.03^a	3.25±0.01^c
SD	1.76	6.21	0.12	0.05
	Total plant yield (g)		Total seed protein content (%)	
Treatments	M_1	M_2	M_1	M_2
Mungbean Var. PDM-11				
Control	9.30±0.17^a	9.21±0.16^c	21.97±0.003^c	21.60±0.30^c
0.1% EMS	10.17±0.66^a	10.32±0.06^b	23.34±0.04^b	23.90±0.21^b
0.2% EMS	10.30±0.62^a	12.65±0.08^a	24.34±0.02^a	25.34±0.61^a
0.3% EMS	9.90±0.53^a	12.34±0.07^a	22.68±0.04^{bc}	24.57±0.62^{ab}
0.4% EMS	9.15±0.40^a	8.33±0.18^d	21.89±0.007^c	22.00±0.15^c
SD	0.51	1.90	1.02	1.64
Urdbean Var. T-9				
Control	7.44±0.08^a	7.34±0.02^d	23.24±0.04^e	23.90±3.33^c
0.1% EMS	8.04±0.46^a	10.00±0.12^a	24.43±0.26^c	26.34±0.06^a
0.2% EMS	8.45±0.38^a	9.39±0.04^b	25.43±0.02^b	24.90±0.10^{ab}
0.3% EMS	7.91±0.43^a	9.07±0.03^c	25.89±0.04^a	26.90±0.08^a
0.4% EMS	7.21±0.02^a	8.34±0.02^d	23.36±0.19^d	22.90±0.15^{ab}
SD	0.49	1.02	1.19	1.66

Each value is the mean (±standard error) of three replicates; SD denotes standard deviation of mean values. The values superscripted with different alphabetical letters are significant to each other at significance level of 0.05 depicted by Duncan's Multiple Range Significance Test analyzed by computer software SPSS 16.0 version (SPSS Inc., Chicago, Illinois 60606)

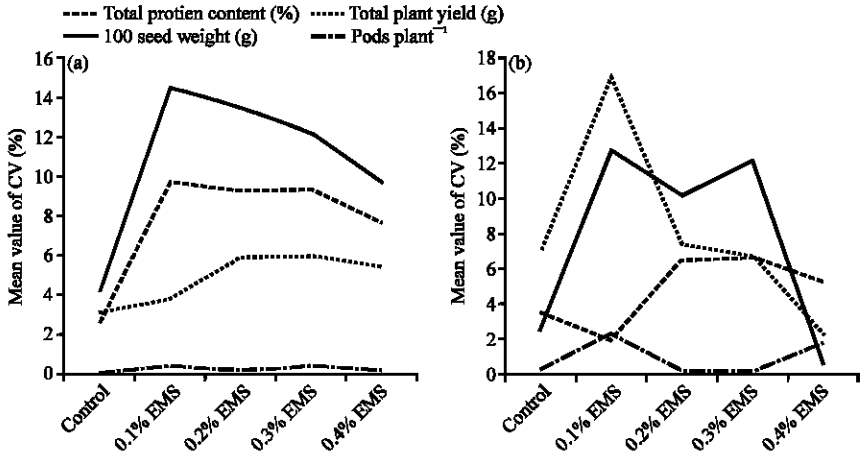

Fig. 1: Comparative effects of mutagen (EMS) concentrations on coefficient of variation (CV) in M_1 generation of (a) mungbean and (b) urdbean

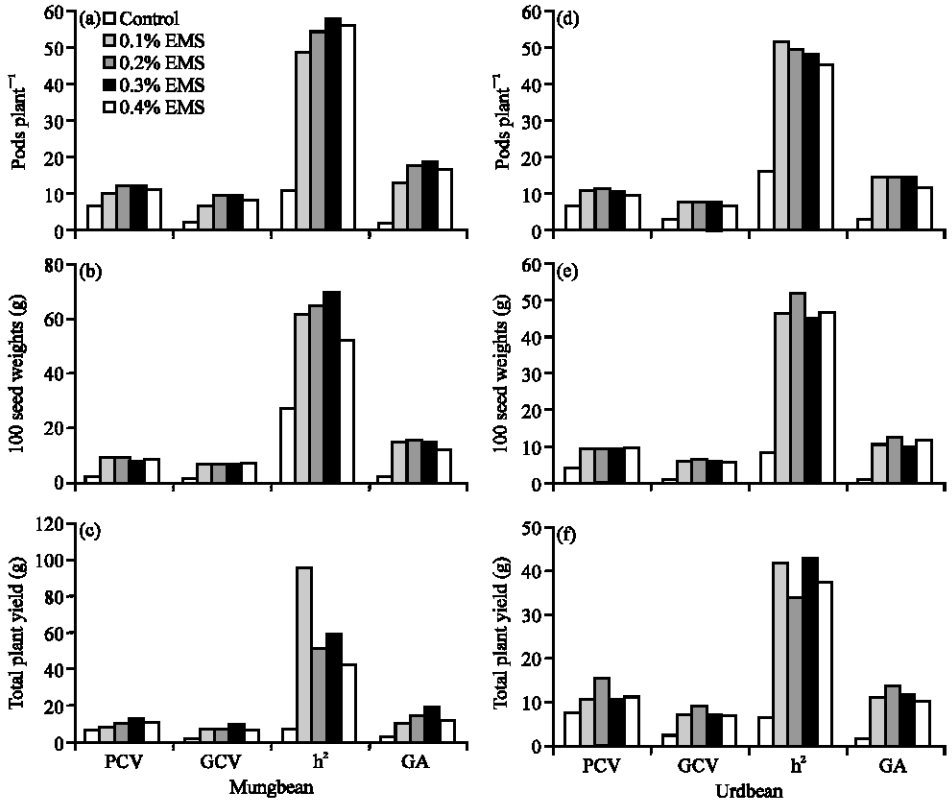

Fig. 2: Comparative estimation of genetic parameters for yields and its components in M_2 generations of mungbean and urdbeen. (PCV: phenotypic coefficient of variation; GCV: Genotypic coefficient; h^2: Heritability in borad sense and GA: Genetic advance as % of mean). (a, d) Pods plant^{-1}, (b, e) 100 seed weight (g) and (c, f) Total plant yield (g)

(14.45%) in mungbean and total plant yield (16.92%) in urdbean at 0.1% of EMS treatment in M_1 generation. Variability was found to be larger in EMS treatments than the control (untreated) population for all characters studied in the present investigation (Fig. 1a, b). Mungbean showed

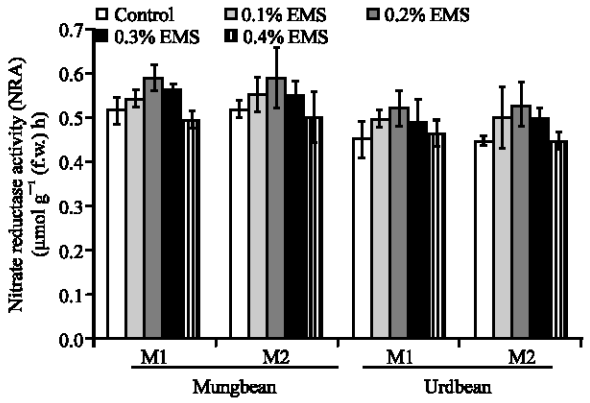

Fig. 3: Comparative study of nitrate reductase activity (NRA) in M_1 and M_2 generations in mungbean and urdbean in leaf tissues at flowering stage

greater variability than urdbean. The genetic parameters for both species were also found to be maximum in mutagenic treated plants over control. Higher concentration (0.4%) of mutagen, on an average, produced minimum variability for all characters in both species of *Vigna*. Genetic advance for pods/plant, 100 seed weight and total yield in M_2 generation was found to be higher at 0.2 and 0.3% of EMS in mungbean whereas it was higher at 0.1 and 0.2% in urdbean. Heritability for total plant yield (96.98%) in mungbean and for pods/plant (51.87%) in urdbean was very high at 1% EMS as compared to all other treatments and characters. PCV and GCV showed similar trends and higher values at 0.2 and 0.3% of EMS in both the species of *Vigna* (Fig. 2a-f).

Nitrate reductase activity (NRA) in both species was also affected in mutagen treated populations. The NRA in mungbean shows higher values as compared to urdbean in both generations. However, among two generations NRA were found to be higher in M_2 generation. The trend in NRA in both species and in both generations showed similarity to that of total seed protein content. The highest values of NRA for mungbean were estimated 0.591 µmol g^{-1} (f.w.)/h and for urdbean 0.531 µmol g^{-1} (f.w.)/h at 0.2% EMS dose, whereas lowest activity of NRA for both species were found at 0.4% EMS in both generations (Fig. 3).

DISCUSSION

Alkylating agents such as EMS induce modifications, through mutagenesis, to be used for generating breeding lines (Lee *et al*., 2003) and are apparent by the results in the present work. Modifications by EMS is mainly due to mispairing and base changes i.e., chemical alterations of nucleotides in a genome (Kim *et al*., 2004). The majority (99%), EMS induces C-to-T changes resulting in C/G to T/A substitution (Greene *et al*., 2003; Kovalchuk *et al*., 2000; Krieg, 1963). EMS mutagenesis generates randomly distributed mutations throughout the genome as shown by Greene *et al*. (2003) in *Arabidopsis*. As a result, chemical mutagenesis can be used not only to search for loss- or gain- of functional mutants but also to understand the role of specific amino acid residues in protein function (Kim *et al*., 2004). Results of many studies suggest that use of chemically induced mutants can also provide useful information for understanding the functions of essential genes by generating weak nonlethal alleles. In the present study the results for the decrease in mean values of various quantitative traits at higher concentrations of EMS in agreement with the hypothesis that, due to mutagenic treatment, mean is shifted to a direction opposite to selection (Bhatia and Swaminathan, 1962), whereas the increase in mean values could

be due to the occurrence of polygenic mutations with cumulative effects (Singh *et al.*, 2001). The changes in the mean values after mutagenic treatments has been reported earlier in many pulse crops including mungbean (Wani *et al.*, 2005; Arulabalchandran and Mullainathan, 2009; Tah, 2006), lentil (Singh *et al.*, 2006) and urdbean (Deepalakshmi and Kumar, 2003). The positive correlation between pods/plant and total plant yield in both species of *Vigna* were found and are in agreement to the works of (Khan *et al.*, 1999) in mungbean. A high degree of correlation also occurred between total seed protein content and NRA. Correlating NRA with protein content and total yield is an effective tool in selecting pure mutant lines at early stage and has been earlier studied by (Kozgar and Khan, 2009) in chickpea and (Aparna *et al.*, 2007) in pigeonpea. Induction of greater variability in polygenic traits might be due to increased mutations and recombination (Singh *et al.*, 2001).

A non-linear relationship was observed between the mutagen concentrations and the variability induced for various traits (Fig. 2). These results are in agreement with earlier reports of Singh *et al.* (2000).

In the present study effect of mutagen on total seed protein content and NRA put an added impetus in selection and have been reported for the first time. It will help and be novel in determining the extent of mutation variability at molecular level in early stages which make it time and cost effective. The difficulty in detecting single-nucleotide polymorphisms or substitution can be overcome by use of targeting induced local lesions in genomes (TILLING). These technologies will allow chemically induced mutant pools to be used for reverse genetics. With help of these automated techniques of rapid detection makes possible to screen a wide range of mutant pools in a short time and to avoid the often laborious process of forward genetic screening (Colbert *et al.*, 2001; Henikoff and Comai, 2003).

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