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Research Article
Gamma Rays and Ethyl Methanesulfonate Induced Early Flowering and Maturing Mutants in Urdbean (*Vigna mungo* (L.) Hepper)

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Abstract

Background and Objective: The nature and extent of genetic variability available within the species forms the basis for effective selection. Present study was aimed at to explore the possibility of inducing genetic variability in M_2 and M_3 generations of urdbean following mutagenesis with individual and combination treatments of gamma rays and ethyl methanesulfonate (EMS) for selecting enviable early flowering and maturing lines for late spring season. **Materials and Methods:** Physically, seeds of two varieties of urdbean were irradiated with 200 and 300 Gy doses of gamma rays at NBRI, Lucknow. For chemical treatments, seeds were treated with 0.2 and 0.3% of EMS for 6 h and for combination treatments, dry seeds of each variety were firstly irradiated with 200 and 300 Gy doses of gamma rays followed by the treatment with 0.2% of EMS. The mutagen treated seeds were sown in complete randomized block design to raise M_1 generation. The M_1 seeds were sown in plant progeny rows to grow M_2 generation. The 10 M_2 progenies showing significant negative deviation in mean values from their respective controls particularly for flowering and maturity were selected to raise M_3 generation. Analysis of variance was performed to assess the extent of induced variation for both the traits. **Results:** Data for days to flowering and maturity in M_3 generation had resulted in reducing the flowering and maturity period by more than four days after mutagenic treatments in both the varieties. Combination treatments were found to be more effective in reducing the flowering and maturity period than the individual treatments of gamma rays and EMS. Genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance (GA) increased manifold in the treated population. **Conclusion:** The quantitative traits (flowering and maturity) exhibited higher genetic variability in M_2 as compared to M_3 generation indicating that potential gain could be achieved through selection in early (M_2) generation.

Key words: Mutagens, early flowering, genetic variability, urdbean, gamma rays, heritability, ethyl methanesulfonate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Urdbean (*Vigna mungo* (L.) Hepper), also known as mash or black gram is an important pulse crop in Southeast Asia and the Indian sub-continent. It is grown in almost all the states of India as kharif (rainy), rabi (post rainy) and zaid (summer) season crop in different agro-ecological systems[1]. Sandy loam soils with good internal drainage are considered ideal for urdbean cultivation.

Improvement of cultivated plants largely depends on the extent of genetic variability available within the species[2,3]. Genetic variability is the most essential pre-requisite for any successful crop improvement program as it provides the spectrum of variants for effective selection[4,5]. Creation of genetic variability followed by screening and selection of best plants is a major target for this crop. Due to lack of sufficient natural variability, conventional methods of plant breeding had limited scope in the improvement of urdbean crop. Since spontaneous mutations occur at very low frequency, artificial induction of mutations facilitates the development of improved varieties at a faster rate[6-8].

The advantage of mutation breeding technique is that it can be applied for changing specific characters in otherwise good varieties by incorporating some useful variations in comparatively shorter period of time[9,10]. So induced mutations supplement plant breeding and confer specific improvement in a variety without significantly altering its otherwise acceptable phenotype[11]. Micro-mutations affecting polygenic characters and each having small effects on the parental genotype might be more useful than macro-mutants because of their buffering ability[12]. The efficiency of mutagenic treatment can be assessed by its potential to produce more useful mutation[13,14]. Thus, for successful development of desirable micro-mutants, information on the efficiency of mutagen and mutagenic treatments for inducing micro-mutations, direction and magnitude of induced variation is required[6,15].

In the past, a lot of study has been undertaken on induced mutagenesis through physical and chemical mutagens in various crop plants[16-23]. However, such information is little scanty in urdbean. In rainy season, urdbean is damaged by various diseases and insect pests which hamper its growth, quality and yield. The problem, however may be trounced if the mutant plants mature before the onset of rainy season. Present investigation was carried out to develop such early mutants of urdbean which could mature before the arrival of rainy season.

MATERIALS AND METHODS

Site of study and varieties used: A field experiment was conducted during the summer (zaid) season of 2008, 2009 and 2010 at Agricultural Farm, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Seeds of two varieties of urdbean (*Vigna mungo* (L.) Hepper) namely T-9 and Pant U-30 were obtained from G. B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India. Both the varieties are well adapted to agro-climatic conditions of Uttar Pradesh including the site of the study.

Experimental design and mutagens used: Dry seeds of each variety with moisture content of 12% were irradiated with 200 and 300 Gy doses of gamma rays from ^{60}CO source at National Botanical Research Institute (NBRI), Lucknow, India. For chemical treatments, healthy seeds of each variety were presoaked for 9 h in distilled water before treating with 0.2 and 0.3% of ethyl methanesulfonate (EMS) for 6 h with intermittent shaking at room temperature of 25±1°C. The solution of EMS was prepared in phosphate buffer of pH 7. After treatment, the seeds were thoroughly washed in running tap water to remove the residual mutagen from seed surface. For combination treatment, dry seeds of each variety were firstly irradiated with gamma rays at 200 and 300 Gy doses and subsequently treated with 0.2% EMS i.e., (200 Gy+0.2% EMS and 300 Gy+0.2% EMS). About 350 pre-soaked seeds from each variety were again soaked in phosphate buffer for 6 h to serve as control.

Three replications of 100-seeds each were sown for every treatment and control in each variety in a randomized complete block design (RCBD). The spacing was maintained at 30 cm (seed to seed in a row) and 60 cm (between the rows) in the field. Recommended agronomic practices were employed for the preparation of field, sowing and subsequent management of the population of urdbean. Twenty-five healthy seeds from each normal looking M_1 plants of different treatments with respective controls in both the varieties were planted in plant progeny rows to raise the M_2 generation. Observations were made on 25-30 normal looking plants of each progeny for each treatment. The progenies segregating for macro-mutations were not considered for such analysis. For raising M_3 generation, such 10 M_2 progenies were selected which showed significant deviation in mean values in the negative direction from the mean values of control particularly for days to flowering and maturity.

Data analysis: Data collected for days to flowering and maturity in M_2 and M_3 generations were subjected to statistical

analysis according to the methods of Singh and Chaudhary[24] and Johnson *et al.*[25]. Critical difference between the means of treated and control population was estimated from the error mean square and tabulated 't' value at 5% level of significance.

RESULTS

Days to flowering: In breeding programs of self-pollinated crops like urdbean, a wide range of genetic variability for quantitatively inherited traits like days to flowering and maturity forms the basis for effective selection. The data recorded for days to flowering in M_2 and M_3 generations are presented in Table 1, 2. A glance at the Tables indicated that ample genetic variability was induced by all the mutagenic treatments in both the varieties. Phenotypic and genotypic coefficients of variation, heritability and genetic advance increased manifold in the treated population. In M_2 generation, the highest phenotypic (11.26%) and genotypic (7.71%) coefficients of variation were recorded with 200 Gy dose of gamma rays in variety Pant U-30, whereas the highest estimate of heritability (53.53%) was observed with combination treatment of 300 Gy+0.2% EMS in variety T-9 (Table 1). In variety Pant U-30, the mean days to flowering were reduced by more than four days with the treatment of 300 Gy+0.2% EMS in M_3 generation (control mean = 41.69, treatment mean = 37.46, shift in mean = -4.23) (Table 2).

Days to maturity: Mean days to maturity was also reduced significantly in most of the mutagenic treatments (Table 3, 4). The combination treatments of gamma rays and EMS proved more effective in reducing the maturity period in both the varieties. Highest coefficients of phenotypic (8.30%) and genotypic (6.00%) variation were recorded with 300 Gy dose of gamma rays in M_2 generation. The estimates of highest heritability (58.78%) and genetic advance (11.39%) were recorded with 200 Gy+0.2% EMS and 300 Gy gamma rays treatments in variety Pant U-30 in M_2 generation (Table 3). For var. Pant U-30, the maturity period was reduced by 4.65 days with 300 Gy dose of gamma rays in M_3 generation (control mean = 69.45, treatment mean = 64.80, shift in mean = -4.65) (Table 4).

In general, the magnitude of genetic parameters for days to flowering and maturity were comparatively higher in M_2 as compared to M_3 generation, indicating that effective selection

Table 1: Estimates of mean values ($\bar{x}$), shift in $\bar{x}$ and genetic parameters for days to flowering in M_2 generation of urdbean

Treatments	Mean±SE	Shift in $\bar{x}$	PCV (%)	GCV (%)	h^2 (%)	GA ($\bar{x}$ %)
Var. T-9						
Control	41.50±0.39	-	6.23	2.98	22.70	3.59
Gamma rays						
200 Gy	40.41±0.29	-1.09	8.45	6.09	52.06	11.58
300 Gy	39.40±0.20	-2.10	8.20	5.92	51.96	11.01
CD (p = 0.05)		1.22				
EMS						
0.2 (%)	40.54±0.30	-0.96	10.23	6.75	43.49	11.56
0.3 (%)	40.26±0.34	-1.24	10.96	7.20	42.95	12.12
CD (p = 0.05)		1.52				
Gamma rays+EMS						
200 Gy+0.2 (%)	39.30±0.22	-2.20	7.84	5.10	42.19	8.67
300 Gy+0.2 (%)	38.63±0.19	-2.87	8.61	6.31	53.53	12.01
CD (p = 0.05)		1.10				
Var. Pant U-30						
Control	41.20±0.36	-	4.98	2.17	18.80	2.35
Gamma rays						
200 Gy	39.80±0.13	-1.40	11.26	7.71	46.63	13.66
300 Gy	40.46±0.19	-0.74	8.29	6.00	52.13	11.34
CD (p = 0.05)		0.76				
EMS						
0.2 (%)	40.50±0.18	-0.70	9.00	6.53	52.56	12.32
0.3 (%)	39.90±0.15	-1.30	8.24	5.80	49.67	10.63
CD (p = 0.05)		1.40				
Gamma rays+EMS						
200 Gy+0.2 (%)	40.36±0.18	-0.84	8.69	6.34	53.06	12.11
300 Gy+0.2 (%)	38.63±0.17	-2.57	8.20	5.14	39.27	8.41
CD (p = 0.05)		1.26				

PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Heritability, GA: Genetic advance, EMS: Ethyl methanesulfonate, Var. T-9: Variety T-9, Var. Pant U-30: Variety Pant U-30

Table 2: Estimates of mean values ($\bar{x}$), shift in $\bar{x}$ and genetic parameters for days to flowering in M_3 generation of urdbean

Treatments	Mean±SE	Shift in $\bar{x}$	PCV (%)	GCV (%)	h^2 (%)	GA ($\bar{x}$ %)
Var. T-9						
Control	41.92±0.13	-	5.01	2.34	21.77	2.77
Gamma rays						
200 Gy	40.11±0.20	-1.81	7.35	4.69	40.57	7.75
300 Gy	39.83±0.45	-2.09	6.90	4.24	37.83	6.75
CD (p = 0.05)		0.53				
EMS						
0.2 (%)	40.32±0.19	-1.60	6.73	4.20	38.96	6.75
0.3 (%)	40.06±0.27	-1.86	7.81	5.09	42.49	8.66
CD (p = 0.05)		0.30				
Gamma rays+EMS						
200 Gy+0.2 (%)	39.67±0.32	-2.25	6.20	3.75	36.69	5.89
300 Gy+0.2 (%)	38.31±0.44	-3.61	7.80	4.98	40.83	8.25
CD (p = 0.05)		0.86				
Var. Pant U-30						
Control	41.69±0.16	-	4.20	1.25	15.42	1.65
Gamma rays						
200 Gy	40.00±0.59	-1.69	6.82	4.12	36.51	6.47
300 Gy	39.52±0.23	-2.17	5.72	3.74	42.86	6.32
CD (p = 0.05)		1.05				
EMS						
0.2 (%)	40.12±0.35	-1.57	7.20	4.36	36.65	6.85
0.3 (%)	39.98±0.40	-1.71	8.13	5.08	39.01	8.38
CD (p = 0.05)		0.67				
Gamma rays+EMS						
200 Gy+0.2 (%)	39.15±0.22	-2.54	6.97	3.91	31.41	5.69
300 Gy+0.2 (%)	37.46±0.19	-4.23	7.30	4.18	32.54	6.15
CD (p = 0.05)		1.08				

PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Heritability, GA: Genetic advance, EMS: Ethyl methanesulfonate, Var. T-9: Variety T-9, Var. Pant U-30: Variety Pant U-30

Table 3: Estimates of mean values ($\bar{x}$), shift in $\bar{x}$ and genetic parameters for day to maturity in M_2 generation of urdbean

Treatments	Mean±SE	Shift in $\bar{x}$	PCV (%)	GCV (%)	h^2 (%)	GA ($\bar{x}$ %)
Var. T-9						
Control	70.03±0.73	-	2.65	1.20	14.32	0.97
Gamma rays						
200 Gy	69.03±0.62	-1.00	6.32	4.37	47.97	7.82
300 Gy	68.49±0.52	-1.54	4.00	2.75	47.00	4.95
CD (p = 0.05)		1.27				
EMS						
0.2 (%)	68.73±0.67	-1.30	6.95	4.98	51.20	9.35
0.3 (%)	66.36±0.49	-3.67	7.22	5.35	54.92	10.28
CD (p = 0.05)		0.87				
Gamma rays+EMS						
200 Gy+0.2 (%)	66.83±0.56	-3.20	6.34	3.98	39.23	6.50
300 Gy+0.2 (%)	66.78±0.68	-3.25	5.93	3.94	44.13	6.87
CD (p = 0.05)		0.92				
Var. Pant U-30						
Control	69.90±0.72	-	2.98	1.20	15.74	1.17
Gamma rays						
200 Gy	68.76±0.65	-1.14	6.23	4.00	41.28	6.73
300 Gy	67.35±0.35	-2.55	8.30	6.00	52.22	11.39
CD (p = 0.05)		2.02				
EMS						
0.2 (%)	68.15±0.32	-1.75	6.21	4.52	52.99	8.51
0.3 (%)	67.03±0.48	-2.87	5.32	3.53	44.36	6.16
CD (p = 0.05)		1.42				
Gamma rays+EMS						
200 Gy+0.2 (%)	67.16±0.45	-2.74	6.20	4.76	58.78	9.46
300 Gy+0.2 (%)	65.91±0.52	-3.99	3.64	2.58	50.61	4.78
CD (p = 0.05)		1.06				

PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Heritability, GA: Genetic advance, EMS: Ethyl methanesulfonate, Var. T-9: Variety T-9, Var. Pant U-30: Variety Pant U-30

Table 4: Estimates of mean values ($\bar{x}$), shift in $\bar{x}$ and genetic parameters for days to maturity in M_3 generation of urdbean

Treatments	Mean±SE	Shift in $\bar{x}$	PCV (%)	GCV (%)	h^2 (%)	GA ($\bar{x}$ %)
Var. T-9						
Control	70.00±0.19	-	2.90	1.06	13.28	0.98
Gamma rays						
200 Gy	68.10±0.35	-1.90	6.89	3.19	21.37	3.82
300 Gy	66.85±0.52	-3.15	3.72	2.43	42.60	4.09
CD (p = 0.05)		1.08				
EMS						
0.2 (%)	67.80±0.61	-2.20	4.58	2.71	35.17	4.22
0.3 (%)	65.80±0.49	-4.20	4.27	2.59	36.63	4.06
CD (p = 0.05)		0.80				
Gamma rays+EMS						
200 Gy+0.2 (%)	67.17±0.32	-2.83	3.20	1.90	35.49	2.96
300 Gy+0.2 (%)	66.00±0.45	-4.00	4.00	2.43	36.78	3.80
CD (p = 0.05)		1.16				
Var. Pant U-30						
Control	69.45±0.13	-	3.20	1.35	17.85	1.42
Gamma rays						
200 Gy	67.45±0.72	-2.00	5.25	3.30	39.34	5.39
300 Gy	64.80±0.35	-4.65	5.00	2.40	22.88	2.90
CD (p = 0.05)		1.64				
EMS						
0.2 (%)	67.15±0.42	-2.30	4.98	2.75	30.67	3.93
0.3 (%)	65.50±0.54	-3.95	4.75	2.93	38.16	4.76
CD (p = 0.05)		1.55				
Gamma rays+EMS						
200 Gy+0.2 (%)	67.35±0.32	-2.10	3.44	2.15	39.03	3.55
300 Gy+0.2 (%)	65.03±0.46	-4.42	3.97	2.49	39.34	4.09
CD (p = 0.05)		1.86				

PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Heritability, GA: Genetic advance, EMS: Ethyl methanesulfonate, Var. T-9: Variety T-9, Var. Pant U-30: Variety Pant U-30

can be made in M_2 generation. Moreover, the combination treatments of gamma rays and EMS were more successful in generating sufficient genetic variability and significant earliness for days to flowering and maturity which could be effectively exploited for urdbean genetic progress in succeeding generations.

DISCUSSION

Induced mutations may be resorted to develop superior genotypes by creating heritable variation in polygenic traits due to their direct and cumulative effect on genetic background[5]. Induction of micro-mutations controlling quantitative traits is important for crop improvement programs. In earlier studies on mutagenesis, it was found that traits differ in their response to mutagenic treatments[26,27]. Variance level may be less responsive in one trait and highly responsive in others[28]. Moreover, the direction of polygenic mutations depends on the genotypic background of the biological material under study[29].

In the present study, the mean flowering time decreased significantly after mutagenic treatments. For var. Pant U-30, flowering was early by more than 4 days with 300 Gy+0.2% EMS treatment in M_3 generation. Flowering depends on a number of physiological changes that take place in the meristem during its transition from vegetative to reproductive phase[9]. Reduction in flowering time accompanied by increase in genetic parameters indicated that variability has been induced in desired direction and would offer the possibility for selecting early flowering mutants in such treatment. The mutation of two dominant genes to their recessive forms could be responsible for early flowering in crop plants.

Data on days to maturity resulted in significant gain in reducing the maturity period by 4.65 days with 300 Gy dose of gamma rays in M_3 generation. Early maturity would be ideal for a crop like urdbean where drought approaches at pod filling stage in summer season. Urdbean is infested by various diseases and insect pests during rainy season which eventually reduces its yield potential. However, the problem may be conquered if the mutant plants mature before the beginning of rainy season. For that reason, its early maturity would be ideal to attain maximum production. The early maturity may be attributed to physiological, biochemical, enzymological and hormonal changes induced by the mutagens[30].

Shamsuzzaman *et al.*[31] in chickpea, Singh *et al.*[32] in lentil and Arulbalachandran *et al.*[33] in urdbean also reported a significant reduction in days to maturity after mutagenic treatments.

The quantitative traits, in the present study, showed a wide range of phenotypic variation. The magnitude of phenotypic variation, however does not reveal the relative amounts of heritable (genetic) and non-heritable (non-genetic) components of variation. This was ascertained with the help of genetic parameters, such as genotypic coefficient of variation, heritability and genetic advance. It is clearly evident from the data that considerable amount of genotypic coefficient of variation was induced by different treatments of gamma rays and EMS alone as well as in combination. The genotypic coefficient of variation for days to flowering and maturity was higher in M_2 as compared to M_3 generation suggesting that these traits have reasonable tendency to stabilize sooner in early generations.

Heritability is of interest to plant breeder as an index of transmissibility. Since the value of heritability depends upon the magnitude of all the components of variance, a change in any one of these may affect it markedly. The traits such as days to flowering and maturity exhibited low to moderate heritability. High heritability for days to flowering and other traits has been reported by earlier workers in *Vigna radiata*[7], *Lathyrus sativus*[34], *Cicer arietinum*[8] and *Vigna mungo*[35]. The disparity in results could be because the heritability is a property not only of a character but also of the population, environment and the circumstances to which the genotypes are subjected to. High heritability was recorded in M_2 as compared to M_3 generation for both the traits. The increased heritability values may be attributed to increased homozygosity of the genes involved[36,37].

Genetic advance is an indicative of expected genetic progress for a particular trait under suitable selection procedure and consequently carries much significance in self-pollinated crops like urdbean. Estimation of heritability along with genetic advance are more helpful than the heritability alone in predicting the resultant effects of selection. This is because the heritability estimates are subjected to genotype-environment interactions[38-40].

By and large, the mutagens utilized have been successful in generating significant genetic variability and earliness for days to flowering and maturity which could be effectively exploited in urdbean improvement programs. Early maturity in mutants makes them more suitable for intercropping practices with greater resistance to yellow mosaic disease and wider adaptability to different agro-climatic conditions.

CONCLUSION

Flowering and maturity period in M_3 generation was significantly reduced by more than four days after individual and combination treatments of gamma rays and EMS in the varieties T-9 and Pant U-30 of urdbean. Days to flower and maturity exhibited higher genetic variability in M_2 as compared to M_3 generation indicating that potential gain vis-à-vis early maturity could be achieved through selection in early (M_2) generation. Early maturity would be ideal for a crop like urdbean where drought usually approaches at pod filling stage in summer and spoils the crop.

SIGNIFICANCE STATEMENT

Results have revealed that combined treatments of gamma rays and EMS have proved more efficient in increasing the genetic variability for days to flowering and maturity in urdbean. The isolated mutants possessed desirable plant architecture and took lesser days to flower and mature as compared to parental lines. These mutants can be evaluated in future generations and after multi-locational trials could be released as early maturing and disease resistant varieties. Hence, the genetic variability induced by gamma rays and EMS alone as well as in combination could be effectively exploited for the improvement of urdbean crop.

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