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Short stature mutants in Urdbean (*Vigna mungo* (L.) Hepper)

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Abstract

The aim of this research was to create genetic diversity in plant height for two urdbean varieties namely T-9 and Pant U-30 by using gamma rays, ethylmethane sulphonate (EMS), and a combination of both treatments. The outcomes revealed that the average plant height values shifted towards negative direction in both M_2 and M_3 generations. Additionally, the genotypic coefficient of variation, heritability, and genetic advance showed a significant increase in the treated population. The treatments involving moderate doses of gamma rays (200 Gy and 300 Gy), EMS (0.2% and 0.3%), and combined gamma rays + EMS (200 Gy+0.2% and 300 Gy+0.2%) were found to be more effective and efficient in generating polygenic variability for plant height. The findings suggest that these treatments could be utilized to obtain urdbean mutants with desirable traits such as shorter stature, resistance to lodging, and higher yield.

Key words : Induced mutagenesis, short stature mutants, genetic variability, urdbean.

Urdbean, scientifically known as *Vigna mungo* (L.) Hepper, holds significant importance as a pulse crop in Southeast Asia and the Indian sub-continent. It is cultivated in various seasons across India, including rainy season (kharif) as a rainfed crop, in rice fallows (rabi) in Eastern and Southern India, and in the summer season (zaid) in Northern India. Urdbean is highly valued for its nutritional value, sustainability, and its ability to fix atmospheric nitrogen. Typically, it is consumed in the form of 'Dal' and serves as a key ingredient in dishes like papad, idly, and dosa. Due to its protein content, it is often referred to as the "poor man's meat".

Urdbean flourishes well in hot and humid environments, thriving best within a temperature range of 25 to 35°C. It can be cultivated in various soil types, ranging from

sandy loam to heavy clay, except for alkaline and saline soils. Loam or slightly heavy soils with a neutral pH are the most suitable for the growth of urdbean. The deep root system and foliage cover of urdbean play a crucial role in preventing soil erosion and effectively competing with the weeds. While irrigation is not necessary during rainy season, it should however be provided during the summer season, taking into account the critical stages and water availability. The frequency and amount of irrigation required depends upon the soil type and weather conditions, with the crop needing irrigation every 10-15 days. Adequate moisture is vital from the flowering to pod development stage.

In India, the total cultivated area for urdbean is 4.14 million hectares, with an annual production of 2.23 million tonnes in 2020-21. However, the average yield of 538 kg/ha (2.2-All-India-Urdbean-and-Mungbean.pdf (icar.gov.in) is relatively low compared to other crops, which poses a challenge in meeting the increasing demand. To overcome this yield deficit, there is a dire need to develop high-yielding varieties with suitable growth habit. Genetic enhancement for yield improvement, synchronization, and tolerance to biotic and abiotic stresses is crucial, considering the limited genetic diversity available in urdbean. Creating genetic variability through mutagenic agents and selecting best plants is a major focus for improving urdbean crop. Mutagenesis has been proven as an effective technique for increasing the rate of mutations, thereby expanding the genetic diversity and widening the scope of possibilities for achieving desired selections[5,7,15]. In particular, inducing micro-mutations in polygenic system that regulates quantitative traits is essential for improving crops. Considering the economic and nutritional importance of urdbean, this research aims to examine the genetic variability for plant height in M_2 and M_3 generations of urdbean varieties T-9 and Pant U-30 after subjecting them to mutagenesis using gamma rays, ethylmethane sulphonate (EMS), and a combination of both treatments, for which there is limited information available.

Two varieties of urdbean (*Vigna mungo* (L.) Hepper) namely T-9 and Pant U-30 were used in the present study. Seeds of both the varieties were obtained from G. B. Pant University of Agriculture and Technology, Pantnagar (Uttaranchal). Both T-9 and Pant U-30 varieties are well-suited to the agroclimatic conditions of Uttar Pradesh, India. Dry seeds of each variety, with a moisture content of 12%, were exposed to gamma rays at doses of 100, 200, 300, and 400 Gy. This irradiation was carried out using Cobalt-60 source at National Botanical Research Institute (NBRI) in Lucknow, Uttar Pradesh, India. The moisture content percentage was determined according to the guidelines set by International Seed Testing Association (ISTA, 1985) which is based on difference between the fresh and dry weights of the seeds.

In terms of chemical treatments, healthy seeds of uniform size from each variety were soaked in distilled water for 9 hours prior to treatment with ethylmethane sulfonate (EMS)- a monofunctional alkylating agent. The concentrations of EMS used were 0.1%, 0.2%, 0.3%, and 0.4%. These treatments were carried out for 6 hours at room temperature (25±1°C) with intermittent shaking. The EMS solution was prepared in phosphate buffer of pH-7. After treatment, the seeds were thoroughly

Table-1. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for plant height (cm) in M_2 generation of urdbean var. T-9

Treatment	Mean±S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
Control	35.24±0.31	-	3.23	1.33	16.92	1.36
Gamma rays						
100 Gy	34.16±0.32	-1.08	3.62	1.56	18.18	1.73
200 Gy	33.64±0.47	-1.60	16.78	10.20	36.91	15.93
300 Gy	31.10±0.43	-4.14	9.79	6.83	48.59	12.38
400 Gy	30.36±0.60	-4.88	10.15	6.39	39.62	10.44
LSD (p = 0.05)		0.48				
EMS						
0.1%	35.45±0.42	+0.21	6.33	4.10	41.83	6.83
0.2%	33.70±0.53	-1.54	8.87	6.35	51.23	11.93
0.3%	31.61±0.24	-3.63	8.78	6.58	55.80	12.72
0.4%	30.99±0.24	-4.25	9.45	4.38	21.58	5.23
LSD (p = 0.05)		0.74				
Gamma rays+EMS						
200 Gy+0.2%	32.56±0.46	-2.68	8.04	5.18	41.69	8.69
300 Gy+0.2%	31.02±0.42	-4.22	8.83	6.09	47.54	10.93
200 Gy+0.3%	30.15±0.15	-5.09	8.80	5.60	40.56	9.25
300 Gy+0.3%	28.16±0.16	-7.08	10.06	6.33	39.50	10.33
LSD (p = 0.05)		1.84				

PCV-Phenotypic coefficient of variation; GCV-Genotypic coefficient of variation; h^2-Heritability; GA-Genetic advance

washed in running tap water to remove any remaining mutagen from the seed surface. For combination treatments, dry seeds of each variety were first irradiated with gamma rays at doses of 200 and 300 Gy, and then treated with 0.2% and 0.3% EMS. This resulted in four combinations: 200 Gy + 0.2% EMS, 300 Gy + 0.2% EMS, 200 Gy + 0.3% EMS, and 300 Gy + 0.3% EMS. Each treatment and control group consisted of 300 seeds.

At Agriculture Farm, Aligarh Muslim University Aligarh, India, three sets of 100 seeds each were planted for both treatment and control groups in each variety. This was done in randomized complete block design (RCBD) to cultivate M_1 generation. The spacing between seeds in a row was maintained at 30 cm, while the distance between rows was 60 cm in the field. Recommended agronomic practices were followed for field preparation, sowing, and subsequent management of the urdbean population. Seeds from M_1 plants

Table-2. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for plant height (cm) in M_2 generation of urdbean var. Pant U-30

Treatment	Mean±S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
Control	35.64±0.36	-	4.01	2.22	30.39	3.17
Gamma rays						
100 Gy	34.43±0.35	-1.21	8.81	6.05	47.17	10.92
200 Gy	34.12±0.37	-1.52	7.57	5.08	44.89	8.76
300 Gy	32.76±0.47	-2.88	8.24	5.93	51.93	12.57
400 Gy	31.75±0.29	-3.89	5.28	3.29	38.29	5.29
LSD (p = 0.05)		0.51				
EMS						
0.1%	35.99±0.39	+0.35	6.32	4.12	42.52	7.00
0.2%	34.81±0.36	-0.83	6.87	5.20	57.44	10.31
0.3%	33.20±0.44	-2.44	4.61	3.18	47.01	5.69
0.4%	32.39±0.32	-3.25	14.98	9.20	37.75	14.63
LSD (p = 0.05)		1.22				
Gamma rays+EMS						
200 Gy+0.2%	33.08±0.46	-2.56	14.62	8.38	32.73	12.36
300 Gy+0.2%	31.90±0.33	-3.74	8.93	5.76	41.62	9.65
200 Gy+0.3%	30.66±0.38	-4.98	15.11	10.75	45.89	20.84
300 Gy+0.3%	28.96±0.22	-6.68	9.20	5.45	34.79	8.21
LSD (p = 0.05)		2.19				

PCV-Phenotypic coefficient of variation; GCV-Genotypic coefficient of variation; h^2-Heritability; GA-Genetic advance

were harvested separately and planted in next season in rows to produce M_2 generation. From each selected M_2 progeny, an equal number of seeds were combined and thoroughly mixed to create a bulk sample. A random sample from this bulk was planted to obtain M_3 progeny. For raising M_3 generation, selected moderate treatments of 200 Gy & 300 Gy of gamma rays, 0.2% & 0.3% of EMS and 200 Gy+0.2% EMS & 300 Gy+0.2% EMS of combination treatments were used. To determine the variance between and within families, analysis of variance (ANOVA) was performed following the method described by Singh and Chaudhary[10]. Data collected for plant height- measured in centimetres from the base to the apex of the plant at maturity in M_2 and M_3 generations were subjected to statistical analysis to evaluate the extent of induced variation. Significance of difference between means of the treated and control populations was tested using least significant difference (LSD)

Table- 3. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for plant height (cm) in M_3 generation of urdbean

Treatment	Mean±S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
Var. T-9						
Control	35.86±0.22	-	3.12	1.17	14.40	1.14
Gamma rays						
200 Gy	34.15±0.53	-1.71	6.35	3.52	30.57	5.00
300 Gy	31.93±0.42	-3.93	7.89	5.14	42.36	8.74
LSD (p=0.05)		0.97				
EMS						
0.2%	34.27±0.69	-1.59	8.32	6.16	54.80	11.85
0.3%	33.32±0.35	-2.54	7.44	4.89	43.25	8.43
LSD (p=0.05)		0.86				
Gamma rays+EMS						
200 Gy+0.2%	33.20±0.62	-2.66	7.36	4.25	33.27	6.38
300 Gy+0.2%	31.95±0.49	-3.91	8.79	4.94	31.64	7.20
LSD (p=0.05)		0.55				
Var. Pant U-30						
Control	36.01±0.31	-	3.95	2.14	29.35	2.99
Gamma rays						
200 Gy	34.42±0.62	-1.59	7.35	4.76	42.03	8.13
300 Gy	33.00±0.51	-3.01	8.21	5.42	43.60	9.33
LSD (p=0.05)		1.41				
EMS						
0.2%	35.12±0.49	-0.89	6.52	4.38	45.08	7.68
0.3%	33.90±0.35	-2.11	4.00	2.30	32.41	3.36
LSD (p=0.05)		0.78				
Gamma rays+EMS						
200 Gy+0.2%	33.48±0.22	-2.53	10.20	7.28	51.16	13.71
300 Gy+0.2%	32.21±0.54	-3.80	8.50	5.34	39.73	8.72
LSD (p=0.05)		1.30				

PCV-Phenotypic coefficient of variation; GCV-Genotypic coefficient of variation; h^2-Heritability; GA-Genetic advance

calculated from error mean square and tabulated 'T' value at 5% level of significance.

Data on plant height in M_2 generation is presented in Tables 1 and 2. The mean values shifted significantly in negative direction in most of the treatments involving gamma rays and EMS, either alone or in combination. Plant height exhibited greater reduction in combined treatments as compared to individual mutagenic treatments of gamma rays and EMS. Table-3 indicates that mean values for plant height in M_3 generation also experienced a significant decrease in both the varieties. The most pronounced reduction in mean plant height was observed in combination treatments of gamma rays+EMS in var. T-9 in M_3 generation. Previous studies have also reported a decrease in plant height following mutagenic treatments in different pulse crops[3,6,16]. However, Singh *et al*.[11] and Arulbalachandran and Mullainathan[1] documented an increase in plant height after treatments with gamma rays and EMS in urdbean. The decrease in plant height may be linked to the suppression of mitotic divisions as proposed by Subba Rao[13] in chickpea.

In M_2 generation, the phenotypic and genotypic coefficients of variation were higher in all the treatments. The highest genotypic coefficient of variation 10.20% at 200 Gy of gamma rays and 10.75% at 200 Gy+0.3% EMS was observed in the varieties T-9 and Pant U-30, respectively. Heritability increased considerably in all the mutagenic treatments in both the varieties. In gamma ray treated population, the highest values of heritability were 48.59% in the var. T-9 and 51.93% in the var. Pant U-30. The highest heritability in the material treated with EMS was 55.80% and 57.44% in the varieties T-9 and Pant U-30, respectively. In the combined treatments of gamma rays + EMS, it was 47.54% in the var. T-9 and 45.89 in the var. Pant U-30. Genetic advance also increased considerably in the treated population. The highest values of genetic advance were recorded with 200 Gy of gamma rays in the var. T-9 and 0.4% EMS and 200 Gy+0.3% EMS in the var. Pant U-30. In M_3 generation, genetic parameters also increased in all the mutagenic treatments. The maximum genotypic coefficient of variation (6.16%), heritability (54.80%) and genetic advance (11.85%) was noticed with 0.2% EMS treatment in the var. T-9. In the var. Pant U-30, the values of such parameters were highest in combination treatment of 200 Gy+0.2% EMS.

There exists a notable discrepancy among researchers regarding the appropriate generations for selecting quantitative traits. Gaul[2] and Tar'an *et al*.,[14] contend that selection for these traits should be delayed until M_3 or subsequent generations following mutagenic treatments. Conversely, several other researchers, including Kharkwal[4], Singh *et al*.,[9], Solanki and Sharma[12], Sheeba *et al*.,[8], and Arulbalachandran and Mullainathan[1], propose that effective selection for polygenic traits can be conducted in early generations, even as early as M_2. The present investigation revealed that selecting progenies based on superior mean in M_2 was exceedingly advantageous. The data unequivocally demonstrates that a substantial amount of variability was induced in the mutagen-treated population in both M_2 and M_3 generations. The variability for plant height was lower in M_3 compared to M_2 generation, suggesting that the trait could

stabilize sooner. Consequently, it is recommended that in urdbean, selection for plant height can be carried out in the early generation.

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