Characterization of Some Macromutations Induced by Single and Combination Treatments of Gamma Rays, EMS and SA in Urdbean (*Vigna mungo* L. Hepper)

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Two improved cultivars of urdbean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 were treated with single and combination doses/concentration of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA). A number of various types of morphological macromutations were induced in M_2 generation. Out of these, 14 mutants from PDU1 and 13 from T9 were identified as true breeding for plant morphology, pod and seed characters and early maturity in M_3 generation. Many macromutants showed significant improvement in yield and other yield components as compared to their parents.

Key Words: Ethyl Methane Sulphonate (EMS), Gamma Rays, Macromutations, Sodium Azide, Urdbean (*Vigna mungo*)

Urdbean (*Vigna mungo* L. Hepper) is an important pulse crop of India grown on an area of about 3.15 million hectares with a production of 1.49 million tonnes. But the productivity is only 473 kg/ha in the country. The productivity of this crop is very low, particularly when compared with pigeonpea and chickpea. Since urdbean is a highly self-pollinated crop, the natural variability available is far less to make selections for its improvement. Induced mutagenesis has immense potential in creating genetic variability among naturally exhausted population which is utilized in selection programme for obtaining desirable improvement. The present study was conducted to induce and isolate useful macromutants which could be utilized for genetic improvement of this crop.

Material and Methods

Two improved cultivars of urdbean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 were used as experimental materials. Three hundred pure, healthy and dry seeds of each cultivar were use for each treatment. The seeds were irradiated with gamma rays at 15, 30, 45 and 60 kR doses from Gamma Cell-200 having a 2000 Curie ^{60}Co source available at the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi, in 1996. For chemical mutagenic treatments, the seeds were pre-soaked in distilled water for 6 h and then treated with ethyl methane sulphonate (0.02, 0.04, 0.06 and 0.08 M concentrations) in freshly prepared phosphate buffer (pH 7.0) or sodium azide (2, 4, 6, and 8 mM concentrations) in phosphate buffer (pH 3.0). For combination treatment, the gamma irradiated seeds at 15-60 kR doses were pre-soaked in distilled water for 6 h and then treated with 0.02 M EMS or 4 mM SA in the manner described earlier. The mutagen treated seeds were thoroughly washed in running water for 1 h to avoid traces of the chemicals, if any.

The mutagen treated seeds along with the untreated control seeds were sown in five rows each measuring 5 m with 30 × 10 cm spacing to raise M_1 generation. The surviving plants were harvested separately and individual plant progenies were raised in M_2 generation. A number of macromutations were induced in various treatments in M_2 generation. The macromutants having sufficient seeds were grown in M_3 generation in Randomized Block Design (RBD) with three replications and they were characterized and also confirmed for their breeding behaviour. The observations were recorded on 10 competitive plants from each replication for various yield and yield components in M_3 generation.

Results and Discussion

Several types of macromutations were induced by different mutagenic treatments in M_2 generation with respect to growth habit, leaf structure, days of mature and days to flower, pod and seed characteristics in both the cultivars, namely, PDU1 and T9. A dose dependent increase in the frequency and spectrum of macromutations was recorded in M_2 generation. A total of 31 and 34 types of macromutants were induced in cvs. PDU1 and T9, respectively. A wide spectrum of viable mutations can be expected in a mutation experiment. The probable causes of these macromutations may be chromosomal changes and most probably point or gene mutations. Most of these macromutations are reported to be

polygenic in nature (Yadav and Singh, 1988; Saini and Mahana, 1989; Thakur and Sethi, 1993).

Fourteen and 13 true breeding macromutants were derived from cultivars PDU1 and T9, respectively, for plant morphology, pod and seed characters and early maturity, were isolated in M_3 generation. These macromutants showed distinct morphological features as compared to their parents (Table 1 and 2). Eleven macromutants derived each from both the cultivars showed significantly higher seed yield than their parents. In case of the remaining macromutants, the performance was either equal or even less than the parents. The characteristic features of these macromutants are described below:

Growth Habit Mutants

The *dwarf, tall, bushy, spreading, tendriller* and *long peduncled* mutants were isolated in M_3 generation, The

Table 1. Mean values of yield and yield attributing traits of macromutants in cv. PDU1 in M_3 generation

S. No.	Macromutant	Mutagen treatment	Plant height (cm)	Days to maturity	Number of branches/ plant	Number of pods/ plant	Pod length (cm)	Number of seeds/ pod	Seed yield/ plant (g)	100-seed weight (g)
1.	Tall	30 kR + 4mM SA	52.50**	79.20	5.36**	36.40**	4.25	5.85	7.50**	4.50
2.	Bushy	45 kR	42.82	81.50	6.90**	34.86**	4.36	5.60	7.30**	4.25
3.	Spreading	30 kR + 4mM SA	40.24	85.50**	7.58**	27.60**	4.35	5.85	7.10**	4.40
4.	Dwarf	30 kR + 4 mM SA	31.10**	75.60**	3.15*	20.15**	4.10	5.58	6.15	4.25
5.	Broad-leaved	0.08 M EMS	38.63*	85.25**	4.50	20.58**	3.90**	5.15**	5.90	4.05
6.	Anthocyanin pigmented	30 kR	44.52	81.36	4.16	22.60	4.05**	5.35**	5.75*	4.20
7.	Hairy	45 kR + 0.02 M EMS	38.36**	82.37	4.52	18.22**	4.40	5.86	5.35**	3.90**
8.	Bold-podded	0.02 M EMS	39.52	82.50	3.92	32.86**	5.05**	6.80**	7.32**	4.82**
9.	Short-podded	0.08 M EMS	44.50	81.85	4.35	35.68**	3.25**	4.90**	6.10	3.85**
10.	Bold-seeded	8 mM SA	45.50**	80.68	4.55	32.87**	4.75**	6.25**	7.25**	5.45**
11.	Small-seeded	45 kR	42.56	82.86	4.55	30.50**	3.90**	4.80**	4.85**	3.05**
12.	Round-seeded	15 kR + 0.02 M EMS	41.96	84.85**	3.90	30.80**	3.95**	4.95**	5.05**	3.50**
13.	Brown-seeded	4 mM SA	46.80**	82.90	4.35	32.80**	4.35	5.70	6.90**	4.50
14.	Early maturing	15 kR + 0.02 M EMS	35.50**	65.00**	4.05	20.15**	4.15	5.56	5.15**	4.15
		Control	42.00	80.50	4.25	25.50	4.30	5.75	6.25	4.30
		SE ±	1.29	1.30	0.296	1.64	0.105	0.136	0.237	0.147

* Significant at 5% level
** Significant at 1% level

Table 2. Mean values of yield and yield attributing traits of macromutants in cv. T9 in M_3 generation

S. No.	Macromutant	Mutagen treatment	Plant height (cm)	Days to maturity	Number of branches/ plant	Number of pods/ plant	Pod length (cm)	Number of seeds/ pod	Seed yield/ plant (g)	100-seed weight (g)
1.	Tall and erect	15 kR + 4 mM SA	44.28**	80.55**	5.15**	28.62**	4.29	5.75	6.75**	4.35
2.	Tendriller	60 kR	30.65**	82.86	2.05**	15.70**	4.05	4.95**	4.58**	4.50**
3.	Bushy	0.08 M EMS	37.85*	83.07	5.90**	32.86**	4.25	5.80	6.50**	4.15
4.	Narrow leaved	4 mM SA	37.26**	82.80	4.25	18.75**	4.26	5.26	4.75**	4.08
5.	Dwarf	45 kR + 4 mM SA	25.60**	78.65**	2.75**	19.80	4.10	5.16**	5.16	4.36
6.	Early maturing	60 kR	32.82*	70.56**	3.65	20.85	4.25	5.75	4.80**	4.46**
7.	Anthocyanin pigmented	15 kR + 0.02 M EMS	38.75**	81.27*	4.05	16.87**	4.60**	5.95**	4.75**	4.35
8.	Hairy	30 kR	32.46**	85.26	3.70	18.26**	4.25	5.65	4.62**	4.26
9.	Bold and long podded	15 kR + 0.02 M EMS	40.81**	84.67	4.50*	30.65**	5.25**	6.95**	6.90**	4.55**
10.	Long-peduncled	30 kR + 4 mM SA	38.52**	86.80**	4.95**	20.26	4.58**	6.05**	5.10	4.10
11.	Bold-seeded	0.08 mM SA	36.75	80.37**	4.25	27.55**	4.75**	6.50**	6.60**	5.35**
12.	Chocolate-seeded	60 kR + 4mM SA	30.56**	86.82**	4.50*	25.86*	4.25	5.62	6.05*	4.35
13.	Short-podded	0.08 M EMS	38.20*	82.80	4.15	30.86**	3.05**	4.30**	4.25**	3.90
		Control	35.25	83.50	3.90	22.50	4.15	5.50	5.50	4.20
		SE ±	1.31	1.09	0.255	1.53	0.127	0.127	0.246	0.090

* Significant at 5% level
** Significant at 1% level

dwarf mutants were characterized by condensed nodes, shorter internodes and lower yield as compared to the control. The plants exceeding the height of the respective controls by at least 10 cm were placed in tall category. The *bushy* mutants were also induced in both the cultivars which were characterized by shorter internodes, compact stature and with higher number of pods. The *spreading* mutant exhibited branching paralled to the ground and covered more area than the normal plants. In the *tendriller* mutant, the leaflets were modified into tendrils. The *long peduncled* mutant had the characteristic of increased peduncle length joining the pods. Similar mutants have been reported by Pande and Raghuvanshi (1988) and Singh and Yadav (1991) in greengram and Thakur and Sethi (1993) and Gautam and Mittal (1998) in blackgram.

Leaf Mutants

The two true breeding leaf mutants, namely, *narrow-leaved* from T9 and *broad-leaved* from PDU1 were isolated in M_3 generation. The *broad-leaved* mutant exhibited increased leaf area and with significantly reduced plant height and number of pods/plants. The *narrow-leaved* mutant showed reduced leaf area with decreased seed yield as compared to the parents. Many types of leaf mutants have been reported in urdbean following mutagenesis (Singh and Raghuvanshi, 1985; Thakur and Sethi, 1993; Gautam and Mittal, 1998).

Early Maturing Mutants

The *early* maturing mutants were isolated from both the cultivars in M_3 generation. These mutants matured 10 to 15 days earlier than the normal period of the control and yielded lower than the respective controls. The *early* maturing mutants have been reported in urd (Sinha, 1988; Gautam and Mittal, 1998) and mung (Singh and Yadav, 1991).

Hairy and Anthocyanin Pigmented Mutants

These included the *hairy* and *anthocyanin* pigmented mutants. The *hairy* mutants had the characteristic of hairy growth all over the above ground parts, particularly on stem and pods. These mutants showed significantly reduced number of pods/plant and low seed yield than the control. This kind of mutant has been reported by Gautam and Mittal (1998) in urdbean. The presence of anthocyanin pigment, particularly on stem and branches of the plant was the characteristic feature of *anthocyanin*-pigmented mutant. This mutant also yielded significantly lower seed yield than its parent. Such mutants have been reported by Tickoo (1987) and Singh and Yadav (1991) in mungbean.

Pod Mutants

The *short-*, *bold-* and *long*-podded mutants were induced by different mutagenic treatments in both the cultivars in M_3 generation. The *short-podded* mutants had relatively less number of seeds per pod with lower yield than the control. Such mutants have been reported by Thakur and Sethi (1993) and Gautam and Mittal (1998) in urdbean. The *bold-* and *long-podded* mutants had significantly increased pod length, greater number of seeds/pod and more seed yield over their parents. Gautam and Mittal (1998) observed such mutants in urdbean.

Seed Mutants

The *brown* and *chocolate* testa colour mutants were isolated in M_3 generation as compared to the black testa colour of the parent varieties. Both the mutants exhibited a significant increase in the seed yield over the respective parents. The *bold*-seeded mutants were induced in both the cultivars which showed significantly increased pod length, number of seeds/pod, 100-seed weight and seed yield as compared to the controls. The *small-* and *round*-seeded mutants were also induced which showed significantly lower yield than the parents. The seed mutants for varied testa colours, shapes and sizes have been reported by Ali Khan and Veerswamy (1974) in redgram and Singh *et al.*, (1982) in greengram. These coloured testa mutants are likely to involve single genes.

Increasing mutation frequency is a very important aspect for improvement of mutation breeding programme. Gamma rays, EMS and SA have been demonstrated in induce mutations by different mechanisms. The combination of these mutagens in appropriate sequences has been shown to cause a synergistic increase in mutation frequency as observed during the present study as well as in the past (Cheng, 1987; Cheng and Gao, 1988). The combined treatments induced a higher mutation frequency, resulting in a higher mutation efficiency and an expansion of the spectrum of morphological mutations, thus, offering more opportunities for selection in mutation breeding practice.

Many mutants such as *tall, bushy, early* maturing, *long-* and *bold-podded, bold-seeded, hairy,* etc. induced during the present study show the desirable characteristics from the breeders' point of view and hold promise for isolation of improved types from their progenies in the later generations.

Acknowledgement

Senior Author (MS) is grateful to the University Grants Commission, New Delhi, for the award of Junior Research Fellowship during the present study.

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