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INDUCED VIABLE MUTANTS IN URDBEAN (*VIGNA MUNGO* (L.) HEPPER)

K. S. USHARANI* AND C. R. ANANDA KUMAR
Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University,
Coimbatore - 641 003, Tamil Nadu, INDIA
e-mail: usharaniagri@gmail.com

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***Corresponding author**

ABSTRACT

The improved cultivar of urdbean namely VBN 4 were treated with single and combination dose/concentration of gamma rays (40kR, 50kR and 60kR) and ethyl methane sulphonate (EMS-50mM, 60mM and 70mM). The viable mutation frequency decreases with increasing doses of gamma rays. The highest frequency of mutants was recorded in 60mM (5.91 per cent) of EMS treatment followed by 60kR+60mM (5.88 per cent) of combination treatment. About 146 mutants were identified. High seed yielding superior plants as a result of increased number of pods ranging from 40-43 were observed in 40kR and 50kR of gamma ray and in 40kR+50mM, 40kR+70mM and 50kR+50mM of combination treatments when compared to the control (20-22 pods). Plants of bushy stature with condensed internodes when compared to control were found among the 50kR and 50mM of gamma ray and EMS population respectively. Dwarf types (10cm-16cm) were also identified among the 40kR, 60kR, 60mM, 70mM and 50kR+50mM, 50kR+60mM and 60kR+70mM population suited for lodging resistant. The present study reveals good scope for isolation of suitable morphological traits mutants induced may be useful in breeding programmes for the improvement of urdbean with agronomically desirable character which force is utilized in future breeding programme.

INTRODUCTION

Blackgram (*Vigna mungo* (L.) Hepper) is a small annual plant and is commonly known in India as urdbean. It is a rich source of protein (20.8 to 30.5%); its total carbohydrates range from 56.5 to 63.7%. It is also a good source of phosphoric acid and calcium. It contains a wide variety of nutrients and is popular for its fermenting action and thus it is largely used in making fermented foods. It is an important pulse crop occupying a unique position in Indian agriculture. Crop improvement of blackgram through hybridization and recombination is very difficult, because of their autogamous nature (Ramya and Nallathambi, 2014). Due to autogamous nature, they lack genetic variability also. For any breeding programme, the genetic variability is important for further crop improvement. Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement and an efficient means supplementing existing germplasm for cultivar improvement in breeding programmes. Shah *et al.* (2008) reported that mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants.

Induced mutagenesis has been successfully used to generate wider variability, particularly for isolating mutants with desirable characters of economic importance (Kharkwal *et al.*, 2004). Although studies on induced mutagenesis in urdbean have been undertaken in the past (Singh and Raghuvanshi, 1987; Gautam *et al.*, 1992; Sharma *et al.*, 2005; Hemavathy and Raveendran, 2005; Kouser *et al.*, 2007; Anbu Selvam *et al.*, 2010 and Bhosale *et al.*, 2013), limited reports are available on induced viable morphological mutations particularly plant habit mutations in urdbean (Arvind Kumar *et al.*, 2007; Senapati *et al.*, 2008; Hemavathy and Balaji, 2008; Gahlot *et al.*, 2010; Bhosale and Hallale, 2011; Wani, 2011 and Ramya *et al.*, 2014). Gustaffson *et al.* (1971) developed a high yielding barley variety with early maturity, high protein contents and stiff straw by mutation breeding techniques. Keeping in view the limited genetic variability available in the germplasm, present investigation was undertaken to understand the nature of induced variability and response of urdbean to different doses of gamma rays and various treatment conditions of EMS. The present investigation was conducted with the objective of to induce and isolate useful plant habit mutations which could be utilized for genetic improvement of this crop.

MATERIALS AND METHODS

Seeds of VBN 4 blackgram were subjected to three different doses of gamma rays *viz.*, 40kR, 50kR and 60kR, EMS 50mM, 60Mm and 70mM and combination treatments *viz.*, 40kR+50mM, 40kR+60mM, 40kR+70mM, 50kR+50mM, 50kR+60mM, 50kR+70mM, 60kR+50mM, 60kR+60mM, 60kR+70mM. To raise M_1 generation, a total of fifteen treatments along with the control were sown in the field at the rate of 150 seeds for each treatment at a spacing of 30 x 15cm at Agricultural College and Research Institute, Madurai in Randomized block design (RBD) with three replications. The M_2 generation was raised from individual M_1 plant (*i.e.*) M_1 plant basis following plant to progeny method. Thirty plants per treatment were forwarded from the M_1 to the M_2 generation. The seeds were sown with adequate spacing. All the recommended cultural measures namely, irrigation, weeding and plant protection methods were carried out during the growth period of the crop. The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M_2 plants particularly from flowering to maturity period. These mutants were classified for deviation from the normal and taking into consideration the most conspicuous characters namely, stature, duration, leaf shape, pod size etc. The frequency and spectrum of viable mutants were calculated on M_2 seedling basis. All the important characters of each mutant were recorded.

RESULTS AND DISCUSSION

Any mutational event may bring large or small change in the phenotype. Such changes in macro mutants have highest significance in plant breeding because they may sometimes give a desired phenotype. A number of new commercial varieties have been originated from induced macro mutants and they proved their usefulness in attaining distinct breeding objectives. It is also possible to induce new features, which do not exist in the available range of variability in a well adapted and high yielding variety. An exact method of estimating induced mutation frequencies must compensate for the bias introduced by factors such as diplontic selection, small progeny size and increased size of mutated sector at higher doses (Nilan *et al.*, 1964). Gaul (1960) concluded that the mutants per 100 M_2 plants will be the best estimate of initial mutation frequency, since it may not distort the functional relationship between dose and mutational response. In the present study, observations on viable mutations were recorded from early seedling stage to maturity. The frequency of viable mutants ranged from 4.14 per cent in 60kR to 5.22 per cent in 40kR for gamma rays treatment. The viable mutation frequency decreases with increasing doses of gamma rays. In EMS treatment the frequency ranged from 2.40 per cent in 50mM to 5.91 per cent in 60mM. A gradual increase in mutation frequency was associated with obtained with the increased in combination treatments, being highest at 60kR+60mM (5.88 per cent) of combination treatment (Table 1 and Fig. 1). The results are akin to the findings of Malarkodi (2008) and Makeen *et al.* (2013) in urdbean.

Table 1: Frequency of viable mutants in induced by gamma rays, EMS and their combination treatments

Treatment	Number of M_2 seedlings		Mutation frequency
	Examined	Showing viable mutants	
Gamma rays (kR)			
Control	342	-	-
40kR	287	15	5.22
50kR	249	11	4.41
60kR	193	8	4.14
EMS (mM)			
Control	328	-	-
50mM	249	6	2.40
60mM	203	12	5.91
70mM	182	8	4.39
Gamma rays + EMS			
Control	328	-	-
40kR+50mM	302	12	3.97
40kR+60mM	254	4	1.57
40kR+70mM	223	11	4.93
50kR+50mM	312	14	4.48
50kR+60mM	276	7	2.53
50kR+70mM	243	4	1.64
60kR+50mM	300	10	3.33
60kR+60mM	272	16	5.88
60kR+70mM	241	8	3.31

In the present investigation viable macro mutations with changes in attributes like stature, duration, leaf, pod and seed mutants were recorded in all the treatments. The highest number of viable mutants was recorded in combination treatments. Similar results were obtained by Malarkodi (2008) in urdbean and Wani (2011) in chickpea. In contrast to this, the highest number of viable mutants was recorded in gamma irradiated population as reported by Palmer (2000) in soybean and Vinita Sharma and Kumar (2003) in bengal gram. In VBN 4, a total of 34, 26 and 86 macro mutants were isolated from gamma ray, EMS and combination treatments respectively (Table 2).

Tall mutants

These mutants were characterized by the long internodes and erect in habit. The height of the mutants was considerably more than the control plants. The mean height of the mutant was 66cm whereas in the control it was 42cm. Tall mutants were observed in 50kR of gamma ray, 50mM of EMS and 40kR+50mM, 50kR+50mM, 50kR+70mM, 60kR+50mM and 60kR+70mM of combination treatments (Fig. 2). Same mutants have been reported by Ahirwar *et al.* (2014) in lentil. The number of primary branches, number of clusters and number of pods was increased.

Dwarf mutants

Reduced plant height is an important trait in plant breeding, mainly because short genotypes are most resistant to lodging

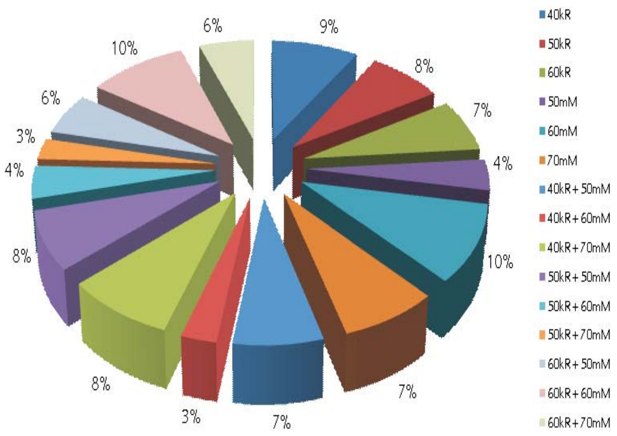


Figure 1: Viable mutation frequency

Table 2: Spectrum of viable mutants in induced by gamma rays, EMS and their combination treatments

Type of mutants	Gamma rays (kR)			EMS (mM)			Combination (gamma rays + EMS)								
	40	50	60	50	60	70	40+50	40+60	40+70	50+50	50+60	50+70	60+50	60+60	60+70
Stature															
Tall and erect	-	1	-	3	-	-	1	-	-	2	-	1	3	-	1
Dwarf	2	-	3	-	2	4	-	-	-	3	1	-	-	-	1
Spreading	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-
Semi spreading	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Compact and bushy	-	1	-	1	-	-	2	-	-	-	1	-	1	-	1
Open type	2	-	-	-	1	-	-	-	-	-	-	-	-	4	-
Twinning	-	-	-	-	-	-	1	2	-	-	-	2	-	-	-
Leaf mutants															
Crinkled & leathery	-	-	-	1	-	4	-	-	-	-	-	-	-	-	-
Small leaf	3	-	-	-	2	-	-	2	-	-	1	-	-	4	1
Big & broad leaf	-	2	2	-	-	-	3	-	4	5	-	-	3	-	-
Narrow leaf	-	1	-	-	1	-	-	-	3	2	1	-	-	2	-
Duration															
Early	-	1	-	-	-	-	-	-	-	-	2	-	-	-	1
Late	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Pod mutants															
Smooth pods	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
More no of pods	2	1	-	-	-	-	4	-	3	2	-	-	-	-	1
Short pods	-	-	-	-	4	-	-	-	-	-	-	-	2	5	-
Hairy pods	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Seed mutants															
Bold seeds	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Small seeds	-	2	-	-	-	-	1	-	-	-	-	-	1	-	-
Brown seeds	4	-	-	-	-	-	-	-	-	-	1	-	-	-	2
Total	15	11	8	6	12	8	12	4	11	14	7	4	10	16	8

Figure 2: Tall mutant (50mM- 60 cm)

Figure 3: Dwarf mutant (60kR- 15 cm)

than standard types (Austin *et al.,* 1980). The existence of dwarf genotypes is common in many plant species and they have been used in several crops for more efficient crop management and increased yield (Abel, 1976). The mutants with mean height of the plant of more than 10cm but less than 16cm were considered as dwarfs in this cultivar. The mean height of the control was 42cm whereas in the dwarf mutant it was 15.2cm. This mutant were recorded in 40kR and 60kR in gamma treatment, 60mM and 70mM of EMS and50kR+50mM, 50kR+60mM and 60kR+70mM of combination treatments (Fig. 3). Similar dwarf mutants have been reported by Singh *et al.* (2005), Singh and Tejeswar Rao (2007), Singh (2007) and Mishra *et al.* (2013) in greengram.

Spreading and semi spreading mutants

Spreading type mutants had creeping habit with longer internodes. The leaf was medium sized and ovate. They appeared only in 50kR+70mM and 60kR+60mM combination treatments. Semi spreading type mutants were observed only at 40kR+70mM of combination treatments. They had less number of pods than control and the internodes were shorter than spreading type. Spreading type mutants have been reported by Ramya *et al.* (2014) in urdbean and Singh *et al.* (2000) in mungbean by the treatment of gamma rays, EMS and ECH (Epichlorhydrin).

Compact and open type mutants

Compact type mutants were obtained at 50kR and in 50mM of gamma ray and EMS treatments respectively. In combination treatments, the mutants found to be appear in 40kR+50mM, 50kR+60mM, 60kR+50mM and 60kR+70mM. They had bushy habit with more number of leaves (Fig. 4). They had on condensed internodes and more number of branches than control. Open type mutants had less number of primary branches than control. The branching angle of such type of mutants was 80º. This mutant was observed at 40kR of gamma ray, 60mM of EMS and in 60kR+60mM of combination treatment.

Twinning mutants

In these mutants, the stem was twinning upto 70cm. They recorded significantly higher values for number of flowers, pod and grain yield as compared to control. These mutants were observed at 40kR+50mM, 40kR+60mM and 50kR+70mM of combination treatment (Fig. 5). Similar type of mutants has been reported by Malarkodi (2008) in urdbean.

Leaf mutants

Leaf mutants observed in M_2 generation include crinkled and

Figure 4: Bushy mutant (60kR+50mM)

Figure 5: Twinning mutant (40kR+50mM)

a. Leaf shape and color variation in M_2 generation

b. Narrow leaf mutant (50kR+60mM)

c. Leaf without (control) and with (60kR+70mM) pigmentation

Figure 6: Leaf mutants

leathery leaf, small, big and narrow leaf mutant (Fig. 6). Occurrence of big and broad leaf mutant was more followed by small leaf mutants. Plants with increased leaf surface may have high biomass yield advantage over the control with medium leaf surface. It implies high leaf area index which may result in higher photosynthetic rate. Colour of the leaf also varied from light green to dark green. Mutants with dark green leaves will be more efficient in photosynthetic activities due to increase in the chlorophyll pigments. Same type of leaf mutants have been reported by Singh (2007) in greengram, Arvind Kumar *et al.* (2007) and Bhosale and Hallale (2011) in blackgram.

The parent had no pigmentation in the leaves. However, in the present investigation, anthocyanin pigmented mutants were recorded in 60kR + 70mM of combination treatment. In these mutants petiole and leaf veins were reddish purple in colour due to the presence of anthocyanin pigment. The presence of anthocyanin pigment may confer selection advantage on such plant as it may likely exhibit better level of insect tolerance. Similar leaf mutants have been reported by Singh and Singh (2002) and Gahlot *et al.* (2010) in urdbean. The variegated leaf and narrow-leaf mutants produced only few pods.

Early and late mutants

Early mutants mature at five days earlier than the control and late mutant types matured 18 to 22 days later than the control. Early mutants were observed at 50kR of gamma ray treatment and in 50kR + 60mM and 60kR + 70mM of combination treatments and late mutants were observed at 40kR of gamma ray and 50mM of EMS treatment. The results are akin to the findings of Karthik (2008) and Ramya *et al.* (2014) in urdbean and Mishra *et al.* (2013) in greengram.

Pod mutants

Hairy pod mutant was characterized by the presence of hairs on the pod surface (Fig. 7). The seeds were medium sized and normal and these mutants are highly resistance to pod borer attack. This hairy pod mutant was found only at 50kR of gamma ray treatment. The pod of the control plants is having smooth pods. The number of pods of the mutants was considerably more than the control plants (Nair and Mehta, 2014). The number of pods of the mutant was 40-43 pods whereas in the control it was 20-22 pods. This mutant was observed at 40kR and 50kR of gamma ray and in 40kR + 50mM, 40kR + 70mM and 50kR + 50mM of combination treatments. Short pod mutants had relatively shorter pods of length 1.5 cm to 2 cm with one or seeds per pod. Short pod mutants were found to appear at 70mM of EMS and in 60kR + 50mM and 60kR + 60mM of combination treatments.

(a) Hairy pods (50kR)

(b) Pod length variation in M_2 generation

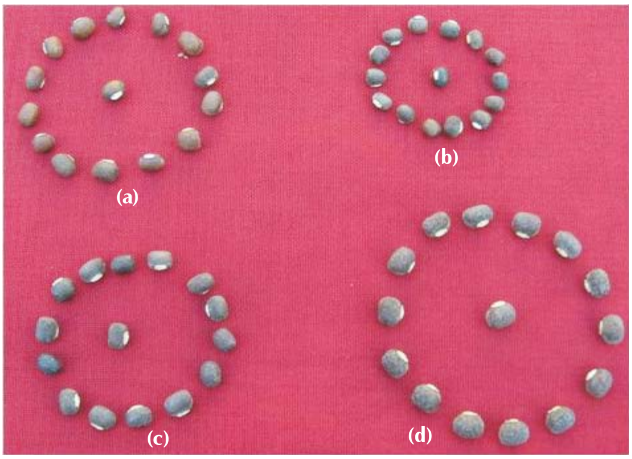
(c) Pods per cluster variation in M_2 generation

Figure 7: Figure showing (a) Hairy pods (50kR), (b) Pod length variation in M_2 generation and (c) Pods per cluster variation in M_2 generation

Short pod mutants have been reported by Laskar and Khan (2014) in Broad bean and Deepalakshmi and Ananda Kumar (2004) in urdbean and hairy pod mutants have been reported by Karthik (2008), Senapati *et al.* (2008), Bhosale and Hallale (2011) and Ramya *et al.* (2014) in blackgram.

Seed mutants

Bold seed mutant had larger pods with thick, bold, bigger seed than normal seeds, and they had normal black seed coat. Small seeded mutant had small sized seeds than the normal seeds. The pods were narrow and possess six to seven seeds per pod. Brown seeded mutants exhibited a characteristic brown seed coat. Bold seeded mutants were observed only at 60kR of gamma rays treatment. Small seed mutants were found to appear at 50kR of gamma ray and in 40kR+50mM and 60kR+50mM of combination treatments. Brown colour seeded mutants were found to be appear at 40kR of gamma ray and in 50kR+60mM and 60kR+70mM of combination treatments (Fig. 8). In blackgram Singh (1996), Malarkodi (2008) and Ramya et al. (2014) were successful in isolating bold seeded mutants. Kadapa (1985) isolated bold seed mutants in greengram. Brown seed mutants were reported earlier by Anbu Selvam *et al.* (2010) and Ramya *et al.* (2014) in blackgram. Similar type of seed coat colour mutant has been reported by Singh *et al.* (2007) in lentil.



Seed size and seed colour variation

(a) Brown seed (b) Small seed (c) Large seed (d) Control seed

Figure 8: Seed mutants

In general, macro mutants play an important role in plant breeding as it may lead to the evolution of new genotypes. Significant morphological variability was created among mutants of urdbean using recombination treatment. The possible cause of these macro mutations may be chromosomal aberrations, small defiencies or duplications and most probably gene mutations. Several workers have reported that these viable mutations were monogenic and recessive in nature controlled by one or more recessive genes. The more frequent induction of positive mutation types by exacting mutagen may be accredited to the information that the genes controlling these traits may be more responsive to either alkylation agents or ionizing radiations. In conclusion, the viable mutants isolated in the present study included mutants with agronomically desirable features which could possibly be utilized in future breeding programmes.

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