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Research paper

Frequency and spectrum of viable mutations in mungbean (*Vigna radiata* (L.) Wilczek)

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ABSTRACT

The mutagenic effect of gamma rays (200Gy, 300Gy, 400Gy, 500Gy, and 600 Gy), ethyl methane sulphonate (0.2%, 0.3%, 0.4%, 0.5%, and 0.6%) and sodium azide (1 mM, 2 mM and 3 mM) on frequency and spectrum of viable mutations in two mungbean varieties *viz*., WGG-42 and LGG-460 were investigated. A wide spectrum of viable mutations for stature, leaf, flowering/maturity duration, cotyledonary leaf, pod, seed size, seed color, and colour stem were identified in the M_2 generation. The spectrum of viable mutants includeda total of 94 mutants in gamma gamma-irradiated population, 132 mutants in EMS and 45 mutants in SA treatments in WGG-42. In LGG-460, a total of 91 mutants in gamma gamma-irradiated population, 118 mutants in EMS treated population, and 45 mutants in SA treatments were observed. Viable mutation frequency increased with an increase in dose/concentration of gamma rays, EMS, and SA treatments in both genotypes. In both the genotypes, EMS showed the highest frequency of viable mutations than gamma rays and SA treatments. These viable mutants could be exploited as a variety or donor for bringing desirable traits into the otherwise well-adapted cultivars.

Key words: EMS, Gamma rays, M_2, Mungbean, SA, Viable mutants

INTRODUCTION

Mungbean(*Vigna radiata* (L.) Wilczek) is an important pulse crop grown in India. It is a short-day, warm-season crop, grown mainly in arid and semi-arid regions. It can be grown invarious croprotation practices, because ofits short-durationnature,wideradaptability, low water requirement, and photo insensitivity (Anil *et al*., 2022). It is a protein-rich stable food containing about 24-28% protein, with high lysine (504 mg/g) content and rich in amino acids, minerals and vitamins, thus meeting the dietary needs of the vegetarian population of the country (Akashi *et al*., 2020). Domestic consumption of mung bean has increased in the recent past because of the rising popularity of Indian ethnic foods and perceived health benefits (Datta *et al*., 2012). Hence, there is an immediate need to improve cultivars with high productivity to meet the current and future demands.

The success of the plant breeding program of any crop largely depends on the wide range of variability present in that crop, but natural variability in this crop is low due to limited natural outcrossing (Wani and Kozgar, 2016). In such cases, the creation of new variability through induced mutagenesis could be an effective means of crop improvement. Mutation techniques are the best methods to enlarge the genetically conditioned variability of a species within a short time. The use of induced mutations using physical and chemical mutagens in crop improvement has been widely accepted by plant breeders as an additional method in plant breeding (Das *et al*., 2021). The frequency of viable mutations serves as an index of the mutagenic sensitivity of various mutagenic agents and their dose effects (Vairam and Ibrahim, 2014). Macro mutations can be used as a variety or donor for bringing desirable traits into the otherwise well-adapted cultivars. Several new commercial varieties have developed 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. Therefore, the present investigation has been undertaken to assess the frequency and spectrum of viable mutations in mungbean to further utilize them in breeding programs aimed at the improvement of mungbean to meet the current and future demands.

MATERIALS AND METHODS

Uniform, healthy and dry seeds of two mungbean varieties *viz.*, WGG-42 and LGG-460 with moisture content of 12%, were irradiated with 200Gy, 300Gy, 400Gy, 500Gy, and 600 Gy of gamma rays at BARC (Trombay, Mumbai). A total of 750 seeds per treatment of both varieties were taken for treatment. For the chemical mutagen, healthy seeds of uniform size of each variety were pre-soaked for 6 h in distilled water and treated with 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% of EMS and 1 mM, 2 mM, and 3 mM of SA for 6 h with intermittent shaking at room temperature of 23 ± 1°C. On the next morning, treated seeds were sown along with control seeds of both the varieties in Randomized Block Design (RBD) at three replications with the spacing of 30 × 10 cm during *kharif*, 2017 for raising the M_1 generation. The recommended agronomic practices and plant protection measures were followed uniformly for all the treatments. The M_2 generation was raised from normal-looking M_1 plants following plant to progeny row method during *rabi*, 2017-18. A total of 376 progenies belonging to the variety WGG-42 and 379 progenies belonging to the variety LGG-460 were grown in Compact Family Block Design (CFBD) with two replications. Each treatment was taken as a family and within treatment, the individual plant progeny was taken as progenies. All the progenies within the family were randomized. Similarly, the family blocks were also randomized. Progenies were sown in single row plots of three meter length adopting a spacing of 30 cm between rows and 10 cm between plants. After every five progeny rows, the corresponding check was planted in a single row. The standard agronomic practices were followed throughout crop growth like that of the M_1 generation. The viable mutants were scored in the M_2 generation based on their phenotypic expression on different characters. They were categorized into several groups including stature, duration, leaf, pod, and seed. The spectrum and frequency of viable mutations were calculated. The frequency of viable mutations was calculated on an M_2 plant population basis as follows.

Mutation frequency (%) =

$$\frac{\text{Number of mutants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

RESULTS AND DISCUSSION

Frequency of viable mutations

Viable mutations were recorded from the early seedling stage to maturity. The frequency of viable mutants computed on an M_2 plant basis is presented in Table 1. In gamma rays treatments, the frequency of viable mutations in WGG-42 ranged between 0.909 (200 Gy) to 1.419 percent (600 Gy), and in LGG-460,it was between 0.824 (200 Gy) to 1.188 percent (600 Gy). Considering the EMS treatments, the frequency of viable mutations in WGG-42 ranged between 1.422 (0.2%) to 2.676 per cent (0.6%) and in LGG-460, it was between 1.133 (0.2%) to 2.409 percent (0.6%). In SA treatments the frequency of

Table 1. Frequency of viable mutations in M_2 generation of WGG-42 and LGG-460

Treatments	Dose	WGG-42			LGG-460		
		Total number of plants examined	Number of plants showing viable mutants	Viable mutation frequency (%)	Total number of plants examined	Number of plants showing viable mutants	Viable mutation frequency (%)
Gamma rays	200 Gy	1650	15	0.909	1820	15	0.824
	300 Gy	1768	17	0.962	1650	17	1.030
	400 Gy	1700	19	1.118	1716	18	1.049
	500 Gy	1668	21	1.259	1750	20	1.143
	600 Gy	1550	22	1.419	1768	21	1.188
EMS	0.2%	1664	24	1.442	1500	17	1.133
	0.3%	1602	25	1.561	1136	18	1.585
	0.4%	1350	26	1.926	1610	26	1.615
	0.5%	1050	25	2.381	1398	28	2.003
	0.6%	1196	32	2.676	1204	29	2.409
SA	1 mM	1700	11	0.647	1502	13	0.866
	2 mM	936	14	1.496	1464	15	1.025
	3 mM	1300	20	1.538	1144	17	1.486

viable mutations in WGG-42 ranged between 0.647 (1 mM) to 1.538 percent (3 mM) and in LGG-460, it was between 0.866 (1 mM) to 1.486 percent (3 mM).

In the present investigation, the frequency of different types of viable mutations was scored at various development stages, particularly from flowering to the maturity period. A mutational event may be accompanied by a larger or smaller change in phenotype and such changes have the highest significance in plant breeding (Brock, 1970; Sigurbjornsson, 1972). Gaul (1964) classified viable mutations as macro and micro mutations, while Swaminathan (1964) grouped them as macro mutations and systemic mutations.In the present study, viable mutation frequency increased with an increase in dose/concentration of gamma rays, EMS, and SA treatments in both the genotypes indicating a positive relationship between dose/concentration of mutagenic treatments and frequency of viable mutations. In both the genotypes, EMS showed the highest frequency of viable mutations than gamma rays and SA treatments. Similar results were also reported by Anand *et al.* (2009) and Vairam and Ibrahim (2014) in mungbean. In the present investigation, both the genotypes of mungbean were found to respond to the mutagenic treatments differently. Such varietal differences in the expression of viable mutation frequency were also observed earlier by Auti (2012) and Arulselvi *et al.* (2016) in mungbean.

Spectrum of viable mutations

The viable mutations observed in M_2 generation in the two genotypes *viz*., WGG-42 and LGG-460 were categorized into different groups such as stature mutants, leaf mutants, duration mutants, cotyledonary leaf mutants, pod mutants, seed size mutants, seed colour mutants etc. A total of 27 types of different viable mutations were identified for all the treatments. The various viable mutants isolated in the M_2 generation under different treatments in both genotypes are presented in Table 2 and 3, respectively. A brief description of each mutant type is given below:

Stature mutants

(i) Tall

These mutants were considerably taller than the respective control plants (Fig 1 and 2). In WGG-42 (Table 2), a total of nine tall mutants were observed in different mutagenic treatments *viz*., 400 Gy (1), 500 Gy (1), and 600 Gy (2) of gamma rays; 0.3% (2) and 0.6% (2) of EMS and 3 mM (1) of SA. The average height of these mutants was 42.63 cm, whereas it was 37.07 cm in the control plants. These mutants were taller at least by 5-6 cm than the control and some of them were also high yielders than the control. In the case of LGG-460 (Table 3), a total of four tall mutants were observed at 0.4% (1), 0.6% (1) of EMS; 2 mM (1), and 3 mM (1) of SA treatments with the average height of 47.50 cm, whereas it was 36.77 cm in the control plants.The increase in plant height was due to the changes in the internodal length and the increase in cell number and cell length or both (Vairam and Ibrahim, 2014).

(ii) Dwarf

The dwarf mutant plants observed in the present investigation were characterized by stunted growth and short internodes, fewer branches, less number of pods, and lower seed yield (Fig 1 and 2). These mutants were very short at the time of maturity and were shorter by 9-12 cm when compared with the mean height of WGG-42 (37.07 cm) and LGG-460 (36.77 cm). In the WGG-42 variety (Table 2), a total of 14 dwarf mutants were recorded and these mutants were observed in most of the gamma rays, EMS and SA treatments. In the LGG-460 variety (Table 3), a total of 14 dwarf mutants were recorded and these mutants were observed in most of the gamma rays, EMS, and SA treatments. The reduction in plant height was mainly due to the short internodal distance manifested in dwarf mutants, as also seen earlier in mungbean (Nandanwar *et al*., 2015).

(iii) Spreading

These types of mutants had longer internodes and spreading habits (Fig 1 and 2). In the WGG-42 variety (Table 2), a total of 16 spreading mutants were recorded and these mutants were observed in most of the treatments of gamma rays, EMS and SA. Whereas, in the LGG-460 variety (Table 3), a total of 12 spreading mutants were observed and these mutants were present in 200 Gy (1) and 400 Gy (1) of gamma rays; 0.2% (3), 0.4% (1) and 0.5% (4) of EMS and 1 mM (1) and 3 mM (1) of SA treatments. Mishra *et al*. (2013) and Vairam and Ibrahim (2014) also reported such types of spreading mutants in mungbean.

(iv) Bushy

These mutants had dense growth with compactly arranged branches and the plant height was extremely reduced giving it a bush-like appearance. These types of mutants were observed

Table 2. Spectrum of viable mutants in M_2 generation of WGG-42

Type of mutants	Gamma rays					EMS					SA			Total
	200 Gy	300 Gy	400 Gy	500 Gy	600 Gy	0.2%	0.3%	0.4%	0.5%	0.6%	1 mM	2 mM	3 mM	
VIABLE MUTANTS														
Stature mutants														
Tall			1	1	2		2			2			1	9
Dwarf		1	1	1		3	4	2			1		1	14
Spreading	1	1		1	1	2	1		2	3	1	2	1	16
Trailing				1			1		1					3
Miniature	2	3	2	6	4	2	4	7	2	2	2			36
Leaf mutants														
Small leaf		1										2	2	5
Tetra foliate leaf		2	1	1	2		3	5	6	6	1	2	7	36
Penta foliate leaf		2	1	1			1	4	2	4	1		6	22
Variegated leaf		1				2			2					5
Serrated leaf		1		1		2	1							5
Duration mutants														
Early maturing		1			1	2				1		2		7
Late maturing		1			1		1	1		2				6
Synchronized maturity			1	1						2	2			6
Cotyledonary leaf mutants														
Unicotyledon	2		1		1		1							5
Tri cotyledon			1											1
Tetra cotyledon		1												1
Pod mutants														
More pod number	1	1	2	2	1	3	2	2	2	1	2		1	20
More cluster number			2			2				1				5
Constricted pods			1	1		2		2	1	3			1	11
Long pod			1	1	1	2			1			2		8
Short pod	5		2		4		2		1	1		2		17
Purple stripped pods		1												1
Seed size mutants														
Bold seeded	2						1							3
Small seeded			1	1	2	2		1	3	1		2		13
Seed colour mutants														
Dark green seeds	1		1	1	1			2	2	1	1			10
Light white seeds	1						1			2				4
Yellowish green seeds				1	1									2
Total	15	17	19	21	22	24	25	26	25	32	11	14	20	271
	Total number of mutants in gamma rays=94					Total number of mutants inEMS=132					Total number of mutants in SA= 45			

only in the variety LGG-460 (Fig 2). The average plant height of the mutants was 22.95 cm. This mutant had a reduced number of pods per plant and seeds per pod and a very low 100-seed weight (2.68 g). They exhibited reduced yield components, thereby giving lower seed yield. They were late in flowering and maturity. A total of nine bushy mutants were recorded (Table 3) and these mutants were observed at 500 Gy (2) of gamma rays and 0.2% (3), 0.3% (1), 0.4% (1), and 0.6% (2) of EMS treatments. Tah (2006) and Singh (2007) studied bushy growth habits that were attributed to a mutagen-induced increase in lateral branches and higher photosynthetic activities in the mungbean. Bushy mutants showed a reduction in yield and yield components hence may not be beneficial for

Table 3. Spectrum of viable mutants in M_2 generation of LGG-460

Type of mutants	Gamma rays					EMS					SA			Total
	200 Gy	300 Gy	400 Gy	500 Gy	600 Gy	0.2%	0.3%	0.4%	0.5%	0.6%	1 mM	2 mM	3 mM	
	Viable Mutants													
Stature mutants														
Tall								1		1		1	1	4
Dwarf	2	1	2		1	2	1			3	2			14
Spreading	1		1			3		1	4		1		1	12
Bushy				2		3	1	1		2				9
Miniature	5	2	3		5		2	5		3	3	4	5	37
Leaf mutants														
Small leaf		1		2	1			2		3	1	2	2	14
Unifoliate leaf							1							1
Tetrafoliate leaf		1	1	2	2			1		2		1	3	13
Pentafoliate leaf		1		2	2			1		2		2	3	13
Variegated leaf							1	1						2
Crinkled leaf							1		2					3
Duration mutants														
Early flowering	1	1	1		2	1	1	1	2	2		2	1	15
Early maturing						1	1	1	2		1			6
Late maturing	2	2	2	3	1			3	2	3	2	1		21
Synchronized maturity						1	1							2
Pod mutants														
More pod number	1	2	2	1	1	2	2	1	3	2	1	1		19
More cluster number		2						2	3	1				8
Constricted pods									3					3
Long pod						1						1		2
Short pod		1	3	2	2	1	1	3		3				16
Seed size mutants														
Bold seeded	1			2			1	1			1			6
Small seeded	2	1	1	1	2	1	1	1	3	1	1		1	16
Seed colour mutants														
Dark green seeds			2		1		1		2	1				7
Light green seeds					1									1
Light white seeds						1	1							2
Brown seeds				1										1
Other mutants														
Purple colour stem		2		2			1		2					7
Total	15	17	18	20	21	17	18	26	28	29	13	15	17	254
	Total number of mutants in gamma rays=91					Total number of mutants inEMS=118					Total number of mutants in SA= 45			

direct profit-making cultivation. Though these mutants may not be usefulfor direct commercial cultivation because of the reduced yield, they may, however, be used inhybridization to transfer some of their useful traits like short stature and more primary branches to other high-yielding varieties of mungbean.

(v) Trailing

These types of mutants were observed only in the variety WGG-42 (Fig 1). A total of three trailing mutants were recorded (Table 2) and these mutants were observed at 500 Gy (1) of gamma rays; 0.3% (1) and 0.5% (1) of EMS treatments. These types of mutants were also observed by Tah (2006) and Mishra and Singh (2014) in mungbean.

(vi) Miniature

In WGG-42 (Table 2), a total of 36 miniature mutants were recorded and these types of mutants

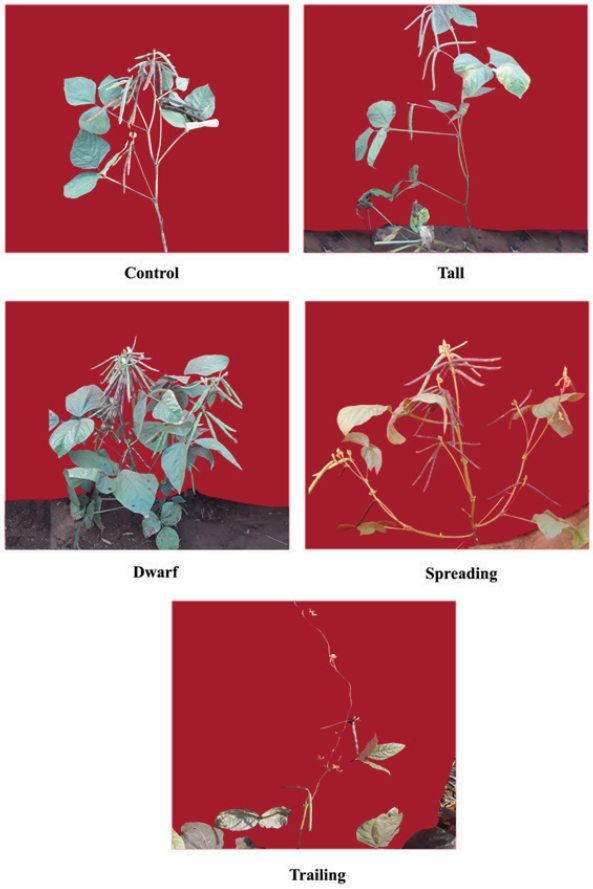

Fig. 1. Stature mutants observed in M_2 generation of WGG-42

were observed in all the treatments of gamma rays; EMS, and at 1 mM (2) of SA treatments. In LGG-460 (Table 3), a total of 37 miniature mutants were recorded and these types of mutants were observed in most of the treatments of gamma rays, EMS, and SA.

Leaf mutants

Leaf mutants *viz*., small leaf, unifoliate, tetrafoliate, pentafoliate, variegated, serrated,and crinkled leafmutantswere observed.Small leafmutants had smaller leaves compared to control plants (Fig 3a and 3b). In WGG-42 (Table 2), five small leaf mutants were found in 300 Gy (1) of gamma rays and at 2 mM (2) and 3 mM (2) of SA treatments. Whereas in LGG-460 (Table 3), a total of 14 small leaf mutants were recorded and these mutants were observed in gamma rays, EMS, and SA treatments. Unifoliate leaf mutants were characterized bysingle leaflets. Unifoliate leaf mutants were observed only in the variety LGG-460 (Fig 3b). In LGG-460 (Table 3), only one unifoliate leaf mutant was found

Fig. 2. Stature mutants observed in M_2 generation of LGG-460

in 0.3% (1) of EMS. Tetra foliate leafmutants were characterized by a leaf divided into four leaflets in contrast to the three leaflets in the control plant (Fig 3a and 3b). In WGG-42 (Table 2), a total of 36 tetra foliate leaf mutants were recorded and these types of mutants were observed in most of the treatments of gamma rays, EMS, and SA. In the case of LGG-460 (Table 3), a total of 13 tetra foliate leaf mutants were recorded and these types of mutants were observed in most treatments of gamma rays, EMS treatments, and SA treatments.Almost all leaf mutants showed a stable phenotype in the subsequent M_3 generation. Leaf mutations could be attributed to mutagen-induced cellular damage, chromosomal breakage, altered mineral metabolism, and the disrupted synthesis and transport of auxin.

Pentafoliate leafmutants showed five leaflets or lobes instead of three leaflets as observed in the control plant (Fig 3a and 3b). In the WGG-42 variety (Table 2), a total of 22 pentafoliate leaf mutants were recorded and these types of mutants were observed in almost all the treatments of gamma rays, EMS, and SA treatments. In the LGG-460 variety (Table 3), a total of 13 pentafoliate leaf mutants were recorded and these types of mutants were present in gamma rays, and SA treatments.Variegated leaf mutants showed the appearance of differently colored zones in the leaves andsometimes on the stems of plants (Fig 3a and 3b). In the variety WGG-42 (Table 2),

five variegated leaf mutants were observed in 300 Gy (1) of gamma rays and at 0.2% (2) and 0.5% (2) of EMS treatments. In the variety LGG-460 (Table 3), twovariegated leaf mutants were observed in 0.3% (1) and 0.4% (1) of EMS treatments.

Serrated leaf mutant was observed only in the variety WGG-42 (Fig 3a). In these mutants, the leaf was characterized by having a margin notched like a saw with teeth pointing toward the apex. A total of five serrated leaf mutants were recorded in WGG-42 (Table 2). These types of mutants were present at 300 Gy (1) and 500 Gy (1) of gamma rays and 0.2% (2) and 0.3% (1) of EMS in WGG-42.Crinkled leafmutants were observed only in the variety LGG-460. A total of threecrinkled leaf mutants were recorded (Table 3) and these types of mutants were observed at 0.3% (1) and 0.5% (2) of EMS treatments. Similar mutants were also reported earlier by Suresh (2014) in mungbean. The leaf mutations obtained in mungbean may be ascribed to the above-cited reasons, which may be useful as gene markers in conventional breeding. These may be useful for understanding the genetic control of leaf formation and the regulation of their size, shape, and form. Though mungbean is trifoliate, an increase in foliation will increase biomass production, which could have a positive impact on seed yield.

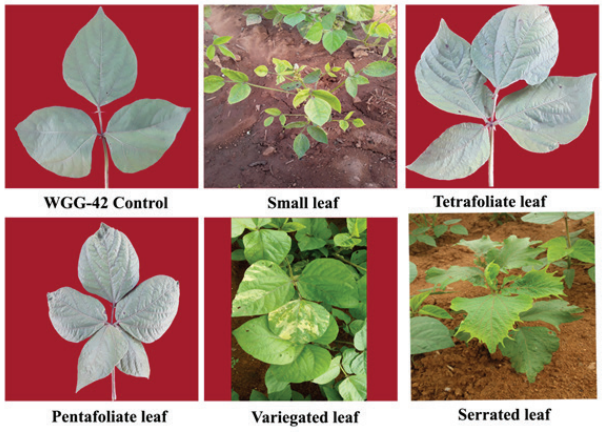

Fig. 3a. Leaf mutants observed in M_2 generation of WGG-42

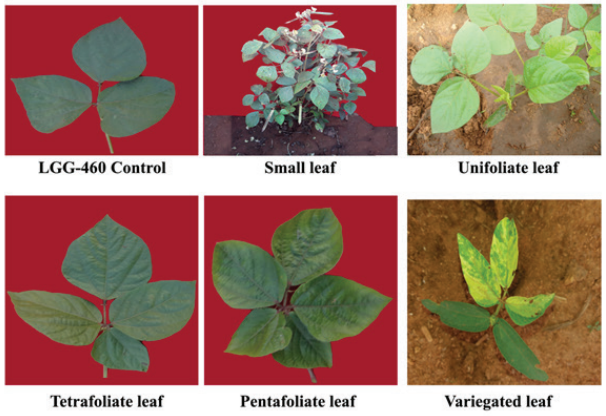

Fig. 3b. Leaf mutants observed in M_2 generation of LGG-460

Duration mutants

(i) Early flowering

In the LGG-460 variety, these mutants flowered 2-3 days earlier than the control. The yield of these mutants was found lower than the control.A total of 15 early flowering mutants were recorded (Table 3) and these mutants were isolated in all the treatments except 500 Gy of gamma rays and 1 mM of SA treatments. Vairam and Ibrahim (2014) and Digbijaya *et al*. (2019) also isolated early flowering mutants in mungbean. In the present investigation, the early mutants of mungbean show pod maturity 2-3 days earlier than the respective control in gamma rays, EMS, and SA treatments. Agronomic traits like early flowering have been always given paramount importance while planning breeding strategies. The early mutants could be very much useful for genecological studies (Gottschalk and Wolff, 1983). The earliness is mainly achieved through rapid growth during the early stages of ontogeny and initiation of first inflorescence.

(ii) Early maturing

In the WGG-42 variety, these mutants matured 2-3 days earlier than the respective control. Yield of these mutants was lower than the control. A total of seven early maturing mutants were recorded (Table 2) and these mutants were isolated at gamma rays treatments (300 Gy and 600 Gy), EMS (0.2% and 0.6%), and SA (2 mM) treatments. In LGG-460, these mutants matured 2-3 days earlier than the control. The yield of these mutants was also found lower than the control.A total of six early maturing mutants were recorded (Table 3) and these mutants were isolated in EMS and SA treatments. Mishra et al. (2013) and Vasantrao (2012) also isolated early maturing mutants through the use of physical and chemical mutagens in their experiments on mungbean.Early maturity in the mutants may be due to the physiological, biochemical, enzymological, and hormonal changes induced by the mutagens. Early maturing mutants possess multiple advantages over the parent variety; these include the ability to escape or tolerate insect damage and prevent insect populations from building up due to the short duration of the reproductive phase (Jackai 1982). Early maturing mutants also possess better

tolerance to drought and thrive in areas receiving less rainfall.

(iii) Late maturing

In WGG-42, the late-maturity mutants were matured 5-6 days later than the control. A total of six late maturing mutants were recorded (Table 2) and these mutants were isolated at 300 Gy (1) and 600 Gy (1) of gamma rays; at 0.3% (1), 0.4% (1), and 0.6% (2) of EMS treatments. In LGG-460, these mutants matured 3-4 days later than control.A total of 21 late-maturing mutants were recorded (Table 3) and these mutants were isolated of gamma rays, EMS, and SA treatments. Similar types of late-maturing mutants were also isolated by Singh and Rao (2007) and Anand *et al*. (2009) in mungbean. The main reasons attributed to the late maturity were inadequate production of flowering hormones, physiological disturbances, enhanced production of a floral inhibitor, and reduced ability to respond to the floral stimulus in the shoot apex (Beveridge and Murfet, 1996). Late or early maturity has agronomic significance as these mutants suit the specific requirement of the breeding strategy (Zakri and Jalani, 1998). The lateness in maturity is worthwhile to prolong the vegetative phase that allows the development of a strong sink which ultimately may enhancethe yield. In addition, the period from flowering to maturity should also be long enough for better seed filling.

(iv) Synchronized maturity

In the WGG-42 variety (Table 2), a total of six synchronized maturity mutants were recorded and these mutants were isolated at 400 Gy (1) and 500 Gy (1) of gamma rays; 0.6% (2) of EMS and 1 mM of SA (2) treatments. In the case of LGG-460 (Table 3), a total of two synchronized mutants were recorded, and these mutants were isolated at 0.2% (1) and 0.3% (1) of EMS treatments. Similar mutants were also reported by Tah (2006) in mungbean.

Cotyledonary leaf mutants

Cotyledonary leaf mutants *viz*., unitri and tetra cotyledon leaf mutants were observed. Uni cotyledon leaf mutants had only one leaf. In the WGG-42 variety (Table 2, Fig 4), a total of five uni cotyledon type mutants were recorded and these mutants were observed at 200 Gy (2), 400 Gy (1), and 600 Gy (1) of gamma rays and 0.3% (1) of EMS treatments. Tri cotyledonmutant was characterized by seedlings with an extra cotyledonary leaf. The

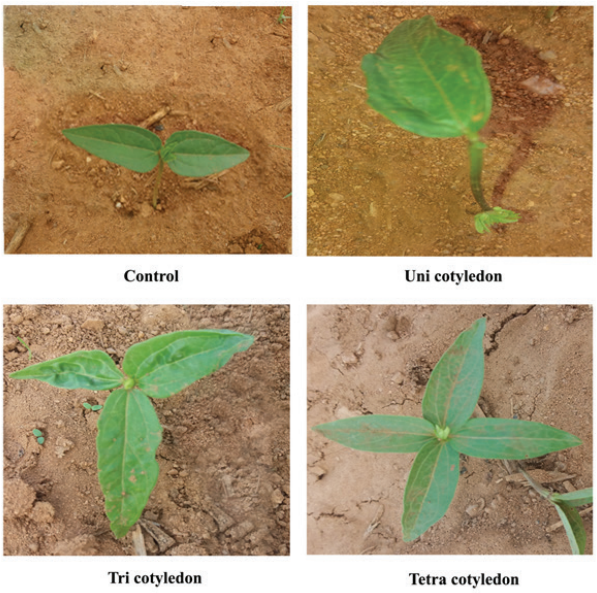

Fig. 4. Cotyledonary Leaf mutants observed in M_2 generation of WGG-42

formation of an extra cotyledonary leaf, on the other hand, indicates the formation of additional leaf primordia or the embryonal cell. In WGG-42 (Table 2, Fig 4), only one tri cotyledon leaf mutant was found in 400 Gy of gamma rays. Tetra cotyledonleaf mutants were characterized in seedlings with an extra pair of cotyledonaryleaves. In the WGG-42 variety (Table 2, Fig 4), only one tetra cotyledon leaf mutant was found in 300 Gy of gamma rays. Similar mutants were also isolated by Wani *et al*. (2014).

Pod mutants

(i) More pod number

In these mutants, the numberof pods per plant was high when compared with the control (Fig 5 and 6). In WGG-42, the mean number of pods per plant was 13.24 in thecontrol, while it was 25.02 in the mutants. A total of 20 mutants having more pod number were observed in all the treatments except 2 mM of SA (Table 2). In LGG-460 (Table 3), a total of 19 mutants had more numbersof podsin all the treatments except 3 mM of SA. The mean number of pods per plant was 20.86 and 35.15 in thecontrol and mutants, respectively. The reports of Mishra *et al.* (2013) and Arulselvi *et al.* (2016) also explained such mutants in mungbean.

(ii) More cluster number

In these mutants, the numberof clusters per plant was high when compared with the control (Fig

5 and 6). In WGG-42 (Table 2), the mean number of clusters per plant was 5.02 and 9.45 in thecontrol and mutants, respectively. In WGG-42 (Table 2), a total of five mutants with more cluster numbers were observed in 400 Gy (2) of gamma rays and 0.2% (2) and 0.6% (1) of EMS treatments. These mutants also had vigorous growth and high-yielding ability. In the case of LGG-460, mean number of clusters per plant was 7.89 and 14.35 in thecontrol and mutants, respectively. A total of eight more cluster mutants were observed in 300 Gy (2) of gamma rays and at 0.4% (2), 0.5% (3), and 0.6% (1) of EMS treatments (Table 3). Similar mutants were also observed by Tah (2006) and Vairam and Ibrahim (2014) in mungbean.

(iii) Constricted pods

In WGG-42 (Fig 5), a total of 11 constricted pod mutants were observed in 400 Gy (1) and 500 Gy (1) of gamma rays; at 0.2% (2), 0.4% (2), 0.5% (1) and 0.6% (3) of EMS and 3 mM (1) of SA treatments (Table 2). In LGG-460 (Fig 6), a total of 3 constricted pod mutants were observed in 0.5% (3) of EMS treatment (Table 3).

(iv) Long pod

In these mutants, the pod length was longer compared to control (Fig 5 and 6). In WGG-42, the mean pod length was 8.35 cm and 9.60 cm in thecontrol and mutants, respectively. In LGG-460, the mean pod length was 7.46 cm and 8.52 cm in the control and mutants, respectively. In WGG-42 (Table 2), a total of eight long pod type ofmutants were recorded and these mutants were observed in 400 Gy (1), 500 Gy (1), and 600 Gy (1) of gamma rays treatments and 0.2% (2) and 0.5% (1) of EMS and 2 mM (2) of SA treatments. Whereas, in LGG-460 (Table 3), a total of two long pod types ofmutants were recorded and these mutants were observed in 0.2% (1) of EMS and 2 mM (1) of SA treatments. Similar mutants were also reported by Dhole and Reddy (2018) in mungbean. Long pod is a useful variation and could be exploited in increasing the number of seeds per pod leading to increased yield potential.

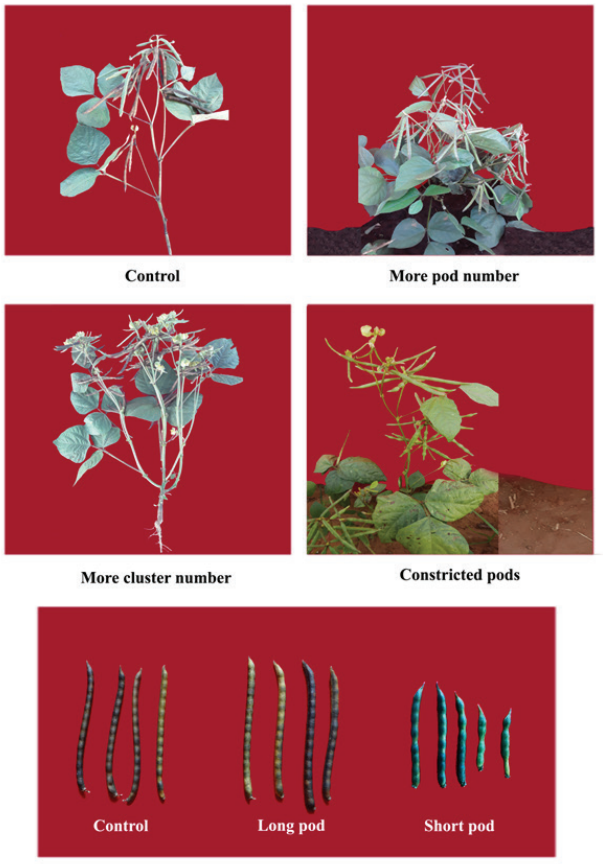

Fig. 5. Pod mutants observed in M_2 generation of WGG-42

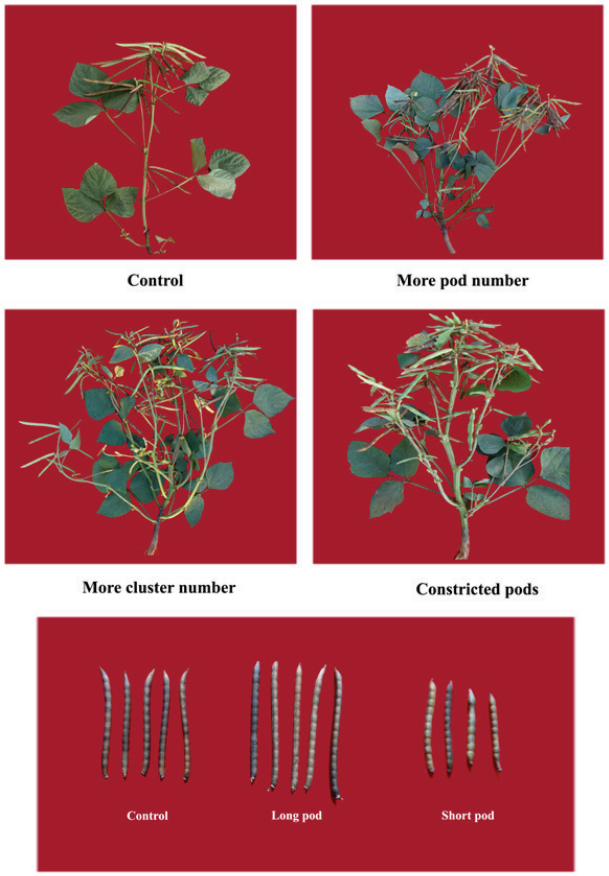

Fig. 6. Pod mutants observed in M_2 generation of LGG-460

(v) Short pod

In these mutants, the pod length was less compared to the control and smaller seed size as compared to the control (Fig 5 and 6). In WGG-42, the mean pod length was 8.35 cm and 7.02 cm in thecontrol and mutants, respectively. In LGG-460, the mean pod length was 7.46 cm and 6.11 cm in thecontrol and mutants, respectively. In WGG-42 (Table 2), a total of 17 short pod mutants were observed in most of the treatments of gamma rays, EMS, and SA treatments. In LGG-460 (Table 3), a total of 16 short pod mutants were observed in most of the treatments of gamma rays and EMS treatments. Similar mutants were also reported by Anand *et al.* (2009) and Balkrushna (2011) in mungbean.

(vi) Purple-stripped pods

A mutant having pods with purple sutures was isolated only in the WGG-42 variety in contrast to green sutures in the control plant. Only one purple-stripped pod mutant was observed at 300 Gy of gamma rays (Table 2). A similar type of mutant was also observed by Suresh (2014) and Arulselvi *et al.* (2016) in mungbean.

Seed size mutants

(i) Bold seeded

The seeds in these mutants were bolder and larger in comparison to the control (Fig 7 and 8). In WGG-42, these mutants exhibited increased 100-seed weight which ranged from 5.25 - 6.20 g against the control plants which were in the range of 4.68-5.38 g. These mutants were vigorous in growth. The maturity duration of these mutants was similar to the control plants. A total of three bold seeded mutants were recorded (Table 2) and these mutants were observed in gamma rays (200 Gy) and EMS (0.3%) treatments. In LGG-460, the 100-seed weight of these mutants ranged from 4.32-4.47 g against the control plants which gave the range of 3.44-3.79 g. A total of six bold-seeded mutants were recorded (Table 3) and these mutants were observed in gamma rays (200 Gy and 500 Gy); EMS (0.3% and 0.4%) and SA (1 mM) treatments. Balkrushna (2011) and Dhole and Reddy (2018) also isolated similar types of bold-seeded mutants in mungbean. The bold-seeded mutants isolated in the present study showed substantial improvement in yield. They could be utilized as donor parents for the bold character or released directly as new cultivars after extensive multi-location trails.

(ii) Small seeded

These mutants had relatively smaller seed sizes and were low-yielders as compared to the control (Fig 7 and 8). In WGG-42, the 100-seed weight of these mutants ranged from 3.06 - 3.32 g against the control plants which gave the range of 4.68 - 5.38 g. A total of 13 small seeded mutants were recorded (Table 2) and these mutants were observed in most of the treatments of gamma rays, EMS, and SA treatments. In LGG-460, the 100-seed weight of these mutants ranged from 2.68 - 2.82 g against the control plants which gave the range of 3.44 - 3.79 g. A total of 16 small seeded mutants were recorded (Table 3) and these mutants were observed in all the treatments of gamma rays, EMS, and SA treatments. Similar mutants were also observed by Vairam and Ibrahim (2014) in mungbean.

Fig. 7. Seed mutants observed in M_2 generation of WGG-42

Seed colour mutants

(i) Dark green seeds

These mutants exhibited dark green seed coat colour in contrast to the normal green colour of the control (Fig 7 and 8). In WGG-42 (Table 2), a total of 10 dark green seed mutants were recorded and these mutants were observed at 200 Gy (1), 400 Gy (1), 500 Gy (1) and 600 Gy (1) of gamma rays and 0.4% (2), 0.5% (2) and 0.6% (1) of EMS and 1 mM of SA (1). In the case of LGG-460 (Table 3), a total of seven dark green colour seed mutants were recorded, and these mutants were observed at 400 Gy (2) and 600 Gy (1) of gamma rays and at 0.3% (1), 0.5% (2) and 0.6% (1) of EMS treatments.

Fig. 8. Seed mutants observed in M_2 generation of LGG-460

(ii) Light green seeds

These mutants exhibited light green seed coat colour as compared to the green colour of the control plants (Fig 8). These types of mutants were recorded only in the LGG-460 variety and these mutants were observed at 600 Gy (1) of gamma rays (Table 3). Similar mutants were also observed by Vairam (2014) in mungbean.

(iii) Light white seeds

These mutants exhibited light white seed coat colour as compared to the green colour of the control plants. However, the 100-seed weight and seed size of these mutants were found lesser than the control (Fig 7 and 8). In WGG-42 (Table 2), a total of four light white seed mutants were recorded and these mutants were observed in gamma rays (200 Gy) and EMS (0.3% and 0.6%) treatments. In the case of LGG-460 variety (Table 3), a total of two light white seed mutants were recorded and these mutants were observed in EMS (0.2% and 0.3%) treatments.

(iv) Yellowish green seeds

These mutants exhibited light yellow seed coat colour as compared to green colour in the control (Fig 7). These types of mutants were observed only in the WGG-42 variety. A total of two yellowish-green seed mutants were recorded (Table 2) and these mutants were observed in 500 Gy (1) and 600 Gy (1) of gamma rays. Similar mutants were also observed by Sweta (2014) in mungbean.

(v) Brown seeds

These mutants exhibited brown-colored seed coats as compared to green colour seed coatsin the control plants (Fig 8). These mutants had very low seed fertility. These types of mutants were observed only in LGG-460. Only one brown colour seed mutant was also observed in 500 Gy (1) of gamma rays (Table 3). Similar mutants were also observed by Vaijanath (2004) in mungbean.

Other mutants

(i) Purple colour stem

This mutant had a purple colour stem (Fig 9). Anthocyanin pigmentation was observed in the stem and petiole of this mutant. Whereas, its

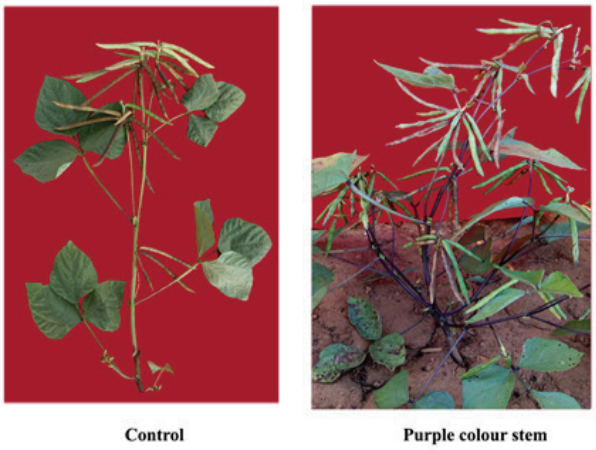

Fig. 9. Purple colour stem observed in M_2 generation of LGG-460

control plants had a green colour stem and petiole. The number of leaves (foliage) was reduced in this mutant and thereby yield potential was also reduced. The seeds of purple-colored stem mutants were dark green with a spotted appearance on it. These types of mutants were observed only in the LGG-460 variety. A total of seven purple colour stem-type of mutants were recorded (Table 3) and these mutants were observed in gamma rays (300 Gy and 500 Gy) and EMS (0.3% and 0.5%). Similar mutants were also observed by Arulselvi *et al.* (2016) in mungbean.

In the present study, the viable mutations isolated showed changes in major characters which could be utilized in future breeding programmes where reshuffling of characters may be tried by conventional breeding methods. However, several of the morphological mutants identified in M_2 generation failed to inherit in M_3 generation. Luo *et al.* (2012) reported that these traits may be controlled by recessive genes or susceptible to the environment. Moreover, whatever changes occurred in the plants due to mutation was an error according to the plants geometry. They tend to rectify it in due course through recombinational events. That is why most of the observed mutants were not inherited in future generations. Thus, evolving a new phenotype with consistent expression through mutation is a chance event rather than a choice.

CONCLUSION

Viable mutants observed in the present investigation like early duration, more number of pods, more number of clusters, more pod length, and seed size mutants may be exploited in hybridization programs to further improve the elite cultivars in mungbean.Viable mutants like pod mutants, seed colour mutants, and purple colour stem mutants observed in the present investigation could be exploited in genetic and molecular studies. Viable mutants like stature, leaf mutants, and cotyledonary mutants were observed, which though seem less important per se, could be exploited in understanding the genetic control of leaf formation and regulation of their size, shape and form.

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