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RESEARCH ARTICLE

Induced morphological mutants and mutagenic efficiency of gamma-irradiated mungbean (*Vigna radiata* (L.) Wilczek) M_2 populations

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Abstract

In the present investigation, three mungbean varieties *viz*., Co 5, Co(Gg) 7 and VBN(Gg) 3 were exposed to six doses (450, 500, 550, 600, 650 and 700 Gy) of gamma rays. Based on probit analysis in the M_1 generation, the LD_{50} was determined to be 600 Gy for all three varieties. A wide range of chlorophyll and morphological mutants, including viable and non-viable types, were observed in the M_2 generation. The results indicated that the frequency of chlorophyll mutations increased at lower doses of mutagenic treatment, showing a slight decline in higher doses of mutagen in all three varieties. Mutagenic effectiveness and efficiency were found to decrease with increasing gamma-ray dose. Based on this investigation, it is exposed that the gamma rays have the potential to generate tremendous variability in mungbean genotypes. Varying degrees of mutant genotypes were observed in the M_2 generation. Most are true breeding genotypes since they were detected in M_3 and other generations. Among these, desirable mutants, *viz*., dwarf type, tall type, synchronized maturity, seed size and early and high yielding mutants, are noteworthy. These mutants can be utilized for further crop breeding programmes to evolve desirable genotypes with improved traits such as higher yield, enhanced pest and disease resistance, greater tolerance to environmental stresses and improved nutritive value. Likewise, mutants with distinct traits give a space to generate novel genotypes in mungbean that are more adaptable to challenging environmental conditions and consumer needs by harnessing genetic variations.

Keywords: efficiency; effectiveness; gamma rays; mungbean; mutation; probit

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), commonly known as green gram, belongs to the family Fabaceae (Leguminosae) and the tribe Phaseoleae. It has a diploid chromosome number 2n=2x=22. Mungbean is a self-pollinated, highly branched, trifoliate leaves covered in sparse hair. It plays a significant role in improving soil fertility (1). Mungbean breeding programs prioritize traits such as early flowering, larger flowers, increased pod counts, higher test weight and uniform ripening, resulting in better harvest ability and higher seed yield. Numerous biotic and abiotic stresses, such as pests and diseases, drought, salinity and waterlogging, negatively impact mungbean's adaptability. These factors affect germination, blooming and fruiting processes, ultimately lowering individual plant yield (2).

Various strategies have been utilized in breeding programs to enhance mungbean production. However, because of certain restrictions on hybridization and selection, developing genotypes with desirable traits is frequently difficult. Conventional breeding techniques have not significantly improved the mungbean because of the narrow genetic base (3). Induced mutation breeding is one of the most effective methods for creating genetic variation in crop plants. Mutation breeding incorporates molecular biology, cytogenetics and biotechnology techniques to induce and identify beneficial mutations. Gamma rays and various physical and chemical mutagens can cause mutations. Gamma rays impact plant growth by changing the cells morphological, physiological, biochemical and genetic characteristics. It is widely known that gamma-ray induction of mutations can create genetic heterogeneity in pulse crops for qualitative and quantitative features (4, 5). The utilization of mutagen is determined by both mutagenic efficacy and mutagenic efficiency (6). Morphological mutants are essential for altering characteristic features of genotypes to create new ideotypes, eventually resulting in new crop varieties. This study aimed to assess the efficiency of gamma-ray mutagenesis in inducing mungbean genetic variability, focusing on identifying viable and non-viable morphological mutants.

Materials and Methods

Plant material and site selection

The field experiment was conducted at the Agricultural Research Station, Vaigai Dam, Tamil Nadu Agricultural University (TNAU), Tamil Nadu, India. The experimental materials comprise three varieties of mungbean (*Vigna radiata* (L.) Wilczek) such as Co 5, Co(Gg) 7 and VBN(Gg) 3, which are susceptible to Mungbean Yellow Mosaic Virus (MYMV). The seed materials were collected from TNAU, Coimbatore and the National Pulse Research Centre (NPRC), Vamban, Tamil Nadu, India. The salient features of mungbean varieties are given below

	Co 5	Co(Gg) 7	VBN(Gg) 3
Plant height (cm) :	100-105	30 - 45	35-55
Habit :	Trailing	Erect	Erect
Colour of stem :	Green	Green	Green
Seeds per pod :	9 - 10	11 - 12	11 - 12
Colour of seeds:	Green	Green	Green
Leaf type :	Trifoliate	Trifoliate	Trifoliate

Gamma irradiation

Gamma irradiation was carried out with 2000 Curie ^{60}Co gamma cells, installed at the Centre for Plant Breeding and Genetics, Coimbatore. Gamma ray doses employed were 450, 500, 550, 600, 650 and 700 Gray (Gy) were applied. Dry, uniform-sized mungbean seeds were irradiated with different doses of gamma radiation. Untreated seeds were used as the control. Treated and control seeds were sown in the experimental field to generate the M_1 generation. The LD_{50} for germination was determined to be 600 Gy in mungbean.

Screening of viable and non-viable mutants in M_2 generation

Using the augmented design, the individually harvested M_1 seeds were sown in the field to raise M_2 generation in separate plant-to-progeny rows. Based on the LD_{50} value, three treatments (550, 600 and 650 Gy) were forwarded to M_2 generation with the spacing of 30x10cm. Throughout the crop cycle, the M_2 progenies were screened multiple times for morphological mutations, including viable and non-viable types. Different chlorophyll mutants (Xantha, chlorina, albino and viridis) were scored from emergence until four weeks in M_2 generation (7, 8).

Statistical analysis

Probit analysis

The LD_{50} gamma radiation values for all three genotypes were determined based on the Probit analysis (9). The probit function is the inverse cumulative distribution function (CDF), or quantile function associated with the standard normal distribution as given in equation 1.

$$\text{Corrected mortality (\%)} = \frac{M_{observed} - M_{control}}{100 - M_{control}} \quad \text{(Eqn. 1)}$$

Mutagenic effectiveness and mutagenic efficiency

Mutation frequency was calculated as the percentage of mutated M2 progenies for both chlorophyll and morphological mutations in each treatment. The mutagenic effectiveness and efficiency were determined using the following Equation 2- 3 formulae (10)

Mutagenic effectiveness % (Gamma rays) =

$$Mp \times 100 / kR \text{ or } Gy \quad \text{(Eqn. 2)}$$

where, Mp = Chlorophyll or viable mutation frequency on M_2 plant basis; kR or Gy = Dose of gamma radiation

Mutagenic efficiency % (Gamma rays) =

$$M \times 100 / L;\ M \times 100 / I;\ M \times 100 / S \quad \text{(Eqn. 3)}$$

where,

M = Chlorophyll or viable mutation frequency on M_2 plant basis

L = percentage of lethality *i.e.,* percentage of reduction in survival of seedlings on 30[th] day

I = percentage of injury *i.e.,* percentage of height reduction of seedlings on 30[th] day

S = percentage of sterility *i.e.,* percentage of reduction in seed or pollen fertility

Results and Discussion

In the current scenario, exploiting variability in legume crops is one of the greatest challenges researchers face. Artificial induction of variability using physical mutagens, especially gamma rays, is a potent tool for scientists. This study aimed to induce genetic variation in mungbean using gamma irradiation to identify high-yielding mutants with potential MYMV resistance.

Probit analysis in M_1 generation

Probit analysis was carried out using seed germination values in all three varieties to determine the Lethal $Dose_{50}$ (LD_{50}) in M_1 generation. Based on probit analysis, the LD_{50} for Co 5, Co (Gg) 7 and VBN (Gg) 3 was 575.43 Gy, 616.60 Gy and 588.84 Gy, respectively (Table 1 & Fig. 1A-C). The LD_{50} for germination was fixed at 600 Gy for gamma rays in mungbean. It was observed that treating seeds with high doses of gamma rays showed reduced germination with a corresponding decline in the growth of seedlings (11). The variation in LD_{50} values for different genotypes of the same species is a common observation in mutation studies depending upon the biological materials, their size, maturity, hardness and moisture content at treatment (12, 13).

Segregation of macro mutants in M_2 generation

The extensive morphological mutations observed in M_2 generations are enumerated in Table 2.

Frequency of chlorophyll mutants

Most mutations are not observed in the M_1 generation, as they are often recessive. Even when detectable mutations occur in the M_1 generation, their frequency is low and detecting them requires a large population size. In the M_2 generation, mutagenic effects, which result from complex interactions between multiple factors, become evident (Table 3).

Table 1. Probit analysis for calculating LD_{50} for mungbean genotypes in M_1 generation

Gamma rays (Gy)	Number of seedlings evaluated in all three varieties	Observed mortality percentage			Corrected mortality percentage			Log_{10} of doses all three varieties	Empirical probit unit			Calculated LD_{50} value		
		Co 5	Co 7	VBN 3	Co 5	Co 7	VBN 3		Co 5	Co 7	VBN 3	Co 5	Co 7	VBN 3
Control	50	12	10	8	-	-	-	-	-	-	-			
450 Gy	50	24	18	22	14	9	16	2.65	3.92	3.66	4.01			
500 Gy	50	32	30	28	23	23	22	2.70	4.26	4.26	4.23			
550 Gy	50	48	42	44	41	36	40	2.74	4.77	4.64	4.75	575.43 Gy	616.60 Gy	588.84 Gy
600 Gy	50	66	50	56	62	45	53	2.78	5.31	4.87	5.08			
650 Gy	50	80	62	64	78	58	61	2.81	5.77	5.20	5.28			
700 Gy	50	90	74	78	89	72	77	2.85	6.23	5.58	5.74			

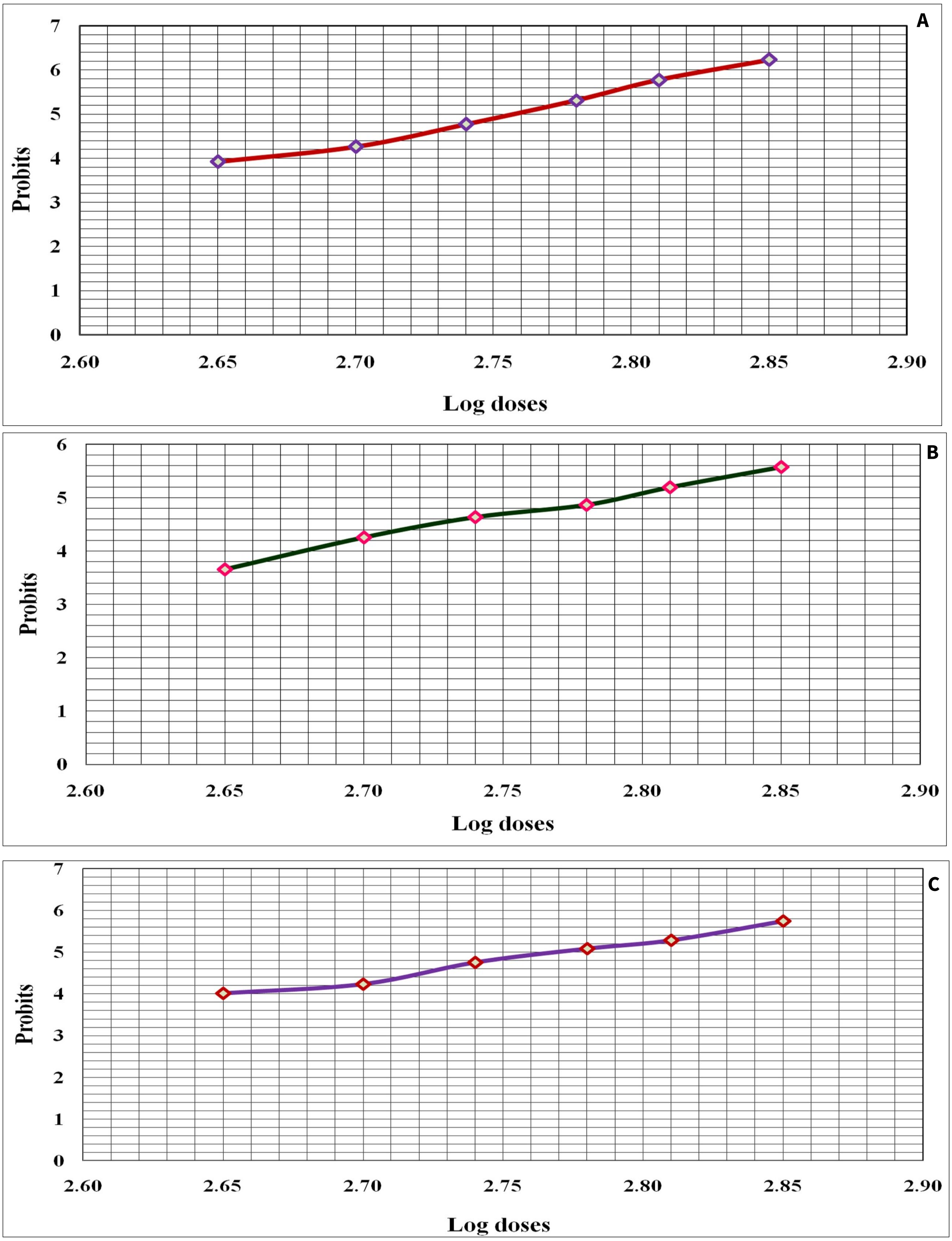

Fig. 1A. Plot of Log doses versus probits for calculation of LD_{50} of Gamma radiation in Co 5**;** B. Plot of Log doses versus probits for calculation of LD_{50} of Gamma radiation in Co(Gg) 7**;** C. Plot of Log doses versus probits for calculation of LD_{50} of Gamma radiation in VBN(Gg) 3.

Table 2. Morphological mutants detected in M_2 generations

Nature of mutants	Viable and non-viable mutants observed in M_2	Nature of mutants	Viable and non-viable mutants observed in M_2
Chlorophyll mutants	Albino	**Stature type mutants**	Dwarf
	Chlorina		Tall
	Xantha		Bushy
	Viridis		Trailing
			Non Trailing
Leaf type mutants	Unifoliate	**Non-viable mutants**	Non flowering mutants
	Tetrafoliate		Sterile mutants
	Pentafoliate	**Pod variation mutants**	Pod Length variation
	Hexafoliate		Hairy pods
	Crinkled		Purple stripped pods
	Serrate	**Color mutants**	Greenish purple hypocotyl colour
	Lobed		Greenish purple corolla
	Variegated		Purple colour stem
Seed variation mutants	Striped seeds	**Duration type**	Early type mutants
	Brown seeds		Late flowering mutants
	Bold seeds		Synchronized maturity

Table 3. Frequency of chlorophyll mutants in M_2 generation

Gamma rays (Gy)	Co 5			Co(Gg) 7			VBN(Gg) 3		
	Seedlings examined	Total number of chlorophyll mutants	Mutation frequency (M)	Total number of plants examined	Total number of chlorophyll mutants	Mutation frequency (M)	Total number of plants examined	Total number of chlorophyll mutants	Mutation frequency (M)
550	1350	49	3.62	3256	129	3.95	2742	102	3.71
600	1282	36	2.81	3158	108	3.41	2656	84	3.16
650	984	10	1.02	2506	30	1.19	2257	20	0.88

In this study, various chlorophyll mutations, including albino, xantha, viridis and chlorina, were observed in the M_2 generation (Fig. 2). The spectrum of albino was higher than the other three chlorophyll mutants. The frequency of chlorophyll mutants was high in 550 Gy dose (3.71 %) of irradiation irrespective of varieties; hence, 550 Gy dose was considered efficient and effective in inducing mutation in mungbean. It was evident that a broad diversity of chlorophyll mutants developed due to biological damage brought on by the mutagens, not due to increased dosage. This result was reinforced by prior works (14-20).

Frequency of viable and non-viable mutants

In general, any mutational event may bring large or small changes in the phenotype. Such changes in macro mutants have the highest significance in plant breeding because they may sometimes give a desired phenotype. In the present investigation, a large number of viable mutants with changes in attributes like stature, duration, leaf, pod and seed mutants were recorded in all the treatments (Fig. 3). A total of 150, 371 and 369 macro mutants were isolated from Co 5, Co(Gg) 7 and VBN(Gg) 3 respectively. The frequency of viable mutants of Co 5 ranged from 2.74 (700 Gy) to 4.96 (550 Gy). The variety Co (Gg) 7 showed a frequency range between 2.08 (700 Gy) and 5.25 (550 Gy), whereas in VBN(Gg) 3, the frequency of viable mutants ranged between 2.66 (700 Gy) and 5.84 (550 Gy) (Table 4). The results indicated that the viable mutation frequency decreased with increasing doses of gamma rays. Likewise, different types of non-viable mutants, completely sterile, semi-sterile mutants and non-flowering mutants have been observed in M_2 populations. In the treated populations, higher doses produced many non-viable mutants. The frequency of non-viable mutants was low in M_2. The total mutation frequency is expressed on the M_2 plant basis. Total mutation frequency was determined by adding the frequencies of chlorophyll and viable mutations (Table 5). The highest total mutation

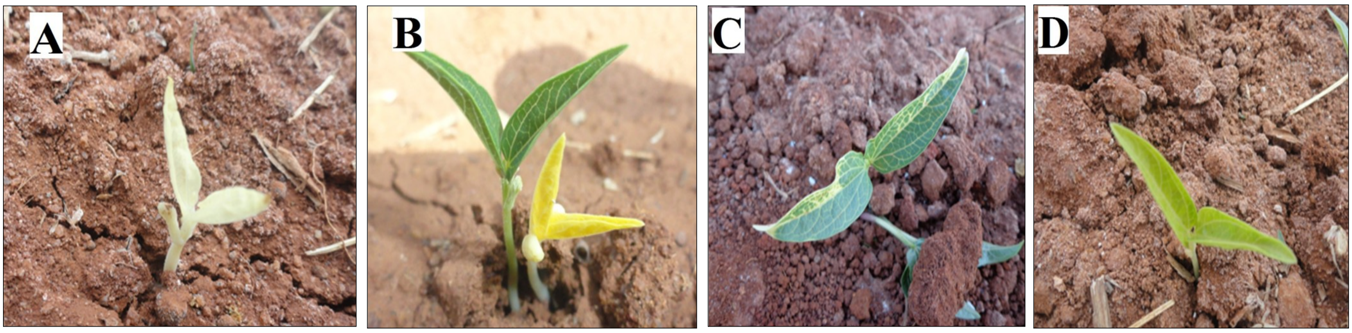

Fig. 2. Diverse classes of chlorophyll mutants perceived in M_2 generation *viz*., A. Albino B. Xantha C. Chlorina D. Viridis.

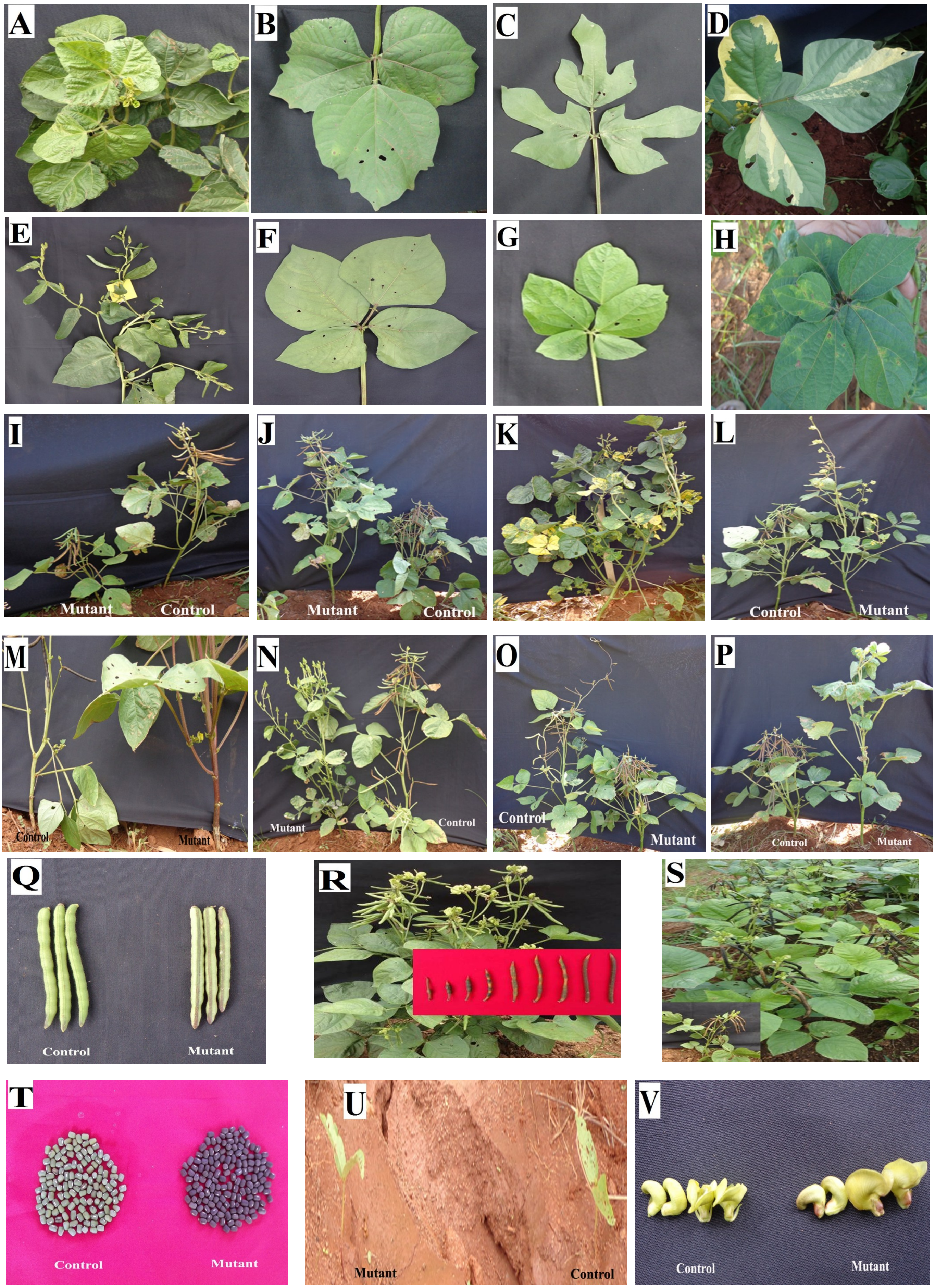

Fig. 3. Various classes of morphological mutants observed in M_2 generations are Crinkled leaf A. Serrated leaf B. Lobed leaf C. Variegated leaf D. Unifoliate leaf E. Tetrafoliate F. Pentafoliate G. Hexafoliate H. Dwarf type I. Tall type J. Bushy type K. Trailing type L. Purple colour stem M. Sterile type N. Non-trailing type O. Non-flowering mutant P. Purple stripped pods Q. Pod length variation R. Synchronized maturity S. Brown seeds T. Greenish purple hypocotyl colour U. Greenish purple corolla V.

Table 4. Frequency of viable mutants in M_2 generation

Gamma rays (Gy)	Co 5			Co(Gg) 7			VBN(Gg) 3		
	Seedlings Examined	Plants showing viable mutants	Mutation frequency (M)	Seedlings Examined	Showing viable mutants	Mutation frequency (M)	Seedlings Examined	Showing viable mutants	Mutation frequency (M)
550	1350	67	4.96	3256	171	5.25	2742	160	5.84
600	1282	56	4.37	3158	148	4.69	2656	149	5.61
650	984	27	2.74	2506	52	2.08	2257	60	2.66

Table 5. Total mutation frequency in M_2 generation

Gamma rays (Gy)	Co 5			Co(Gg) 7			VBN(Gg) 3		
	Frequency of chlorophyll mutants	Frequencyof viable mutants	Total mutation frequency	Frequency of chlorophyll mutants	Frequencyof viable mutants	Total mutation frequency	Frequency of chlorophyll mutants	Frequency of viable mutants	Total mutation frequency
550	3.62	4.96	8.58	3.95	5.25	9.20	3.71	5.84	9.55
600	2.81	4.37	7.18	3.41	4.69	8.10	3.16	5.61	8.77
650	1.02	2.74	3.76	1.19	2.08	3.27	0.88	2.66	3.54

frequency was observed at 550 Gy of Co 5 (8.58), Co(Gg) 7 (9.20) and VBN(Gg) 3 (9.55), followed by 600 Gy, *i.e*., 7.18 (Co 5), 8.10 (Co(Gg)7) and 8.77 (VBN(Gg) 3) respectively. The lowest total mutation frequency was recorded at 650 Gy of Co 5 (3.76), Co(Gg) 7 (3.27) and VBN(Gg) 3 (3.54). It was found that the mutagenic treatment 550Gy of gamma rays was highly efficient in inducing viable mutants in all three mungbean varieties. In mungbean, the viable mutants were segregated towards the traits *viz*., stem colour, leaf shape, leaf counts (unifoliate, bifoliate, tetrafoliate, pentafoliate & hexafoliate), flower colour, seed size, tall, dwarf, bushy, prostrate, synchronized maturity, early maturity and high yielding mutants. Further, sterile mutants and non-flowering mutants also were identified in this investigation. Among these different mutant genotypes, notable types, viz., dwarf type, seed size, synchronized type and tall and high-yielding mutants, are valuable to plant breeders. Bold-seeded mutants are a helpful variant that can be used to improve the size and number of seeds per pod, improving the genetic potential of the yield. Mutants in flower colour can be used as genetic markers in many breeding experiments. Likewise, tetrafoliate, pentafoliate and hexafoliate leaf types are taking advantage of increasing photosynthetic rate, which may ultimately increase the yield. Polygenes were revealed to be responsible for the several morphological mutants that bred true in subsequent generations, including tall, dwarf, semidwarf, bushy, prostate and bold-seeded types (21). Notable types of mutants were reported in previous studies (15, 22-24). Most viable mutants in this investigation were true-breeding because it was also observed in the M_3 generation. It can be utilized for future mungbean crop breeding programmes.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness and mutagenic efficiency are two critical factors in mutant breeding programs. The worth of a mutagen in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens) but also on its mutagenic efficiency (mutation about undesirable changes like sterility, lethality, injury, etc.).

The effects of effectiveness and efficiency of different mutagenic treatments based on chlorophyll mutants are shown in Table 6 & Fig. 4. Based on chlorophyll mutants, the mutagenic effectiveness of Co 5 varied from 0.16 to 0.66 per cent. In contrast, Co(Gg) 7 varied from 0.18 to 0.72 per cent and 0.14 to 0.67 per cent was present in VBN(Gg) 3 mutants. The mutagenic effectiveness showed a trend inversely proportional to the increasing dose of mutagens in mungbean. The mutagenic efficiency was recorded higher in 550 Gy of gamma irradiation in Co 5 based on lethality (14.78), injury (11.13) and sterility (25.77). In the case of Co(Gg) 7, the efficiency was found to be higher at the dose of 550 Gy based on

Table 6. Mutagenic effectiveness and efficiency based on chlorophyll and viable mutants in M_2 generation

Genotypes	Gamma rays (Gy)	Per cent survival reduction at 30th day (Lethality)		Per cent height reduction at 30th day (Injury)		Per cent pollen fertility reduction (Sterility)		Mutation frequency (M)		Effectiveness (M × 100) / Gy or C × t		Efficiency					
												(M × 100) / L		(M x 100) / I		(M x 100) / S	
		Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.	Chl. mut.	Vl. mut.
Co 5	**550**	24.50	24.50	32.53	32.53	14.05	14.05	3.62	4.96	0.66	0.90	14.78	20.24	11.13	15.25	25.77	35.30
	600	35.02	35.02	39.94	39.94	21.60	21.60	2.81	4.37	0.47	0.73	8.02	12.48	7.03	10.94	13.01	20.23
	650	48.40	48.40	47.90	47.90	24.55	24.55	1.02	2.74	0.16	0.42	2.11	5.66	2.13	5.72	4.15	11.16
Co(Gg) 7	**550**	31.37	31.37	26.61	26.61	17.25	17.25	3.95	5.25	0.72	0.95	12.59	16.74	14.84	19.73	22.89	30.43
	600	36.56	36.56	35.48	35.48	22.76	22.76	3.41	4.69	0.57	0.78	9.32	12.83	9.61	13.22	14.98	20.61
	650	47.37	47.37	43.78	43.78	30.32	30.32	1.19	2.08	0.18	0.32	2.51	4.39	2.72	4.75	3.92	6.86
VBN(Gg) 3	**550**	32.88	32.88	33.11	33.11	18.01	18.01	3.71	5.84	0.67	1.06	11.28	17.76	11.20	17.64	20.60	32.43
	600	40.16	40.16	42.36	42.36	24.72	24.72	3.16	5.61	0.53	0.94	7.87	13.97	7.46	13.24	12.78	22.69
	650	46.37	46.37	49.73	49.73	29.38	29.38	0.88	2.66	0.14	0.41	1.90	5.74	1.77	5.35	3.00	9.05

Chl. mut. - chlorophyll mutants; Vl. mut. - viable mutants

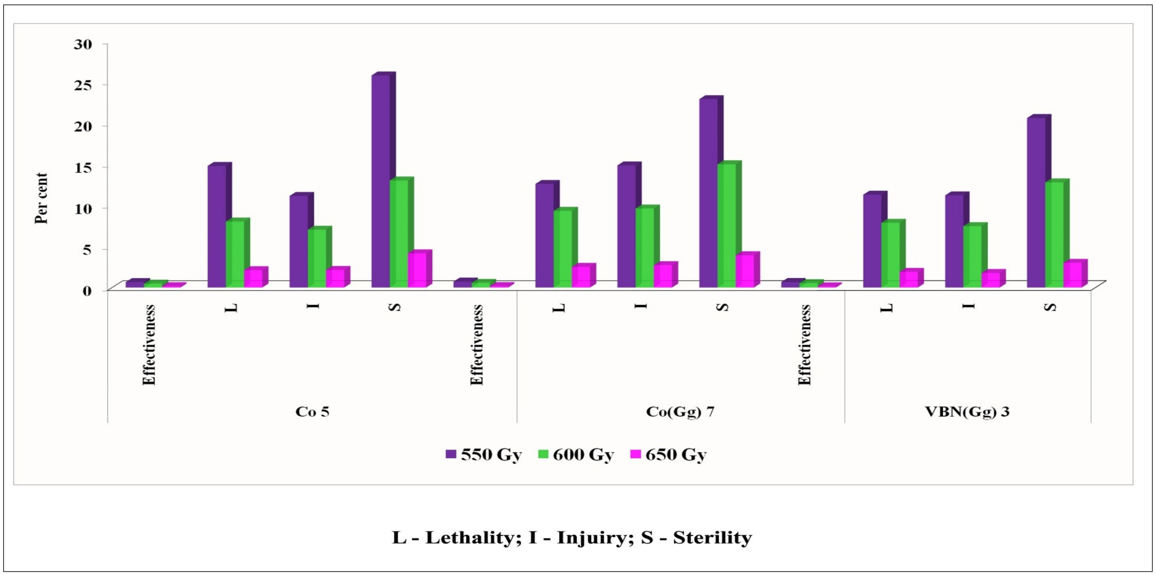

Fig. 4. Mutagenic effectiveness and mutagenic efficiency based on chlorophyll mutants in M_2 generation.

lethality (12.59), injury (14.84) and sterility (22.89). The same pattern of mutagenic efficiency was found in VBN (Gg) 3 at the dose of 550 Gy based on lethality (11.28), injury (11.20) and sterility (20.60).

The effectiveness and efficiency based on the viable mutants of Co 5, Co(Gg) 7 and VBN(Gg) 3 were calculated based on M_2 seedlings (Table 6 & Fig. 5). In Co 5, the mutagenic effectiveness ranged from 0.42 to 0.90 per cent. The variety Co(Gg) 7 exhibited a range between 0.32 and 0.95 per cent, whereas in VBN(Gg) 3, the mutagenic effectiveness ranged from 0.41 to 1.06 per cent. The highest efficiency of gamma rays in inducing viable mutations based on lethality (20.24), injury (15.25) and survival (35.30) at 550 Gy in Co 5. Then the same pattern also occurred in Co(Gg) 7, the highest efficiency was present at 550 Gy based on lethality (16.74), injury (19.73) and survival (30.43), whereas in VBN(Gg) 3, the efficiency was recorded to be higher at the dose of 550 Gy based on lethality (17.76), injury (17.64) and sterility (32.43). In the current state, the proportion of mutagenic effectiveness and efficiency decreased with the increase in the dose of the mutagen in all three mungbean varieties. Based on lethality, injury and sterility in M_2 generation, it was noticed that a lower dosage of gamma-ray is the most effective and efficient in causing mutations rather than a dosage with a higher concentration in mungbean.

The mutation breeding programme describes a high mutation rate with minimal deleterious effects. Generally, the mutagen that gave the higher mutation rate also induced a high degree of lethality, sterility and other undesirable effects (8). Higher gamma-ray irradiation doses resulted in a broad range of genetic diversity in blackgram plant features. Most of the viable macro mutants were observed in the M_3 generation. Also, it is exposed that it was proper breeding (25). Various morphological mutants are purified for genetic study in future generations and valuable traits for further improvement in mungbean are identified (26). Based on the mutagenic effectiveness and efficiency of gamma rays, they were found to be more effective in mungbean (27). The mutagen induces structural changes in the chromosomes, restricting pairing and resulting in univalence formation. Those structural changes in the chromosomes will affect the sterility of the crop plants. The uninterrupted relationship of pollen and ovule sterility with

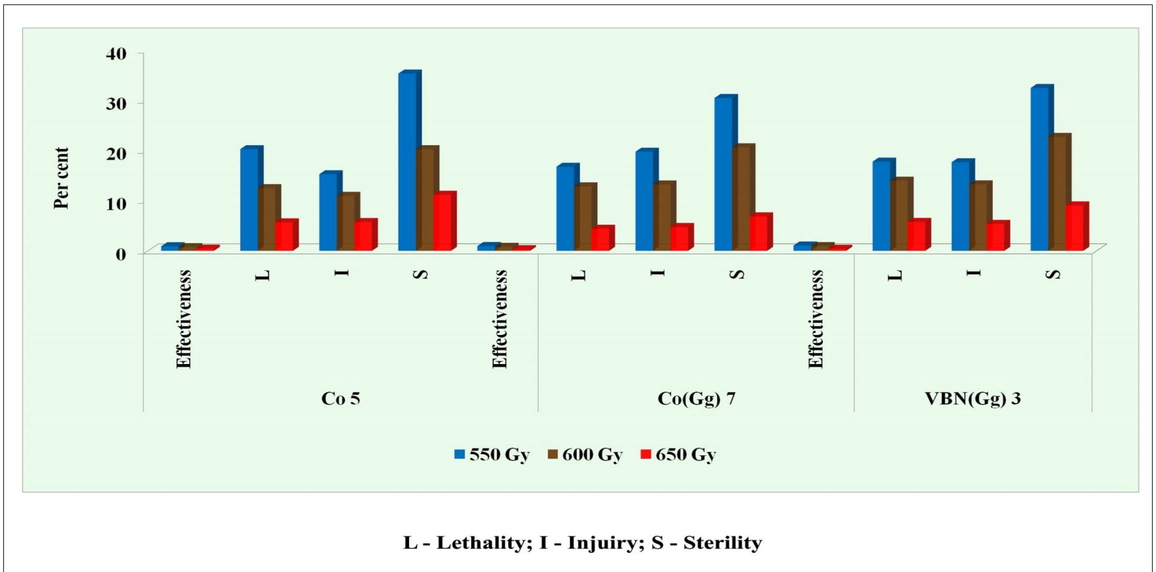

Fig. 5. Mutagenic effectiveness and mutagenic efficiency based on viable mutants in M_2 generation.

higher doses of gamma rays in *Vigna mungo* was reported (28). More than one mutation in a single is entitled as multiple mutations. The various mutations discovered in mungbean and put forth mutagenic agents with higher mutagenic efficiency may accumulate many mutations in a single plant. Multiple mutants are getting attention to generate superior varieties in any crop. Several discoveries were revealed on the effectiveness and efficiency of mutagens based on lethality, injury and sterility in several crop species, including mungbean (29-33).

Conclusion

In a broad sense, mutational events can cause significant or minor phenotypic variations. Gamma-ray was found to be the most effective mutagen for creating genetic variability in the case of mungbean. Since they occasionally provide a desired phenotype, such modifications in macro and micro mutants are of utmost importance in plant breeding. Induced viable mutants have produced a variety of new commercial types that have demonstrated their value in achieving specific breeding goals. This study discovered many useful viable and beneficial mutants in the M_2 generation and it is true-breeding since it was observed in M_3 and later generations. These possibly viable mutant genotypes can be utilized for future breeding programmes in such a way as to obtain an improved variety in mungbean.

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Authors' contributions

SS, AS, DV and JS contributed to the conceptualization and methodology of the study. SS, AS and JS were responsible for the investigation. Resources were provided by SS, AS and DV. Validation was carried out by SS, MD, AS and JS. The base draft of the manuscript was written by SS, MD, ASand JS. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest

Ethical issues: None

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