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Selection of Genotype and Mutagens in M1 Generation for Establishment of Mutation Breeding Programme in Mungbean [*Vigna radiata* (L.) Wilczek]

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A B S T R A C T

An experiment was conducted using seeds of two mungbean verities *viz*., WGG-42 and LGG-460 treated with various doses/ concentrations of gamma rays (200, 300, 400, 500 and 600 Gy) and ethyl methane sulphonate (0.2%, 0.3%, 0.4%, 0.5% and 0.6%) and sodium azide (1 mM, 2 mM and 3 mM) to find the LD_{50} dose by using probit analysis in M_1 generation. LD_{50} dose is important to understand the sensitivity of various genotypes to the critical dose of mutagens creating 50 percent mortality. LD_{50} value for gamma rays in WGG-42 and LGG-460 was 565.63 Gy and 448.33 Gy, respectively. Similarly, LD_{50} value for EMS in WGG-42 and LGG-460 was 0.46% and 0.42%, respectively. Whereas, LD_{50} value for SA in WGG-42 and LGG-460 was 2.72 mM and 2.01 mM, respectively. The effect of mutagens on biological parameters like germination (%), shoot length and root length studied under laboratory conditions in M_1 generation. The germination (%), shoot length and root length decreased progressively with increasing doses of gamma rays, EMS and SA in both the varieties of mungbean. There is a difference of LD_{50} values of mutagens among different varieties which indicated that there is a varied sensitivity for different mutagens on WGG-42 and LGG-460. The variety LGG-460 is found more sensitive to various mutagens when compared to WGG-42.

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] (2n=22) is one of the most important leguminous crops in Asia. It belongs to the family *Fabaceae*. It occupies the third position after chickpea and redgram among legume crops and contributes nearly 15% of the total pulse production and is the cheap source of proteins (24%) and carbohydrates (38-50%) and rich in aminoacids particularly with lysine, minerals and vitamins, thus meeting the dietary needs of the vegetarian population of the country. It is a short duration pulse crop grown in varied conditions across all three crop seasons *viz*., *kharif*, *rabi* and *summer* in different parts of the country, as a sole or intercrop for grain and green manure (Robertson *et al.*, 2004). It is widely cultivated in the tropics and subtropics for human

consumption and animal feed in addition as a cover crop and as supplemented crop in cereal based cropping system (Tah, 2006). It increases soil fertility due to its ability to fix nitrogen together with soil bacteria and it is also relatively tolerant to nutrient deficiency and drought.

In the world, India is the largest producer of mungbean with an area covered 4.07 million hectares, with an average production of 21.65 lakh tonnes and productivity of 467 kg ha^{-1}. In Andhra Pradesh, the area covered under mungbean is about 1.24 lakh hectares with an average production and productivity of 0.82 lakh tonnes and 661 kg ha^{-1}, respectively (Anonymous, 2017-2018).

Though, mungbean is considered to be an important pulse crop in India and also in Andhra Pradesh, the average productivity is 599 kg ha^{-1} against its yield potential of over 1300 kg ha^{-1} (Indiastat, 2016-17). The major constrains in achieving higher yield of this crop are lack of genetic variability, poor harvest index absence of suitable ideotypes for different cropping systems and susceptibility to diseases.

Improvement of any cultivated crops is largely depends on the extent of genetic variability available within the species. In green gram a large part of genetic variability has been eroded due to its continuous cultivation in marginal and sub-marginal land and its adaption to survival fitness rather than yield. Further, hybridization in this crop is difficult due to its small cleistogamous flower (Mishra and Singh, 2014).

The induced mutations have been found quite effective in generating useful variation for polygenically controlled traits. Mutation breeding can, thus, be a valuable supplement to conventional breeding methods in creating variability and particularly for seed yield and its component characters as well as to rectify the minor defects in otherwise agronomically superior variety.

Mutation induction has become an establishment tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al.*, 2002).

Mutation may produce spontaneously or it may be brought about either by using chemical mutagen or by physical mutagen. The rate of spontaneous mutations in nature is very low. Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid *et al.*, 2000). Among physical muatgens, gamma rays directly penetrate the plant tissue and are partially ionizing. Depending upon the radiation level, they can damage or modify important components of plant cells and affect the morphology, anatomy, biochemistry and physiology of plants. Among the chemical mutagens EMS generates mostly SNPs. EMS used to produce a high density of point mutations, causing a variety of lesions including nonsense and missense mutations. The effect of treatment with EMS is highly predictable; G: C->A: T transition changes represent the majority of induced mutations in most organisms. Sodium azide (NaN_3) is highly effective in producing mutations in plants and microbial species. Sodium azide is reported to be most efficient and effective in inducing Chlorophyll-deficient, morphological and bio-chemical mutants in barley (Konzak *et al.*, 1965). Sodium azide was found to be effective in

inducing polygenic variability and it could be effectively utilized for yield improvement in mungbean (Lavanya *et al.,* 2011).

The success of mutation using mutagens depends on its dose. Low dose cannot cause mutation and therefore there are no changes in mutated seeds, but high dose can cause death of the mutated seeds, sterility, and other deleterious effect. Thus, we must first determine the LD_{50} (lethal dose), a dose that causes 50% mortality to the seeds or a safe dose where 50% of the seeds can survive. Similarly, the sensitivity of the genotype in a crop species also varies with different mutagens. Hence, understanding the effective dose and the genotype sensitivity to the mutagens is highly useful to establish successful mutation breeding programme in a cost effective way. Therefore, this study was carried out to find LD_{50} values for various mutagens by using probit analysis in addition to select the effective genotype and dose of various mutagens.

Materials and Methods

Dry, healthy and uniform sized seeds of two mungean genotypes *viz*., WGG-42 and LGG-460 were subjected to one physical mutagen *i.e*. gamma irradiation at the doses of 200, 300, 400, 500 and 600 Gy and two chemical mutagens *viz*., ethyl methane sulphonate (EMS) at 0.2%, 0.3%, 0.4%, 0.5% and 0.6% concentrations and sodium azide (SA) at 1 mM, 2 mM and 3 mM concentrations. For each dose of physical and chemical mutagens, 75 seeds were treated, to fix the LD_{50} value in the laboratory. Seeds were pre-soaked for 6 h in water initially (Malarkodi, 2008). Then, the seeds were immersed for 6 h in the requisite concentration of mutagen ethyl methane sulphonate and sodium azide with intermittent shaking. To ensure a uniform absorption of the mutagen, the volume of mutagen solution was maintained at 10 times proportion to that of the seed volume. The whole treatment was carried out at a room temperature of 28±1°C for 4 h after washing in running water and untreated seeds were used as control.

The treated seeds of gamma rays, ethyl methane sulphonate and sodium azide and control seeds were kept in petriplates for germination following CRD with three replications to determine the LD_{50} dose.

Germination of seeds was carefully observed every day and the emergence of radicle, which was taken as an index for germination was recorded by counting the number of seeds germinated after 5 days of sowing in each petridish and the percentage of germination was calculated as follows:-

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germination}}{\text{Total number of seeds kept for germination}} \times 100$$

Based on the germination percentage (%), the LD_{50} values for the three mutagens *viz*., gamma radiation, EMS and SA for the two genotypes *viz*., WGG-42 and LGG-460 were determined by using probit analysis (Finney, 1971, 1978). The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

Length of the shoot from cotyledonary node to the tip of the shoot, root length from the cotyledonary node to tip of primary root were also measured in cm on 5^{th} day after placing seeds in each petridish for each treatment for each genotype. The data were analyzed as per the standard procedure (Panse and Sukatme, 1961) to understand the effect of the variety and mutagen doses for these parameters.

Results and Discussion

Estimation of lethal dose (LD_{50})

In the present investigation, two mungbean genotypes namely WGG-42 and LGG-460 were selected to study the effect of different doses of gamma rays, EMS and SA to determine LD_{50} dose. The LD_{50} values were determined with the help of probit analysis for the physical and chemical mutagens used based on their germination of both varieties. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. The results obtained for observed mortality percentage for gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA) treated population of WGG-42 and LGG-460 are presented in Table 1 and 2, respectively. The LD_{50} value for gamma rays in WGG-42 was 565.63 Gy and in LGG-460 it was 448.33 Gy (Figure 1 and 2). Similarly, the LD_{50} value for EMS in WGG-42 was 0.46% and in LGG-460 it was 0.42% (Figure 3 and 4) and LD_{50} value for SA in WGG-42 was 2.72 mM and in LGG-460 it was 2.01 mM (Figure 5 and 6). These results indicated that the variations in LD_{50} value between both the genotypes were observed in case of gamma ray treatment. Variations in LD_{50}, between different varieties of same species is a common phenomenon in mutation study as the resistance offered by the biological material mainly depends on size, maturity and moisture content at the time of treatment. Such variations in LD_{50} were also observed by Tah (2006) in mungbean and Jain and Khandelwal (2008) in blackgram. Similarly, minor differences were also observed in LD_{50} dose between both the genotypes incase of EMS and SA treatments. These minor differences might be due to the similarity in physical characters of the seeds in both the varieties. Similar results were also observed by Surender and Vanniarajan (2014) and Veni *et al.,* (2016) in blackgram.

Effect of mutagens in M_1 generation

Effect of different treatments of gamma rays, ethyl methane sulphonate and sodium azide on biological parameters such as germination percentage, shoot length and root length under laboratory conditions in M_1 generation was also studied and presented as follows.

Germination percentage (%)

The data on seed germination percentage in M_1 generation for various mutagenic treatments in both the genotypes under laboratory conditions are presented in Table 3. In all the treatments of gamma rays, EMS and SA, the germination percentage recorded was lesser than their respective control. Germination percentage was found to decrease with an increase in dose/ concentration of the mutagens in both the varieties. In the variety WGG-42, germination percentage in gamma ray irradiated population ranged from 44.67 (600 Gy) to 86.67% (200 Gy). While, in EMS treated population it ranged from 36.67 (0.6%) to 88.89% (0.2%). Similarly, in SA treated population it ranged from 45.33 (3 mM) to 86.11% (1 mM). In case of LGG-460, the germination percentage ranged from 28.67 (600 Gy) to 92.00% (200 Gy) in gamma ray irradiated populations and in EMS treated population it ranged from 27.33 (0.6%) to 82.00% (0.2%). Similarly, in SA treated population it ranged from 31.67 (3 mM) to 74.00% (1 mM).

In the variety WGG-42, per cent reduction in germination percentage in gamma rays irradiated population ranged from 10.65 (200 Gy) to 53.95 (600 Gy) and in EMS treated population it ranged from 8.36 (0.2%) to 62.20 (0.6%). Similarly, in SA treated population it ranged from 11.23 (1 mM) to 53.27 (3 mM). In case of LGG-460, per cent reduction in germination percentage in gamma rays irradiated population ranged from 6.12 (200

Gy) to 70.74 (600 Gy) and in EMS treated population it ranged from 16.33 (0.2%) to 72.11 (0.6%). Similarly, in SA treated population it ranged from 24.49 (1 mM) to 67.68 (3 mM).

In WGG-42, reduction in germination percentage was more in EMS treatments as compared to gamma rays and SA treatments, whereas in LGG-460 the reduction was more in EMS treatments as compared to gamma rays and SA treatments.

In the current study, germination percent decreased with increase in the dose/concentration of mutagenic treatments in both the varieties. A greater reduction was observed at higher dose in both the varieties. However, germination was less affected by a lower dose/concentration of mutagens. Such a dose dependent decrease in germination percentage was also observed by Nandanwar *et al.,* (2001), Khan and Wani (2006), Mori *et al.,* (2016) and Rukesh *et al.,* (2017) in mungbean. The decrease in germination at higher doses of the mutagen treatment might be due to the disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both (Singh *et al.,* 1997). Delay in the one set of mitosis (Yadav, 1987) and chromosomal aberrations, enzyme activity such as catalyse lipase and hormonal activity results in reduced germination (Ananthaswamy *et al.,* 1971).

Shoot length (cm)

Effect of various mutagens on shoot length of WGG-42 and LGG-460 in M_1 generation under laboratory condition was presented in Table 4. In all the mutagenic treatments, the mean shoot length was lesser than their respective control. An increase in dose/concentration of the mutagens led to a decrease in shoot length in both the genotypes.

In WGG-42, the mean shoot length ranged from 5.63 (600 Gy) to 16.53 cm (200 Gy) in gamma ray treatments, while 8.52 (0.6%) to 15.20 cm (0.2%) in EMS treatments and 12.45 (3 mM) to 15.51cm (1 mM) in SA treatments.

In case of LGG-460, the shoot length ranged from 13.59 (600 Gy) to 20.77 cm (200 Gy) in gamma ray treatments, while 10.70 (0.6%) to 16.03 cm (0.2%) in EMS treatments and 11.45 (3 mM) to 15.41 cm (1 mM) in SA treatments.

In WGG-42, the per cent reduction in shoot length ranged from 7.84 (200 Gy) to 68.60 (600 Gy) in gamma ray treatments, while it is ranged from 15.27 (0.2%) to 52.53 (0.6%) in EMS treatments and 13.56 (1 mM) to 30.58 (3 mM) in SA treatments. In case of LGG-460, the per cent reduction in shoot length ranged from 2.64 (200 Gy) to 36.27 (600 Gy) in gamma ray treatments, while, it is ranged from 24.83 (0.2%) to 49.84 (0.6%) in EMS treatments and 27.77 (1 mM) to 46.30 (3 mM) in SA treatments. In WGG-42, reduction in shoot length was more in gamma rays treatments as compared to EMS and SA treatments, whereas in LGG-460 reduction was more in EMS treatments as compared to SA and gamma rays treatments.

In the present investigation, the reduction in the shoot length was caused by the higher dose/ concentrations of physical as well as chemical mutagens, while lower doses of physical and chemical mutagens exhibited higher shoot length. Similar results have also been reported by Kumar and Mishra (1999), Singh and Kole (2005), Lavanya *et al.,* (2011) and Kuldeep and Singh (2013) in munbean. Reduction in shoot length might be attributed to changes in levels of auxin and ascorbic acid and to physiological and biochemical disturbance or chromosomal aberrations, changes in enzymatic activity and impaired mitosis in the meristemic zone of growing seedlings.

Table.1 Probit analysis for calculating LD_{50} in WGG-42

		Number of seeds evaluated	Number of plants killed	Observed mortality percentage	Corrected mortality percentage	Log $_{10}$ of doses	Empirical probit unit
Control		75	3	4.00	-	-	-
Gamma rays	**200 Gy**	75	10	13.33	9.72	2.30	3.70
	300 Gy	75	19	25.31	22.22	2.48	4.24
	400 Gy	75	25	33.33	30.55	2.60	4.49
	500 Gy	75	36	48.00	45.83	2.70	4.90
	600 Gy	75	41	54.66	52.77	2.80	5.07
Ethyl methane sulphonate (EMS)	**0.2%**	75	9	12.00	8.33	-0.70	3.62
	0.3%	75	20	26.66	23.61	-0.52	4.28
	0.4%	75	35	46.67	44.44	-0.40	4.86
	0.5%	75	44	58.66	56.94	-0.30	5.17
	0.6 %	75	48	64.00	62.50	-0.22	5.32
Sodium azide (SA)	**1 mM**	75	11	14.64	11.11	0.00	3.78
	2 mM	75	29	38.66	36.11	0.30	4.64
	3 mM	75	42	56.65	54.16	0.48	5.10

Table.2 Probit analysis for calculating LD_{50} in LGG-460

		Number of seeds evaluated	Number of plants killed	Observed mortality percentage	Corrected mortality percentage	Log $_{10}$ of doses	Empirical probit unit
Control		75	2	2.66	-	-	-
Gamma rays	**200 Gy**	75	6	8.00	5.48	2.30	3.40
	300 Gy	75	19	25.33	23.28	2.48	4.27
	400 Gy	75	34	45.30	43.83	2.60	4.84
	500 Gy	75	44	58.66	57.53	2.70	5.19
	600 Gy	75	53	70.66	69.86	2.80	5.52
Ethyl methane sulphonate (EMS)	**0.2%**	75	14	18.64	16.43	-0.70	4.02
	0.3%	75	18	24.00	21.91	-0.52	4.23
	0.4%	75	37	49.33	47.94	-0.40	4.95
	0.5%	75	46	61.31	60.27	-0.30	5.26
	0.6 %	75	54	72.00	71.23	-0.22	5.56
Sodium azide (SA)	**1 mM**	75	20	26.66	24.65	0.00	4.31
	2 mM	75	35	46.67	45.20	0.30	4.88
	3 mM	75	52	69.33	68.49	0.48	5.48

Table.3 Effect of mutagen on germination percentage (%) in M_1 generation of WGG-42 and LGG-460

Treatments	WGG-42			LGG-460		
	Germination Percentage (%)	Per cent over control	Per cent reduction	Germination Percentage (%)	Per cent over control	Per cent reduction
Control	97.00	100	-	98.00	100	-
Gamma rays						
200 Gy	86.67	89.35	10.65	92.00	93.88	6.12
300 Gy	74.00	76.29	23.71	74.00	75.51	24.49
400 Gy	66.33	68.38	31.62	54.67	55.79	44.21
500 Gy	52.33	53.95	46.05	40.33	41.15	58.85
600 Gy	44.67	46.05	53.95	28.67	29.26	70.74
Mean	**64.80**	**66.80**	**33.20**	**57.93**	**59.12**	**40.88**
Ethyl methane sulphonate (EMS)						
0.2%	88.89	91.64	8.36	82.00	83.67	16.33
0.3%	73.33	75.60	24.40	75.56	77.10	22.90
0.4%	53.00	54.64	45.36	50.67	51.70	48.30
0.5%	41.33	42.61	57.39	38.67	39.46	60.54
0.6 %	36.67	37.80	62.20	27.33	27.89	72.11
Mean	**58.68**	**60.49**	**39.51**	**54.84**	**55.96**	**44.04**
Sodium azide (SA)						
1 mM	86.11	88.77	11.23	74.00	75.51	24.49
2 mM	62.22	64.14	35.86	53.33	54.42	45.58
3 mM	45.33	46.73	53.27	31.67	32.32	67.68
Mean	**64.56**	**66.55**	**33.45**	**53.00**	**54.08**	**45.92**

Table.4 Effect of mutagen on shoot length (cm) in M_1 generation of WGG-42 and LGG-460

Treatments	WGG-42			LGG-460		
	Shoot length (cm)	Per cent over control	Per cent reduction	Shoot length (cm)	Per cent over control	Per cent reduction
Control	17.94	100	-	21.33	100	-
Gamma rays						
200 Gy	16.53	92.16	7.84	20.77	97.36	2.64
300 Gy	15.40	85.84	14.16	19.03	89.23	10.77
400 Gy	12.80	71.35	28.65	17.05	79.93	20.07
500 Gy	7.65	42.62	57.38	15.20	71.26	28.74
600 Gy	5.63	31.40	68.60	13.59	63.73	36.27
Mean	**11.60**	**64.67**	**35.33**	**17.13**	**80.30**	**19.70**
	SE(d): 0.37 CD(0.05): 0.84			SE(d): 0.71 CD(0.05): 1.61		
Ethyl methane sulphonate (EMS)						
0.2%	15.20	84.73	15.27	16.03	75.17	24.83
0.3%	14.41	80.30	19.70	13.70	64.23	35.77
0.4%	11.23	62.62	37.38	12.63	59.23	40.77
0.5%	10.03	55.93	44.07	11.60	54.38	45.62
0.6 %	8.52	47.47	52.53	10.70	50.16	49.84
Mean	**11.58**	**66.21**	**33.79**	**12.93**	**60.63**	**39.37**
	SE(d): 0.36 CD(0.05): 0.83			SE(d): 0.38 CD(0.05):0.86		
Sodium azide (SA)						
1 mM	15.51	86.44	13.56	15.41	72.23	27.77
2 mM	13.55	75.51	24.49	13.55	63.51	36.49
3 mM	12.45	69.42	30.58	11.45	53.70	46.30
Mean	**13.84**	**77.12**	**22.88**	**13.47**	**63.15**	**36.85**
	SE(d): 0.38 CD(0.05): 0.96			SE(d): 0.45 CD(0.05): 1.13		

Table.5 Effect of mutagen on root length (cm) in M_1 generation of WGG-42 and LGG-460

Treatments	WGG-42			LGG-460		
	Root length (cm)	Per cent over control	Per cent reduction	Root length (cm)	Per cent over control	Per cent reduction
Control	12.52	100	-	14.98	100	-
Gamma rays						
200 Gy	12.05	96.27	3.73	14.49	96.71	3.29
300 Gy	10.37	82.80	17.20	14.09	94.04	5.96
400 Gy	8.49	67.78	32.22	13.01	86.87	13.13
500 Gy	5.41	43.24	56.76	11.79	78.73	21.27
600 Gy	3.15	25.13	74.87	9.57	63.86	36.14
Mean	**7.89**	**63.05**	**36.95**	**12.59**	**84.04**	**15.96**
	SE(d): 0.34 **CD(0.05): 0.78**			SE(d): 0.31 CD(0.05): 0.72		
Ethyl methane sulphonate (EMS)						
0.2%	10.20	81.47	18.53	12.29	82.06	17.94
0.3%	9.23	73.75	26.25	10.03	66.98	33.02
0.4%	7.03	56.12	43.88	8.49	56.65	43.35
0.5%	6.10	48.72	51.28	7.36	49.13	50.87
0.6 %	4.33	34.61	65.39	5.93	39.61	60.39
Mean	**7.38**	**58.94**	**41.06**	**8.82**	**58.89**	**41.11**
	SE(d): 0.38 **CD(0.05): 0.86**			SE(d): 0.44 CD(0.05): 1.00		
Sodium azide (SA)						
1 mM	11.89	94.94	5.06	9.95	66.44	33.56
2 mM	8.23	65.76	34.24	8.93	59.64	40.36
3 mM	7.31	58.41	41.59	7.21	48.11	51.89
Mean	**9.14**	**73.04**	**26.96**	**8.70**	**58.06**	**41.94**
	SE(d): 0.26 **CD(0.05): 0.65**			SE(d): 0.39 CD(0.05): 0.97		

Fig.1 Plots of Log doses versus probits for calculation of LD_{50} of gamma radiation in WGG-42

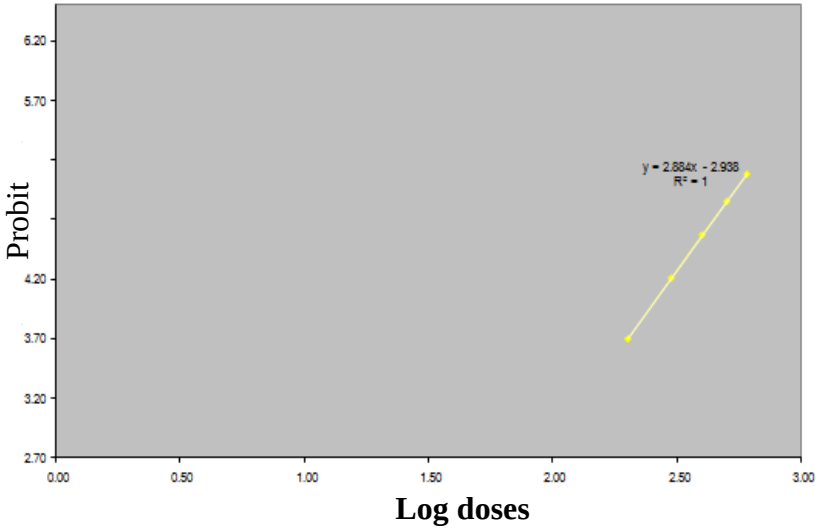


Fig.2 Plots of Log doses versus probits for calculation of LD_{50} of gamma radiation in LGG-460

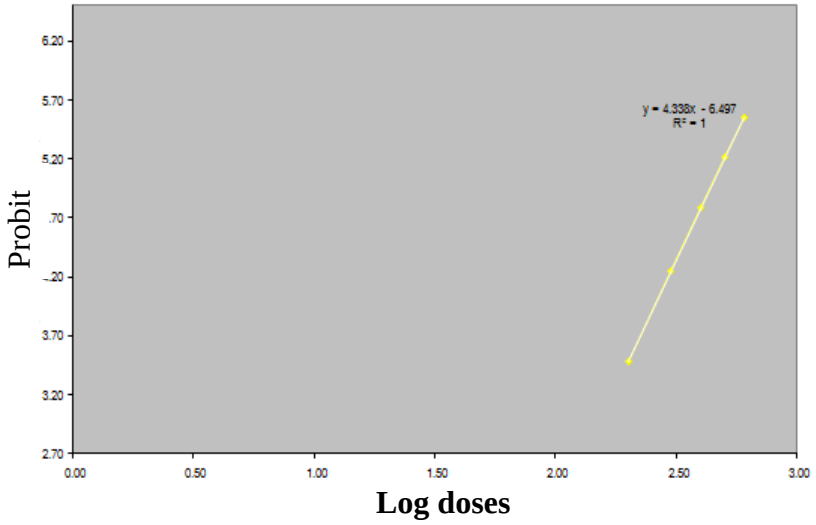


Fig.3 Plots of Log concentration versus probits for calculation of LD_{50} of EMS in WGG-42

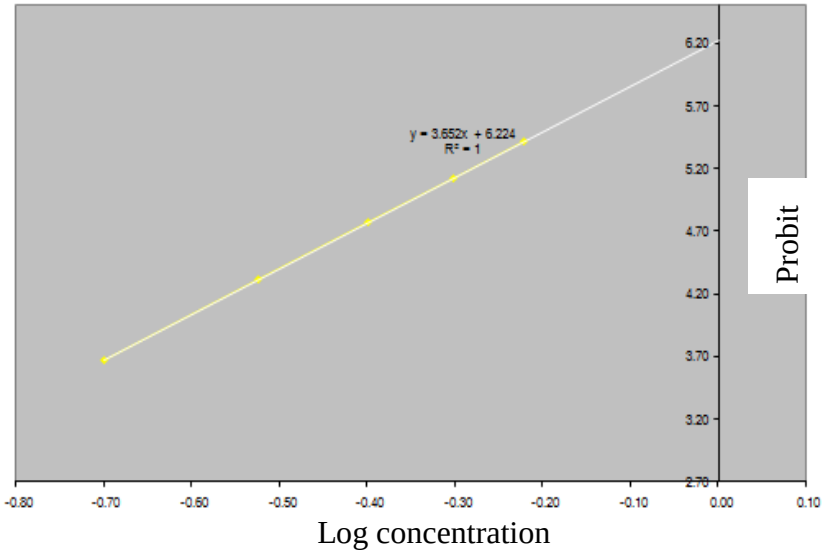


Fig.4 Plots of Log concentration versus probits for calculation of LD_{50} of EMS in LGG-460

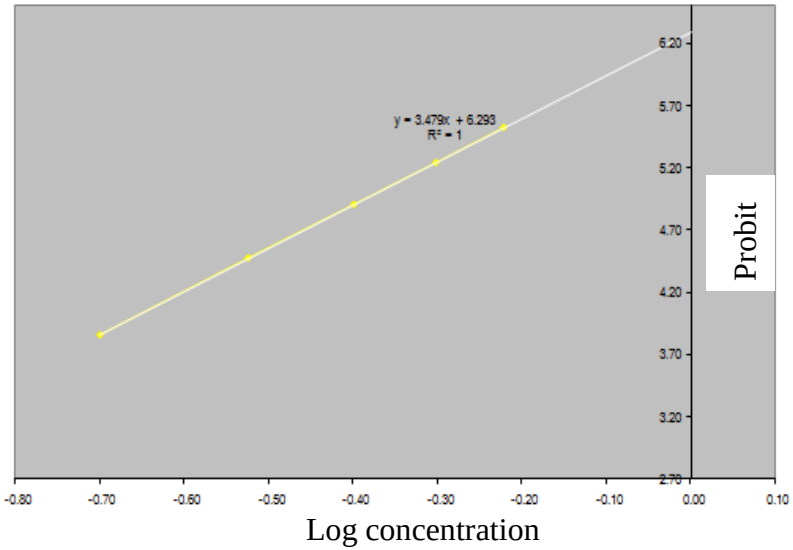


Fig.5 Plots of Log concentration versus probits for calculation of LD_{50} of SA in WGG-42

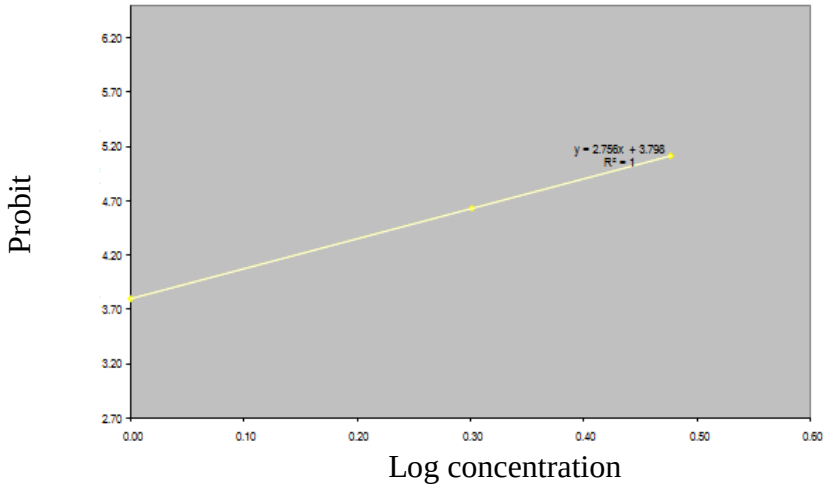
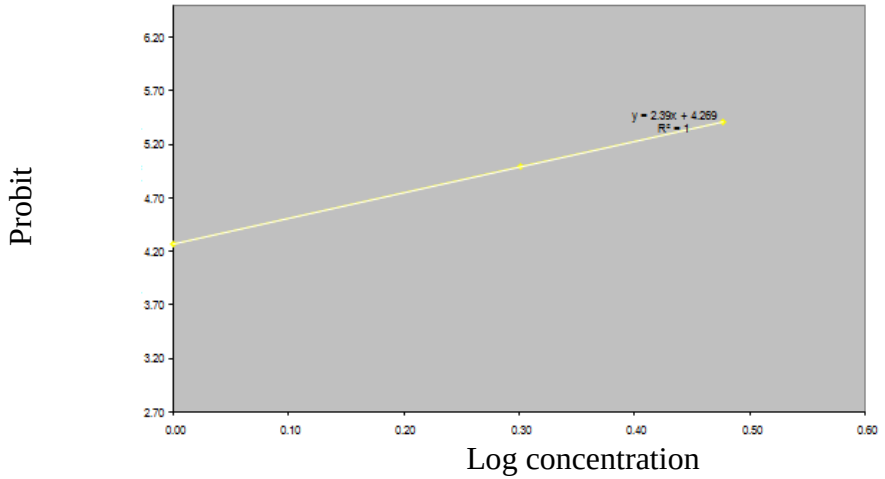


Fig.6 Plots of Log concentration versus probits for calculation of LD_{50} of SA in LGG-460



It might be due to decrease in respiratory quotient in the seedlings obtained from treated seeds. Similar type of findings for plumule length was also reported earlier by Sujay and Singh (2001) and Hemavathy (2015) in munbean.

Root length (cm)

Effect of various mutagens on root length of WGG-42 and LGG-460 in M_1 generation under laboratory condition was presented in Table 5. In all the treatments of gamma rays, EMS and SA the mean root length recorded was lesser than their respective control.

The mean root length reduced progressively with the increasing dose/ concentration of mutagens and a greater reduction was observed at higher dose in both the genotypes. In WGG-42, the mean root length ranged from 3.15 (600 Gy) to 12.05 cm (200 Gy) in gamma ray treatments, while 4.33 (0.6%) to 10.20 cm (0.2%) in EMS treatments.

Similarly, 7.31 (3 mM) to 11.89 cm (1 mM) in SA treatments. In case of LGG-460, the mean root length ranged from 9.57 (600 Gy) to 14.49 cm (200 Gy) in gamma ray treatments, while 5.93 (0.6%) to 12.29 cm (0.2%) in EMS treatments. Similarly, 7.21 (3 mM) to 9.95 cm (1 mM) in SA treatments.

In WGG-42, the per cent reduction in root length ranged from 3.73 (200 Gy) to 74.87 (600 Gy) in gamma ray treatments, while it is ranged from 18.53 (0.2%) to 65.39 (0.6%) in EMS treatments and 5.06 (1 mM) to 41.59 (3 mM) in SA treatments. In case of LGG-460, the per cent reduction in root length ranged from 3.29 (200 Gy) to 36.14 (600 Gy) in gamma ray treatments, while 17.94 (0.2%) to 60.39 (0.6%) in EMS treatments and 33.56 (1 mM) to 51.89 (3 mM) in SA treatments. In WGG-42, reduction in root length was more in gamma rays treatments as compared to EMS and SA treatments, whereas in LGG-460 reduction was more in EMS treatments as compared to gamma rays and SA treatments.

In the present study, higher reduction in root length was observed at higher dose/ concentration of mutagens in both the genotypes. The reduction in root length increased with increase in doses/ concentration of mutagens in both the genotypes. Similar results of reduction in root length were also observed by Kumar and Mishra (1999), Singh and Nalinikanth (2008) and Lavanya *et al.,* (2011). Severe reduction in plumule to radicle length and physiological injuries of radicles indicates the effective use of mutagenesis. Singh and Kole (2005) opined that the root inhibition arises primarily from the effect of mutagens on meristems by arresting the synthesis of growth stimulating auxins and consequent inhibition of cell division.

From the present study, it can be concluded that three mutagens showed an inhibitory effect on germination, shoot length and root length in both the varieties *viz*., WGG- 42 and LGG-460 under laboratory condition in M_1 generation. Increase in dose/concentration of the three mutagenic treatments resulted in decrease of germination percentage (%), shoot length and root length were observed in both the genotypes. The LD_{50} dose based on seed germination (%) for gamma rays was at 565.63 Gy in WGG-42 and for LGG-460 it was 448.33 Gy. Similarly, the LD_{50} value for EMS in WGG-42 and LGG-460 was at 0.46% and 0.42%, respectively. Whereas, the LD_{50} value for SA in WGG-42 was at 2.72 mM and 2.01 mM, respectively. The variety LGG-460 was found more sensitive to various mutagens when compared with WGG-42. These findings would greatly help for cost effective selection of variety and mutagens for successful future mutation breeding programme in mungbean crop improvement.

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