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Response of gamma irradiation on germination and seedling growth of green gram var. GAM 8

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ABSTRACT

Being a pulse crop, green gram has huge scope in crop improvement in terms of productivity and other yield related parameters. Genetic variability is a preferable option for breeders in breeding programme for varietal development programme and others crop improvement aspect. The present investigation was framed to create the variability in mung bean var. GAM 8 during the seedling period through gamma (γ) irradiation. The significant variation was observed in germination percentage and it was found that dose dependent relationship between the germination rate and dose. Significantly minimum germination percentage (22.38 %) was observed in 700 Gy but optimal lethal dose (LD50) calculated through Probit analysis based on germination percentage was revealed at 540.26 Gy. Data revealed that γ irradiation had significantly reduced the seedling growth parameters such as shoot length (10.05 cm), shoot dry weight (19.68 mg), seedling length (13.90 cm), vigour index I (311.01) and II (509.01) was registered in 700 Gy while minimum root length (3.83 cm) was occurred in 600 Gy γ irradiation. Growth reduction (GR) 50 and 30 with respect to the seedling length was occurred respectively at 1093.79 and 1469.74 Gy. Therefore, this finding as the source of genetic variability would be used in future breeding and crop improvement programme like enhancement of yield potentiality and stress management in mung bean var. GAM 8.

Introduction

Green gram [*Vigna radiata* (L.) Wilczek] is commonly known as mung bean and believed to originated from India and Central Asia. This crop is belonging to the subgenus Ceratotropis in the Leguminosae genus *Vigna* and the family Fabaceae. It is self-pollinated legume crop as the petals enclosed the stamen and stigma. Being a legume crops the crop productivity rate is very low as compared to cereals and it has huge scope in genetic enhancement on account of small genome size (579 Mb) (Arumuganathan and Earle, 1991), short life cycle (around 80 days) and close genetic relationship to other legumes (Kim *et al*., 2015). It is good source of protein (24 %) and these types of proteins are easily digested (Yi-Shen *et al*., 2018). Besides this, iron and folate are abundantly present in mung bean. Recently more emphasis has been initiated using molecular breeding approaches by incorporating next generation sequencing technologies (Kumar *et al*., 2021). Researchers are also approaching to

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increase the productivity of mung bean through various techniques including mutation breeding. However, in mung bean normal breeding methods are not much progress in yield enhancement strategy due to low genetic variability. So far use of several bioagents particularly different species of *Trichoderma* have been advocated for not only in enhancement in seed germination and plant growth promotion in different crops (Kumar *et al.*, 2019) but also their capabilities in disease management (Kumar *et al.*, 2013; Kumar and Sahu, 2015; Jain *et al.*, 2017; Kharte *et al.*, 2022), and as biofertilizers (Srivastava *et al.*, 2009). However, limited success has been achieved due to inconsistency of these bioagents in natural field conditions. Mutation is one of the oldest techniques used in crop improvement and has been using various physical or chemical forms of mutagens in this technique. X and γ (gamma) rays are the most commonly use physical mutagens in mutation breeding. It has been revealed that yield improvement through γ irradiation in *Brassica juncea* (Khatri *et al.*, 2005), trait specific enhancement in mung bean (Tah, 2009) and soybean (Pavadai *et al.*, 2010) and even in seed germination and seedling growth variation in chickpea (Shah *et al.*,2008) and mung bean (Bonde *et al.*, 2020) was revealed earlier. Selection of mutagen with their desirable dose relay to the effectiveness in mutation and it is the source of variability. Higher the variability in existing germplasms or varieties is an opportunity for plant breeders for healthier and wider selection and formulate advance breeding programme in varietal development policy. Dose or concentration of mutagen varies with varieties; median lethal dose (LD50) is an important tool to fix the concentration of mutagen in mutation breeding. LD50 indicate that optimum dose of mutagen causing 50% of mortality of seed (maximum variability). For examples, LD50 value of mung bean cv. K-851 and Sona was identified as 54.06 and 53.20 kR, respectively (Tah 2006). Beside this, 50% growth reduction (GR50) is also consider by many researchers to be noted down in mutation (Khalil *et al.*, 2014). Because LD50 value signified only 50% germination but those germinated plants may or may not reach up to maturity level, survival of mutant is very much necessary for proper generation of M1. Therefore, in mutation breeding programme identification of LD50 along with GR50/GR30 is very meaningful. On this background, the present investigation was framed to create variability at seedling stage and determination of LD and GR50 in mung bean var. GAM 8.

Material and Methods

Green gram variety GAM 8 was released from Anand Agricultural University (AAU), Gujarat in the year 2021 and popularly known as Hira Moti. Seed of said mung bean variety were treated with 9 doses of γ irradiation (*viz*., 200, 250, 300, 350, 400, 450, 500, 600 and 700 Gy) at BARC, Mumbai for the estimation of germination percentage and seeding growth parameters as influenced by γ irradiation. The experiment was conducted in *kharif* 2022 at Medicinal and Aromatic Plants Research Station, AAU, Gujarat. The treated seed along with control were sown in germination tray filled with soil and cocopeat (1:1) in triplet repetitions under the open condition. The final germination percentage was recorded at 9th days after sowing (DAS) and those seeds that attained of 0.5 cm plumule length was considered as germination (Maguire, 1962). The said parameter was calculated using the formula given by Anon., (1999).

$$Germination\ percentage = \frac{Total\ No.of\ seed\ germinated}{Total\ no.of\ seed\ sown} x\ 100$$

Moreover, other seedling parameters which included shoot and root length (cm), shoot and root dry weight (mg), seedling length (cm) and vigour index I and II were also assessed at same period. The VI I and II was calculated by determining germination percentage, length and weight of seedling (Ali *et al.*, 2018).

$$Vigour\ index - I = Germination\ percentage\ x\ Seedling\ length$$

$$Vigour\ index - II = Germination\ percentage\ x\ Seedling\ dry\ weight$$

Moreover, from the above germination percentage non-germinated seed was calculated and then it was converted into mortality rate over the control. The corresponding Probit value was generated using Probit table (Robertson *et al.*, 2017) and analysed regression in Microsoft excel sheet. LD50 was calculated using regression equation (Y=mx+C) based on the Probit value. For the calculation of GR50 and GR30, seedling length trait was selected and converted by considered control treatment value as 100%. The method of "Analysis of Variance" was Completely Randomized Design (CRD) and treatment means of all characters studied was further compared by means of critical differences at 5% level of significance employing "F test'. The C.V.% was also worked out using standard statistical analysis given by Mungikar, 1997.

Results and Discussion

Germination percentage: In the present investigation, mung bean treated with γ irradiation was showed significant variation in germination and it was decreased with increased in radiation concentration. Significantly maximum germination percent (86.39 %) was registered under the control while it was minimum in 700 Gy (22.38 %) (Table 1-2). The present finding was contrast with an earlier result that germination was increased with increased in mutation dose in mung bean var. (TARM 1) (Bonde *et al*., 2020). They revealed that maximum germination percentage was occurred in 350 Gy (95.9 %) followed by decreased through dose increased. However, in present finding there was gradual decreased in germination and this phenomenon was same with the result of green gram seed mutated with sodium azide (Lavanya *et al*., 2011) and gamma irradiation (Rukesh *et al*., 2017). The higher in dose may inhibit the metabolic function of the cells resulting in damage of certain cells and even of seed embryo. With the fact that there is increase in chromosomal damage with the increase in dose (Kiong *et al*., 2008) resulting its effects on proteins expression and functioning of cells finally preventing in germination (Cheng *et al*., 2010).

Table 1. Effect of γ irradiation on germination and seedling quality parameters of mung bean var. GAM 8

γ Irradiation	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot dry wt. (mg)	Root dry wt. (mg)	Seedling length (cm)	VI I	VI II
0 (Control)	86.39	15.10	4.90	28.67	3.35	20.00	1728.07	2764.33
200 Gy	80.83	12.40	4.70	21.23	3.30	17.10	1381.62	2764.33
250 Gy	80.02	15.77	4.53	27.00	3.07	20.30	1625.00	2406.82
300 Gy	76.50	16.67	4.90	28.23	3.00	21.57	1650.08	2388.43
350 Gy	75.74	15.13	4.43	24.10	3.23	19.57	1481.66	2069.74
400 Gy	69.16	14.83	4.13	28.17	3.30	18.97	1312.71	2178.19
450 Gy	62.24	12.33	4.27	26.87	3.23	16.60	1033.39	1873.00
500 Gy	57.71	10.63	3.87	24.07	3.15	14.50	835.16	1571.46
600 Gy	47.96	10.10	3.83	23.74	3.11	13.93	667.29	1286.29
700 Gy	22.38	10.05	3.85	19.68	3.11	13.90	311.01	509.01
S.Em±	1.79	0.27	0.14	0.46	0.07	0.30	38.41	61.73
C.D. (5 %)	5.28	0.81	0.42	1.36	NS	0.89	113.30	182.10
CV	4.71	3.56	5.74	3.17	3.96	2.96	5.53	5.62

Table 2. Mean square of various germination related parameters of mung bean var. GAM 8

Source of variation	df	Germination	Shoot length	Root length	Shoot dry wt.	Root dry wt.	Seedling length	VI I	VI II
γ irradiation	9	1119.24	18.68	0.52	28.95	0.039	24.17	673991	1258565
Error	20	9.62	0.22	0.06	0.64	0.015	0.27	4425	11431

#df = Degree of freedom

Estimation of median lethal dose (LD50): The LD50 value of γ irradiation of mung bean var. GAM 8 was analysed based on the non-germinated seed through Probit value analysis. The dose response curve based on Probit value were presented in Fig. 1 and its analysis of variance is presented in Table 3. Descriptive statistical data analysis showed statistically significant differences indicated that changes in the independent variables (dose) correlated with shifts in the dependent variable (germination percentage) and relative strength of different independent variables' effects on dependent variable was moderate, r^2 =0.68 (Fig. 2). Mortality percentage based on non-germinated seed and corresponding Probit value are presented in Table 4. According to the regression equation, in this variable the LD50 was occurred at 540.26 Gy [x= (5.0+2.46)/2.73]. So, this γ irradiation dose (540 Gy) was recognised as the optimum dose with respect to 50% germination percentage using this mutagen in mung bean var. GAM 8. However, it was reported that LD50 value varies with species, genotypes, varieties, types of mutagen and nature of treatment and procedure (Parthasarathi *et al.*, 2020). In previous finding showed that LD50 value as 250 Gy of gamma rays in balck gram var. TNAUCo(Bg)6 (Ramya *et al.*, 2014), 450 Gy in green gram var. CO 6 and CO 8 (Rukesh *et al.*, 2017), 375.52 Gy in green gram var. TARM 1 (Bonde *et al.*, 2020), 5888.4 ppm of EMS mutagen in short day Indian onion cv. Bhima Dark Red (Singh, 2021) and in sesame 53.4 % of survival was found in 450 Gy (Kumari *et al.*, 2016).

Seedling parameters: Analysis of variance of all seedling quality parameters analysed in present investigation except root dry weight was found significant effect as affected by γ irradiation in mung bean var. GAM 8 (Table 1). Significantly better seedling quality parameters was found in control. This was indicated that γ irradiation has significant impact on seedling growth in mung bean var. GAM 8. Shoot length was increased from 200 (12.40 cm) to 300 Gy (16.67 cm) dose of γ irradiation followed by gradually decreased. However, root length was found significantly maximum in control as well as in 300 Gy (4.90 cm) and beyond this concentration root length was found started decreased, highest reduction was found in 600 Gy (3.83 cm). Similarly, it was revealed that shoot dry weight was increased

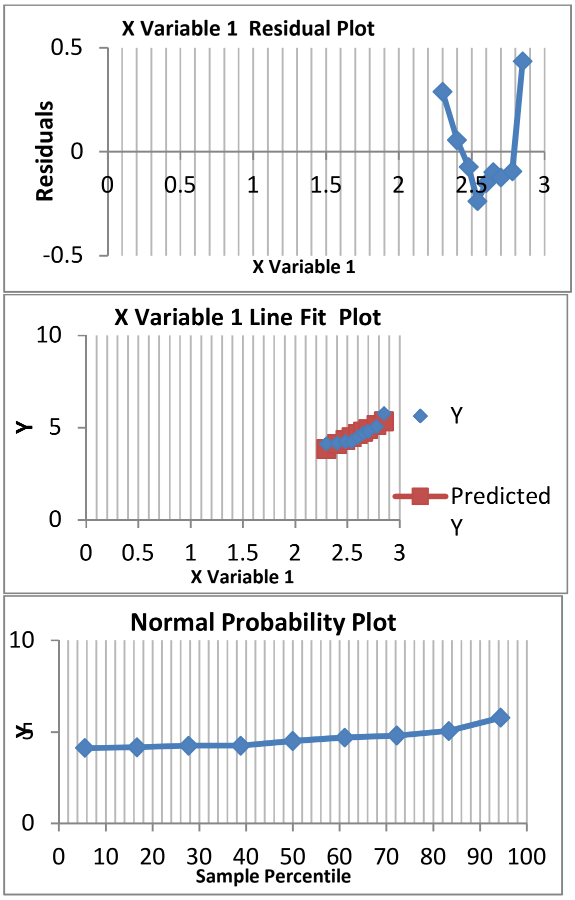

Fig.1. Relation curve of γ irradiation dose and mortality (based on non-germinated seed) of mung bean var. GAM 8 generated through Probit analysis

with increased in dose up to 300 Gy (28.23 mg) after that it was showed reduction in weight. However, maximum shoot dry weight was occurred in control (28.67 mg). Significantly maximum vigour index I (1728) and II (2764) was registered under the control but maximum reduction was found in 700 Gy over the control. The significant reduction of seedling quality parameters during the germination period owing to γ irradiation have been explained by various researchers in different crops. Earlier it was reported that used of less dose mutagen in mutation treatment showed higher in shoot length in sesame (Kumari *et al.*, 2016). Similarly, Bonde *et al.*, 2020 also showed higher in shoot, root and seedling length over the control. However, in present investigation showed contrast of seedling length as the reduction of length was noted in mutated seedling. It could be resulting that whatever dose used in present studies injured to the meristematic tissue as the outcomes of genetic damage or injury.

Table 3. Analysis of variance of probit analysis

Source of variation	df	SS	MS	F	Significance F
Regression	1	1.90012	1.90012	33.72692	0.000658
Residual	7	0.394369	0.056338		
Total	8	2.294489			

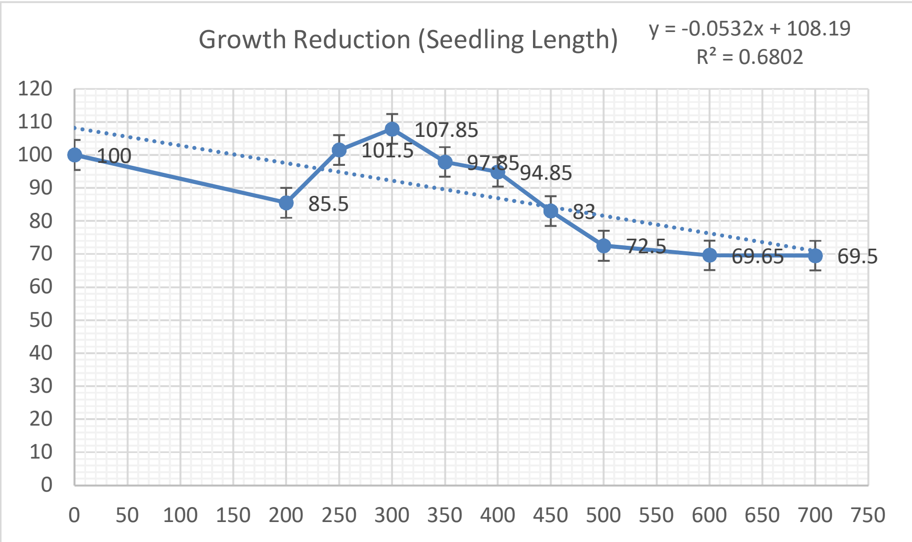

Fig. 2. GR calculation based on converted seedling length value [GR50, x= -58.19/-0.0532 = 1093.79 Gy; GR30, x=1469.74 Gy]

Table 4. Probit analysis for LD50 concentration of γ irradiation on Mung bean var. GAM 8

γ irradiation dose	log10 concentration	Motility percent (Based on non-germinated seed)	Probit value	LD50
200 Gy	2.30	19	4.12	540.26 Gy
250 Gy	2.40	20	4.16	
300 Gy	2.48	24	4.25	
350 Gy	2.54	24	4.25	
400 Gy	2.60	31	4.50	
450 Gy	2.65	38	4.69	
500 Gy	2.70	42	4.80	
600 Gy	2.78	52	5.05	
700 Gy	2.84	78	5.77	

Similar report was also given by Senapati *et al*., 2008 and Ramya *et al*., 2014 in black gram. Further it was showed maximum vigour index (2231.55) in mung bean var. TARM 1 mutated with 450 Gy dose of γ irradiation over the control (1820.72) (Bonde *et al*., 2020). In case of vigour index II declined in various field crops was revealed in spinach, field pea, garden pea and wheat but increased in okra with exposed to γ irradiation (Singh *et al*., 2014). Therefore, response to mutation is directly corresponds to the types of crop and mutagen dose.

Determination of growth reduction (GR50 and GR30) calculation: Variable degree of seedling length response was observed in different γ doses and its converted value by considering control as 100 % is given in Table 5 & 6 and it was used for GR analysis. The maximum reduction of seedling growth length was revealed in 700 Gy (69.5 %).

According to the regression equation shown in Fig. 2 (y =-0.0532x + 108.19), GR50 and GR30 was calculated based on the seedling length and it was occurred at 1093.79 and 1469.74 Gy respectively. The reduction in growth is might be due to the damage of compounds related to the plant metabolism, such as proteins, chlorophyll, auxins and ascorbic acid potentially inhibiting the growth of the seedlings (Kiong *et al.*, 2008).

Table 5. Results of probit analysis as the γ irradiation on mung bean var. GAM 8

Parameter	Coefficients
Intercept	-2.45574
X variable 1	2.733549

[Regression Equation, y=mx+c, x= (5.0+2.46)/2.73 =2.73 = Antilog of 2.73 = 540.26 Gy]

Table 6. Determination of GR50 and GR30 based on converted seedling length value by considering control as 100 %

γ irradiation dose	Seedling length (cm)	Converted value
0 (Control)	20.00	100
200 Gy	17.10	85.5
250 Gy	20.30	101.5
300 Gy	21.57	107.85
350 Gy	19.57	97.85
400 Gy	18.97	94.85
450 Gy	16.60	83
500 Gy	14.50	72.5
600 Gy	13.93	69.65
700 Gy	13.90	69.5

Conclusion

From the above results, it can conclude that γ irradiation treatment in mung bean var. GAM 8 had a positive response in creating variation in terms of germination percentage and seedling growth parameters. The LD50 and GR50 with respect to the germination percentage and seedling length was occurred respectively at 540.26 and 1093.79 Gy. Considering all points in mung bean var. GAM 8 could be create variability with γ irradiation and above said doses can be used by breeders to create new mutant line as well as in crop improvement programme of mung bean.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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