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Lethal dose (LD_{50}) fixation and sensitivity of Fenugreek (*Trigonella foenum-graecum* L.) to gamma radiation for induction of mutation

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop, mainly used as a spice and leafy vegetable crop in many parts of the world. Classical breeding in fenugreek is restricted due to its low genetic variability and small flower size which hamper manual emasculation and pollination. Mutation breeding is an effective way to enrich genetic variability in crop plants. An experiment was conducted to determine the lethal dose of the physical mutagen gamma rays in fenugreek. The dry seeds of fenugreek were exposed to different doses of gamma rays i.e. 150Gy, 200Gy, 250Gy, 300Gy and 350Gy. These irradiated seeds were sown in the Petri plates with non-irradiated seeds (control). As the dose of gamma rays increased, there was a decrease in germination percentage, seedling survival, root length, shoot length and vigour index. Among five doses of gamma rays, the maximum seed germination was observed at lowest dose 150Gy (93%), followed by 200Gy (83%), 250Gy (76%), 300Gy (76%) and 350Gy (64%). The seedling survival was decreased from 90% (in control) to 56% in 350Gy dose of gamma rays. The gamma rays dose of 150Gy gave stimulatory effect on seedlings growth. The growth parameters were dose dependent, as the dose of gamma rays increased from 200Gy to 350Gy. The gamma rays dose of 350Gy showed 64% seeds germination and 56% of seedlings survival. Therefore, it is concluded that the LD_{50} dose for fenugreek is close to 350Gy. This information would be highly useful for initiating mutation breeding programme in fenugreek.

Keywords: Fenugreek, Growth, LD_{50}, Mutagenesis, Seed germination, Seedling survival

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L) is anautogamous annual herbaceous crop, also known as methi, bird's foot and Greek hayseed. The crop belongs to the family Fabaceae and have basic chromosome number $2n$ =16. Fenugreek is a dicotyledonous plant with branched stems, trifoliate leaves, which bear white flowers and produce golden yellow seeds. The crop is native to the Indian subcontinent and the Eastern Mediterranean region, but now it is grown as a spice in most part of the world. It is an important condiment crop grown during winter (*rabi* season) in Northern India. It is also grown for green fodder and vegetable purposes. Fenugreek has huge medicinal properties such as anti-diabetic, cure digestive disorders, lowering blood sugar and cholesterol level, anticancer, antimicrobial etc (Wani and Kumar, 2016). It is also arich source of protein, minerals and vitamins (Leela and Shafeekh, 2008). The extracts of spices including fenugreek are promising sources of alternative medicine with high free radical scavenging ability and can also be used for therapeutic purposes (Choudhary *et al.*, 2017). Fenugreek seeds are considered to be of commercial interest as a source of a steroid diosgenin and sapogenins, which is of importance to the pharmaceutical industry (Mehrafarin *et al.*, 2010; Agarwal *et al.*, 2015). In India, the major area concentrated in Rajasthan and Gujarat. The total area under fenugreek cultivation in India is about 2.27 lakh ha with the production of 2.48 lakh tons. India also exporting the fenugreek seed worth of Rs 233 crore annually (DASD, 2015-16).

Crop improvement in fenugreek has been attempted through selection, hybridization and mutation breeding. The presence of genetic variability is necessary for the crop improvement. The conventional breeding in fenugreek restricted due to lack of genetic variability, the small size of flower that hampers hand emasculation and pollination etc. The variability available to the breeders comes from spontaneous or artificially induced mutations. Since the natural variability present in this crop is very low, hence the alternate approach to generate variability would be an induced mutation. Selection and mutation breeding have been advocated for fenugreek (Basu *et al.*, 2008; Giridhar *et al.*, 2016). The majority of the current improvement on fenugreek has focused on these two approaches. Most of the induced mutations are recessive and can be observed to segregate in a 3:1 genetic ratio in diploid crops. Manha *et al.* (1994) indicated that diosgen in content in species of fenugreek can be increased through mutation breeding. Mutation breeding is important when a desirable character is not available in the germplasm that normally could be used as a source for hybridization and selection. Though the frequency of desirable heritable

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changes from artificial mutagenesis is usually quite low, the probability of success can be increased by screening higher numbers of mutants for the desired morphological traits (Basu *et al*., 2008; Verma *et al*., 20017a). Several approaches can be used to generate variability, however, use of ionizing radiation requires special facilities to reduce the risk to human health and to avoid uncontrolled chromosome breakage.

In crop improvement by induced mutation, it is important to determine a suitable dose/concentration of mutagen for a particular crop and variety, which can be employed for inducing maximum variability. Induced mutation by use of either physical or chemical mutagen is one way of creating variation in crop plants. The physical mutation comprises of ionising radiation viz., particulate (alpha rays, beta rays, fast neutrons and thermal neutrons) and non-particulate also called as electromagnetic radiation (X-rays and gamma rays). The Gamma rays have been widely used for the improvement of various traits of crops (Piri *et al*., 2011). According to Kovács and Keresztesa (2002), gamma rays are considered in most penetrating in comparison to other radiations. Gamma rays are electromagnetic radiation that initiates and inhibit the growth of the plant. Gamma rays interact with cell internal component and release free radicals and these free radicals either damage or modify the differentiation process, morphology, physiology and bioactive components depending on applied dose. Gamma rays are known to affect the plant growth and development by inducing cytological, genetical, biochemical, physiological and morphological changes in the cell and tissues. Whereas, a low dose can enhance the physiological activities of cells in the plant by ameliorating germination and plant growth rates increase stress resistance and improve crop yield. Since fenugreek is a predominantly self-pollinated crop and determinate trait by recessive genes (Malhotra, 2011) we believe that mutation breeding can be used to generate mutant plant with determinate growth habit without losing beneficial adaptations and other agronomic traits in the base of population. The success of mutation using gamma rays depends upon its dose. The mutagenic effectiveness decreased with the increase in dose of mutagen indicative of anegative relationship between them. A low dose of mutagens may not cause mutation hence, there are no changes in mutated seeds, but higher dose can cause the death of the mutated seeds, sterility and other deleterious consequences. Therefore, it is necessary to first determine the LD_{50} (lethal dose) that cause 50% mortality to the seeds or a safe dose where 50% of seeds can survive. LD_{50}differs between species and varieties of the crops. Mutagenic efficiency was highest at the lowest dose and it decreased with the increase in dose (Ganapathy *et al*., 2008). Therefore, optimizing the optimum dose of gamma irradiation is very important to generate the desired variability in plants.

MATERIALS AND METHODS

Plant materials and mutagen treatment

The well dried, fully matured, disease and insect free with auniform shape, size and colour, fenugreek seeds (cv-AFg-3) were collected from ICAR- National Research Centre on Seed Spices, Ajmer, Rajasthan. The seeds of fenugreek were irradiated at Bhabha Atomic Research Centre (BARC), Mumbai. The seeds were placed in gamma chamber and exposed to different doses of gamma irradiation viz. 150Gy, 200Gy, 250Gy, 300Gy and 350Gy using ^{60}Co as a radiation source. Non- irradiated dry seeds were taken as control.

Growing of M_1 plants and growing condition

The experiment was carried out in three replicates in Petri dishes on filter paper bed to check the germination and survival percentage. Each replication contains 30 seeds and germinates on filter paper imbibed in distilled water. Distilled water was constantly applied twice a day. All the sprouted seeds were considered as germinated either the resulting seedlings were normal or abnormal.

Observations recorded

Following observations were recorded:

Per cent seed germination: Seed germination was recorded treatment wise on the day when the control showed 100 percent germination. The emergence of plumule and radicle was considered as germination.

Per cent seed germination = (Number of germinated seeds / Total number of seeds) X 100

Per cent survival: The per cent survival was calculated using the formula given below:

$$\text{Per cent survival} = \left(\frac{\text{Number of plants survived (after 30 DAS)}}{\text{Total number of seeds}}\right) \times 100$$

Seedling height (cm): Seedling height was recorded from randomly selected seedlings per replication.

Root-length (cm): Root length was recorded from randomly selected seedlings per replication.

Shoot Length: Shoot length was recorded from randomly selected seedlings per replication.

The Seedling Vigor Index (SVI): Seedling Vigor Index was calculated by using follow ingformula.

VI = root length + shoot length × germination percentage

Statistical analysis

The recorded data were analyzed statistically using OPSTAT software provided online by CCS Haryana Agriculture University, to draw inferences and the results were interpreted accordingly. The graphs were prepared by using Microsoft Office Excel- 2007.

RESULTS AND DISCUSSION

Seed germination and seedling survival affected by different doses of gamma rays

The data presented in Fig. 1 shows a reduction in seed germination and plant survival with the increase in dose of gamma raysin fenugreek. The germination was recorded on 2nd, 4th and 6th days after sowing. Data presented in Table 1 shows that the stimulation in seed germination with anincrease in gamma rays dose of 150Gy after 2 days to 6 days of sowing. The result showed that increased doses of gamma irradiation significantly affected the mean germination as compared to control treatment. The seed germination on 2nd days of sowing was maximum (24) at 150Gy followed by control (23). After 150Gy the seed germination was reduced as the gamma rays dose increased as compared to control and lowest (18) germination were recorded at a highest dose 350Gy followed by 250Gy (19), 300Gy (20) and 200Gy (22). At the 6th day of sowing, all seed were germinated and no further seed germination was recorded in any treatment. The maximum seed germination was recorded on 150Gy (28), followed by 200Gy (25), 250Gy (23), 300Gy (23) and 350 Gy (20).A perusal of data presented in Fig. 1 with regards to germination percent and survival percent. Seed germination was 96% in control while, among the different doses of irradiation, maximum seed germination was noticed at a lowest dose 150Gy (93%), 200Gy (83%), 250Gy (76%), 300Gy (76%) and 350Gy (64%). The survival of seedlings was also found to be decreasing with increase in dose of gamma rays in fenugreek. The maximum seedlings survival was recorded at lowest (86%) dose of gamma rays (150Gy) while as the dose of gamma rays increased the seedlings survival decreased. The highest dose 350Gy was recorded minimum (56%) seedlings survival.

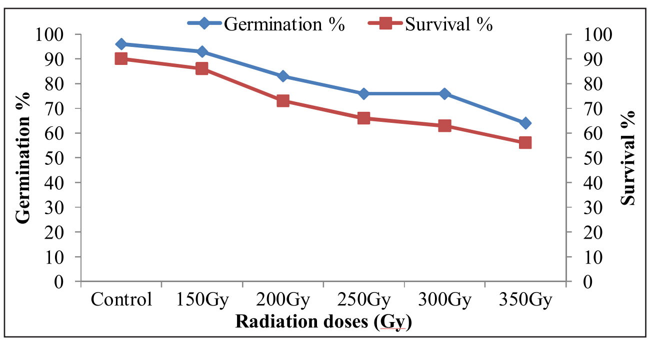

Fig. 1: Effect of exposure to gamma rays on seed germination and seedling survival in fenugreek

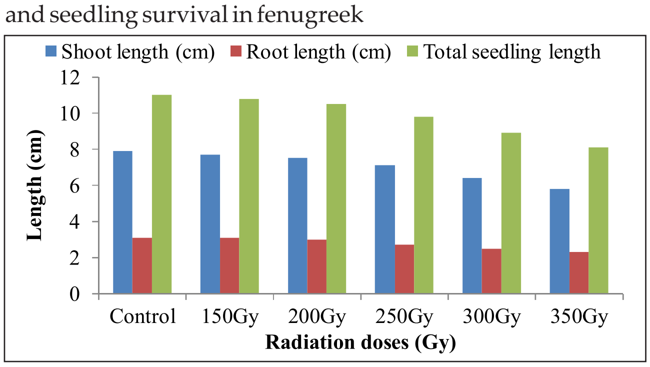

Fig. 2: Effect of gamma rays on shoot length, root length and total seedling length in fenugreek

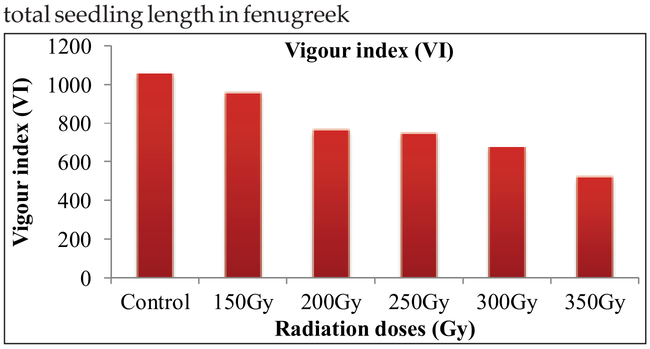

Fig. 3: Vigour index of fenugreek seedlings influenced by different doses of gamma rays.

Table 1: Effect of different doses of gamma rays on germination rate of fenugreek seeds after each 48hr till 6 days of sowing

Treatment	No. of germinated seeds		
	2 days	4 days	6 days
Control	23	27	29
150 Gy	24	28	28
200 Gy	22	24	25
250 Gy	19	22	23
300 Gy	20	21	23
350 Gy	18	20	20

The relationship between the dose of gamma rays and germination percentage in fenugreek was inversely proportional. As the dose of gamma rays increases the germination and survival rate decrease. The decrease in seed germination at a high dose may be attributed to the damage of cell constituents at the molecular level or due to altered enzymatic activity. Micco *et al.* (2011) have correlated the decrease in seed germination with abnormalities in mitotic cycles and in the metabolic pathway of the cell. Higher exposures were usually inhibitory, whereas some authors refer to the concept of hormes is, the stimulation of different biological processes (e.g. faster germination, increased growth of roots and leaves), that occurs when seeds are subjected to pre-irradiation with low doses of a radiation source (Zimmermann *et al.*, 1996; Thapa, 1999). The stimulating causes of gamma-ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated with a low dose (Abdel-Hady *et al.*, 2008; Mohajer *et al.*, 2014).This could be due to the enhanced rate of respiration or auxin metabolism in seedlings. The inability of seeds to germinate at higher doses of gamma-rays has been attributed to disruption and disorganisation of the tunica or seed layer that is directly proportional to the intensity of exposure to gamma-rays. Bashir *et al.* (2013) also reported that the germination percentage and percent survival decreased with an increase in dose/concentration of the mutagens in fenugreek. They concluded that lower treatments of mutagens have influenced less biological damage and would be suitable for inducing desirable mutations. The decline in germination and survival percent may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovács and Keresztes, 2002). Some of the seedlings were germinated but they died in further growth due to the injury caused by gamma rays. In a study, it was found that mutagenic treatment severely affected germinability in M_1 generation (Basu *et al.*, 2008; Siddiqui *et al.*, 2008) in fenugreek. The relationship between mutagenic dose and germination percentage was inversely proportional has been recorded by Sarada *et al.* (2015) in coriander; Sikder *et al.* (2013) in tomato; Verma *et al.* (2017b) in cumin and Verma *et al.* (2017c) in fennel. The inhibition of seed germination at higher doses may be due to injurycaused in seed tissue, chromosomes and subsequent mitotic retardation and the severity of the injury depend on the doses/concentration of mutagens used (Preussa and Britta, 2003; Piri *et al*., 2011; Verma *et al*., 2017c).

Seedlings growth of fenugreek affected by different doses of gamma rays

It is evident from the Fig. 2 and 3 that gamma rays had a highly significant impact on shoot length, root length, total seedling length and seedling vigour index. The results presented in Table 2 revealed that at 6th days of sowing the shoot length increased (3.6 cm) in the dose of 150Gy as compared to control (3.3 cm). While the shoot length was decreased with increased in irradiated doses and maximum increased were recorded at the dose of 200Gy (2.8 cm) followed by 250Gy (2.6 cm), 300Gy (2.6 cm) and 350Gy (2.4 cm) when compared to control. Similar trends were recorded till 14h days of sowing. From the data recorded, it was observed that gamma rays had a highly significant impact on root length (Table 3).

Table 2: Effect of different doses of gamma rays on shoot length of fenugreek

Treatment	Shoot length (cm)				
	6 days	8 days	10 days	12 days	14 days
Control	3.3	4.9	5.8	7.0	7.9
150 Gy	3.6	5.0	6.2	7.1	7.5
200 Gy	2.8	3.6	4.4	6.2	7.2
250 Gy	2.6	3.4	4.0	5.5	7.1
300 Gy	2.6	3.3	3.8	5.2	6.4
350 Gy	2.4	2.9	3.6	4.9	5.8

Likewise, shoot length, root length was higher (1.9 cm) at 200 Gy (2.0 cm) and 150 Gy and compared to control (1.8 cm) on 6th days of sowing. After 200Gy the root length was decreased in irradiated plants. The root length was higher as compared to control on 8th, 10th and 12th days of sowing at the dose of 150 Gy. The data presented in Fig. 2 signifies mean value of shoot length, root length and total seedling length after 14th days of sowing. According to the results obtained in this study shoot length, root length, total seedling length and vigourindex decrease significantly in proportion with the increase in applied dose of gamma rays. After 14th days the lowest shoot length was observed 5.8 cm at 350Gy whereas, the highest shoot length was observed 7.7cm at 150Gy. In control, the shoot length was observed 7.9cm after 14 days. These doses are grouped and called as inhibitory (negative growth). The root length was also recorded at different interval till 14th days. The root length also decreased with increase in doses of gamma irradiation. After 14th days the lowest root length was observed 2.3cm at 350Gy and highest root was observed 3.1cm at 150Gy which was at par with control (3.1 cm). The vigour index decreased with the increased in the dose of gamma rays when compared to control. Maximum (957.9) vigour index was recorded at the lowest dose (150Gy) of gamma rays. Highest dose (350Gy) of gamma rays recorded minimum vigour index (518.4).LD_{50} (the safe dose at which half of the planting material survive) was calculated on the bases of 50% reduction of seed germination, seedling survival as well as reduced in seedling growth parameters. The investigation exhibited that survival percentage of fenugreek decrease with the increase in the doses of gamma rays were used to find out the value of LD_{50}. The result indicated that the average survival percentage decreased with increasing the doses of gamma irradiation. The LD_{50} was closed to 350 on which 64 percent seed germination, 56% seedlings survival and a considerable reduction in seedlings growth were recorded.

Table 3: Effect of different doses of gamma rays on root length of fenugreek

Treatment	Root length (cm)				
	6th days	8th days	10th days	12th days	14th days
Control	1.8	2.6	2.7	2.9	3.1
150 Gy	1.9	2.9	3.0	3.1	3.1
200 Gy	2.0	2.7	2.8	2.9	3.0
250 Gy	1.2	2.3	2.6	2.7	2.7
300 Gy	1.7	2.3	2.5	2.7	2.5
350 Gy	1.1	2.1	2.5	2.6	2.3

The result shows that the shoot length and root length fenugreek at 150Gy and 200Gy treatment were not significantly different from control treatment but at 250Gy treatment was moderately and 350Gy treatment show drastic different from control. The shoot length and root length are the most important parameter because root is in direct contact with water and shoot supply it to the rest of the plant. Our results lend support from the report of earlier workers. The inhibition of plant growth by gamma- rays may be related to auxin and DNA synthesis. The primary radiation block auxin synthesis the auxin is requiring for the formation of nucleic acid. The high dose of exposure that caused growth retardation could be attributed to the cell cycle arrest during somatic cell division and/or various damages in the entire genome (Preussa and Britta, 2003). In one more report the higher efficiency of a lower dose of gamma rays could be described to the fact that seedling injury, lethality and sterility increased with increase in mutagens dose at a faster rate than the mutations. Inhibition effect by gamma-irradiation may be related to auxin and DNA biogenesis in a relationship which showed that DNA is required for and is previously synthesized sequentially to auxin formation, the radiation block occurring in the formation of nucleic acid. The primary radiation block is in auxin synthesis, the auxin required for the formation of DNA.

The effect of radiation is an undefined entity in reaction essential for both DNA and auxin synthesis (Krasaechai and Yu, 2009). Plants growth and development do not proceed at constant or fixed rates during the completion of life cycle. Plant development is a term that includes a broad spectrum of processes by which plant structures originate and mature as growth occurs. Any change in growth pattern will ultimately affect maturity and yield. Gamma irradiated seed has been found to exert pronounced effects on plant growth and yield (Verma *et al*., 2017 b and c). The gamma irradiation can have stimulatory effects on some phenotypic characters and can enhance the yield of plants in terms of growth and economic yield.

CONCLUSION

The selection of mutagenic dose is very crucial in mutation breeding experiments and the optimal dose is aimed to induce maximum beneficial mutation. The present study revealed that the lethal dose in fenugreek to be 350Gy dose of gamma rays under laboratory conditions. The result of the experiment indicated that high dose of gamma radiation reduced germination percentage, seedling survival, shoot length, root length and plant vigour. The high doses of 350Gy gamma rays were more lethal as compared to all other doses. The gamma rays dose of 150Gy was given the stimulatory effect of seed germination of plant growth. The present investigation clearly demonstrated that induced mutation can be successfully utilized to create genetic variability when it is desired to improve specific traits in fenugreek. The 64% seed germination and 56% seedling survivability was recorded at the dose of 350Gy which was very close to $LD_{50}$and can be chosen for the improvement programme of fenugreek through induced mutation by gamma rays.

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