Int J Seed Spice, 15(1), June, 2025: 74-81 DOI: https://doi.org/10.56093/IJSS.v15i1.6

Gamma irradiation induced powdery mildew disease resistance in fenugreek (*Trigonella foenum-graecum* L.)

Shyam Sunder Meena[1*], R.D. Meena[1], R.S. Meena[1], Y.K. Sharma[1], Sanjay Kumar[1], N.K. Meena[1], S.S. Meena[1] and Ravi Y[1]

Abstract

Fenugreek variety "Ajmer Fenugreek-3" was irradiated with gamma rays at 250, 300 and 350 Gy for inducting disease resistance in this well adapted high yielding variety. Gamma irradiation caused a reduction in germination of M_1 seed by 8.2, 17.3 and 26.5% and a decrease in seedling length by 10.3, 23.3 and 42.2% at doses of 250, 300 and 350 Gy respectively, as compared to control. From M_1 generation more than eight hundred single plants from each irradiation dose were harvested individually and plant to row progeny were sown to raise M_2 generation. In M_1 and M_2 generation a wide range of variants showed abnormal phenotypes *viz*., stunted growth, absence of branching, narrow and elongated leaves, unusual thick leaves, dwarfness, albinism, and occurrence of terminal or apical flowers instead of the typical axillary flowers. However, in the M_3 and M_4 generations, the frequency of abnormal variants was found negligible. Eighty-seven progenies exhibited seed yield more or equal to parental variety and possessing resistance for powdery mildew and downy mildew were selected for further evaluation in M_3 generation. On the basis of yield performance and disease resistance nineteen progenies were selected from M_3 generation. Thirteen promising M_4 progenies along with three checks varieties were sown in replicated trial for evaluating yield performance, uniformity and stability. Four mutants *viz.* AFg-3/300/207, AFg-3/300/84, AFg-3/350/48 and AFg-3/350/22 exhibited up to 13.5 % higher seed yield coupled with powdery mildew resistance as comparted to parental variety. The powdery mildew scoring of these mutants ranged from 6.7-14.7% as compared to 42.8-68.7 % in check varieties. All the mutants showed uniformity for most of the targeted traits. The variability generated in the present investigation can either be directly utilized as a new variety after evaluation for uniformity and stability or it may serve as donner germplasm for further varietal improvement programs.

Reviewed by
Dr. Bhusan Kale
Navsari Agricultural University
Navsari, Gujarat
Email: bhushan.kale@nau.in

***Correspondence**
Shyam Sunder Meena
ssmeena123@gmail.com

Received: 25 August 2025
Revision: 15 September 2025
Accepted: 22 September 2025

Citation
Meena, Shyam S., Meena, R.D., Meena, R.S., Sharma, Y.K., Kumar, S., Meena, N.K., Meena, S.S., Ravi, Y. 2025. Gamma irradiation induced powdery mildew disease resistance in fenugreek (*Trigonella foenum-graecum* L.). *Int J Seed Spice*.15 (1): 74-81

DOI
https://doi.org/10.56093/IJSS.v15i1.6

Affiliation
[1]ICAR-National Research Centre on Seed spices, Ajmer (Rajasthan), India-305206

Keywords: Fenugreek, powdery mildew, downy mildew, disease resistance, gamma irradiation

Introduction

Fenugreek (*Trigonella foenum-graecum* L.), is a versatile annual legume crop utilized as aspice, vegetable, forage, medicinal and industrial purposes. It is believed to have originated in the Mediterranean region. Apart from India, fenugreek is extensively cultivated in Argentina, Egypt, Morocco, Algeria, Ethiopia, Lebanon. In India, its cultivation is predominantly concentrated in Rajasthan, Gujarat, Madhya Pradesh and Uttar Pradesh. Rajasthan is the leading producer, accounting nearly 80% of the total area under fenugreek cultivation in India.

Fenugreek seed and leaves are used to decrease blood sugar and lower blood cholesterol in humans and animals. It is also good source of fibre, iron, calcium, and zinc even more than the regular food items (Srinivasan, 2006). Gum of fenugreek has also been used as a food stabilizer, adhesive, emulsifier, to produce various types of bakery and extruded products (Wani and Kumar, 2018). It also used in crop rotation for the purpose of enhancing soil fertility as air nitrogen fixer in the soil (Acharya *et al*., 2006).

Under the umbrella of AICRP on Spices, many high yielding varieties of fenugreek has been developed from different State Agricultural Universities and ICAR-National Research Centre on Seed Spices, Ajmer. Some of varieties released with moderate resistance against downy mildew and powdery mildew diseases but with due course of time the resistance level of these varieties has reduced considerably (Gopal Lal, 2018). Drivable variability is present for Agro-morphological traits and various biotic stresses in this crop which has been well documented under different cultivation conditions of the country (Malhotra, 2011; Sindhu *et al.* 2017; Desai *et al*., 2022; and Meena *et al.,* 2022: Demelash, 2023). Although genotypes with resistance for almost biotic stresses are available in germplasm assemblage but invariably the resistant genotypes are poor seed yielder (Prakash and Saharan 1999).

The major pivotal disease causes for low productivity are powdery mildew (*Erysiphe polygoni*), downy mildew (*Peronospora trigonellae*), Cercospora leaf spot (*Cercosporatraversiana*), root rot (*Rhizoctonia solani*), damping off (*Pythium aphanidermatum*), Fusarium wilt (*Fusarium oxysporum*) etc. The seed yield of fenugreek crops adversely impacted by these diseases that exerts a substantial impact on the entire plant components and loss of its output. Fenugreek in India can have a 20-70% yield loss as a result of the complicated disease pathogens (Malhotra, 2011 and Prakash and Saharan 2002). Powdery mildew, has a negative impact which is characterized by the appearance of white floury patches on the surface of leaves and other aerial parts of the plants and causes significant loss in its productivity and quality. The later stage of the fenugreek crop becomes serious when pod formation takes place (Prakash and Saharan 2002 and Mulat 2017). Since the AFg-3 variety lacks resistance against powdery and downy mildew diseases, it was subjected to mutation breeding for further improvement against these biotic stresses. Although, source of resistance for pivotal biotic stresses are available in the available gene pool but manual hybridization in this crop requires great attention and even after successful pollination, the seed set is not guaranteed. This limitation hampers introgressive hybridization programme in fenugreek to transfer specific genes in high yielding genetic background. Therefore, mutation breeding was opted to supplement the high yielding cultivars by inducting resistance against the biotic stresses.

On the other hand, the manual hybridization prosses in this crop is much difficult due to cleistogamous flowers which gives meagre chance of cross pollination to transfer specific genes from resistant genotypes. In these circumstances mutation breeding is one of the foremost techniques used to overcome the genetic bottleneck of widely cultivated varieties. With the advent of various means of mutagenesis, novel mutations have been identified and are being used in crop improvement programmes.Mutation breeding studies is being done in fenugreek for the past three decades in India and many promising mutants have been identified for higher yield and other agro-morphological traits (Raje *et al*. 2002 and Kaushik and Dashora, 2001). Mutation proved to be an effective approach for generating highly variable lines and it also led to the identification of some unique mutants As evident from Chaudhary and Singh (2001) where they identified the determinate mutant in fenugreek in M_2 generation from RMt-1 (indeterminate type) treated with ethyl methane sulphonate (EMS) at 0.10% for 4 h.). Mutation breeding also serves as an effective tool to generate new genetic variation within the existing gene pool of fenugreek. It enables the utilization of large number of alleles simultaneously to improve a particular trait of interest (Raina *et. al*., 2016). Using this technique, a total of 3450 varieties have been released worldwide including 350 varieties in India. Several crop varieties have been developed through isolation of mutation using gamma rays (Jambhulkar, 2007). Hence, mutation breeding programme was initiated in fenugreek to induce disease resistance in a well-adapted variety, thereby contributing to the improvement of existing cultivars.

Materials and Methods

Fenugreek variety "Ajmer Fenugreek-3" (AFg-3) is a high yielding and well adapted prevailing variety, developed at ICAR-National Research Centre on Seed Spices, Ajmer. It was notified in 2016 for cultivation in all major fenugreek growing regions of the India.

On the basis of previous studies, it has revealed that gamma dose of 350 Gy may be considered as dose close to LD_{50} for fenugreek seed. In the present research programme apart from 350 Gy two lower doses viz., 250 and 300 Gy were also used, as it believed that underdose can also produce few desirable mutants (Bashir, *et al.,* 2013, Anuradha *et al.,* 2017).

More than 30,000 dry seed of AFg-3 were irradiated separately with three doses of Gamma rays *viz*. 250, 300 and 350 Gy using ^{60}Co as the radiation source at BARC, Mumbai during October 2021. The non irradiated dry seeds from the same lot were taken as control. M_1 seed of each treatment separately were subjected to germination test under laboratory for study the reduction in germination due to irradiation at different doses. M_1 seed of each treatment were planted separately in wide planting space as compared to recommended spacing at research farm of ICAR-National Research Centre on Seed Spices, Ajmer during 2021-22. All recommended package of practices were followed in all generation to grow ideal crop establishment . More than eight hundred individual plants were harvested from each treatment separately from M_1 generation. During 2022-23 plant to row progenies of all the individually harvested plants were planted in paired row of 4.0 m length to raise M_2 generation and maintained through pedigree method. All the plant progenies were rigorously observed for disease reaction against downy mildew and powdery mildew and other trait specific variants throughout the cropping period. In the M_2 generations individual mutated plants exhibiting variations in plant growth habit, leaf morphology, branching pattern, podding behaviour and chlorophyll content was identified and isolated. All progenies were also observed for specific traits *viz.* early maturity, more branches, a greater number of pods/plant, more seed/pod, short plant height etc. Simultaneously, disease reaction for downy mildew and powdery mildew was determined by disease scoring and converted as Percentage Disease Index (PDI) as per Prakash and Saharan,1999. Disease scoring for downy mildew and powdery mildew was done by following 0-4 rating scale [(0 immune (I); 1-15 resistant (R); 16-30 moderately resistant (MR); 31-50 for susceptible (S) and <51 for susceptible (HS).

A large number of individual selected plants which carrying traits of interest from M_2 generation were subjected plant to row progenies test in paired to row of 4.0 m length to raise M_3 generation during 2023-24. In M_3 generation a few progenies exhibiting segregation while some progenies exhibiting substantial homogeneity for powdery mildew and downy mildew resistance. Individual resistant plants were isolated from the segregating progenies based on their disease reaction while, bulk selection was carried out in progenies that showed substantial homogeneity for resistance to powdery mildew and downy mildew. Individual plants selected from segregating progenies of M_3 were was subjected to plant to row progeny test. Based on disease resistant reaction and seed yield performance, promising progenies were identified from M_3 generation and initial yield trial were conducted in RBD with plot size of 12.0 sqm during 2024-25. Analysis of variance were studied to know the differences between mutants and parental line for agro-morphological traits as well disease reaction.

Results and Discussion

Effect on germination and seedling growth

The data depicted in Table 1 shows reduction in seed germination and seedling growth with the increase dose of gamma radiation in fenugreek. Different doses of gamma radiation essentially affected the mean germination and seedling growth as compared to control. The irradiated seeds (M_1) were subjected to a germination test within fifteen days of irradiation. Germination reduced by 8.2, 17.3 and 26.5% while seedling length was reduced by 10.3, 23.3 and 42.2% in irradiated seed at 250, 300 and 350 Gy respectively, as compared to control. Among the germinated seeds, seedling growth was reduced by about 40% at 350 Gy dose. Thus, the combined effect of reduction in germination and reduction in seedling growth resulted total estimated reduction percent was ranged from 44 to 62%. Therefore, a dose of 350 Gy of gamma irradiation in fenugreek seeds may be considered as the $LD_{50.}$ The findings are almost similar with the results reported by Anuradha *et. al.* 2017 and Bashir *et al.* 2013.

Effect of mutagens on quantitative and qualitative traits Gamma rays irradiated seeds were space planted in field during October, 2021 and plants were observed during the entire growth period for detection of variability. In M_1 and M_2 generation several variants were observed, showed abnormal phenotypes viz., stunted growth, absence of branching, extreme narrow & elongated leaves, plant with very thick leaves, dwarfness, albinism, and presence of terminal or apical flowers instead of the typical axillary flowers (Fig.1).However, several mutated plants either died before reaching maturity or exhibited very poor seed set therefore, these plants were largely not carried forward. In contrast, the frequency of abnormal variants in M_3 and M_4 generations was negligible. Bashir, *et al.*, (2013) studied comparative effects of physical and chemical mutagens in fenugreek and revealed that gamma rays was more effective than EMS and SA in reducing germination, survival percentage and seedling length. Similarly, Yadav and Krishna, (2013) observed that gamma rays caused more sterility than chemical mutagens and gamma rays (70 kR) was most efficient dose in cumin.

Screening of Genotypes for Powdery Mildew Resistance

From M_1 generation more than 800 individual plants from each irradiation treatment were harvested and threshed separately. During 2022-23 plant to row progeny of all individual harvested plants were sown in paired row and rigorous screening for powdery mildew downy mildew disease was done. Disease scoring for downy mildew and powdery mildew were recorded by following 0-4 rating scale (Prakash and Saharan 1999). From M_2generation (300 and 350 Gy irradiation), 86 progenies, exhibiting seed yield equal or higher than parental variety coupled with disease resistance were identified and maintained using pedigree method. In M_3 generation, certain progenies exhibited segregation into resistant and susceptible plants while others showed substantial homogeneity for powdery mildew resistance. Based on yield performance and disease resistance nineteen progenies were selected from M_3 generation. Progenies found homogenous for disease resistance were harvested in bulk, while, individual resistant plants were further selected from segregating progenies (Fig. 2). Kakani *et.al.* (2012) isolated single stem mutant from M_2 generation of UM-344 treated with 40 kR gamma rays and rosette leaf mutant from M_3 generation of variety RMt-305 treated with 25 kR gamma rays. Similarly, Choudhary and Singh (2001) identified a determinate type mutant plant in fenugreek using EMS treatment at 0.10 % concentration.

Evaluation of resistant mutants for yield performance

Thirteen M_4 mutant progenies along with three check varieties of fenugreek were evaluated in a RBD to assess yield performance and uniformity. These mutants were selected based on their superiority in seed yield (> 10%) coupled with significantly lower disease score for powdery mildew (ranged from 5.9-12.9) as compared to 42.8-68.7 % in check varieties (Table 2).

Analysis of variance (ANOVA) revealed significant difference for seed yield, test weight, no. of pods/plant, powdery mildew and downy mildew disease scoring, whereas, days to maturity, pod length and seeds/pod were not significantly differed. Mutant AFg-3/300/207, AFg-3/300/84, AFg-3/350/48 and AFg-3/350/22 exhibited up to 13.5 % higher seed yield coupled with powdery mildew resistance as comparted to parental variety. The powdery mildew scoring of all tested mutants ranged from 6.7-14.7% as compared to 42.8-68.7 % in check varieties. Mutant AFg-3/300/131 and AFg-3/350/22 were observed moderately resistant for downy mildew resistant. All the mutants in M_4 generation exhibited a high degree of uniformity for most of the targeted traits. The promising mutants will be further assessed through multi location trials to evaluate their yield performance, uniformity and stability.

The present study demonstrates that gamma radiation induced mutagenesis generated, substantial variability in the fenugreek population, with several mutant lines exhibited valuable traits that are rare among germplasm collections. The variability being generated under the present investigation can be utilized as a variety directly or a donner germplasm for further varietal improvement. The mutants can also serve as valuable resources to understand the nucleotide variations and to identify genes responsible for the expression of target traits. Molecular analysis of these mutants may further facilitate to identification of markers that are tightly linked with the mutated regions.

Acknowledgement

Research was supported by the Indian Council of Agricultural Research, Department ofAgricultural Research and Education, Government of India. Authors are also grateful to Dr. Sanjay J. Jambhulkar and his team, Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai for irradiation and valuable suggestions for handling the mutated generation.

Table 1: Effect of gamma irradiation on seed germination and seedling

Variety	Gamma irradiation dose (Gy)	Germination (%)	% reduction in germination over control	Seedling length (cm)	% reduction in seedling length over control
Ajmer Fenugreek -3 (AFg -3)	250	90	8.2	10.4	10.3
	300	81	17.3	8.9	23.3
	350	72	26.5	6.7	42.2
	Control	98	NA	11.6	NA

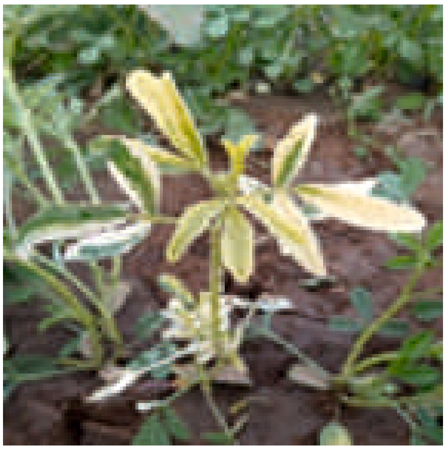
Table 2: Performance of promising fenugreek mutants for yield, yield attributes and diseases incidence

S.N.	Entry	Seed yield (kg/ha)	% increase over AFg-3	Test weight (g)	Days to maturity	No. of pod/ plant	Pod length (cm)	No. of seeds /pod	Downy Mildew		Powdery mildew	
									Scoring (%)	Reaction	Scoring (%)	Reaction
1.	AFg-3/300/161	2112	0.4	15.6	138	59.3	10.2	19.7	40.3	S	12.9	R
2.	AFg-3/300/219	2067	-1.5	14.7	141	70.0	9.7	19.2	37.9	S	11.1	R
3.	AFg-3/350/48	2378	11.5	14.9	139	56.7	9.8	17.8	33.4	S	11.8	R
4.	AFg-3/300/204	2145	1.8	14.1	140	62.3	9.0	19.8	42.7	S	7.9	R
5.	AFg-3/350/254	2135	1.4	13.9	142	60.0	9.0	19.3	38.7	S	8.9	R
6.	AFg-3/300/117	2110	0.3	14.7	139	59.1	8.8	18.0	26.4	S	12.9	R
7.	AFg-3/300/131	2134	1.3	16.4	142	57.5	9.0	18.7	17.4	MR	5.9	R
8.	AFg-3/350/143	2113	0.5	12.4	140	49.5	9.3	19.3	42.9	S	6.7	R
9.	AFg-3/350/22	2321	9.1	14.1	140	56.0	9.7	17.8	18.9	MR	11.9	R
10.	AFg-3/300/84	2367	11.0	16.9	140	64.0	9.5	19.7	68.7	HS	14.7	R
11.	AFg-3/300/219	2140	1.6	14.1	141	65.0	9.8	18.5	67.9	HS	11.1	R
12.	AFg-3/300/207	2406	13.5	16.1	140	62.7	9.2	18.8	44.3	S	7.9	R
13.	AFg-3	2132	0.0	14.6	142	57.3	9.3	19.2	59.3	HS	68.7	HS
14.	Hisar Sonali	2090	-3.5	15.4	142	64.3	9.7	18.3	44.5	S	57.3	HS
15.	Rmt-361	2044	-2.4	15.1	140	62.7	9.5	18.2	36.7	S	42.8	S
	Mean	**2116**		**13.5**	**142**	**59.9**	**9.3**	**18.8**	**46.5**	**-**	**35.3**	**-**
	Sem (±)	**41.1**		**1.04**	**0.34**	**4.74**	**0.32**	**0.50**	**5.44**	**-**	**4.24**	**-**
	CD (5%)	**115.7**		**2.6**	**NS**	**13.4**	**NS**	**NS**	**15.3**	**-**	**12.0**	**-**

Mutant with narrow and long leaf

Mutant with narrow and long leaf

Mutant with albinism white leaf



Mutant with albinism pale yellow stem/leaf

Mutant with very dense leaf apical

Mutant with terminal flowering

Fig. 1: Morphological mutants detected in M1 and M2 generation

Powdery mildew susceptible check variety (Left) and resistant mutant line (Right)

Selection for powdery mildew resistance individual plants in M2-M4

Fig. 2: Selection of powdery mildew resistance individual plants in M2-M4

Conflict of Interest

The authors declare that they have no conflict of interest.

Reference

Acharya, S., Anchalee, and Saikat, B. 2006. Improvement in the nutraceutical properties of fenugreek (*Trigonella foenumgraecum* L.) *Songklanakarin J Sci Technol*, 28(1): 1-9.

Anuradha P. Patel, Poonam Verma P., Sharda Choudhary, S and Arvind Kumar Verma A.K. 2017. Lethal dose (LD_{50}) fixation and sensitivity of Fenugreek (*Trigonella foenum-graecum* L.) to gamma radiation for induction of mutation. *J Agri Search*, 4(4): 237-241

Bashir Shagufta, Aijaz, A. Wani and Irshad, A. Nawchoo. 2013. Studies on mutagenic effectiveness and efficiency in Fenugreek (*Trigonella foenum-graecum* L.). *Afr J Biotechnol*, 12(18): 2437-2440.

Chaudhary, A.K. and Singh, V.V. 2001. An induced degeminate mutant in fenugreek *(Trigonella foenum-graecum L.). J Spices Aromat Crops*, 10(1): 51-53.

Demelash, A. Book chapter: Resistance Sources for Powdery Mildew and BreedingStrategies for Improvement in Fenugreek. Advances in Agriculture 2023.

Desai, S., Gangadharappa, P.M., Hiremath, J.S., Sandhyarani N., Pushpa TN and Vijayakumar R. 2022. Performance of potential fenugreek genotypes for seed yield and its attributing traits in Northern dry zone of Karnataka. *Pharma Innov J* SP-11(6): 259-263.

Gopal, L., 2018. Scenario, importance and prospects of seed spices:a review. *Curr Investig Agric Curr Res*, 4(2): 491-498.

Jambhulkar, S.J. 2007. Mutagenesis: Generation and Evaluation of Induced Mutations. *In*: Advances in Botanical Research (Rapeseed Breeding) Vol. 45 Elsevier Publication Ltd. USA. Ed. Gupta, P.C. pp: 418-428.

Kakani, R.K., Saxena, S.N., Meena S.S., Sharma, Y.K., Meena, R.S. and Krishna Kant. 2012. Two unique induced mutants identified in fenugreek for growth habit and leaf characteristics. *Int J Seed Spices,* 2(1): 66-68.

Kaushik, S.K. and Dashora, S.L., 2001. Variability assessment in M_7 line of fenugreek (*Trigonella foenumgraecum* L.) *J Spices Aromat Crops*, 10(1): 45-47.

Malhotra, S. 2011. Fenugreek (*Trigonella foenum-graecum* L.). Genetic Resources, Chromosome Engineering, and Crop Improvement, CRC Press, Boca Raton, FL, USA, Advances in Agriculture.

Meena, R.S., Meena, N.K., Saxena, S.N, Verma, A.K., Ravi, Y. and Bagra, S.K. 2022. Estimation of genetic variability, correlation and divergence studies on seed yield and its attributing characters in fenugreek (*Trigonella foenum-graecum* L.). *International Journal Seed Spice.* 12 (2): 63-69

Mulat, Y.W. 2017. Effects of powdery mildew (*Leveillula Taurica* and E*rysiphe polygoni*) on yield and yield components of fenugreek in the mid-altitudes of bale, South eastern Ethiopia. *Journal of Plant Sciences.* 5(2): 65-67.

Prakash, S. and Saharan, G. 1999. Sources of resistance to downy and powdery mildew of fenugreek. *Indian J Mycol Plant Pathol,* 36: 95-99.

Prakash S. and Saharan, G. 2002. "Estimation of losses in yield offenugreek due to downy and powdery mildew" *Haryana J Hortic Sci,* 31(1/2):133-134.

Raina, R., Laskar, S., Khursheed. 2016. Role of mutation breeding in crop improvement-past, present and future. *Asian Res J Agric,* 2(2): 1-13.

Raje, R.S., Singh, D. and Singhania, D.L. 2002. Inheritance of giant mutant type in fenugreek (*Trigonella foenum-graecum* L.) *J Spices Aromat Crops,* 11(2): 141-142.

Sindhu A., Tehlan, S.K. and Chaudhury, A. 2017. Analysis ofgenetic diversity among medicinal therapist *Trigonella foenum-graecum* L. genotypes through RAPD and SSR markers. *Acta Physiol Plant,* 39(4):100–115.

Srinivasan, K., 2006. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. *Food Rev Int,* 22(2): 203-224.

Wani, S.A. and Kumar, P., 2018. Fenugreek: a review on its nutraceutical properties and utilization in various food products. *J Saudi Soc Agric Sci*, 17: 97-106.

Yadav, S.K. and K Ram Krishna R. K. 2013. Effectiveness and Efficiency of Physical and Chemical Mutagens on Cumin (*Cuminum cyminum* L.) *Int J Plant Res,* 26(1): 44-49.