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Estimation of induced variability in M_2 generation of fennel (*Foeniculum vulgare* Mill.)

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Abstract
Mutagenesis involving ionizing radiations have been extensively used for development of genetic and breeding resources with novel characteristics. Genetic variability in fennel for agronomically important traits is low. Thus this study was aimed at generating genetic variability in Rajasthan Fennel-125 variety of fennel using the most potent physical mutagen i.e. gamma rays. The seeds were exposed to different doses of gamma rays (150, 175, 200, 225 and 250 Gy). The M_1 and subsequent M_2 generations were raised in the field. In M_2 generation, large numbers of morphological mutants were identified. Observations were recorded in each of selected putative mutants in M_2 generation (from seedling to harvesting stage). Many of the selected variants showed comparatively slow growth and variation in chlorophyll content. The chlorophyll variegation involved either whole plant or was restricted to part of leaves. In chlorophyll mutants, the leaf colour ranged from yellow to pure white. Huge variability was also observed with respect to plant height in selected variants. The selected putative mutants showed variation in flowering time, number of primary branches, number of umbels per plant, number of seeds per umbel and days to maturity. Though plant yield in many of the mutants were low in comparison to the parents, the ideotype of the variants were good with respect to plant height and branching pattern. Many of the hitherto indentified mutants exhibited yield superiority over the parent. The identified variants would be further evaluated for their superiority and the breeding nature in M_3 & M_4 generation to ascertain the nature of mutation.

Keywords: Chlorophyll mutants, Fennel, Gamma rays, Genetic variability, M_2 generation, Mutagenic effects

1. Introduction
Fennel (*Foeniculum vulgare* Mill.) is an important aromatic and medicinal herb belonging to the family Apiaceae with basic chromosome number of 2n=22. It is largely used for flavoring liquors, bread, fishes, salads, soups, cheese and in manufacturing of pickles, perfumes, soaps, cosmetics, cough drops and as good source of antioxidants value [1, 2, 3]. The growth habit of fennel ranges from annual to biennial or perennial and is native to the Mediterranean areas [4]. The major fennel producing countries include India, Argentina, China, Indonesia, Russia, Japan and Pakistan. The annual fennel grows to a height of up to 2.5 m with hollow stems and bears yellow flowers and feathery leaves under arid and semi-arid parts of India. The flowers are produced in terminal compound umbels and leaves growing up to 40 cm long are finely dissected with the ultimate filiform segments being about 0.5 mm wide. The fruit has a (4-10 mm long) dry seed splitting into two halve [5]. The fennel cultivation in India is spreads over an area of 76000 ha with production of 129350 tons and productivity of about 17.02 q/ha [6]. The yield of fennel is abysmally low and there is an immense need to increase the present yield. The long duration of fennel ranging from 180 to 220 days makes it vulnerable to crop damage due to sudden occurrence of natural hazards and also makes them resource demanding. Their tall stature (>2 meter) makes them susceptible to lodging and reduces the yield and quality of crop [7].

The genetic improvement in fennel crop has largely been attempted through conventional breeding method i.e selection. Conventional breeding in fennel has its own limitations including low variability in available germplasm, minute flower size and cross pollination promoted by protandry. Narrow genetic variability exists in fennel for different traits, particularly for short duration with dwarf/semi dwarf growth [7]. Very few efforts have been made to improve fennel crop through genetic manipulation. Induced mutations have been widely used in a number of crops and species to generate broad spectrum of genetic variability [8]. Gamma rays are low linear energy transfers and inducing chromosomal alterations at high doses [9]. Low dose ionising irradiations have been used for inducing genetic variability through mutation breeding programmes of various crops [10].

Gamma irradiation has been used in mutation breeding primarily to induce mutation for setting desired traits in plants and to increase genetic variability in crops, which having low genetic base. By using gamma rays many high yielding mutant varieties have been developed world wide, which are resistant to biotic and abiotic stresses with improved quality [11]. The success of mutation breeding programme largely depends on selection of promising mutants based on phenotypic characters [12]. The probability of the occurrence of phenotypic mutation is extremely low and only rare dominant mutations can be identified in M_1 generation [13]. Mutant characters are generally governed by recessive genes [12] and selection of such mutants can be done in segregating generation (M_2) [14]. Moreover, the yield of mutagen could be perceived by M_2 generation as a result of pollen sterility, reduction in plant height, late and/or early flowering, curled leaves and days to maturity etc [7, 12]. In segregating population, visual screening is the most effective approach to indentify and select mutations based on morphological characters. The appearance of chlorophyll mutants in M_2 population is the most dependable index for evaluating genetic effect of mutagenic treatments [15]. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromeres or on proximal segments of chromosomes [16]. The screening of mutants for yield and yield attributing traits could be done on the basis of plant height, time to flowering, more number of branch, maturity period, disease resistance etc [17]. The induced mutations can quickly generate variability in quantitatively and qualitatively inherited traits in crops. Hence, the objective of the present study was to induce somatic variability in fennel for important traits and to identify agronomical superior mutants in M_2 population.

Materials and methods

Plant materials and method: M_1 seeds of fennel cultivar Rajasthan Fennel-125 were used to raise M_2 population at ICAR- National Research Centre on Seed Spices, Ajmer, Rajasthan. M_1 seeds were produced from growing irradiated dried seeds of the fennel (cv. Rajasthan Fennel-125) at different doses of gamma rays (150 Gy, 175 Gy, 200 Gy, 225 Gy and 250 Gy) in gamma chamber containing CO^{60} as a source at BARC, Mumbai. Seeds of control samples were also sown in M_2 generation. Total of 5097 plants were raised from all five doses of gamma rays. The segregating M_2 population was observed for frequency and spectrum of chlorophyll mutations (from germination to maturity). The spectrum of chlorophyll mutations was classified as per the described method [18] with modifications and also for any type of phenotypic variability. At maturity each of the putative mutant plant was harvested individually to advance for next generation.

Growing condition: M_1 generation was sown in the month of October during 2015-16 under field condition and plot size kept 3x2 m. All recommended packages of practices were followed. The identified variants mutants were bagged at the flowering stage to avoid cross pollination.

Observations recorded and statistical procedure: The M_2 generation was screened for phenotypic variations from germination to harvesting. The frequency of the mutant plants out of the total number of individuals in M_2 generation was calculated. To identify probable mutants following phenotypic observations were recorded: plant habit (erect, semi erect and spreading), variation in leaves (chlorophyll mutants), early plant vigour (poor, good and very good), plant height (short stature, up to main umbel and up to top of the plant), size of the leaf sheath (small, big), number of primary branches, number of secondary branches, branching pattern, foliage at full bloom stage (profuse, sparse), days to appearance of flower, days to full maturity, number of umbels per plant, number of umbellate per umbel, number of seeds per umbel, seed yield per plant, test weight (1000 seeds) etc. Only selected characters of the putative mutants have been presented in this paper. The recorded observations were statistically analyzed to determine the degree of significance for the variations.

Results

Frequency and spectrum of chlorophyll mutants

The M_1 population consisting of a total 5097 plants from five different doses of gamma rays (150, 175, 200, 225 and 250 Gy). A large spectrum of chlorophyll mutants were observed at seedling emergence (Table 1). Mutants were identified in whole population based on morphological traits and plants exhibiting chlorophyll variegation were observed. Among total 5097 M_1 plants, 63 plants showed different types of chlorophyll mutants were observed. Chlorophyll mutants were observed to the extent of 5.86% in M_2 generation. Among the different doses, the highest percentage (1.59%) of chlorophyll mutants were recorded in 225 Gy followed by 200 Gy (1.47%) and the lowest was recorded in 250 Gy (0.88%). Different types of chlorophyll mutants appeared in the segregating generation. Xantha type of chlorophyll mutants (Fig. 1a) appeared in which half of the leaves were devoid of chlorophyll and such leaves became completely white thereafter. Likewise albino type mutants (Fig. 1 b&c) completely devoid of chlorophyll were indentified that die in due course of time. Chlorina type mutants (Fig. 1d) were present in all the doses, whereas viridi (Fig. 1e) type of mutants also appeared in all doses except 175 Gy. Striata (Fig. 1f) and irregular chlorophyll leaf pattern on mutants leaves (light green/ yellowish green) appeared in the M_2 population. In the further growth yellow leaves and irregulars leaves mutants become normal while the complete plant devoid of chlorophyll mutants were died.

Identification and selection of putative mutants in M_2 under field conditions

The whole surviving M_2 generation were screened for any type of variability and putative mutants were selected from these segregating population. A total of 108 mutants were identified based on different morphological characters (Fig. 2). Plants carrying putative mutation affect plant height, flowering time, number of primary branches, number of umbels per plant, number of seeds per umbel and days to maturity were identified. The maximum number of mutants were selected from the gamma rays dose of 225 Gy (35) followed by 200 Gy (29), 250 Gy (19), 175 Gy (14) and 150 Gy (11). The identified and selected mutants were grouped into different groups to know the extent of variability created through gamma rays. In M_2 population, many plants showed unique phenotypic traits (Fig. 3a-f). Such phenotypic expression consisted of ultra dwarf plant type (Fig. 3a), no branching, prostrate growth (Fig. 3b) and plant with single umbel (Fig. 3c). There were some zigzagged single stem (early flowering) mutants (Fig. 3d), thin stem (Fig. 3e) mutants with higher number of primary and secondary branches were also noticed in the M_2 population (Fig. 3f).

The mutants were grouped based on phenotypic characters as enlisted in Table 2. By grouping based on plant height revealed that mutants could be classified into six different groups. The maximum number (26.85%) of mutants was of short type (51-100 cm) followed by dwarf type (10-50 cm) accounting for 21.30% of the selected mutants. Medium dwarf plant is a desirable trait in fennel and 13.89% of selected mutants were of medium height (101-150 cm) with good number of primary and secondary branches. Ultra dwarf mutant having plant height less than 10 cm with no branching and early flowering formed 12.96% of total identified mutants. Long type mutants (8.33%) with plant height more than 175 cm were also isolated. The time taken for appearance of flowering is presented in Table 2. The flowering time in 6.48% of the mutants identified was less than 50 DAS and classified as very early flowering mutants. Some mutants (13.89%) flowered between 50-60 DAS, while 62.04% flowered between 61-90 DAS and some (17.59%) of the mutants flowered very late (after 90 DAS). Mutation affecting number of primary branches per plant (Table 2), 4.63% of mutants did not produce primary branches and only main stem were emerged (Fig. 3a). About 17.59% of mutants showed relatively less number (1-5) of primary branches, while 32.41% of mutants were recorded medium number of primary branches (6-10). Nearby 45.37% of mutants produced more than 10 primary branches. The number of umbels is one of the important yield attributing traits in fennel crop and it was improved in some selected mutants (Table 2). Among the selected mutants, 4.63% recorded single umbel per plant, 12.04% of mutants recorded 2-20, 25.93% produced 21-50, 40.74% showed high (51-150) and 16.67% of the mutants produced very high (more than 150) number of umbels per plant. With regards to seeds per umbel, majority (41.67%) of mutants fall under medium (101-500) category (Table 2), while 17.59% of the mutants recorded high number (501-700) of seeds per umbel. Very high number (>700) of seeds per umbels were observed in 6.48% of the mutants. Spectrum of mutation affecting maturity period were isolated in M_2 generation. Early mutants maturing less than 150 DAS accounted for 8.33% of mutants, while 62.96% of the mutants matured between 150 and180 DAS. About 28.70% of mutants matured late taking more than 180 days. For almost all growth and yield attributing characters variation was observed in both directions i.e. positive and negative in comparison to parent variety ascertaining the efficiency of gamma rays to induced genetic variability in fennel crop.

Discussion

Frequency and spectrum of chlorophyll mutants

Chlorophyll mutation frequency is an indication in assessing effectiveness of a mutagen, genetic effects of a mutagen and in estimation of mutational events in crop plants. The appearance of chlorophyll mutants during seedling stage was indicator for gamma rays efficiency in fennel crop. The scoring of chlorophyll mutation frequency in M_2 generation is one of the most reliable measures for evaluating the mutagenic efficiency and effectiveness in the plants [19]. From a breeder's point of view, the frequency of chlorophyll mutants expressed as per cent of M_2 population is more realistic and helpful. Hence, results were explained on M_2 plant basis. The reports of chlorophyll mutants in large number of crops could be ascribed to various causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching taking place due to deficiency of carotenoids [20, 21]. Our results are in confirm with previous findings where mutation affecting chlorophyll, growth period and maturity period have been identified in M_2 population which was the sign of efficiency of gamma rays for the induction of mutation [22]. Chlorophyll deficient chimeras in M_1 generation and their segregation in M_2 generation are often observed in an induced population. Several authors have reported the occurrence of different types of chlorophyll mutations such as albina, xantha, chlorina, viridis, etc [12, 23].

Identification and selection of putative mutants under field conditions

Mutation breeding provides a powerful means of creating new and useful variability in crop plants both in qualitative and quantitative characters. Morphological characterizations of germplasm/mutants are necessary for the efficient use of the material through conventional methods or modern techniques. The success of induced mutation also depends on the selection of putative mutants based on phenotypic characters [12]. The findings on plant morphological and reproductive parameters showed that gamma rays irradiated plants can alter the flowering and maturity in either positive or negative direction resulting in sufficient variability in the treated population that can be utilized for selection of early or late flowering plants for further improvement of this versatile crop. The flowering and maturity in the case of induced populations were consistently shifted in the direction of earliness. It is valuable in obtaining varieties associated with escape from pests, drought and other stress injuries that occur in standing crop during cropping season. The results obtained in M_2 generation revealed the possibility of increasing seed yield and yields components through irradiation with gamma rays in fennel. Improvement of agronomic characteristics of fennel by using gamma radiation has been reported [7] and the gamma irradiation is important for inducing genetic variability and mutants can be selected in segregating population [24].

Plant height is one of the important parameters which needs to be addressed in fennel crop. The fennel germplasm is deficient in dwarf plant type. In segregating population a number of the variants identified showed dwarfism. In a report, induced genetic variability in wild type pepper and numbers of mutant selected in segregating population (M_2 generation) showed dwarfism [12]. Dwarfism is an enviable character for breeding programs to reduce to lodging problem during maturity time in fennel. In the present investigation, the mutation created dwarfism and we recorded a plant with height less than 10 cm with short internodes and green leaves. Dwarf mutants were also reported earlier in pepper [25], which might be due to the inhibition of the elongation of epidermal cells, or a defect in gibberellic acid biosynthesis [26]. Discovery of recessive, monogenic mutations causing dwarfism is very important to allow for easier identification of the genes that control plant growth [27].

Days to flowering and maturity in case of irradiated populations were consistently shifted towards earliness. The M_2 generation was analysed for variability generated for early flowering and maturity as compared to parent plant. The results revealed that M_2 progenies of fennel significantly differed among themselves and also as a group from the control plant (parent variety) in respect to flowering. The early flowering was recorded in several mutants as compared to control (56 DAS). In our results, it was found that nine mutants reached maturity before 150 DAS. Similar results were reported by other worker [28] and they observed significant variation for days to flowering and maturity in

coriander through induced mutation.

The number of primary branches between the selected mutants varied in both, positive and negative direction. Maximum number of primary branches (>10) mutants were obtained whereas no primary branches were recorded in five mutants. These characters significantly contributed in yield of crop. A wide range of variability for number of primary branches was observed in several lines of cumin [29]. One line showed high yield/plant than the check variety, indicating the possibility of further yield improvement by mutation breeding. In M_2 generation of fennel, 1575 macromutants identified namely, thick stem, slender stem, pigmented stem, dwarf, elongated pinnae, narrow pinnae and early flowering etc [30]. These lines were advanced by selfing and evaluated in M_6 generation along with control. The eight phenotypic traits including plant height, number of primary branches/plant, total branches/plant, number of compound umbels/plant, number of umbels/plant, number of umbellate of first inflorescence, seed yield and harvest index were improved.

In case of number of umbels improved significantly from the parent plant. The mutants recorded maximum number of umbels per plant as compared to parent variety. It was also seen that a number of progenies exhibiting superiority in many characters as compared to control [7, 29]. This allowed for possible identification of even better progenies through selection. Yield is a complex character, which depends on it main components, *viz.,* number of primary and secondary branches, number of umbels per plant, number of umbellate per umbel, number of seeds per umbel etc. These components are further dependent for the expression or reveal morphological and developmental traits, which are interrelated with each other. Therefore, the parents selected in the breeding program, aimed at increasing seed yield should possess wide range of genetic variation for the above said morphological and developmental characters. Besides, it would be of interest to know the magnitude of variation due to heritable component, which in turn would be a guide post in selection for the improvement of a population. In other words, for the improvement of any crop species, the knowledge of genetic variability for characters of economic importance and their heritability and genetic advance is of utmost importance in further breeding programmes. The high yielding progenies resulting from mutation breeding have been earlier reported in coriander [28, 31] and black cumin [32].

Table 1: Effect of gamma irradiation on the frequency of the chlorophyll mutations in M_2 generation of fennel.

Gamma rays dose (Gy)	Number of plants study	Total chlorophyll mutants	Spectrum of chlorophyll mutations						Frequency of chlorophyll mutations (%)
			Albino Type	Xantha	Chlorina	Viridi	Striata	Others (light green/ yellowish green)	
150	693	7	2	0	1	1	1	2	1.01
175	661	6	1	1	2	0	0	2	0.91
200	1223	18	1	4	3	3	1	6	1.47
225	1386	22	1	1	4	7	2	7	1.59
250	1134	10	1	0	2	2	0	5	0.88

Table 2: Phenotypic characters of the selected mutants in M_2 generation of fennel.

Group	Number of mutants in M_2 generation	Percent
Plant height (cm)		
Ultra Dwarf (<10 cm)	14	12.96
Dwarf (10-50 cm)	23	21.30
Short (51-100 cm)	29	26.85
Medium (101-150 cm)	15	13.89
Normal (151-175 cm)	18	16.67
Long (>175 cm)	9	8.33
Flowering time (days)		
Early (<50)	7	6.48
Medium (50-60)	15	13.89
Normal (61-90)	67	62.04
Late (>90)	19	17.59
Number of primary branch per plant		
No branching (0)	5	4.63
Less branch (1-5)	19	17.59
Medium branch (6- 10)	35	32.41
More branched (>10)	49	45.37
Number of umbel per plant		
Very less (1)	5	4.63
Less (1-20)	13	12.04
Medium (21-50)	28	25.93
High (51-150)	44	40.74
Very high (>150)	18	16.67
Number of seeds per umbel		
Very less (<10)	16	14.81
Less (10-100)	21	19.44
Medium (101-500)	45	41.67
High (>501-700)	19	17.59
Very high (>700)	7	6.48
Days to maturity		
Early (<150)	9	8.33
Medium (150-180)	68	62.96
Late (>180)	31	28.70

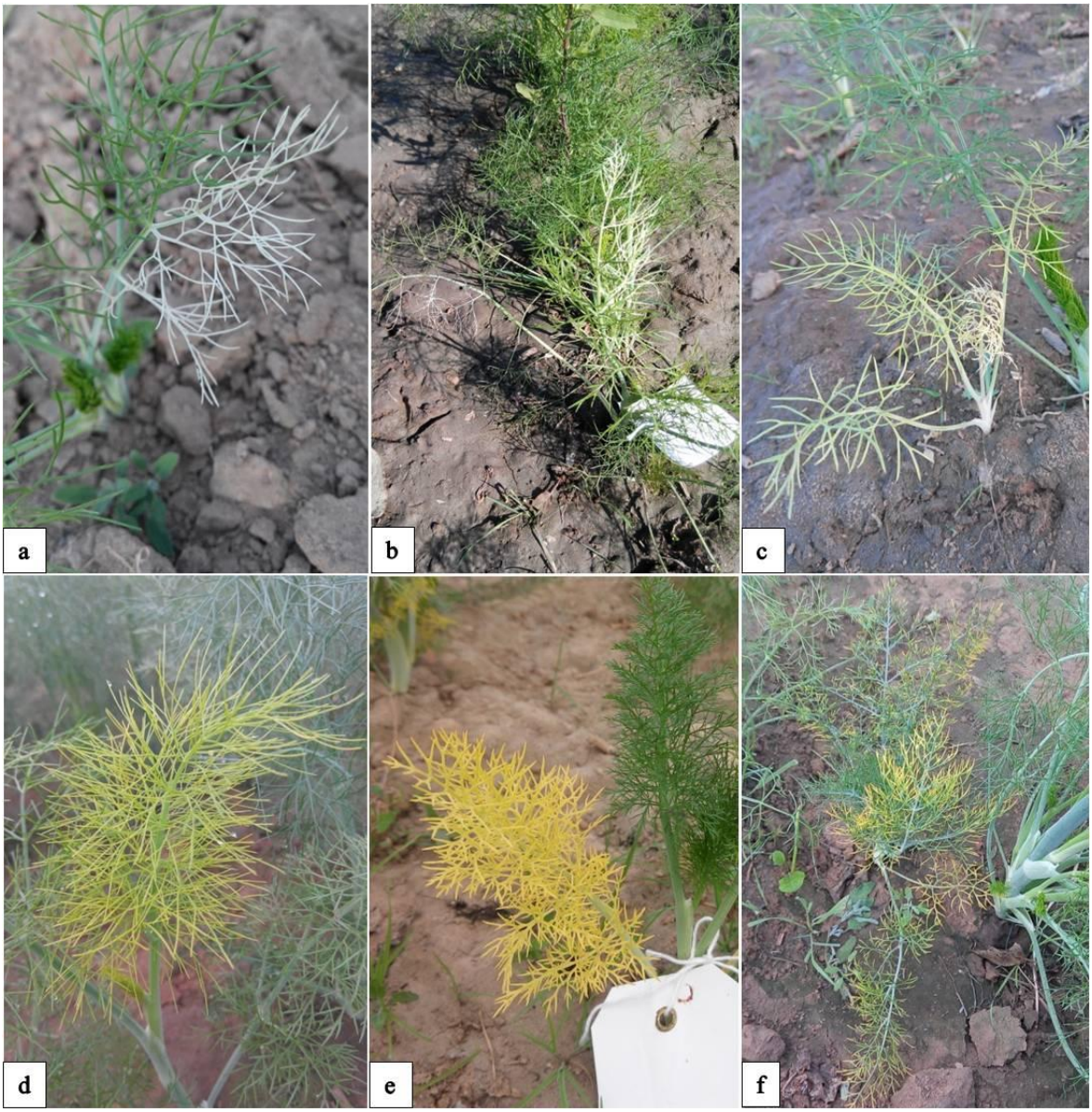

Fig 1: Appearance of different types of chlorophyll mutants during the seedling stage in segregating population (M_2). a; xantha type; b&c; albino type (completely white plant), d; chlorina mutant (light green colour), e; viridi type, f; striata

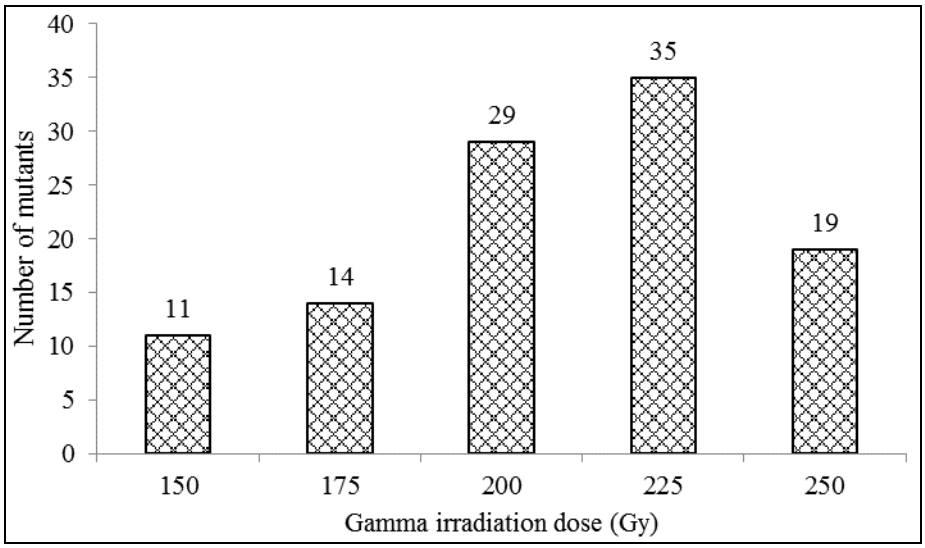

Fig. 2: Total numbers of putative mutants selected from different doses of gamma irradiation in M_2 generation.

Fig. 3: Phenotypic appearance mutant in M_2 generation of fennel: a; ultra dwarf mutant, b; horizontal growth, c; single umbel type mutant, d; zigzag type of mutants, e; thin plant mutant, f; early flowering mutant

Conclusion

The present study revealed that the utility of gamma rays in inducing genetic variability in fennel crop. The irradiation had significant effect on induction of chlorophyll mutants in M_2 generation. Maximum number of mutants were identified at 225 Gy followed by 200 Gy. The mutants varied in different morphological characters which are generally absent in available germplasm. The gene which is responsible for dwarfism and early maturity in fennel can be identified and used to develop short stature fennel varieties.

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