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Effect of acute gamma radiation exposure on seed germination, survivability and seedling growth in cumin cv. Gujarat Cumin-4

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Abstract

Cumin production and productivity is very low because of lack in inherent capacity and susceptibility to wilt and blight. This crop has low genetic variability for different essential traits and improvement of this crop through conventional breeding approaches is very tedious. The induction of genetic variability through physical mutagen could be aviable approach to generate variability in cumin. An experiment was carried out to determine the LD_{50} of gamma radiation in cumin for identifying most effective dose. In first experiment cumin seeds were irradiated with different gamma rays doses (100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy). At the lowest dose (100 Gy) of gamma ray maximum seed germination (90.47%) was obtained while the highest dose (500 Gy) resulted in minimum seed germination (9.52%). The dose of 300 Gy recoded 35.71% germination and 4.76% seedlings survival, respectively. In the latter growth stage none of the seedlings survived at the dose of 400 Gy and 500 Gy. At lower dose of 100 Gy maximum number of leaves (7), number of roots per plant (5.00), root length (4.27 cm), shoots length (2.9 cm) and fresh weight per plant (0.009 g) were observed. The seed germination, seedlings survivability and growth parameters were adegnate at 100 Gy and 200 Gy doses of gamma ray. To know the LD_{50} more precisely another experiment was carried out on the basis of preliminary experiment. In this experiment 5 different doses were selected from 100 Gy to 200 Gy giving the interval of 25 Gy. The seed germination and seedlings survival decreased as the radiation dose increased. The maximum seed germination, was noticed at 100 Gy (95.5%) followed by 125 Gy, 150 Gy, 175 Gy, and 200 Gy. The seedlings growth parameters like number of leaves, root length, shoot length and fresh weight per plant reduced around 50% at 200 Gy dose of gamma radiation. Among the five doses of irradiation the LD_{50} was close to 200 Gy which caused around 55% death of seedlings, 45% seedlings survival and around 50% reduction in plant growth. Hence, it is concluded that 200 Gy may be the optimum dose for creating useful mutation in cumin.

Key words : *Cuminum cyminum*, gamma ray, growth, irradiation, LD_{50}, mutagenesis

Introduction

Cumin (*Cuminum cyminum* L.) is an annual herbaceous spice cum medicinal plant. This crop belongs to the family apiaceae and having the basic chromosome number 2n = 14. The crop mostly grows in Mediterranean and Asian region and probably the Mediterranean region in the native place of cumin crop. The cumin crop is dicotyledonous and allogamous with hermaphrodite flowers (Sastry and Anandaraj, 2013). The cumin seeds have abundant industrial value as it had been used as a medicinal and spice plants since ancient times (Rathore *et al.*, 2013). Cumin is the most popular spice crop in the world, after black pepper and India is the largest producer, consumer and exporters of this crop (Hashemian *et al.*, 2013). Cumin is a highly valuable crop and growing area is mainly concentrated in Gujarat and Rajasthan. The total area is around 701560 ha with the production of 372290 tonnes. The total export of seed spices is worth of more than Rs 2800 crore annually out of which cumin alone contributed more than Rs 1500 crore (Spice Board of India, 2015-16). Trade and use of the crop is limited to natural areas that produce cumin crop (Avatar *et al.*, 1991). The productivity is low due to inherently low yield potential and susceptibility to diseases (Sharma, 1994). For the cultivation and supply to industrial and other uses farmers required to grow high yielding variety with high industrial compound contents (Gohari and Saeidnia, 2011; Al-Snafi, 2016).

The success of breeding programmes in any crop depends on the variability present in the gene pool for the essential traits. The induced mutations have been found pretty

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effective in generating useful variation for polygenically controlled traits in many crops. The physical mutagens like gamma rays are known to be the most popular mutagen because of their simple use, high penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002). After the discovery that physical mutagens can induce mutation, many plant breeders and geneticists started observing the use of radiation induced mutations for altering plant characters (Ahloowalia *et al.*, 2004). During the last seventy years, more than 3218 mutant varieties have been officially released (Mutant Variety Database, FAO/IAEA, 2016). Mutation induced by radiation was the most commonly used method to develop direct mutant varieties (89%). Gamma rays were employed to develop 64% of the radiation induced mutant varieties, followed by 22% by X-rays (Maluszynski *et al.*, 2000). Radiation has been the most frequently used method for crop improvement whereas the use of chemical mutagens was relatively infrequent. In several mutation derived varieties, the changed traits have resulted in synergistic effect on increasing the yield and quality of the crop and better consumer acceptance (Ahloowalia *et al.*, 2004).

Although mutations are beneficial for producing variability in populations, the treatments themselves can be detrimental and can cause a reduction in germination, growth rate, plant vigour and pollen and ovule fertility in a plant (Micke and Donini, 1993). If the variability is low in the population/ germplasm, then it is necessary to create variability for crop improvement. The variability is created through recombination techniques in general, but its difficult in cumin, due to small flower size, lack of genetic variability and non availability of any type of sterility (Sastry and Anandaraj, 2013). There is immense need of high yielding varieties, which is not possible without generating variability in cumin and also in other seed spices. Induced mutation can be an effective approach to generate genetic variability in cumin. Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen (time × concentration/dose), while mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in M_1 generation (Konzak *et al.*, 1965; Ambavane *et al.*, 2015). The mutagenic effectiveness decreased with the increase in dose of mutagen that indicates negative relationship between effectiveness and amount of mutagens. The mutagenic efficiency was maximum at the lowest dose and it reduced with the increase in dose of mutagens (Ganapathy *et al.*, 2008). However, for initiating mutation breeding programme in any crop to create the desired variability, the knowledge of optimum dose of mutagen is essential. It is essential to decide the appropriate dose of mutagen for a crop and or variety which can be employed for inducing maximum variability through mutations in mutation breeding programme. The growth parameters including percent seed germination, seedling growth are some of the usually used criteria for determining mutagenic sensitivity in a particular crop and variety (Lal *et al.*, 2009). Therefore, an experiment was conducted to determine the LD_{50} dose in cumin crop more accurately.

Materials and methods

The aim of present investigation was to determine the lethal dose (LD_{50}) of gamma radiation in cumin cv. Gujarat Cumin- 4. The experiment was carried out at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai. The dry seeds of cumin crop were used for the irradiation purpose. In first step of experiment, a preliminary experiment was conducted to know the sensivity of cumin to gamma radiation in which, cumin seeds irradiated with gamma rays at the doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy at Bhabha Atomic Research Centre, Mumbai. In second step of the experiment, based on preliminary results the cumin seeds were irradiated with gamma rays of 100 Gy, 125 Gy, 150 Gy, 175 Gy and 200 Gy to determine the exact $LD_{50.}$ The irradiated seeds along with non irradiated seeds were grown in controlled condition (Panasonic modelMLR – 352 H- PE) on blotting paper. Following different parameters related to determine LD_{50} were recorded.

Percent 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.

$$\text{Percent seed germination} = \frac{\text{No. of germinated seeds}}{\text{Total number of seed}} \times 100$$

Percent seedling survival: The percent survival of seedling was calculated after 30 DAS using formula given below :

$$\text{Percent survival} = \frac{\text{No. of plant survived}}{\text{Number of seed sown}} \times 100$$

Seedling height (cm): Seedling height was recorded from 15 randomly selected seedlings per replication after 30 DAS sowing.

Root-length (cm): Root length was recorded from 15 randomly selected seedlings per replication after 30 DAS sowing.

Shoot Length: Shoot length was recorded from 15 randomly selected seedlings per replication after 30 DAS sowing.

Fresh weight of plants: Freshly uprooted seedlings were placed on blotting paper to soak the surface water and then weighted on an electronic balance and mean value was calculated for each treatment.

Experiments were carried out in completely randomized design and each treatment was replicated thrice. All the data were analyzed using the statistical software SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The means were separated by Duncan Multiple Range Test (DMRT) at 5% level of significance. The graphs were prepared by using Microsoft Office Excel- 2013.

Results and discussion

Determination of sensivity of cumin to gamma radiation dose

The data pertaining to seed germination and seedlings survival is presented in Fig. 1 which revealed that as the irradiation doses increased, both percentage of seed germination and seedling survival decreased. The seed germination was 100% in non irradiated seeds, while after imposing irradiation the germination reduced by 9.53% in 100 Gy, 30.94% in 200 Gy, 64.29% in 300 Gy, 83.33% in 400 Gy and 90.48% in 500 Gy as compared to control. Among the doses of irradiation, 400 Gy and 500 Gy were more lethal. The plumule and radical emerged at the dose of 400 Gy and 500 Gy but in later growth stages failed to transform into complete seedling (Fig. 2). With regards to the survival of seedlings, the survival of seedlings decreased with increase in dose of gamma radiation. In control the 100% seedlings survived but the irradiated seedlings reduced in its survival after germination. The seedlings survival was reduced by 38.10% at 100 Gy, 58.52% at 200 Gy, 95.24% at 300 Gy and none of the seedlings survived at the dose of 400 Gy and 500 Gy. Highly significant differences were observed among the lower and higher doses of irradiation on number of leaves, root length, shoot length and fresh weight per plant. Dose dependent reduction in percent of seed germination and percent seedlings survival was observed in present study. The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). Micco *et al.*, (2011) have correlated seed germination with abnormalities in mitotic cycles and in metabolic pathways of the cells. The reduction in germination and survival may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovacsand and Keresztes, 2002). Bashir *et al.* (2013) also reported that the seed germination percentage and percent survival decreased with an increase in dose/concentration of the mutagens. They concluded that lower treatment of mutagens have influenced less biological damage and would be suitable for inducing desirable mutations. The present findings are in agreement with the above mentioned reports. Similar findings were also reported wherein, higher doses of gamma radiation reduced germination percentage and survival in fennel (Verma *et al.*, 2017) and coriander (Sarada *et al.*, 2015).

From the data recorded, it was observed that gamma radiation had a highly significant impact on number of leaves, number of roots per plant, root length, shoot length and fresh weight per plant (Table 1 & Fig. 2). The maximum number of leaves (7), number of roots per plant (5.00), root length (4.27 cm), shoots length (2.9 cm) and fresh weight per plant (0.009 g) were observed at lower dose 100 Gy followed by 200 Gy and 300 Gy. Since the seedling growth was observed at the gamma radiation dose 300 Gy but the survival of the seedlings was very low (4.76%) while at the dose of 400 Gy and 500 Gy the all the

Table 1. Effect of acute exposure of different doses of gamma rays on growth characters of M_1 seedlings.

Dose (Gy)	Number of leaves	No. of roots per plant	Root length (cm)	Shoot length (cm)	Fresh weight per plant (g)
0 (Control)	7.67^{d}	9.00^{d}	5.70^{d}	3.17^{d}	0.010^{d}
100	7.00c^{d}	5.00^{c}	4.27^{c}	2.90^{c}	0.009c^{d}
200	6.00^{c}	3.33^{b}	3.53^{b}	2.80^{c}	0.008^{c}
300	2.33^{b}	2.00a^{b}	1.57^{b}	1.19^{b}	0.004^{b}
400	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.000^{a}
500	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.000^{a}

Same superscript letters in a column do not differ significantly when compared by DMRT test at 5% level of significance

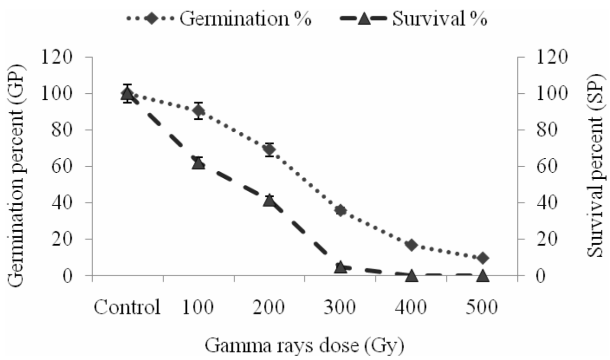

Fig 1. Mutagenic effect of gamma rays on percent seed germination and percent seedling survival in cumin cv. GC-4

germinated seedlings failed to survive (Fig. 2). The high dose irradiation that caused growth inhibition could be ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome as reported by Preussa and Britta (2003). Similar results were obtained by Yadav and and Ramkrishna, 2013 in *Cuminum cyminum*.

Damage in M_1 generation plants and determination of LD_{50}

Based on preliminary experiment in which 38.10% reduction observed in seedling survival at 100 Gy followed by 200 Gy (58.52%) and 300 Gy (95.24%). In order to determine the LD_{50} dose in cumin more accurately, 5 doses were selected between 100 Gy to 200Gy by giving 25 Gy intervals. The results showed reduction in seed germination and seedlings survival with the increase in dose of gamma rays (Fig. 3). Seed germination was 100% in control, while among the different doses of gamma rays, the maximum seed germination was noticed at lower dose 100 Gy (95.50%) followed by 125 Gy (90.90%), 150 Gy (75.00%), 175 Gy (72.00%) and 200 Gy (68.18%). The seedlings survival was decreased with increase in the dose of gamma radiation. The 45.45 percent seedlings survived at highest dose of 200 Gy of gamma radiation. With regards to seedling growth parameters, it was recorded that increased in the radiation dose, the growth parameter were decreased. Control recorded the highest shoots length (7.27 cm), root length (22.47 cm), number of leaves (10.67) and fresh weight per plant (0.0017 g) followed by irradiated seedlings (Fig. 4&5). The shoots length (3.63 cm) and root length (10.67 cm) were decreased at radiation dose 200 Gy and found 49.93% and 47.48% reduction in shoot and root growth over control (Fig. 4). The reduction in the number of leaves and fresh weight were also recorded. The 49.95% reduction in number of leaves and 52.95% reduction in fresh weight per plant was recorded at the gamma ray dose of 200 Gy as compared to control (Fig. 5). The reduction in plant survival is ascribed to damage cause at cytogenetical and physiological levels (Sato and Gaul, 1967). Srivastava *et al.*, (2011) suggested that the reduction in seedling survival may be due to the hindrance caused by the mutagen on different metabolic pathways of the cells. In an another report Sarada *et al.*, (2015) find that increase in the dose of gamma rays significantly decreased seedlings growth parameters in coriander. Same results was obtained by Verma *et al.* (2017) in fennel who reported that decreased in the root length, shoot length, number of leaves and fresh weight of plant when the gamma rays dose increased.

The results of the experiments indicated that high dose of gamma radiation reduced germination percentage, seedling survival, shoot length, root length, number of

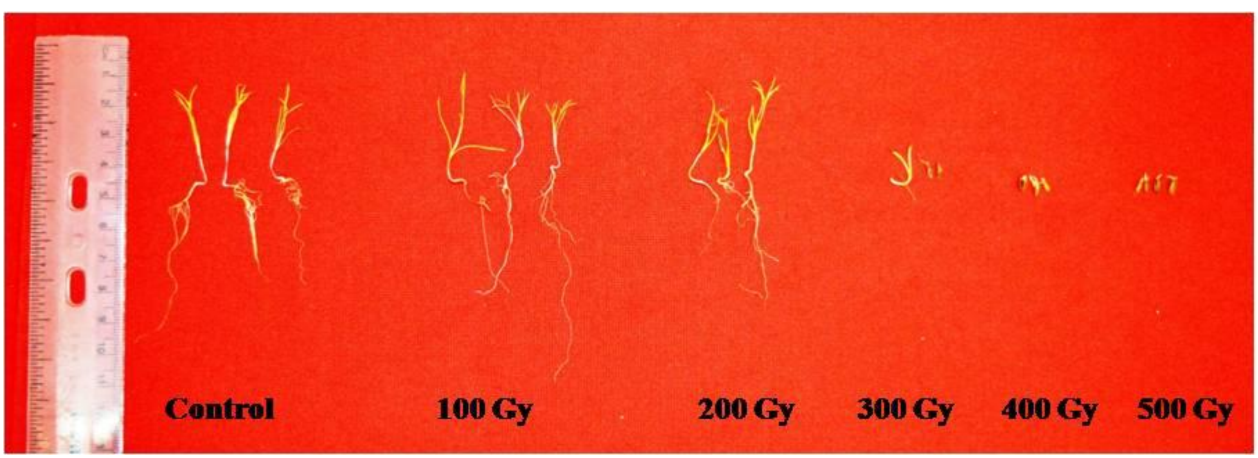

Fig. 2. Effect of different doses of gamma irradiation on growth of cumin cv. GC- 4

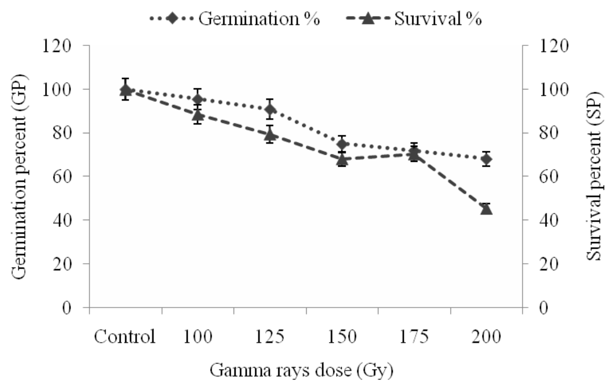

Fig 3. Mutagenic effect on percentage of seed germination and seedlings survival in M_1 generation of cumin cv. GC-4

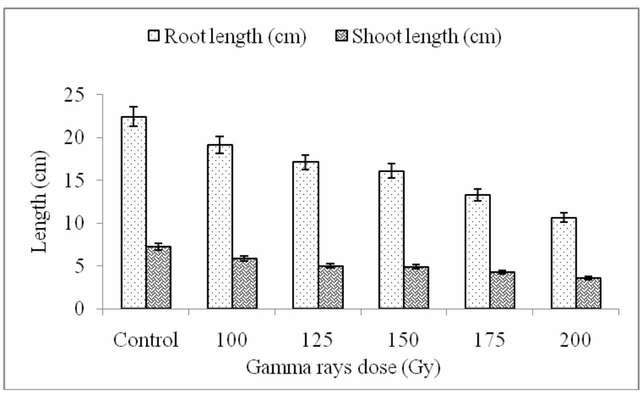

Fig 4. Effect of acute exposure of gamma rays on root and shoot length in cumin cv. GC-4

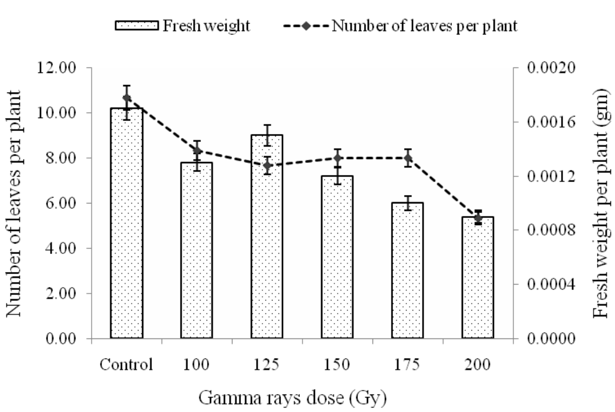

Fig 5. Mutagenic effect of acute exposure of gamma radiation on fresh weight and number of leaves in cumin cv. GC-4.

leaves and fresh weight of plant drastically. The high doses of 300 to 500 Gy gamma rays were more lethal. 200 Gy of gamma radiation was found to cause near about 50% seedling survival and 50% reduction in root length, shoot length and fresh weight per plant. It is expected that 200 Gy of gamma ray would be the optimal dose for inducing useful mutation in cumin.

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