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DETERMINATION OF LETHAL DOSE FOR GAMMA RAYS AND ETHYL METHANE SULPHONATE INDUCED MUTAGENESIS IN OKRA (*Abelmoschus esculentus* (L.) Moench.)

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SUMMARY

Induced mutation by gamma rays and Ethyl Methane Sulphonate has been found to be a very useful technique for crop improvement. Chemical mutagens such as EMS and physical mutagens such as gamma rays, X- rays have been broadly used to cause a majority of practical variations in several crops. Therefore, a study was undertaken to determine LD_{50} and effect of mutagens on germination, seedling length and survival percentage of seedlings derived from mutated seeds of okra variety P-8 by using EMS and gamma rays to create variability for desirable traits. Seeds were exposed to different doses of gamma radiations using ^{60}Co as the radiation source at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. The seeds were also treated using EMS. The treated seeds along with control were kept for germination in petri plates under seed germinator and also sown in the greenhouse. Based on probit curve from survival of treated material, the LD_{50} dose for gamma rays and EMS were 75 kR and 1.4%, respectively. With the increase in concentration of EMS, a decrease in survival was observed. Seedling length and survival percentage also decreased with the increase in gamma dose. Therefore, standardized dose of EMS and gamma rays can be tested in other varieties or lines of okra to create variability for further selection.

Key words: Gamma rays, ethyl methane sulphonate, okra, LD_{50}, germination percentage, seedling growth.

Key findings: Lethal dose estimation of both gamma rays and EMS is important for plant breeders to induce mutation, because LD_{50} values determines the effect of mutation and is different between species, varieties and genotypes. Per cent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. The survival rate and seedling length was highly reduced with increasing dose/concentration of mutagens. The LD_{50} in this study can be used by various breeders to obtain the desired characteristics in P-8 variety of okra.

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INTRODUCTION

Mutation breeding has been widely used for the improvement of probable traits of various crop plants. The key strategy in mutation breeding is to promote the well-adapted plant varieties by altering 1 or 2 major agronomic metrical traits which limit their productivity or enhance their

quality and potential source of creating variability (Novak and Brunner, 1992). It is a powerful and effective tool being used to study the nature of function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). It offers significant increase in vegetable crop production and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution (Kharkwal and Shu, 2009). Mutagenic agents have been used to induce useful phenotypic variations in plants for more than 70 decades (Anitha *et al*., 2005) and more than 2543 mutant cultivars from 175 plant species have been officially released in 50 countries all over the world (Chopra, 2005; Bhat *et al*., 2007). China, India, and Japan are the 3 countries, who had released the largest number of mutant varieties in the world. In India, 343 varieties have been released in 57 different crops (Kharkwal and Shu, 2009). Several factors such as properties of mutagens, temperature, pH, duration of treatment, pre- and post-treatment temperature, etc. influence the effect of mutagens. Mutations are induced by physical and chemical mutagen treatment both in seed and vegetative propagated crops. The mechanism of mutation induction is that the mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations occur randomly and are heritable. Gamma rays and EMS are commonly used mutagens in plant breeding programmes, because these are known for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gossal, 2002). The toxicity of EMS may vary depending on the species and other mutagens or post-treatments with antioxidants may be worth considering (Henikoff and Comai, 2003). The dose assessment of chemicals is determined by varying the concentration and duration of treatment, solvent used or pH of the solution (Jain, 2010).

Chemical mutagens and ionizing radiation have been used for long time as the plant mutagens in breeding research and genetic studies (Guenet, 2004). Production of mutants by chemical or irradiation mutagenesis is fairly economical. Chemicals mainly cause point mutations, therefore are perfect for production of missense and nonsense mutations. However, ionizing radiations normally cause chromosomal rearrangements and deletions (Bhat *et al*., 2007). The use of physical and chemical mutagens help to improve many traits of agronomical importance in major crops such as *Cymopsis tetragonoloba* (Arora and Pahuja, 2008), beans (Khan, 2009), capsicum (Nascimento *et al*., 2015) and paprika (Kumar *et al*., 2012). Induced mutations are necessary to enhance the rate of genetic variability.

Okra (*Abelmoschus escluentu*s (L.) Moench.) also called as lady's finger or bhindi, is one of the most common and principal vegetable crops grown year round in the country including Himachal Pradesh. Due to its high nutritive value and long post-harvest life, it has captured a prominent position among the export oriented vegetables. Even though okra is cultivated to a larger extent throughout the country, there is ample scope of enhancing its productivity further. There is not much variability in okra in the Indian subcontinent and most of the available varieties are highly susceptible to yellow vein mosaic virus. During okra breeding, while developing superior crop varieties with increased productivity phenotypically and agronomically important traits are given due weightage. As a result, genetic diversity is lost through fixation, genetic sweeps and increased dependence of breeders on smaller sets of superior genotypes, creating successive bottlenecks (Varshney, 2013).

Induced mutagenesis using an array of physical and chemical mutagens, offers a possibility for the induction of desirable changes in various attributes, which can be exploited as such or through recombinant breeding. It is possible to change one or few undesirable traits without altering an otherwise superior agronomic base. Mutagenesis also enriches the germplasm with the newly arisen and hitherto unknown genotypes. To avoid excessive loss of actual experimental materials, radio/chemical sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive) doses before massive irradiation of similar materials are accepted.

Lethal dose, the percentage of test organisms that killed by a specific dosage (of chemicals or radiation), half will die at LD_{50} and is considered as a dose at which highest frequency of mutation occurs. The present study is aimed at determining the optimum lethal dose (LD_{50}) of two mutagens viz., Gamma rays as physical mutagen and EMS as chemical mutagen for creating genetic variability in okra.

MATERIALS AND METHODS

In this investigation a very popular and well adapted cultivar 'P-8' of okra was chosen to study the effect of gamma rays (^{60}Co) and Ethyl Methane Sulphonate (EMS) on survival percentage and length of okra seedlings. The study was conducted at vegetable farm of Chaudhary Sarwan Kumar Himachal Pradesh KrishiVishvavidyalaya, India during June 2014. 'P-8' seeds were chosen because of its high economic value and resistance to yellow vein mosaic virus. Its yield potential is about 100-150 q/ha. The moisture content of seeds was measured using non-digital moisture meter PM-600 at the department of Seed Science and Technology, CSK HPKV Palampur (H.P.), India. A chemical mutagen, EMS and a physical mutagen, ^{60}Co were used in the present investigations to induce mutations in the selected plant material and to achieve genetic variability.

Dry seeds of okra cultivar 'P-8' having moisture content of 11.1% were irradiated with 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 and 85 kR of gamma rays. Gamma irradiation was conducted using ^{60}Co gamma source (Gamma chamber, Bhabha Atomic Research Centre, Trombay, Mumbai), India. Also for EMS mutagenesis, healthy and mature disease free seeds were selected and soaked in distilled water for 6 hours. Water was decanted and then dried in shade for few hours. Fresh solution of Ethyl Methane Sulphonate (Hi-media, Mumbai) was prepared in phosphate buffer at pH 7.0 in different concentrations (0.2, 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.4 and 1.6%). Seeds were incubated at room temperature for 6 hours followed by decanting of EMS. Finally, seeds were rinsed with tap water for 12 hours to wash out chemical residues.

The experiment was organised in completely randomized design (CRD) with 3 replications. Based on gamma radiation and EMS mutagenesis, hundred seeds of each treatment along with untreated seeds as control were placed in petri plates over moistened germination paper under seed germinator at 25 ± 1^0C. The treated seeds were also sown in the polyhouse. The germination percentage and seedling length of seeds germinated in seed germinator were recorded after 12 days. Germination was considered to have occurred when the hypocotyls emerged out from the seed. The seedling length was measured with the help of scale after 12 days of germination. LD_{50} values were determined with the help of probit analysis based on survival rate of the okra plants after treatment with different doses/concentration of gamma rays and EMS as compared with untreated control. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. The LD_{50} also vary and depends upon factors such as moisture content and the type of variety used. Data so obtained were subjected to analysis of variance having significant level of 5% ($\alpha \leq 0.05$) after transformation of data following the computer software package "CPCS", developed by Singh and Cheema (1985) as per the procedure suggested by Gomez and Gomez (1984). When statistical differences were found, the least significant difference (LSD) was used to compare the means both at 5% and 1% significance levels.

RESULTS AND DISCUSSION

Determination of lethal dose

The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering 1 or 2 major agronomical traits which limit their productivity or enhance their quality. The success of mutation breeding greatly depends upon the rate of mutation, the number of plants screened and mutation efficiency. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive)

doses before massive irradiation of similar materials are accepted.

In this study, from the probit curve analysis the LD_{50} value for gamma rays and EMS were recorded as 75 kR and 1.4%, respectively (Figures 1 and 2). Norfadzrin *et al*. 2007 treated okra seeds with different concentrations of gamma rays and determined 770 Gy as appropriate dose to induce mutations in okra. In concert with previous study on radiation mutation (Kiong *et. al*., 2008), survival of plants to maturity depends on the nature and extent of chromosomal damage. The present findings are also similar to Kangarasu *et al*. (2014) in cassava.

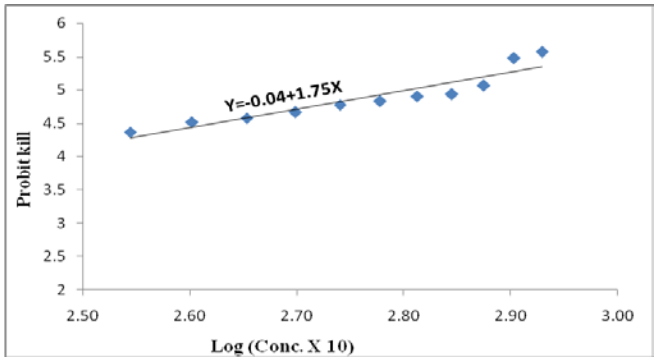

Figure 1. Probit analysis for calculation of LD_{50} of gamma irradiation.

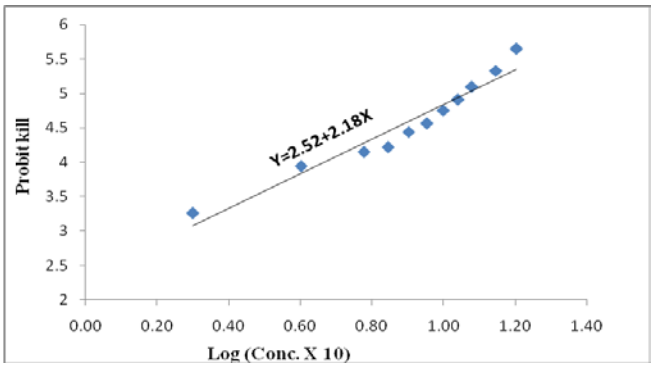

Figure 2. Probit analysis for calculation of LD_{50} of gamma irradiation.

Impact of mutagenesis on survival and seedling length

Survival percentage of okra variety 'P-8' under different dose concentrations were calculated based on the survival of seedlings after treatment and compared with control. There was an abnormal reduction in the survival of seedlings with the increase in gamma dose and EMS. Conversely, there was an increase in the survival reduction in higher doses as compared to lower doses (Table 1). Data analysis on number of seeds that survived showed an attendant decrease in survival significantly with the increase in concentration of EMS. According to Table 2, the results obtained indicate that significant reduction in survival occurred with corresponding increase in EMS concentration. Although the reduction in survival was high in gamma radiation as compared to EMS treatment. This is evident that chemicals produce only point mutations, whereas radiations normally cause chromosomal rearrangements and deletions (Bhat *et al*., 2007). Reduction in

germination inability, plant growth and survival was due to increasing frequency of chromosomal harm with increasing radiation dose or mutagen concentration (Kumar *et al*., 2004, Kiong *et al*., 2008, Bashir *et al*., 2013).

Table 1. Mean values of survival and seedling length after gamma irradiation.

Treatments (kR)	Survival (%)		Seedling length (cm)
	Actual	Survival	
Control	100	80 (8.99**)	20.0
35	100	70 (8.41**)	18.0
40	100	50 (7.12**)	16.5
45	100	70 (8.41**)	14.0
50	100	70 (8.41**)	13.5
55	100	40 (5.09**)	12.8
60	100	46 (6.83**)	12.0
65	100	49 (7.05**)	11.2
70	100	50 (6.37**)	10.5
75	100	39 (6.30**)	9.50
80	100	0 (1.00)	8.70
85	100	10 (3.31*)	7.50
CV (%)	-	10.95	17.05
$CD_{(0.05)}$	-	1.18	3.68

Figures in the parentheses are square root transformed
* Significant at 5% level of significance
** Significant at 1% level of significance

Table 2. Mean values of survival and seedling length after treatment with EMS.

Treatments (%)	Survival (%)		Seedling length (cm)
	Actual	Survival	
Control	100	96 (4.56**)	20.0
0.2	100	93 (4.53**)	15.0
0.4	100	83 (4.41**)	12.5
0.6	100	71 (4.23**)	11.5
0.7	100	35 (3.54**)	10.0
0.8	100	33 (3.49**)	9.00
0.9	100	13 (2.26)	8.00
1.0	100	15 (2.70*)	7.50
1.1	100	33 (3.48**)	7.20
1.2	100	18 (2.86**)	6.50
1.4	100	10 (2.26)	6.00
1.6	100	30 (3.39*)	6.00
CV (%)	-	10.20	34.26
$CD_{(0.05)}$	-	0.597	5.83

Figures in the parentheses are log transformed
*Significant at 5% level of significance
**Significant at 1% level of significance

In this study, least values of 39 and 10% survival rate were observed in the seeds irradiated with the highest dose of 75 and 85 kR gamma rays respectively. In case of EMS, least germination percentage of 10% was observed at higher dose of 1.4% (Tables 1 and 2). The decrease in okra germination with increasing dosage could be attributed to the occurrence of seeds without

completely developed embryos (Omar *et. al*., 2008). The survival rate of control plants was certainly higher because their seeds were not exposed to any mutagenic treatment. At a certain level of mutagenic dose, the plant can grow at early stage of growth, but cannot survive at certain duration probably due to DNA breakage and inability to repair them. Failure in seed germination was recorded in treated plants with a dose of 85 kR might be due to the delay or inhibition in physiological processes viz., enzyme activity, hormonal imbalance and inhibition of meiotic process necessary for seed germination (Ananthaswamy*et.al.,* 1971) owing to application of gamma rays. Moreover, the decrease in the percentage of germination at higher doses may be due to the disturbances caused at physico-chemical level of cells, chromosomal damage or due to the combined effect. This is in close conformity with the earlier findings of Horn and Shimelis (2013) in cowpea, Monica and Seetharaman (2015) in garden bean and Umavati and Mullainanthan (2015) in chickpea.

According to results obtained in this study (Tables 1 and 2), seedling length decreased significantly in proportion with increase in applied doses of either gamma rays or EMS as compared to control. The least seedling length7.5 cm and 6.0 cm has been recorded when 85 kR of gamma dose and 1.4, 1.6% concentrations of EMS has been applied, respectively as shown in Plate 1.

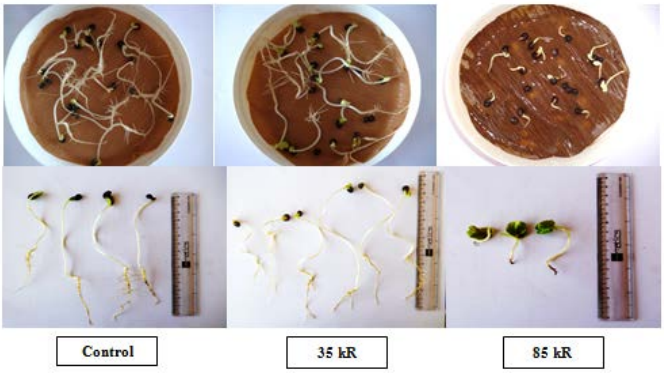

Figure 1a. Petri plates showing germination percentage as well as seedling length in untreated (control), lower dose (35kR) and higher dose (85kR) of gamma rays.

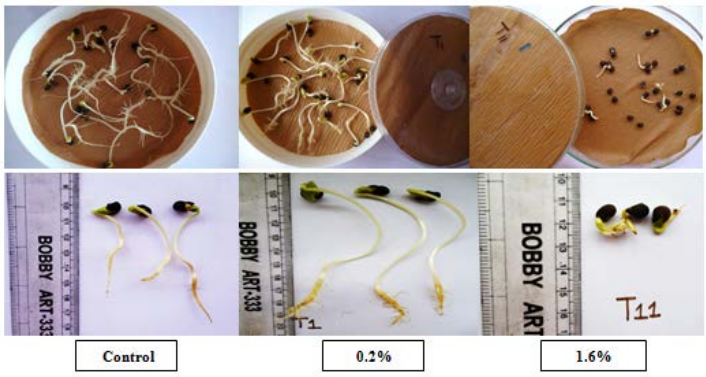

Figure 1b. Petri plates showing germination percentage as well as seedling length of untreated (control), lower dose (1.2%) and higher dose (1.6%) of EMS.

EMS mutated seeds Gamma rays irradiated seeds

Figure 2. Combined effect of mutagens on seedling growth.

The combined effect of mutagens (gamma rays and EMS) on seedling growth is shown in Plate 2. The results showed that the differences among mutation treatments considerably influence the survival and seedling length. There was a significant decrease in the level of survival, seedling length with the increase in the either gamma rays or concentration of EMS comparing to control. To identify the biological influences of different physical and chemical mutagens, seedling length is mostly utilized as an index (Bhat *et al*., 2007). It has been shown that a linear dependency exists between seedling length and the dosage of physical or chemical mutagens. In concept with this observation, our findings show that decreases in seedling length were because of increases in EMS concentration or gamma radiation doses. The findings of radio sensitivity study were also confirmed from the results obtained by Wi *et al*. (2007).

CONCLUSION

Determination of LD_{50} value for any mutagen is necessary to produce maximum viable mutants with minimum damage to the plant. The LD_{50} dose based on the reduction in survival after treatment with different doses of gamma rays and different concentration of EMS for the okra cultivar P-8 were 75 kR and 1.4%. In addition, the optimum dose based on the reduction in survival and growth parameters were 1.2-1.6% of EMS concentration and 65-85 kR of gamma rays to create maximum variability with minimum numbers of undesirable mutants. Increasing gamma rays and EMS dose decreased germination percentage, survival percentage and seedling length of okra. These optimum mutagen doses determined for the okra genotype could be useful while formulating okra mutation breeding programme for improvement of specific traits in okra.

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