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# MEDIAN LETHAL DOSE ESTIMATION OF GAMMA RAYS AND ETHYL METHANE SULPHONATE IN BELL PEPPER (*Capsicum annuum* L.)

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## SUMMARY

Induced mutation is an efficient tool to improve a crop through creation of variability but it has been rarely exploited in improving bell pepper. This research was aimed to determine the lethal dose ($LD_{50}$) and effect of gamma rays and ethyl methane sulphonate (EMS) on germination, root length, shoot length, seedling length, speed of emergence and seedling vigor index derived from mutagen treated seeds of bell pepper cultivar 'California Wonder', to create variability for desirable traits. Seeds were exposed to different doses of gamma rays using $^{60}Co$ gamma cell at Bhabha Atomic Research Centre, Trombay, Mumbai, India. Another set of presoaked seeds were treated with freshly prepared solution of EMS. The treated seeds including control were sown in the nursery beds in a green house in completely randomized design (CRD) with 5 replications. Observations showed that germination percentage, seedling root length, seedling shoot length, speed of emergence and seedling vigor index decreases with increasing dose of gamma rays and EMS. Higher doses of gamma rays (19kR and 22kR) and EMS (2.0% and 3.0%) had profound effect on these variables due to seed injury and resulted in poor growth of seedlings. Based on probit curve, $LD_{50}$ dose for gamma rays and EMS were 17.8 kR and 1.6%, respectively. Higher gamma rays and EMS doses had negative effect on the morphological characteristics and growth parameters of the seedlings derived from mutagen treated seeds. Therefore, consistent dose of gamma rays and EMS can be tested in other varieties or lines of bell pepper to generate variability for novel selection.

**Key words:** Lethal dose, gamma rays, ethyl methane sulphonate (EMS), induced mutation, survival, *Capsicum annuum* L. var. *grossum* cv. California Wonder

**Key findings:** $LD_{50}$ value of gamma ray irradiation and EMS is important for plant breeders to induce mutation, because $LD_{50}$ value determines the effect of mutation in plant and is different between species, varieties and genotypes. The $LD_{50}$ in this study can be used by capsicum breeders to use these mutagens in California Wonder cultivar to obtain the mutant plants.

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## INTRODUCTION

Mutation induction is of great potential value for plant improvement. Pepper (*Capsicum annuum* L.) originated in South America and spread into Asia and Africa (George, 1985). Sweet pepper is now commonly grown crop in India because of its preferred consumption in many food cuisines. Mutation is a change of DNA sequence in a gene or a change of chromosome and is responsible for genetic variation. It can occur spontaneously or resulting from exposure to radiations and chemicals. Chemical mutagens (ethyl methane sulphonate, and diethyl sulphonate and sodium azide) and irradiations (gamma rays, X-rays and fast neutrons) have been widely used to induce a large number of functional variations in crop plants (Umavathi and Mullainathan, 2015).

Chemicals induce mainly point mutations, and are thus ideal for producing missense and nonsense mutations, which would provide a series of change-of-function mutations. On the other hand, ionizing radiations normally induce chromosomal rearrangements and deletions. Gamma rays are commonly used in plant breeding programs because these are known for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gosal, 2002; Roslim *et al*., 2015). Different types of morphological mutations were isolated through chemical mutagenesis in *Capsicum annuum* (Gandhi *et al*., 2014; Aruldoss and Mullainathan, 2015). The use of physical and chemical mutagens helped to improve many traits of agronomical importance in Capsicum (Nascimento *et al*., 2015) and paprika (Kumar *et al*., 2012). As a result of induced mutation and improved management and agronomic inputs over the past years, significant increase of major crops including bell pepper varieties have been reported (Daskalov and Baralieva, 1992; Pillai and Abraham, 1996; Swaminathan, 1998; Ariraman *et al.,* 2014).

Higher doses inevitably bring about mortality, high pollen and seed sterility and deleterious mutations. To avoid excessive loss of actual experimental materials, radio/chemical sensitivity test must be conducted to determine $LD_{50}$ (the safe dose at which half of the planting material survive) doses before massive irradiation/chemical treatment of similar materials are accepted (Talebi *et al.*, 2012). $LD_{50}$ is the dose at which highest frequency of mutation occurs. This study is aimed to determine optimum lethal dose ($LD_{50}$) for two mutagens gamma rays (physical mutagen) and ethyl methane sulphonate (chemical mutagen) in bell pepper.

## MATERIALS AND METHODS

The genetically pure seeds of commercial bell pepper cultivar 'California Wonder' seeds with 90% germination were chosen for mutation induction. The moisture content of the seeds was measured using moisture analyzer (AND MX-50) at the Department of Seed Science and Technology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSK HPKV), Palampur, HP, India. A chemical mutagen, Ethyl Methane Sulphonate (EMS) and a physical mutagen, gamma rays ($^{60}CO$) were used.

One hundred seeds of cultivar 'California Wonder' were exposed to different doses (0.5 kR, 1.0 kR, 3.0 kR, 5.0 kR, 8.0 kR, 11kR, 13kR, 16kR, 19kR and 22kR) of gamma rays, using $^{60}CO$ gamma source at Bhabha Atomic Research Centre, Trombay, Mumbai, India. For EMS mutagenesis, the 100 seeds were first soaked in distilled water for 12 hours. Water was decanted and dried in shade. Fresh solution of Ethyl Methane Sulphonate (Hi-media, Mumbai) was prepared in phosphate buffer at pH 7.0 in 0.1%, 0.25%, 0.50%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% and 3.0% concentrations and treatment was given for 6 hours at $30 \pm 1$°C with intermittent shaking. Finally rinse the treated seeds with running tap water for 6 hours to wash out the chemical residues. The treated seeds were sown in the nursery beds along with control in completely randomized design (CRD) with 5 replications during 2011 and 2012 at the Department of Vegetable Science and Floriculture, CSK HPKV, Palampur, India. Data were observed on various parameters *viz.*, germination percentage, root length, shoot length, seedling length, seedling vigor index and speed of emergence. Water was applied manually to maintain moisture at field capacity. Pesticides, herbicides and fertilizers were not applied in order to avoid any interference during the study. Data on seed germination was recorded 15 and 30 days after sowing. Seedling root length, seedling shoot length was measured using scale. Seedling vigor index and speed of emergence were calculated using the following formulae.

## Germination

The observations on germination were taken on 15th day and 30th day after sowing by visually counting the number of germinated seeds for each treatment.

## Seedling growth

Primary shoot, root and seedling length were recorded for 10 randomly selected seedlings from each treatment.

## Seedling vigor index

The seedling vigor index was calculated according to ISTA, (Hangarter, 1997) by using following formula:

$$\text{Seedling vigor index} = \frac{\text{Seedling length (cm) x Germination}}{100}$$

## Speed of emergence

The speed of emergence was calculated according to following formula:

$$\text{Speed of emergence} = \frac{\text{Number of germinated seeds 15 days after sowing}}{\text{Number of seedling emergence 30 days after sowing}} \times 100$$

## $LD_{50}$

$LD_{50}$ was assayed by calculating the seedlings that survived after nursery period. The above data regarding survival percentage was subjected to probit analysis to determine $LD_{50}$.

## Statistical analysis

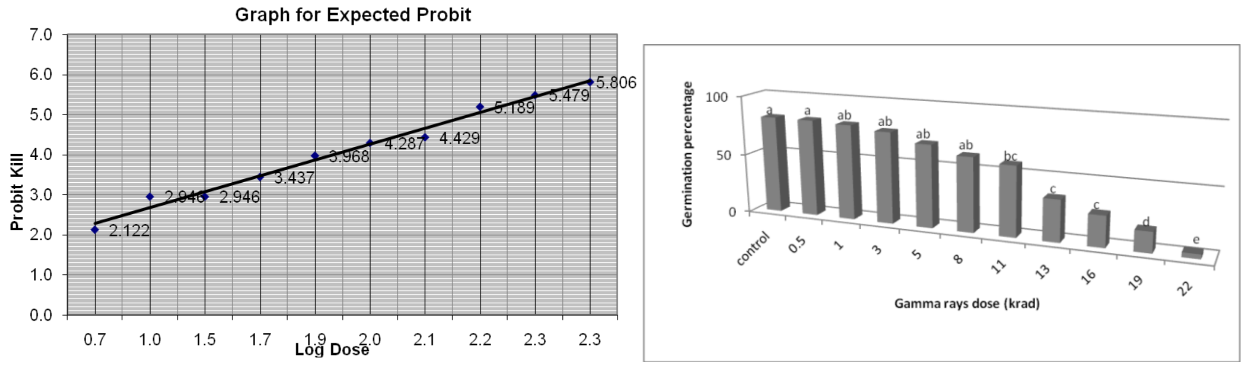
The experiment was arranged in a completely randomized design with 5 replications of 100 seeds each. Experimental data and means in each trait were compared and analyzed by Tukey's Test using Statistical Analysis System Version 9.1 (SAS).

# RESULTS AND DISCUSSION

## Determination of lethal dose

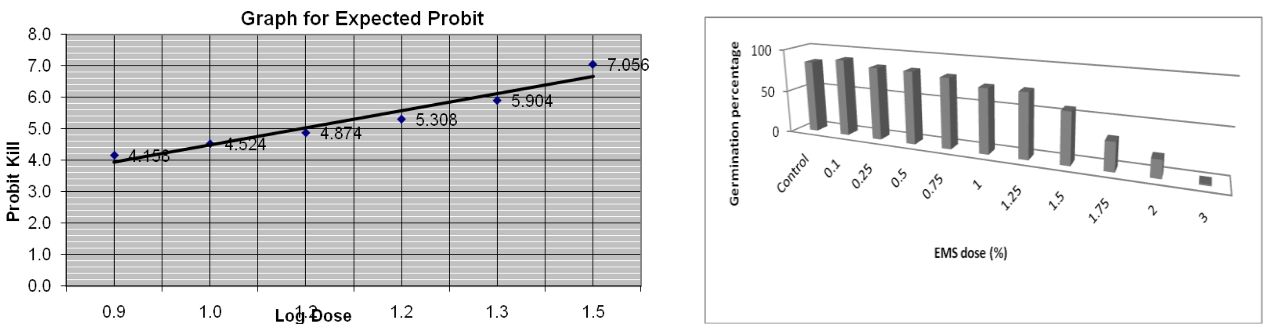
The mortality of plants increased linearly with the increase in gamma rays and EMS doses. The probit curve analysis revealed the $LD_{50}$ value for gamma rays and EMS were 17.8 kR (Figures 1a, b) and 1.6% (Figures 2a, b) , respectively. It means that 50% of plant population would be dead if exposed to 17.8 kR and 1.6% of gamma rays and EMS, respectively. Gaswanto *et al.* (2016) gave a series of gamma radiations to chilli seeds and determined a range between 422.64 to 629.68 Gy to induce desirable mutations. The $LD_{50}$ for chilli seeds was determined in various researches as 445 Gy (Omar *et al*., 2013), 40 kR gamma rays and 30 mM EMS (Aruldoss and Mullainathan, 2015). Previous studies on physical and chemical mutagens revealed that survival of plants to maturity depends on the nature and extent of chromosomal damage (Adamu and Aliyu, 2007; Khan and Goyal, 2009; Nascimento *et al*., 2015; Monica and Seetharaman, 2016).

Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in germination ability, plant growth and survival. Furthermore, genes near the centromere are more prone to mutagenic treatment than those located farther away. In another study, chlorophyll mutants were frequently observed among EMS treatment group but were rare among those treated with physical mutagens. The stimulating effect of physical mutation on germination may be credited to the activation of RNA or protein synthesis during the early stage of germination (Chopra, 2005).




a b

**Figure 1.** Effect of gamma irradiation on seed germination of bell pepper var. California Wonder.




a b

**Figure 2.** Effect of EMS on seed germination of bell pepper var. California Wonder.

**Impact of mutagenesis on survival and growth variables**

Estimation of lethal dose was done on the basis of measurement of different characters *viz*., survival (%), root length (cm), shoot length (cm), seedling length (cm), seedling vigor index and speed of emergence in $M_1$ generation.

The lower doses (0.5 kR to 11kR) of gamma rays and EMS (1% to 1.5%) in comparison with control did not affect germination, but contrary was the case of the higher doses i.e. 13 kR to 22 kR of gamma rays and 1.75% to 3% of EMS were so pronounced that their effect in particular inhibited seed germination (Tables 1 and 2). The higher doses had significant effect on germination, root length, shoot length, seedling vigor index and speed of emergence in physical mutagen 19kR, 22 kR and chemical mutagen 1.75%, 2% and 3%. Gandhi *et al*. (2014) in chilli found that increasing concentration of EMS and DES (Di-ethyl Sulphonate) showed decrease in morphological and yield characters like germination (%), plant height (cm), primary and secondary branches per plant, days to first flowering, fruit length (cm), fruit girth (cm), fruits per plant, seeds per fruit, seed weight per fruit (g), 100 seed weight (g) and pericarp: seed ratio. Similarly, Aruldoss and Mullainathan (2015) showed a dose dependent decrease for most the characters plant height (cm), primary and secondary branches per plant, number of leaves, days to first flowering, fruits per plant,

**Table 1.** Effect of gamma irradiation on seed germination and growth of bell pepper seedlings.

| Dose ( kR ) | Germination (%) | | Root length (cm) | Shoot length (cm) | Seedling length (cm) | Seedling vigor index | Speed of emergence (%) |
|---|---|---|---|---|---|---|---|
| | DAP15 | DAP30 | | | | | |
| Control | 76.4$^{a}$ | 81.5$^{a}$ | 3.68$^{a}$ | 5.05$^{a}$ | 8.73$^{a}$ | 7.13$^{a}$ | 93.8$^{a}$ |
| 0.5 | 76.0$^{a}$ | 81.4$^{a}$ | 3.46$^{ab}$ | 4.31$^{ab}$ | 7.77$^{ab}$ | 6.31$^{ab}$ | 93.3$^{a}$ |
| 1 | 74.6$^{ab}$ | 80.0$^{ab}$ | 3.16$^{abc}$ | 4.24$^{ab}$ | 7.41$^{ab}$ | 5.91$^{abc}$ | 93.23$^{a}$ |
| 3 | 70.4$^{abc}$ | 76.8$^{ab}$ | 2.93$^{abc}$ | 4.20$^{ab}$ | 7.13$^{abc}$ | 5.36$^{bcd}$ | 91.65$^{ab}$ |
| 5 | 67.2$^{bc}$ | 75.6$^{ab}$ | 2.86$^{abc}$ | 4.15$^{abc}$ | 6.89$^{abc}$ | 5.13$^{bcde}$ | 88.88$^{ab}$ |
| 8 | 65.6$^{cd}$ | 74.1$^{abc}$ | 2.71$^{abc}$ | 4.03$^{abc}$ | 6.86$^{bc}$ | 5.05$^{bcde}$ | 88.52$^{ab}$ |
| 11 | 63.4$^{cd}$ | 71.7$^{bc}$ | 2.67$^{abc}$ | 3.90$^{abcd}$ | 6.58$^{bcd}$ | 4.7$^{cde}$ | 88.39$^{ab}$ |
| 13 | 57.8$^{de}$ | 66.2$^{c}$ | 2.62$^{abc}$ | 3.69$^{bcd}$ | 6.31$^{bcd}$ | 4.15$^{def}$ | 87.52$^{ab}$ |
| 16 | 55.2$^{e}$ | 65.6$^{c}$ | 2.61$^{bc}$ | 3.40$^{bcd}$ | 6.01$^{bcd}$ | 3.92$^{ef}$ | 84.1$^{b}$ |
| 19 | 37.8$^{f}$ | 50.2$^{d}$ | 2.51$^{bc}$ | 2.98$^{cd}$ | 5.5$^{cd}$ | 3.21$^{f}$ | 75.27$^{c}$ |
| 22 | 17.4$^{g}$ | 36.4$^{e}$ | 2.24$^{c}$ | 2.65$^{d}$ | 4.89$^{d}$ | 1.78$^{g}$ | 47.83$^{d}$ |
| Means | 60.17 | 72.48 | 3.40 | 3.87 | 6.72 | 4.79 | 93.23 |

In columns, means followed by different letters are statistically different by the Tukey's test ( $P < 0.05$).

**Table 2.** Effect of Ethyl Methane Sulphonate (EMS) on seed germination and growth of bell pepper seedlings.

| Dose (% EMS) | Germination (%) | | Root length (cm) | Shoot length (cm) | Seedling length (cm) | Seedling vigor index | Speed of emergence (%) |
|---|---|---|---|---|---|---|---|
| | DAP15 | DAP30 | | | | | |
| Control | 77.4$^{a}$ | 84.4$^{ab}$ | 4.22$^{a}$ | 5.03$^{a}$ | 9.25$^{a}$ | 7.82$^{a}$ | 91.71$^{a}$ |
| 0.1 | 81.8$^{a}$ | 90.0$^{a}$ | 3.61$^{ab}$ | 4.32$^{ab}$ | 7.93$^{ab}$ | 7.13$^{ab}$ | 90.89$^{ab}$ |
| 0.25 | 76.4$^{ab}$ | 84.4$^{ab}$ | 3.61$^{ab}$ | 4.28$^{ab}$ | 7.89$^{ab}$ | 6.65$^{abc}$ | 90.52$^{ab}$ |
| 0.5 | 75.6$^{ab}$ | 84.0$^{abc}$ | 3.23$^{abc}$ | 4.27$^{ab}$ | 7.50$^{abc}$ | 6.31$^{bc}$ | 89.93$^{ab}$ |
| 0.75 | 72.2$^{abc}$ | 80.8$^{abc}$ | 2.88$^{bc}$ | 4.16$^{ab}$ | 7.04$^{bcd}$ | 5.67$^{cd}$ | 89.36$^{ab}$ |
| 1 | 63.8$^{bc}$ | 73.6$^{bc}$ | 2.74$^{bc}$ | 3.83$^{ab}$ | 6.57$^{bcde}$ | 4.81$^{de}$ | 86.68$^{abc}$ |
| 1.25 | 60.0$^{c}$ | 72.8$^{c}$ | 2.61$^{bc}$ | 3.52$^{bc}$ | 6.13$^{bcde}$ | 4.40$^{def}$ | 82.42$^{bc}$ |
| 1.5 | 45.8$^{d}$ | 58.0$^{d}$ | 2.57$^{bc}$ | 3.41$^{bc}$ | 5.98$^{cde}$ | 3.87$^{ef}$ | 78.96$^{c}$ |
| 1.75 | 21.8$^{e}$ | 45.4$^{e}$ | 2.55$^{bc}$ | 3.11$^{bc}$ | 5.65$^{de}$ | 2.56$^{fg}$ | 48.02$^{d}$ |
| 2 | 6.6$^{f}$ | 19.8$^{f}$ | 2.29$^{c}$ | 2.52$^{c}$ | 4.81$^{e}$ | 2.01$^{g}$ | 33.33$^{e}$ |
| 3 | 0.00$^{f}$ | 1.6$^{g}$ | 0.038$^{d}$ | 0.02$^{d}$ | 0.06$^{f}$ | 0.003$^{h}$ | 0.00$^{f}$ |
| Means | 52.79 | 63.07 | 2.76 | 3.50 | 6.26 | 4.66 | 71.07 |

In columns, means followed by different letters are statistically different by the Tukey's test ( $P < 0.05$).

fruit length (cm), fruit girth (cm), average dry fruit weight (g) and 100 seed weight (g) in gamma rays and EMS treated seeds in *Capsicum annuum*. In tomato, Sikder *et al*. (2015) revealed that seed germination, seedling height and pollen fertility in $M_1$ generation reduced steadily with the increasing doses of both mutagens gamma rays and EMS.

In terms of plant survival, irradiated bell pepper seeds with 0.5 kR, 1 kR, 3 kR, 5 kR, 8 kR and 11 kR gamma rays and 0.1%, 0.25%, 0.5%, 0.75%, 1% and 1.25% EMS had no significant effect compared with control (Tables 1 and 2). However, seeds treated with 13 kR, 16kR, 19kR and 22 kR gamma rays; 1.5%, 1.75%, 2% and 3% of EMS were severely affected while the plants derived from treating bell pepper seeds with 16 kR, 19 kR and 22kR gamma rays and 1.5%, 1.75% and 2% EMS could not survive with time. Rajesh *et al*. (2014) used different concentrations of EMS in tomato to induce point mutations at higher frequency at random location in the genome and identified $M_2$ plants with morphological and developmental altered phenotype as compared to control. Laskar *et al*. (2016) found that the rate of survival and fertility in $M_1$ plants of tomato was highly affected due to increased mutagenic treatment by using EMS and hydrazine hydrates (HZ).

In case of EMS, 3% had lethal effect on root length, shoot length, seedling length, seedling vigor index and speed of emergence unlike the effects of lower doses (Table 2). The decrease in bell pepper germination with increasing dosage could be attributed to the occurrence of seeds without completely developed embryos (Omar *et al*., 2008). Reduction in root length, shoot length, seedling length, seedling vigor index and speed of emergence occurred with each corresponding increase in the concentration of EMS and gamma rays. The symptoms frequently observed in the low or high doses treated plants are due to enhancement or inhibition of germination, seedling growth and other biological responses (Wi *et al*., 2007).

Regarding the physical mutation study by Wi *et al*. (2007), a hypothesis was presented that low dose irradiation will induce growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells. Plants can easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition. In contrast, the high dose treatment that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/ or various damages in the entire genome. In this study, variability was measured by mean values of the root length, shoot length, seedling length, seedling vigor index and speed of emergence which decreased with increases in the concentration of EMS and gamma rays. According to a physical mutation study, when irradiation is sufficient to reduce the rooting percentages, the root lengths do not exceed a few millimeters in length (Chaudhuri, 2002). Hence, due to metabolic disorders in the seeds after irradiation treatment, the seeds are unable to germinate.

The fact that bell pepper seeds which were treated with lower doses of gamma rays and EMS grow better than those exposed to higher doses (Figures 3a, b), which suggests that more the seeds were exposed to gamma rays and EMS, the poorer will be the growth performance of the crop and this was so because mutagen may cause a block in cellular DNA, hence causing plant growth to stop or slow (Roslim *et al*., 2015; Gaswanto *et al*., 2016). Mokobia and Anomohaman (2004) carried out an investigation on maize, okra and groundnut to determine the effect of gamma irradiation on germination and growth and found decrease in number and the growth rate of germinated seeds with increase in the exposed radiation dose.


**Figure 3a.** Effect of gamma radiation on germination and growth vigor.


**Figure 3b.** Effect of Ethyl Methane Sulphonate (EMS) on germination and growth vigor.

The survival rate of the control plants was certainly higher because their seeds were not exposed to mutagens. At certain level of gamma rays and EMS, the plant can grow at early stage of growth but cannot survive after some duration; probably due to DNA breakage and inability to repair them. As in case of this study, plants exposed to 2%, 3% EMS and 19kR, 22kR gamma rays started to grow well at the early stage but they could not survive after 30 days of planting. The seedling root length, shoot length, speed of emergence and seedling vigor index decreases with increasing mutagen doses (Tables 1and 2) may be attributed to poor water and nutrient utilization; as poor plant growth and development leads to inefficient utilization of these essential resources.

## CONCLUSION

Lethal dose for any mutagen is essential to generate highest practicable mutants with lowest damage to the plant. The $LD_{50}$ dose based on survival percentage of the seedlings after treatment with diverse doses of gamma rays and different concentrations of EMS for the bell pepper cultivar California Wonder were 17.8 kR and 1.6%, respectively. In addition, the optimum dose based on the reduction in survival and growth parameters were 1.5-1.7% of EMS concentration and 16-19 kR of gamma rays to generate maximum variability with least number of unwanted mutants. Increasing gamma rays and EMS dose decreased the germination percentage, root length, shoot length, seedling length, speed of emergence and seedling vigor index of bell pepper. Generally, higher gamma rays (16kR, 19kR and 22kR) doses and higher concentration of EMS (1.75%, 2% and 3%) had prominent/lethal effect on the morphological and growth characteristics of bell pepper seedlings. These optimal mutagen doses determined for the bell pepper genotype could be useful while formulating mutation breeding programme for enrichment of meticulous traits in bell pepper.

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## REFERENCES

Adamu AK, Aliyu H (2007). Morphological effects of sodium azide on tomato (*L. esculentum* Mill). *Sci. World J.* 2(4): 9-12.

Ariraman M, Gnanamurthy S, Dhanavel D, Bharathi T, Murugan S (2014). Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon Pea (*C. cajan* (L.) Millsp.). *Int. Lett. Nat. Sci.* 21: 41-49.

Aruldoss T, Mullainathan L (2015). Studies on effect of induced mutagenesis on *Capsicum annuum* (L.) var K1 in $M_1$ generation. *J. Chem. Bio. Phys. Sci*. 5(1): 347-352.

Auerbach C, Robson JM (1946). Chemical production of mutations. *Nature* 157(3984): 302.

Chahal GS, Gosal SS (2002). Principles and procedures of plant breeding. Alpha Science International Ltd., pp. 399-412.

Chaudhuri SK (2002). A simple and reliable method to detect gamma irradiated lentil (*Lens culinaris* Medik) seeds by germination efficiency and seedling growth test. *Radiat. Phys. Chem.* 64(2): 131-136.

Chopra VL (2005). Mutagenesis: Investigating the process and processing the outcome for crop improvement. *Curr. Sci.* 89 (2): 353-359.

Daskalov S, Baralieva D (1992). Orangeva Kapia: a new cultivar in sweet pepper with increased beta carotene (provitamin A) content. *Mutation Breed. Newslett.* 39: 9-10.

Falque M (1994). Pod and seed development and phenotype of the $M_1$ plants after pollination and fertilization with irradiated pollen in cacao (*T. cacao* L.). *Euphytica* 75: 19-25.

Gandhi ES, Sridevi A, Mullainathan L (2014). The effect of ethyl methane sulphonate and diethyl sulphonate on chilli (*C. annuum* L.) in $M_1$ generation. *Int. Lett. Nat. Sci*. 10: 18-23.

Gaswanto R, Syukur M, Purwoko BS and Hidaya SH (2016). Induced mutation by gamma rays irradiation to increase chilli resistance to begomovirus. *Agrivita* 38 (1): 24-32.

George RAT (1985). Vegetable seed production. Longman group UK. Ltd., England. pp. 229-234.

Hangarter RP (1997). Gravity light and plant form. *Plant Cell Environ.* 20(6): 796- 800.

Heiser CB (1995). Peppers. In: J. Smart, N.W. Simmonds, eds., Evaluation of crop plants ($2^{nd}$ ed.). Longman Scientific and Technical, Harlow, UK. pp. 449-451.

Jain SM (2010). Mutagenesis in crop improvement under the climate change. *Romanian Biotechnol. Lett.* 15(2): 89.

Khan S, Goyal S (2009). Improvement of mungbean varieties through induced mutations. *Afr. J. Plant Sci.* 3: 174-180.

Kumar A, Ponnuswam V, Sundar ST (2012). Effect of induced chlorophyll mutation, mutagenic efficiency and effectiveness of gamma rays and EMS in paprika (*C. annuum* L.) cv. Bydagi Kaddi. *Indian J. Hort.* 69: 60-64.

Laskar RA, Chaudhary C, Khan S, Chandra A (2016). Induction of mutagenised tomato populations for investigations on agronomic traits and mutant phenotyping. *J. Saudi Soc. Agric. Sci.* http://dx.doi.org/10.1016/j.jssas.2016.01.002

Mokobia CE, Anomohanran O (2004). The effect of gamma irradiation on the germination and growth of certain Nigerian agricultural crops. *J. Radiol. Prot.* 25: 181-188.

Monica S, Seetharaman N (2016). Effect of gamma irradiation and ethyl methane sulphonate (EMS) mutagenesis in early generation of garden bean (*L. purpureus* (L.)) Sweet var. *typicus*). *Int. J. Adv. Sci. Techn. Res*. 6(3): 398-410.

Nascimento KS, Rego MM, Nascimento AM, Rego AR (2015). Ethyl methane sulphonate in the generation of genetic variability in Capsicum. *Acta Hort.* 1087: 249-254.

Omar SR, Ahmed OH, Saamin S, Majid NMA (2008). Gamma radio-sensitivity study on chilli (*C. annuum*). *Am. J. Applied Sci*. 5(2): 67-70.

Pillai PS, Abraham S (1996). Improvement of fruit characters and yield in sweet pepper by mutation induction. *Mutation Breed. Newslett*. 42: 17-18.

Rajesh N, Prashanth PJE, Basha PO (2014). Development of mutant genetic sources of tomato *S .lycopersicum* L. cv. Arka Vikas. *Ann. Biol. Res*. 5(11): 1-6.

Roslim DI, Herman A, Fiatin I (2015). Lethal dose 50 ($LD_{50}$) of mungbean (*V. radiata* L. Wilczek) cultivar Kampar. *SABRAO J. Breed. Genet*. 47(4): 510-516.

Sikder S, Ravat VK, Basfore S, Hazra P (2015). Isolation of induced mutants using gamma ray and ethyl methane sulphonate in tomato (*S. lycopersicum* L.). *Electr. J. Plant Breed*. 6(2): 464-471.

Sniezko R, Visser T (1987). Embryo development and fruit set in pear induced by untreated and irradiated pollen. *Euphytica* 36: 287-294.

Swaminathan MS (1998). Crop production and sustainable food security. In: V.L. Chopra, R.B. Singh and A. Verma, eds., Crop productivity and sustainability. Shaping the future, IBH Publishing Co Pvt. Ltd., New Delhi, pp. 3-18.

Talebi AB, Talebi AB, Jafarpour M (2012). Identify the lethal dose of EMS and gamma radiation mutagenesis in rice MR219. 2nd Int. Conf. Environ. Sci. Biotechnol., IPCBEE, Singapore.

Umavathi S, Mullainathan L (2015). Physical and chemical induced mutagenesis study for identifying lethality dose in chick pea (*Cicer arietinum* L.) Var. Co–4. *Int. Lett. Nat. Sci*. 35: 1-5.

Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee JW, Kim YS (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron* 38(6): 553-564.