Determination of Proper Gamma Radiation Dose in Mutation Breeding in Eggplant (*Solanum melongena* L.)

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Abstract
This study was conducted to determine the proper dose of gamma irradiation for the mutation breeding program of eggplant (*Solanum melongena* L.). To serve the purpose, seeds of BR-12 and BR-16 eggplant genotypes were subjected to different doses of gamma rays (0, 80, 120, 160 and 180 Gy) with Cobalt 60 (Co-60) by irradiation and some characteristics of M_1 plants, germination rate (%), shoot length (cm), hypocotyls length (cm), plant fresh weight (gr/plant), plant dry weight, shoot fresh weight (mg/plant), shoot dry weight (mg/plant) and leaf width (cm) and leaf length (cm), were studied. The experimental results revealed that doses of gamma rays used to develop new generation and create variation were varied in accordance with genotypes and different doses lead to significant reductions in the inspected characters of two eggplant genotypes. Applicable appropriate dose without any reduction, for BR-12 genotype was determined as 160gy, no sufficient reduction was observed for effective dose for BR-16 genotype.

Key- Words: *Solanum melongena* L., Eggplant, mutation breeding, efficient dose, gamma ray, LD_{50}

1. Introduction

Eggplant is the sixth most produced crop (48424295 tons) in the world [2]. In Turkey, it is an important vegetable as it comes after tomato and pepper with 826 941 ton production in *Solanaceae* Family [5]. Eggplant is a low calorific valued plant (25 kcal/100g) and is a very rich plant in terms of minerals and fatty acids such as Ca, Mg, and P [25]. Its common use as a traditional medicine for treatment of many diseases [16] and its varied, vide range of use lead for the increase of its production and consumption.

80% of population indigent countries live on agriculture and they heavily suffer from the climate change. It is reported that, the portion of areas under the negative impact of water scarcity and drought has increased to 13% from 6% during last 30 years [3]. Changing conditions had an urge plant breeder to practice on some avant-garde methods such as genetic engineering and mutation breeding, as an addition to traditional methods. Like in other crops there is a genetic bottle-neck due to traditional breeding studies. Genetic creating a mutation may lead to the possibility of creating a desired feature(s) which does not available in the nature or has been lost during the evolution. With the mutation practices, chromosomes are broken or genes are changed [18]. Instead of waiting for a natural mutation for years, creating a mutation with different tools may a lot contribute to breeding studies in different aspects. Ionized radiation use in mutation works was started in early 20th Century (1928). The use of induced radiation has had an important role for the development of superior featured plant species around the world for a long time in the last 50 years. According to the data of International Atomic Energy Agency, there are 3424 mutant species existing [4]. There are 313 mutant species allowed to be produced in India and 12 of these are all vegetables including only one variety of eggplant [9]. Around the world, vegetables form only the 3% of the mutant products that legally permitted to train [15]. As investigating the current studies, it is clearly seen that, many *Fusarium oxysporum* f sp. *cubense* (FOC) 4 race resistant banana varieties, long fruity strawberry varieties, early fruits apricot varieties and dwarf apple varieties with basic agronomical features, are developed [12]. Studies are still being conducted for many areas such as management of soil and water, decrease of post-harvests losses, and minimize the pesticide use, improvement of fruit quality features and increase of prolificacy. Mutation practice is an alternative for the genetically modified organisms (GMOs) techniques in terms of easy application, being non-hazardous, not subjected to any specific legislation and low cost for breeding studies.

The most important point of mutation breeding is to select the suitable mutagen and develop a method to determine the mutants [26]. Physical mutagens are more advantaged than chemical mutagens on not needing of washing/treating the material for removal of the mutagen after practice [13] and not forming waste. An additionally, chemical mutagens are very dangerous chemicals and for the reason working material should be disposed very carefully and laboratories should be subject to detoxification in every detail. The health risks created by physical mutagens are comparatively less than chemical mutagens. Selection of the mutagen is about the researcher's current conditions such as mutagen attainability, cost of application and laboratory infrastructure [15]. Radiation practice, which is a physical mutagen, is widely used method in mutation works. The 90% of obtained mutants were created through this application (64% with gamma-rays, 22% with X-rays) [11, 12]. High-dose mutagen applications are more effective in mutation practices for providing the possibility to create more mutant individuals. Yet, the highest applicable dose is not used as uncalled mutations that lead for infertility and the loss of plant life might occur [14]. Slow rate growth or seedling damages that seen after seed germination is usually an indicator of that the plant was damaged genetically. Seed germination might be seen in high-dose practices, however, the high dose lead the loss of plant life afterwards [17]. The key factor in irradiating the plant material is the dose amount that determines the radiation absorbed by the plant. Determination of suitable mutagen and dosage combination were aimed in previous studies conducted on many vegetables, fruits and field crops [9]. As a starting point, it is needed to determine LD_{50} (Lethal Dosage) value to define exact mutation dose [20]. The plants sensitivity to irradiation varies according to species, cultivar and the plant's physiological conditions [8]. Mutation dose much more important than mutagen type. No matter what mutagen is used, firstly, appropriate dose of the mutagen should be determined before a large-scale application.

Therefore, the aim of the present study is to determine the appropriate radiating dose for eggplant, which is a very important species of vegetables.

1. Materials and Methods

1.1. Plant Materials and Methods

In the study, BR-12 and BR-16 eggplant seeds provided from Gento Seed Corporation were used. The seeds used in the experiment were counted and placed in transparent petri dishes as 100 seeds for each control group and radiated at 0, 80, 120, 160, 200 and 240 Gy under Co-60 source (150 Gy/h) in Akdeniz University Gamma Rays Department. Irradiated seeds were sowed in viols containing a 1: 1 rate mixture of peat and perlite. Seedlings were grown under controlled conditions in greenhouses belonging to Gento Seed Corporation. Germination rates of seeds are determined from the 7th day after the sowing until the 14th day. The plants' heights are measured on the plants harvested with root at the 14th day, when the first leaf development is over. The effective dose (LD_{50}) amount is determined by measuring the dose that decreases the plant height down to 50% [23]. To determine the effects of gamma rays at different levels on plant development, the values of germination percentage, shoot length, hypocotyls length, plant fresh weight, plant dry weight, shoot fresh weight, shoot dry weight, leaf length and width were measured.

2.2. Statistical Analysis

The experiment was designed according to completely randomized with three replications and data analysis was carried out using the analysis of variance and SAS statistical computer package ($p \leq 0.05$) [22].

3. Results and Discussion

In this study, the highest germination percent was obtained through on control group activities for both genotypes. As seen in Figure 1, with the increase of irradiation dose, germination rates decreased. Reduction of germination rates were observed as from 100% to 88% (240 Gy) on BR-12 genotype and from 99% to 92% (240 Gy) on BR-16 genotype. In the study conducted by Arunal *et al* [6], 100 Gy applications led the seed germination rates to decrease approximately 50% on all 5 eggplant genotypes they studied. In another study conducted on cluster bean species, 800 Gy application was needed to decrease the germination at 50% [27]. Increased gamma rays on *Solanum* *macrororpon*, known as African Eggplant, decreased the germination percentage [10] and high dose gamma rays on *Solanum nigrum* L. ssp. *villosum*, which is also a member of *Solanaceae* family, decreased both germination percentage and plant survival rate [19]. Differences between present study and previous studies may be due to the differences on the being of the DNA synthesis and seed metabolism [7].

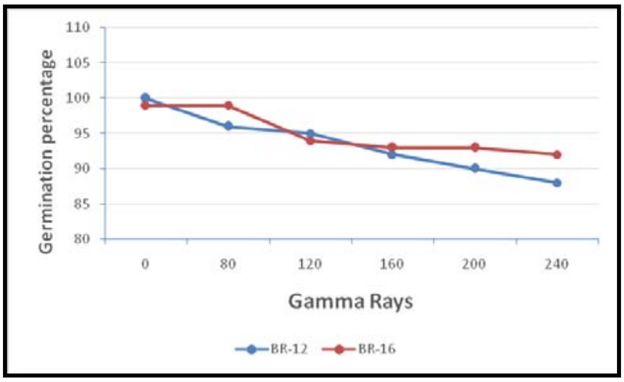

Figure 1. Effect of gamma rays on germination percentage in M_1 generation.

Srivastava and Roy [24] examined 5-40 krad gamma radiation effect for some development features on two eggplant cultivars and found that both cultivars reacted in the same way two different doses. Two eggplant cultivars, KT3 and BG, and while KT3 variation was affected 100% lethal under 40 krad gamma radiation, it was only 73% for BG. However, Velu *et al* [27] obtained only 50% seed germination decrease on cluster bean cultivars with 800 Gy applications. These two results indicate that cultivar and varieties react mutation doses differently and differentiation of varieties has a major role on determining the effective dose in mutation studies.

Shoot length of the genotypes, like as germination percentages, decreased in parallel with increasing doses of gamma ray. However, in both eggplant genotypes, the efficiency of gamma-ray doses in shoot length showed variable trend with rising dose. BR-12 Genotypes decreased 50% in 160 Gy application, yet, these applications had a 66% decrease on BR-16 genotypes at a maximum level (Table 1). Thus it is thought that the application dose for BR-16 is not enough and the doses should be increased. Effective dose is determined as the dose reducing shoot leght 50%. Afterwards 10% above or below of effetive dose is used in field trials [23]. Similar to shoots length results, hypocotyls length also decreased 41.5% on BR-12 species, while the dose applied on BR-16 species was inadequate (Table 2).

Table 1. Comparison of shoot length (cm) on different doses of gamma ray in M_1 generation.

Dose / Geno	0	80 Gy	120 Gy	160 Gy	200 Gy	240 Gy
BR-16	20.41a	19.09b	18.21b	16.85c	14.38d	13.58d
BR-12	19.09a	15.28b	12.63c	9.47d	7.62e	5.36f

Table 2. Comparison of hypocotyls length (cm) on different doses of gamma ray in M_1 generation.

Dose / Geno	0	80 Gy	120 Gy	160 Gy	200 Gy	240 Gy
BR-16	4.60a	4.45a	4.42ab	3.88b	3.58c	3.02d
BR-12	4.77a	4.08b	3.02c	1.98d	1.79d	1.35e

In present study, as the other traits the fresh and dry shoot weight of genotypes decreased in accordance with increase gamma ray dose (Table 3, Figure 2 and Figure 3). It is known that, mutation studies are not only conducted under field conditions but also in *in vitro* conditions. Alikamanoğlu [1], applied different gamma doses on sugar beet meristems and measured regenerated plants weight when they were dry and wet. Experiment results revealed that weight loss decreased with the increase of application dose. And the reduction was used to determine the optimum dose. Among different application doses, 20 Gy dose led to 30% weight loss was the optimum dose. Also considering 30% weight loss, the optimum dose is 120 Gy for BR-12 genotype and 160 Gy for BR-16 genotype.

Table 3. Shoot fresh weight (g) of the genotypes on gamma rays

Dose / Geno	0	80 Gy	120 Gy	160 Gy	200 Gy	240 Gy
BR-16	3.021 A	2.765 b	2.735 B	2.371 c	1.923 d	1.705 e
BR-12	2.514 A	1.971 B	1.462 C	0.985 d	0.574 e	0.241 f

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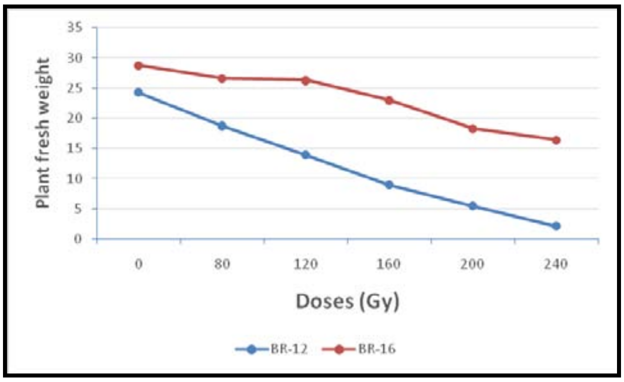

Figure 2. Plant fresh weight of various doses of gamma rays in M_1 generation.

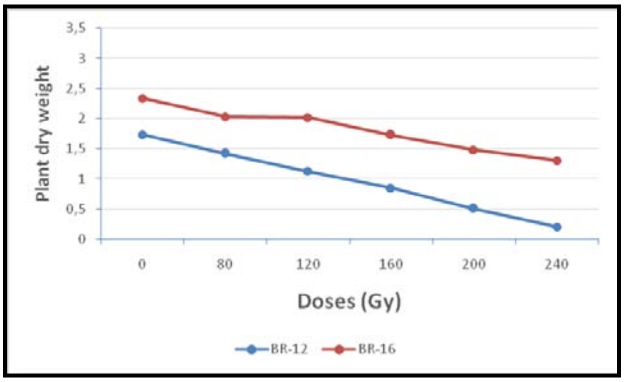

Figure 3. Effect of gamma rays on plant dry weight in eggplant genotypes.

Roychowdhury *et al* [21], analyzed the mutations by using 3 different chemical mutagens on 10 different eggplant genotypes and reported that all 3 mutagens decreased leaf amount. In a similar way, in a study conducted on a cluster bean species by [27], an increase on both gamma rays and EMS dose led to a decrease on leaf number and plant height. Comparing to present study, it is possible to say that the decrease on leaf length and width is related to mutagen activity (Table 4, Table 5).

Table 4. Average leaf width (cm) of the genotypes in M1 generation.

Dose / Geno	0	80 Gy	120 Gy	160 Gy	200 Gy	240 Gy
BR-16	4.550 a	4.430 ab	4.365 ac	4.040 bc	3.910 c	3.350 d
BR-12	4.600 a	4.300 a	3.620 b	3.100 c	2.200 d	1.450 e

Table 5. Average leaf length (cm) of the genotypes in M1 generation.

Dose / Geno	0	80 Gy	120 Gy	160 Gy	200 Gy	240 Gy
BR-16	6.510 A	6.050 ab	5.940 Bc	5.850 Bc	5.470 C	4.630 D
BR-12	6.570 A	5.970 b	5.260 C	4.140 D	3.260 E	1.940 F

As an addition, Zeerak *et al* [28] have reported that, gamma rays are more effective than EMS and gamma + EMS combination on obtaining usable eggplant mutants. For an effective mutagenesis on eggplant, they suggested radiation practices as having low biologic damage and ability to create a more effective mutation by applying high dose.

4. Conclusion

Determining the mutagen and its optimum dose in mutation breeding studies are the key factors for success. Results of the present study indicate that 160 Gy irradiating is the optimum dose for BR-12 genotype. On the other hand BR-16 genotype needed higher dose, indicating further studies are needed to verify different eggplants genotypes react in similar ways.

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5. References

[1] Alikamanoğlu S, Efficiency of the Gamma Irradiation in the Induction of *in vitro* Somatic Mutations, *Journal of Cell and Molecular Biology*, 1, 2002, p. 19-24.

[2] Anonymous a, *FAO Production Year Book*, 2012 (http://www.fao.org/).

[3] Anonymous b, European Environment Agency (EEA) Report. Urban adaptation to climate change in Europe, No: 2, 2012, (http://www.klimatilpasning.dk/media/5367/eea-report-2-2012.pdf).

[4] Anonymous c, International Atomic Energy Agency Report 2011, 2012,

(https://www.iaea.org/sites/default/files/publications/reports/2011/anrep2011_full.pdf)

[5]Anonymous, Türkiye İstatistik Kurumu, 2013, (http://www.tuik.gov.tr/).

[6] Arunal J., Prakash M and Sunil Kumar B, Studies on Effect of Physical and Chemical Mutagens on Seedling Charecters in Brinjal (*Solanum melongena* L.), *International Journal of Current Research*, vol. 3, 2010, pp.038-041.

[7] Bhosle S. S and Kothekar V. S, Mutagenic Efficiency and Effectiveness in Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub), *Journal of Phytology*, Vol. 2, No. 6, 2010, p: 21–27.

[8] Britt A. B, DNA Damage and Repair in Plants, *Annual Reviews Plant Physioogy*, 47, 1996, p. 75-100.

[9] Chopra V. L, Mutagenesis: Investigating the Process and Processing the Outcome for Crop Improvement, *Current Science*, Vol. 89, No: 2, 2005, p: 353-359.

[10] GIRISHA, B. R, Breeding for Shoot and Fruit Borer (*Leucinodes arbonalis* Guene) Resistance in Brinjal (*S. melongena* L.) Through Interspecific Hybridization and Mutation, *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Bangalore, 1993.

[11] Jain S. M, Major mutation-assisted plant breeding programs supported by FAO/IAEA, *Plant Cell, Tissue and Organ Culture*, 82, 2005, p: 113–123.

[12] Jain S. M, Mutagenesis in Crop Improvement under the Climate Change, *Romanian Biotechnological Letters*, Vol. 15, No. 2, 2010, p: 88-106.

[13] Khan S. J., Khan H. U., Khan R. D., Iqbal M. M. and Zafar Y, Development of Sugarcane Mutants Through *in vitro* Mutagenesis, *Pakistan Journal of Biological Sciences*, Vol. 3, No. 7, 2000, p: 1123-1125.

[14] Koornneef M, *Molecular Plant Biology*. Gene Identification: Classical Mutagenesis in Higher Plants, 2002 p: 1-13. Edited by Philip M. Gilmartin and Chris Bowler, Oxford University Press.

[15] Mba C, Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture, *Agronomy*, 3, 2013, p: 200-231.

[16] Naujeer H. B, Morphological diversity in eggplant (*Solanum melongena* L.), their related species and wild types conserved at the National gene bank in Mauritius, *Master thesis*, 2009, Swedish Biodiversity Centre, p: 74.

[17] Nouri H., Kiani D and Khani M. A. H, Investigation of Mutagenic Effects of Various Doses of Gamma Ray on Seed Germination Traits of Pinto Bean Cultivar of Khomein, *Annals of Biological Research*, Vol. 3, No. 10, 2012, p: 4977-4979.

[18] Novak F. J. and Brunner H, Plant Breeding: Induced Mutation Technology for Crop Improvement. *IAEA Bull 4*, 1992, : 25-32.

[19] Ojiewo C. O., Agong S. G., Murakami K., Tanaka A., Hase Y and Masuda M, Male-sterility Induced by γ-ray Irradiation of African nightshade (*Solanum nigrum* L. ssp. *villosum*) seed, *Journal of Horticultural Science and Biotechnology*, 80, 2005, p: 699- 704.

[20] Predieri S, Mutation Induction and Tissue Culture in Improving Fruits, *Plant Cell, Tissue and Organ Culture*, 64, 2001, p: 185–210.

[21] Roychowdhury R., Roy S and Tah J, Estimation of Heritable Components of Variation and Character Selection in Eggplant (*Solanum melongena* L.) for Mutation Breeding Programme, *Continental J. Biological Sciences*, Vol. 4, No. 2, 2011, p: 31–36.

[22] SAS, SAS Institute Inc. SAS /STAT. *Guide for personal computers. version 6*, 4thed.vol.2, 1985, Cary, NC, USA.

[23] Sağel Z., Peşkircioğlu H and Tutluer M. İ, Nükleer Tekniklerin Bitki Islahında Kullanılması, *VIII. Ulusal Nükleer Bilimler ve Teknolojileri Kongresi*, 15-17 Ekim 2003, s: 14, Kayseri.

[24] Srivastava D. P and Roy S. K, Effect of Gamma Irradiation on Dry Seeds of *Solanum melongena* L.: Observations on the M_1Generation, *Israil Journal of Botany*, Vol. 30, No. 2, 1981, p: 89-94.

[25] Tomar, B. S. and Kalda, J. S, Production system. Is eggplant nutritious? *TVIS Newsletter*, Vol. 1, No. 1, 1998, p : 26.

[26]Ukai Y, Effectiveness and Efficiency of Mutagenic Treatments, *Gamma Field Symposia* Number 45, 2006, p: 1-15, Japan.

[27]Velu S., Mullainathan L and Arulbalachandran D, Induced Morphological Variations in Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub), *International Journal of Current Research*, Vol. 1, No. 1, 2012, p: 48-55.

[28] Zeerak N. A, Mutagenic Effectiveness and Efficiency of Gamma Rays and Ethyl Methanesulphonate in Brinjal, *Journal of Nuclear Agriculture and Biology*, Vol. 21, No. 2, 1992, p: 84-87.