EURASIAN JOURNAL OF AGRICULTURAL RESEARCH

Determination of Effective Mutagen Dose for Lettuce (*Lactuca sativa* var. *longifolia* cv. Cervantes) Seeds

Şule SARIÇAM[1*], K. Yaprak KANTOĞLU[2], Ş. Şebnem ELLİALTIOĞLU[3]

[1] Transitional Zone Agricultural Research Institute, Department of Horticulture, Eskişehir, Turkey
[2] Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Unit of Agriculture, Ankara, Turkey
[3]Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey

*Corresponding Author: sule.saricam@tarim.gov.tr

Abstract

In plant breeding strategies induced mutagenesis has become an effective way of supplementing the existing germplasm and improving new varieties. Mutation can be induced by chemical and physical mutagens or by combination. The commercial importance and production of lettuce, which is the most popular of the local salad crops, is increasing in Turkey. Lettuce is an important vegetable commodity and in demand by the local markets throughout the year. This study was carried out to get database for mutation breeding studies on lettuce cv. Cervantes. For this aim, 0, 50, 100, 200, 300, 400, 500 and 600 Gray (Gy) doses of Co^{60} (gamma-rays) were treated on lettuce seeds as a physical mutagen. 30 seeds were used for each dose. Thirty days after treatment, germination and shoot developing of cloves were determined. The Effective Mutagen Dose (EMD_{50}) calculated by linear regression analyses. According to results, 372.66 Gy dose was found as EMD_{50}.

Keywords: Lettuce, mutation breeding, Co^{60}, gamma ray, EMD_{50}, *Lactuca sativa*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a major fresh vegetable and its leaves are commonly found in salad mixtures and sandwiches. In some eastern countries like China and Egypt, stems instead of leaves of lettuce are consumed, either cooked, raw, pickled, dried, or as a sauce. Some less common uses for lettuce include a cigarette without nicotine made from lettuce leaves, edible oil extracted from seeds of a primitive lettuce (Mou, 2008) Salads and lettuce leaves, which are among the vitamins and minerals and appetizing vegetables that are consumed in our table, contain 94-95% water. It is easy to grow in all regions because it is a cool climate vegetable. Gradually increased marketing of ready to use salad mixtures and different leaf colors, shapes like diversity point out the increased demand of upcoming period in this product group. Approximately two-thirds of the total production in the world is carried out by Asian countries.

China is the largest lettuce producer country, meeting nearly half of world production. Production rate of Turkey was 233 662 tons for cos lettuce, 179 712 tons for leaf lettuce and 65 068 tons for iceberg (head salad) in 2016 (Anonymus, 2016).

Turkey lettuce seed production was 12.965 tons in 2015. In the case for seeds of plants from the salad group, we are dependent on foreign countries, and it is an important fact that this results in losses due to foreign exchange rates every year. Annually 15 000 kg of foreign variety vegetable seeds for salads are purchased from abroad and approximately $ 3.6 million USD is paid. The production rate is increasing day by day as vegetables, especially when freshly consumed from salads, have a positive effect on human health due to their high nutritional values.

When the breeding studies are examined to develop new varieties in the world, it is seen that many different techniques are used alone or in combination. There are some studies in the breeding studies conducted from the past to the present day like early flowering and head deformations besides yield criterion, resistance to diseases (especially Downy Mildew), and development of miniature head type (Waycott et al. 1995), tolerance to herbicides and early harvesting in the salad group, which has a prescription for human health and nutrition (Mou, 2011). In addition to the studies starting with classical hybridization, new varieties are being developed by utilizing in vitro tissue culture techniques such as hypocotyl and leaf culture Grube et al. 2003; Ohki et al. 2012).

In addition to the discovery of the efficacy of recombinant DNA technology, studies on the use of biotechnological methods in the last 30 years continue to work on disease-resistant, high-yield transgenic (GMO) varieties (Dayton et al. 2012). Studies carried out to obtain transgenic plants, especially in the salad group, have shown that hypocotyl and leaf parts give the best results and the regeneration capacity of these parts is very high (Okubara et al. 1994).

However, especially due to the adverse effects that GMOs that are rapidly spreading in the market can create in human health in the future, researchers are expanding the existing genetic pools and creating new variations from which high-quality F_1 hybrid parental candidates are obtained by using nuclear techniques (Masuda et al. 2004).

When Nuclear-based studies are compared with the studies on obtaining genetically modified plants which are very popular today, this technique requires a lower cost than the transgenic plant line which also created many disputes and arguments all around the World, doesn't create any disadvantages in terms of human health, and makes it possible to reach a definitive result in a shorter time. The mutation breeding technique provides a significant advantage in the development of characters Anonymus, 1977; Sağel et al. 2002; Waycott et al.) that are managed by a single gene and exhibit simple inheritance, especially in self-fertilized plants, in studies conducted predominantly during the last 50 years.

The frequency of the mutation that occurs as a result of chemical mutagenesis in the physical compartment is 10^3 times higher than naturally occurring mutations. Lettuce breeding studies are focused on leaf-shaped, tight head, presence or absence of anthocyanins, resistance to diseases and insects, good quality in stress conditions, short growth period, yield, adaptation to different regions and environmental conditions (De Vries, 1997; Mou, 2011)

As a result of the mutation breeding trials in the salad group species, according to the records of the International Atomic Energy Agency (IAEA), the "Giantgreen" mutant variety was registered in 1966, following it "Evergreen" which is late-flowered, "Novogodnii" which gives a high yield in low light intensity and is rich in vitamin C, "Ice Cube", "Blush" and "Mini Green" varieties which have miniature head features, "Satilo" which has different leaf types. Very important data was obtained involving important deletions caused by ethyl methane sulfonate (EMS) on C/G pairings and T/A pairings resulting from the susceptibility of salad group varieties to mutations caused by both natural or physical (fast neutron, gamma rays) and chemical mutagen applications (Mou, 2011).

To obtain successful results, it is important to know the susceptibility of the selected variety to mutations and, by determining the "Effective Mutation Dose" (EMD_{50}), to start the studies with the correct irradiation dose for the genotype to be studied. In the first phase of the work we have planned to create a new variation in the Cervantes variety with the method of mutation breeding; Effective Mutation Dose (EMD_{50}) was determined by determining the effects of different gamma ray doses applied to the Cervantes variety on seed germination and plant growth.

MATERIALS and METHODS

Genetically a homogene seed of *L. sativa cv*. Cervantes containing 4.60% moisture, which has valuable biological and economic traits in the Mediterranean region, was used as the starting material.

After the amount of moisture in the seeds to be used in the experiment is determined, seeds were irradiated with a gamma irradiation device which is used in experimental irradiation with the Isotope brand, the Ob-Servo Sanguis Co-60 Research Irradiator model (dose rate: 407 Gy / h); at Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Department of Technology. Different irradiation doses (0, 50, 100, 200, 300, 400, 500 and 600) by ^{60}Co gamma rays radiator were used for seed irradiation to determine the effects on seed germination and shoot development.

The irradiated material were planted in a greenhouse belonging to the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Unit of Agriculture, in plastic pots containing :1:1 (soil:manure:peat) ratios of soil mixture. The experiment was arranged in 10 seeds / pot, with three replications per treatment.

Measurements were made to determine seedling height, root length, fresh weight, dry weight and seed germination ratio to determine the effects of different gamma ray doses on plant development at 30 days after planting (Figure 1). The obtained data were subjected to linear regression analysis to determine the dosage of $EMD_{50,}$ defined as the dose, which reduces the seedling growth and mean rooting height of control plants by 50%.

Figure 1. Germination of gamma-ray treated plants after 30 days

.

RESULTS and DISCUSSION

After irradiation at 8 different doses with Co^{60} gamma ray source, 80% germination occurred in the control at the end of the counts made 30 days after sowing in sowing seeds, 90% at 50 Gy dose, 76.67% at 100 Gy dose, 86.67% at 200 Gy dose, 43.35% at 300 Gy dose, 76.67% at 400 Gy dose, 83.33% at 500 Gy dose and 83.30% at 600 Gy. The germination rate of 90% of the 50 Gy dose is an expected effect of a low dose application which has been caused by the stimulating effect of germination (Anonymous 1977). During the measurements made, only root formation was observed in the seeds from the 500 dose. It has been observed that at a dose of 400 Gy or more, where no shoot development other than hypocotyl development was provided, shoot development appeared at a lower rate and the shoots germinated from the seeds germinated in the 30 days following planting have decreased due to the increased radiation dose (Figure 2). The obtained data were shown to be in parallel with the research (Mou, 2011) conducted to produce variability in the Evergreen variety treated with 420 Gy dose.

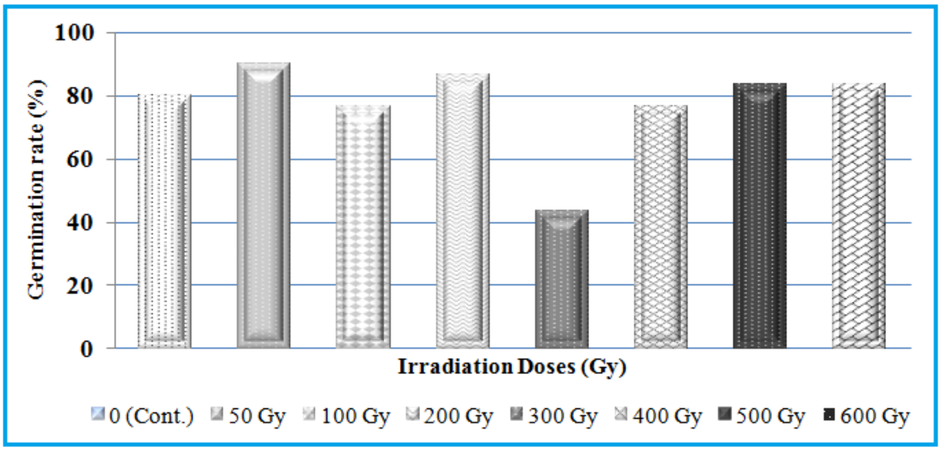

Figure 2. Relative germination rate of seeds treated with different gamma-ray doses.

As can be seen from Table 1 as a result of the measurements made on the 30th day after irradiation, significant reductions in seedling height and root length depending on the increasing irradiation dose was observed. According to results there was a negative correlation between seedling height and irradiation doses. The root length was 3.86 cm in the control, 3.05 cm in the 50 Gy dose, 3.72 cm in the 100 Gy application, 3.38 cm in the 200 Gy application, 2.96 cm in the 300 Gy application, 2.13 in the 400 Gy application, while it was 1.69-1.77 cm in the 500 and Gy 600 applications respectively.

Table 1. Seedling height, root length, fresh weight, dry weight, germination values in the plants obtained from seeds irradiated at different doses (seedling size = 13,705 +(-0,0203) x dose, R^2= 1)

Irradiation Dose (Gy)	Seedling Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	% Germination
0 (Control)	12.28	3.86	7.15	0.31	80.00
50	11.18	3.05	4.14	0.18	90.00
100	13.29	3.72	9.23	0.35	76.67
200	11.18	3.38	3.76	0.15	86.67
300	9.36	2.96	1.29	0.07	43.35
400	4.88	2.13	0.48	0.03	76.67
500	2.20	1.69	0.21	0.02	83.33
600	1.53	1.77	0.14	0.02	83.33
Regression Rate According to Mean Seedling Height: 372.66 Gy					

In the same way, mean seedling height was determined as 12.28 cm in control and 11.18 cm in 50 Gy. While average seedling height was found to be very low in measurements made after the 400 Gy dose was applied on the 30th day depending on increasing doses (Table 1, Figure 3). It has been reported in a study that 150 and 300 Gy dosing applications caused chromosomal degradation in irradiated lettuce seeds while no significant effects of dose applications between 0-75 Gy were observed (Franco et al. 2015).

As a result of the research, it was determined that 372.66 Gy was an effective mutation dose due to the regression analyses made from the 8 different doses applied to the seeds, and that the seeds irradiated at this dose could form plants with a survival rate of 50%.

Based on the data obtained, EMD_{50}, and 10% lower and upper values of this dose (330-410 Gy), may be used as an effective dose in mutation breeding studies for the Cervantes variety in order to create variability depending on the source power to be used in irradiation and the amount of moisture contained in the seeds.

Figure 3. Appearance of seedlings obtained from lettuce seeds being irradiated in different dose on the 30th day

ACKNOWLEDGMENT

This study was presented as abstract at the International Conference on Agriculture, Forest, Food Sciences and Technologies conference held in Cappadocia / Nevşehir on May 15-17, 2017.

REFERENCES

Anonymus 1977. "Manual on Mutation Breeding". International Atomic Energy Agency, Technical Report Series No:119, Vienna. 290p.

Anonymus 2016. Turkish Statistical Institute, https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul.

Anonymus, 1977. "Manual on Mutation Breeding". International Atomic Energy Agency, Technical Report Series No:119, Vienna. 290p,

Dayton Wilde H., Chen Y., Jiang P. and Bhattacharya A. 2012. "Targeted mutation breeding of horticultural plants", Emir. J. Food Agric. 24 (1): 31-41.

De Vries I. M.1997. "Origin and domestication of *Lactuca sativa* L. genetic resources and crop evolution", 44, 165–174.

Franco C. H., Santos H.M., Silva L. P., Arthur V. and Silva R. G. M. 2015. "Mutagenic Potential of Lettuce Grown from Irradiated Seeds", Scientia Horticulturae 182, 27–30.

Grube R. C., Brennan E. B. and Ryder E. J. 2003. "Characterization and genetic analysis of a lettuce (*Lactuca sativa* L.) mutant, weary, that exhibits reduced gravitropic response in hypocotyls and inflorescence stems", Journal of Experimental Botany, 54: 385, 1259-1268.

Masuda M., Agong S., Tanaka A., Shikazono N. and Hase Y. 2004. "Mutation spectrum of tomato seed induced by radiation with helium ion beams and coal", Acta Hort. 637, 257-262.

Mou, B., 2011. "Mutations in Lettuce Improvement", International Journal of Plant Genomics, 2011(3): 723518.

Mou B. 2008. Lettuce. Handbook of Plant Breeding, Vegetables I, Asteraceae, Brassicaceae, Chenopodicaceae and Cucurbitaceae, Page: 85-126.

Ohki S. and Hatashita M. 2012. "Mutation breeding by ion beam in lettuce (*Lactuca sativa* L.) using an *in vitro* regeneration", Research and Development Department Proc, 7th ISHS on In Vitro Culture and Horticultural Breeding (Ed.: D. Geelen) Acta Hort. 961, 285-290.

Okubara P. A., Anderson P. A., Ochoa O. E. and Michelmore R. W. 1994. "Mutants of downy mildew resistance in *Lactuca sativa* (lettuce)", Genetics, 137: 3, 867–874.

Sagel Z., Peskircioglu H., Tutluer I., Uslu N., Senay A., Taner K.Y., Kunter B., Sekerci S. and Yalcin S. 2002. "Bitki Islahında Mutasyon ve Doku Kültürü Teknikleri", III. Ulusal Mutasyon Kursu Kurs Notları, TAEK, ANTHAM Nükleer Tarım Radyobiyoloji Bölümü, Ankara.

Waycott W., Fort S. B. T and Ryder E. J. 1995, "Inheritance of dwarfing genes in *Lactuca sativa* L.", Journal of Heredity, 86:1, 39–44.