Turkish Journal of Biology

Volume 36 | Number 4 Article 5

1-1-2012

Induction of salt-tolerant potato (Solanum tuberosum L.) mutants with gamma irradiation and characterization of genetic variations via RAPD-PCR analysis

ORKUN YAYCILI

SEMA ALİKAMANOĞLU

Turk J Biol
36 (2012) 405-412

doi:10.3906/biy-1110-14

Induction of salt-tolerant potato (*Solanum tuberosum* L.) mutants with gamma irradiation and characterization of genetic variations via RAPD-PCR analysis

Orkun YAYCILI, Sema ALİKAMANOĞLU
Department of Biology, Faculty of Science, İstanbul University, 34459 Vezneciler, İstanbul - TURKEY

Received: 14.10.2011 ● Accepted: 24.02.2012

Abstract: Salt-tolerant mutants of potato (*Solanum tuberosum* L. 'Marfona') were obtained via gamma irradiation. Node explants of the Marfona potato plant were treated with various dosages of gamma irradiation, and the M_1V_2 and M_1V_3 clonal generations were developed. Selection of salt-tolerant mutants was accomplished by in vitro selection media containing 50, 100, and 125 mM NaCl. Molecular-level differences between the control and mutant plants were elucidated using RAPD-PCR method, and the polymorphism rate according to the selected primers was calculated as 89.66%. Genetic distances between the controls and mutants were also calculated, and related dendrograms were produced. On average the mutants were genetically 27.5% different from the control plants. The greatest difference encountered between the control and mutants was 47%, which was detected in mutant plants produced by 20 or 30 Gy gamma irradiation and regenerated in selection medium containing 100 mM NaCl.

Key words: Gamma radiation, in vitro mutagenesis, mutation breeding, potato, RAPD-PCR, salt stress

Introduction

Salinity is an environmental stress factor that impacts 25% of agricultural land. It has become a serious problem particularly in agricultural regions with the greatest crop yield potential such as the Mediterranean Basin, California, and Southeast Asia. Unless measures are taken it is estimated that by the year 2050 50% of agricultural lands could be suffering from excessive salinity, causing considerable damage to plant growth (1-3).

In addition to preventing growth, salt stress can decrease yield and quality, eventually causing abrupt plant death. It is particularly crucial to assess the maximum salinity tolerance of economically important plants grown in the regions where salinity levels cannot be lowered significantly (4-8).

Improving plants via mutation can lead to the development of varieties that are more tolerant of or resistant to environmental stress factors such as salinity. In particular, somatic mutations are highly valuable for mutant production in vegetative plants. There are several reports about somatic mutation induction to produce desired mutants, and thus new varieties (9,10). Another approach in vegetative plant development is to combine mutagenesis and in vitro methods. This combination has proven effective in increasing plant variation. In addition, the desired genotype was produced in a shorter time and in smaller fields; consequently, selection procedures were facilitated (9-18). A search of the literature demonstrates that, in direct and indirect mutation studies conducted in several species, 2570 mutant

varieties have been obtained. Among them, 1023 were produced via gamma irradiation while 19 were produced by gamma irradiation combined with in vitro techniques. As of today, 6 mutant varieties have been developed in potato through mutation breeding; however, only 1 of these varieties was created via gamma irradiation (19).

In mutation-based development using in vitro cultivation methods in vegetative plants, in order to produce a wide variety of mutants with the desired characteristics (without genetic exchanges), plant parts must be clonally reproduced (i.e., M_1V_1, M_1V_2, M_1V_3 generations created) (14,17). In these generations mutant individuals with the desired characteristics are easily detected via stability tests (16,20,21).

Potato (*Solanum tuberosum* L.), a vegetative plant grown for its starch-rich tubers, is the fourth most important agricultural crop after rice, wheat, and corn, with a yearly production of 300 million tons (22,23). Economically, it is the most important tuberous plant, and potato plant varieties are usually very sensitive to environmental stresses such as temperature changes, drought, and salinity due to their sparse and short root systems. There is significant loss in plant growth and product yields when potato is grown in soil that contains 20-35 mM concentrations of NaCl. When compared to other agricultural plants such as pepper and corn, the potato plant is more resistant to salinity; however, it is less resistant than tomato, rice, soy, and barley (22,24).

In the current work, the aim was to induce somatic mutations in vegetatively growing potato plants via gamma irradiation and to demonstrate the molecular-level differences among mutants using the random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) method.

Materials and methods

Materials

Potato plant tubers (*Solanum tuberosum* L. 'Marfona') were obtained from the Aegean Agricultural Research Institute Directorate of the Ministry of Agriculture and Rural Affairs in Menemen, İzmir, Turkey and stored at 4 °C. These tubers were then incubated in the dark at room temperature for 2 weeks until 5-6 cm-long shoots appeared (25).

Explant production

Shoots formed by the tubers were surface-sterilized by placing them in 70% ethanol for 2 min and 5% hypochloride solution for 10 min. Then they were rinsed 3 times with distilled water, dried with sterile drying paper, and planted in MS (26) medium containing 30 g/L saccharose. The shoots were incubated for 10 days at 26 °C in growth chambers with 16 h light/8 h dark periods, and the node explants used in the study were obtained (25).

Potato tissue culture and irradiation of the explants

Node explants from Marfona potato variety were planted in MS medium containing 0.5 mg/L ZR and 1.5mg/L IAA. To create the M_1V_1 generation, explants were irradiated with 0, 5, 10, 15, 20, 25, 30, or 50 Gy gamma radiation by a cesium-137 (Cs^{137}) gamma source with an activity of 6.5 Gy/min (16,25,27).

Generation of the M_1V_2 and M_1V_3 plants

In order to form large populations from which to select mutants with the desired characteristics, individuals of the M_1V_1 generation were vegetatively reproduced, and M_1V_2 and M_1V_3 generations were created.

Treatment with NaCl concentrations

In order to determine the sensitivity of Marfona potato variety against NaCl and choose the selection medium to be used in the study, explants were planted in regeneration media containing 0, 50, 100, 125, 150, 175, or 200 mM of NaCl. The regeneration ratios of the 28-day-old cultures were then evaluated (Table 1), and growth media containing 50, 100, or 125 mM NaCl were chosen as selective media for selection of the plants with salinity tolerance.

Molecular analysis

Molecular differences between the control and salt-tolerant mutants of Marfona potato variety were demonstrated using the PCR-based RAPD technique.

Genomic DNA isolation and analysis

The plant DNA extraction kit from Fujifilm (QuickGene DNA tissue kit S) was used for genomic DNA isolation from the leaf samples of control and salinity-tolerant individuals of Marfona potato variety.

Table 1. Regeneration ratios of the node explants of Marfona potato variety grown in selection medium containing various concentrations of NaCl on day 28.

NaCl concentration (mM)	Number of explants	Regeneration ratio (%)
0 (control)	40	93
50	40	63
100	40	33
125	40	20
150	40	10
175	40	0
200	40	0

Amplification conditions

For PCR amplification, randomly selected oligonucleotide primers (Operon Technologies, Alameda, CA, USA) were used (Table 2). A PCR experiment was set up using 50 ng genomic DNA, 2.5 mM $MgCl_2$, 0.1 mM dNTP, 0.4 µM primer, and 0.5 U Taq DNA polymerase in a total volume of 50 µL. The PCR was designed as 40 cycles of 1.5 min at 94 °C, 1 min at 36 °C, and 3 min at 72 °C. PCR products were then run on a 1.7% (w/v) agarose gel in TBE buffer at 90 V. Each PCR amplification was repeated at least 3 times. After separation, RAPD bands were examined and documented under UV.

Statistical analysis and determination of genetic distance

Plant height and leaf count data of the gamma-irradiated or non-irradiated (control) 28-day-old cultures of Marfona potato plants were produced. The data were analyzed by one-way ANOVA, and statistically significant data were compared using the Student Newman-Keuls test (28).

In order to determine the genetic distance between the control and mutant plants of Marfona potato, during RAPD-PCR analysis numerical values of 1 and 0 were assigned to the amplified and non-amplified RAPD bands, respectively. These values were then used in clustering analysis in SPSS to form a dendrogram demonstrating the genetic distance among the mutant plants and the controls (29-32).

Results

Effect of gamma irradiation on tissue cultures

Sensitivity of the Marfona potato plant variety towards irradiation was demonstrated with respect to regeneration ratios, average plant heights, average leaf counts, and percentage of root formation (Table 3). The dosage of radiation that decreased the regeneration ratio and average leaf count by 30% (ED_{30}) was 18 Gy, while the dosage that decreased these measures by 50% (ED_{50}) was 26 Gy. The average plant height decreased by 30% when irradiated with 15 Gy, while it decreased by 50% when irradiated with 20 Gy. Evaluation of the root formation ratio

Table 2. Primers used in RAPD analysis and number of total and polymorphic bands in the amplified primers.

Primer code	Name of the primer	Primer sequence (5'……3')	Number of total bands	Number of polymorphic bands
P12	OPH-12	ACGCGCATGT	6	3
P13	OPH-13	GACGCCACAC	6	6
P14	OPH-14	ACCAGGTTGG	2	2
P16	OPH-16	TCTCAGCTGG	4	4
P17	OPH-17	CACTCTCCTC	6	6
P19	OPH-19	CTGACCAGCC	5	5

Table 3. Physiological parameters of the 28-day-old cultures of control and mutated node explants.

Irradiation level	Number of explants	Regeneration rate (%)	Average plant height (cm)	Average leaf count	Root formation (%)
No irradiation (control)	40	93	4.61 ± 0.27 a*	4.8 ± 0.26 a	60
5 Gy	40	90	3.91 ± 0.26 ab	5.3 ± 0.35 a	53
10 Gy	40	83	3.43 ± 0.28 b	4.7 ± 0.38 a	47
15 Gy	40	73	3.05 ± 0.32 bc	4.6 ± 0.48 a	47
20 Gy	40	60	2.27 ± 0.33 cd	2.6 ± 0.35 b	37
25 Gy	50	50	1.95 ± 0.3 d	2.53 ± 0.38 bc	23
30 Gy	60	30	1.83 ± 0.37 d	1.6 ± 0.33 c	13
50 Gy	50	6.5	0.21 ± 0.12 e	0.23 ± 0.13 d	0

* In terms of plant height and average leaf count differences among control and irradiated plants, values marked with different letters denote a significance of at least 0.05 by Student Newman-Keuls method;
±: standard error.

revealed that the radiation dosages that decreased root formation by 30% and 50% were 18 Gy and 23 Gy, respectively.

Production of salt-tolerant somatic mutants

In order to select the salt-tolerant mutants, following plantation of M_1V_3 generation node explants into selection media with 50, 100, 150, and 125 mM concentrations of NaCl, plant formation rates on day 28 were recorded; they were 17%, 14%, and 12% in the 15, 20, and 30 Gy gamma irradiated groups, respectively (Table 4).

Determination of genetic differences via RAPD-PCR method

Among the 10 RAPD primers examined in this study, 6 were used for amplification of the samples belonging to Marfona potato variety (Table 5). The highest number of amplified bands was found in mutant #15 (20 bands), while in the control plant 11 bands were observed. The average polymorphism ratio of the 6 primers used was estimated as 89.66% (Figure 1).

Table 4. Selection of salt-tolerant explants in the M_1V_3 generation.

Radiation dosage	M_1V_3				
	NaCl concentration in the media			Salt-tolerant mutants produced	
	50 mM	100 mM	125 mM	Amount	(%)
	Number of explants				
0 Gy (control)	40	40	40	0	0
5 Gy	40	40	40	0	0
10 Gy	30	30	30	0	0
15 Gy	40	40	40	20	17
20 Gy	40	40	40	17	14
25 Gy	30	30	30	0	0
30 Gy	40	40	40	14	12

Table 5. Classification of the 19 salt-tolerant mutants.

	Numbers of the salt-tolerant mutant plants																		
	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#11**	**#12**	**#13**	**#14**	**#15**	**#16**	**#17**	**#18**	**#19**
Gamma irradiation dosage (Gy)	15	15	20	20	30	15	15	15	20	20	30	15	15	15	20	20	30	15	30
NaCl concentration (mM)	125	125	125	125	125	100	100	100	100	100	100	50	50	50	50	50	50	100	100

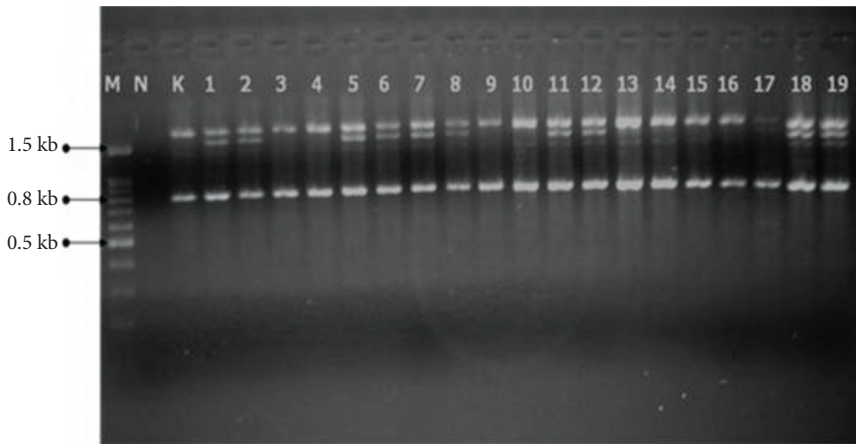

Figure 1. RAPD profiles obtained from the OPH-12 primer. M: marker, N: negative, K: control.

Upon evaluation of the RAPD results from all primers used, the genetic distance of mutants #10 and #11 from the control plants was 47%, while the distance of mutants #3 and #16 from the control plant was 5% (Table 6).

Upon evaluation of the dendrograms demonstrating genetic distances, mutants #3, #4, and #16 of the Marfona potato variety appeared to be very close the control; mutants #5, #9, #10, #11, #17, #18, and #19 were very distant. In particular, all of the mutants obtained via 30 Gy gamma irradiation were genetically very distant from the control plant (Figure 2).

Discussion

Due to their genotypic differences, plants respond differently to irradiation dosages. Higher doses of radiation cause chromosomal damage in plant meristematic cells, deceleration of the cell cycle, and delay of mitosis, which significantly affect overall plant regeneration and development. While an increase in radiation doses boosts mutation frequency, it also increases damage to the plant (14,18,20,21,25,33). Therefore, selection of the correct dosages in mutation studies is very important. In mutation studies with vegetative plants, ED_{50} dosage is usually taken as the upper limit, while in plant improvement studies

Table 6. Genetic distance between the mutants and control plants.

	C	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19
C	0																			
#1	0.25	0																		
#2	0.13	0.12	0																	
#3	0.05	0.22	0.18	0																
#4	0.13	0.28	0.25	0.09	0															
#5	0.46	0.21	0.33	0.44	0.33	0														
#6	0.26	0.1	0.21	0.23	0.29	0.23	0													
#7	0.18	0.17	0.13	0.14	0.22	0.39	0.19	0												
#8	0.31	0.04	0.19	0.28	0.33	0.27	0.1	0.23	0											
#9	0.37	0.24	0.4	0.33	0.4	0.3	0.33	0.47	0.3	0										
#10	0.47	0.53	0.5	0.43	0.5	0.58	0.6	0.6	0.58	0.33	0									
#11	0.47	0.33	0.3	0.44	0.5	0.3	0.42	0.26	0.39	0.5	0.5	0								
#12	0.27	0.17	0.13	0.24	0.3	0.3	0.26	0.18	0.23	0.37	0.47	0.26	0							
#13	0.27	0.33	0.3	0.24	0.22	0.39	0.33	0.27	0.39	0.58	0.47	0.37	0.27	0						
#14	0.12	0.19	0.23	0.17	0.23	0.38	0.27	0.28	0.24	0.27	0.56	0.55	0.36	0.36	0					
#15	0.29	0.33	0.38	0.33	0.38	0.49	0.33	0.42	0.37	0.43	0.67	0.64	0.48	0.48	0.18	0				
#16	0.09	0.25	0.22	0.05	0.04	0.39	0.26	0.18	0.31	0.37	0.47	0.47	0.27	0.18	0.2	0.36	0			
#17	0.33	0.22	0.18	0.3	0.36	0.28	0.31	0.24	0.32	0.33	0.43	0.22	0.24	0.43	0.42	0.53	0.33	0		
#18	0.46	0.25	0.3	0.43	0.48	0.35	0.26	0.36	0.31	0.47	0.47	0.37	0.18	0.27	0.44	0.55	0.46	0.43	0	
#19	0.31	0.21	0.19	0.28	0.19	0.13	0.23	0.23	0.27	0.48	0.58	0.3	0.15	0.23	0.38	0.49	0.23	0.28	0.23	0

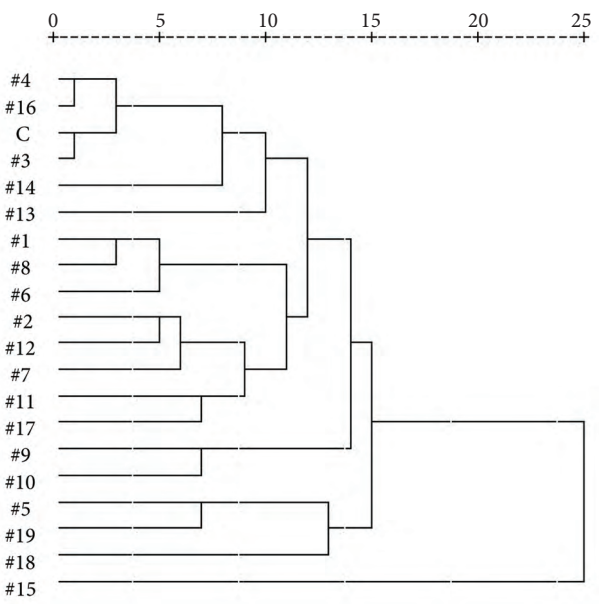

Figure 2. Dendrogram of the control and mutants based on distances produced by RAPD-PCR.

dosages around ED_{30} are preferred (18,34). In our study the salt-tolerant mutants were also obtained with an irradiation dosage around ED_{30}.

Regardless of genetic differences among plants, for somatic mutation induction radiation doses applied to plant cells and tissues for in vitro tissue culture studies must be around 20 Gy (35). In various in vitro mutation studies with potato plants the effective dose was 20 Gy, and it was noted that higher doses could be lethal (16,25).

During somatic mutation studies in tissue cultures using micropropagation techniques, M_1V_3, M_1V_4, and M_1V_5 generations are formed, and mutant plants with the desired characteristics can be successfully selected in vitro (14,17,18). In order to induce somatic mutations in potato plant in the current study, tissue cultures were formed using node explants, and these cultures were then gamma-irradiated. With controls and mutants, a total of 650 explants of M_1V_2

generation were formed from 360 M_1V_1 explants, and 1024 explants of M_1V_3 generation were formed from M_1V_2. Various studies reported that stability tests of salinity and temperature-tolerant mutants were generally conducted on plants of the M_1V_3 generation (10,25).

A total of 51 salt-tolerant mutants (20, 17, and 14 mutant plants created by 15 Gy, 20 Gy, and 30 Gy gamma irradiation, respectively) were detected in selection media; these mutant plants grew significantly better than the controls. Nevertheless, salt-tolerant mutants could not be induced in the experimental group exposed to 25 Gy gamma irradiation. It can be assumed that the gene mutations in this group of plants occurred in the regulatory regions responsible for suppressing genes that play a role in salinity tolerance by preventing or increasing transcription and/or translation (36).

Statistical evaluation demonstrated that the salt-tolerant mutants obtained by induction of somatic mutations via gamma irradiation exhibited, on average, 27.5% genetic difference from control plants.

While the physical damage caused by irradiation can be evaluated by studying physiological parameters in the M_1V_1 generation, hereditary changes in living organisms can only be assessed in later generations. Genetic changes in organisms exposed to irradiation may vary from one cell to another. These changes may consist of differences in DNA repair mechanisms (pre-replicative or during replication) as well as changes in the regulation of gene expression (transcriptional, posttranscriptional, or translational) (36,37). Thus, even within a group of the same type of plant irradiated with a given dosage, the formation of different genotypic and phenotypic characters can be expected.

Among the mutant plants created via 20 Gy gamma irradiation in the current study, mutant #3 was genetically only 5% different from the control plants, while mutant #10 was 47% different. According to the results of RAPD-PCR analysis, it was obvious that genotypic variations developed even within a group of plants exposed to the same level of gamma radiation.

In conclusion, salt-tolerant potato plants were successfully created for in vitro tissue cultures via mutation induction using 15, 20, and 30 Gy gamma irradiation. The genetic distances between the mutant and control plants were demonstrated using RAPD-PCR analysis. The data produced are valuable for selection, plant development, and characterization of gene sources in future studies of this plant.

Acknowledgements

This study was carried out with the support of the İstanbul University Institute of Science and Engineering. It is taken from the PhD thesis, Induced Somatic Mutation with Gamma Radiation in Potato (*Solanum tuberosum* L.) Tissue Culture and was supported financially by the İstanbul University Research Fund (project no. T-791/27122005).

Corresponding author:

Orkun YAYCILI

Department of Biology,

Faculty of Science,

İstanbul University,

34459 Vezneciler, İstanbul - TURKEY

E-mail: oyaycili@istanbul.edu.tr

References

1. Pareek A, Singla-Pareek S, Sopory SK et al. Analysis of salt stress-related transcriptome fingerprints from diverse plant species. In: Varshney RK, Tuberosa R. eds. Genomics-Assisted Crop Improvement: Vol. 1: Genomics Approaches and Platforms. Springer; 2007: pp. 267-287.
2. Blumwald E, Grover A. Salt tolerance. In: Halford NG. ed. Plant Biotechnology: Current and Future Uses of Genetically Modified Crops. John Wiley & Sons Ltd.; 2006: pp. 206-22.
3. Leblebici Z, Aksoy A, Duman F. Influence of salinity on the growth and heavy metal accumulation capacity of *Spirodela polyrrhiza* (Lemnaceae). Turk J Biol 35: 215-220, 2011.
4. Munns R, Richards RA. Recent advances in breeding wheat for drought and salt stresses. In: Jenks MA et al. eds. Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer; 2007: pp. 565-585.

5. Mba C, Afza R, Jain SM et al. Induced mutations for enhancing salinity tolerance in rice. In: Jenks MA et al. eds. Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer; 2007: pp. 413-454.
6. Müftüoğlu NM, Dardeniz A, Sungur A et al. Bazı sofralık üzüm çeşitlerinin tuza toleranslarının belirlenmesi. S Ü Ziraat Fakültesi Dergisi 20: 37-42, 2006.
7. Sen A, Alikamanoglu S. Effects of salt stress on growth parameters and antioxidant enzymes of different wheat (*Triticum aestivum* L.) varieties on in vitro tissue culture. FEB 20-2a: 489-495, 2011.
8. Sairam RK, Tyagi A. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci 86: 407-421, 2004.
9. Saleem MY, Mukhtar Z, Cheema AA et al. Induced mutation and in vitro techniques as a method to induce salt tolerance in Basmati rice (*Oryza sativa* L.). Int J Environ Sci Tech 2/2: 141-145, 2005.
10. Das A, Gosal SS, Sidhu JS et al. Induction of mutations for heat tolerance in potato by using in vitro culture and radiation. Euphytica 114: 205-209, 2000.
11. Lu G, Zhang X, Zou Y et al. Effect of radiation on regeneration of Chinese narcissus and analysis of genetic variation with AFLP and RAPD markers. Plant Cell Tiss Org 88: 319-327, 2007.
12. Szarejko I, Forster BP. Doubled haploidy and induced mutation. Euphytica 158: 359-370, 2007.
13. El-Sayed OE, Rizkalla AA, Sabri SRS. In vitro mutagenesis for genetic improvement of salinity tolerance in wheat. Res J Agr Biol Sci 4: 377-383, 2007.
14. Hewawasam WDCJ, Bandara DC, Aberathne WM. New phenotypes of *Crossandra infundibuliformis* var. Danica through in vitro culture and induced mutations. Tropical Agricul Res 16: 253-270, 2004.
15. Lee IS, Kim DS, Lee SJ et al. Selection and characterizations of radiation-induced salinity-tolerant lines in rice. Breeding Sci 53: 313-318.
16. Saif-ur-Rasheed M, Asad S, Zafar Y et al. Use of radiation and in vitro techniques for development of salt tolerant mutants in sugarcane and potato. In Vitro Techniques for Selection of Radiation Induced Mutations Adapted to Adverse Environmental Conditions. IAEA Vienna, IAEA-TECDOC-1227, ISSN 1011-4289, 2001.
17. Ahloowalia BS, Maluszynski M. Induced mutations – a new paradigm in plant breeding. Euphytica 118: 167-173, 2001.
18. Alikamanoğlu, S. Efficiency of the gamma radiation in the induction of in vitro somatic mutations. J Cell Molec Biol 1: 19-24, 2002.
19. FAO/IAEA: Database of mutant variety and genetic stocks. Available from: URL: www.mvd.iaea.org [accessed 10 March 2009].
20. Gulsen O, Uzun A, Pala H et al. Development of seedless and *Mal Secco* tolerant mutant lemons through budwood irradiation. Sci Hortic 112: 184-190, 2007
21. Zhen HR. In vitro technique for selection of radiation induced mutants of sweet potato. In Vitro Techniques for Selection of Radiation Induced Mutations Adapted to Adverse Environmental Conditions. IAEA Vienna, IAEA TECDOC 1227, ISSN 1011-4289, 2001.
22. Byun M, Kwon H, Park S. Recent advances in genetic engineering of potato crops for drought and saline stress tolerance. In: Jenks MA et al. eds. Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer; 2007: pp. 713-737.
23. Nhut DT, Nguhen NH, Thuy DTT. A novel in vitro hydroponic culture system for potato (*Solanum tuberosum* L.) microtuber production. Sci Hortic 110: 230-234, 2006.
24. Manrique LM. Potato Production in the Tropics. Manrique International Agrotech. Honolulu; 2000.
25. Sharabash MT. Radiation induced variation in potato for tolerance to salinity using tissue culture technique. In Vitro Techniques for Selection of Radiation Induced Mutations Adapted to Adverse Environmental Conditions. IAEA Vienna, IAEA-TECDOC-1227 ISSN 1011-4289, 2001.
26. Murashige T, Skoog F. A revised medium for rapid growth and bioassays tobacco cultures. Physiol Plant 15: 473-497, 1962.
27. Gosal SS, Das A, Gopal J et al. In vitro induction of variability through radiation for late blight resistance and heat tolerance in potato. In Vitro Techniques for Selection of Radiation Induced Mutations Adapted to Adverse Environmental Conditions. IAEA Vienna, IAEA-TECDOC-1227 ISSN 1011-4289, 2001.
28. Zar JH. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey; 1984.
29. Abbas SJ, Rasool G, Shah SRU et al. Analysis of genetic diversity in Pakistani potato cultivars by using randomly amplified polymorphic DNA (RAPD) primers. Am Eurasian J Sustain Agric 2: 50-53, 2008.
30. Atak C, Alikamanoğlu S, Açık L et al. Induced of plastid mutations in soybean plant (*Glycine max* L. Merrill) with gamma radiation and determination with RAPD. Mutat Res 556: 35-44, 2004.
31. Babaoğlu S, Açık L, Çelebi A et al. Molecular analysis of Turkish *Alyssum* L. (*Brassicaceae*) species by RAPD-PCR and SDS-PAGE methods. Gazi University J Sci 17: 25-33, 2004.
32. Wolf K, Rijini J. Rapid detection of genomic variability in *Chrysanthemum* using random primers. Heredity 71: 335-341, 1993.
33. Toker C, Yadav SS, Solanki IS. Mutation breeding. In: Yadav SS, McNeil DL, Stevenson PC. eds. Lentil: An Ancient Crop for Modern Times. Springer; 2007: pp. 209-224.
34. Predieri S, Di Virgilio N. In vitro mutagenesis and mutant multiplication. In: Jain SM, Häggman H. eds. Protocols for Micropropagation of Woody Trees and Fruits. Springer; 2007: 323-333.
35. Donini P, Sanino A. Induced mutation in plant breeding: current status and future outlook. In: Mohan Lain S, Brar DS, Ahloowalia BS. eds. Somaclonal Variation and Induced Mutations in Crop Improvement. Kluwer Academic Publishers; 1998: pp. 255-291.
36. Lüleyap H. Moleküler Genetiğin Esasları. Nobel Kitabevi. Adana; ISBN: 978-605-397-005-7, 2008.
37. Kumar G, Kumar Rai P. Genetic repairing through storage of gamma irradiated seeds in inbred maize (*Zea mays* L.). Turk J Biol 33: 195-204, 2009.