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Morphological, Physiological and Molecular Changes in *Solanum tuberosum* L. in Response to Pre-sowing Tuber Irradiation by Gamma Rays

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Abstract Tuber of two varieties of potato named Silana and Daimont were irradiated with control, 10 and 30 Gy of gamma rays before planting to investigate its effects on morphological, physiological criteria and DNA profiles. The effects of γ-irradiation on stored tubers were also investigated. It was observed that irradiation treatments improved vegetative characters in Silana and most of the physical properties were increased in response to treatment with 10Gy. But, in Daimont variety, the physical properties were inconsistently affected. Total phenols showed insignificant changes in the two varieties as affected by gamma irradiation and storage. A higher level of Ca, Cl, Fe and Na were detected in Silana as compared with corresponding contents in Daimont variety. Gamma irradiation treatments induced changes in DNA profile within the two varieties used.

Keywords: *potato, Solanum tuberosum, storage, minerals, phenols, TSS, DNA*

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1. Introduction

Potato (*Solanum tuberosum* L.) is one of the unique and most potential crops having high productivity and supplementing major food requirement in the world after wheat, rice and maize. It constitutes nearly half of the worlds annual output of all root and tuber crops and has always remained in the top ten since last twenty years. Potatoes contain significant levels of important antioxidants, including phenolic compounds, flavonoids and carotenoids [1]. It has high nutritive value and rich in vitamins A, B and C besides several minerals such as calcium, phosphorus and iron [2,3]. Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry, and physiology of plants depending on the irradiation level [4,5,6]. Low doses of irradiation have been reported to stimulate plant growth [7]. Radiation - induced mutations have been extensively used for improve crop plants. Studies conducted with several plant species indicated that, 2570 mutant have been obtained; among them, 1023 were produced via gamma irradiation [8]. Molecular markers are important tools for precisely detecting the effects of gamma irradiation since they identify genetic polymorphisms at DNA level and have been used to study genetic dissimilarity in many crop species [9,10,11]. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology [12]. Thus, this work aims to investigate the effect of γ- rays on morphological, physiological changes in plants as well as in the produced tubers and their DNA profile. The changes in the tubers after storage for 90 days were also investigated.

2. Materials and Methods

Two potato varieties (Daimont and Silana) were obtained from the Agriculture Research Center, Giza, Egypt. The two varieties, were irradiated with (0, 10 and 30 Gy), Irradiation facility used was Indian Gamma Cell Research Irradiator (60Co). The dose rate was 1Gy Sec-1. At the time of the experiment, irradiated and un-irradiated tubers were planted in sand- loamy soil in the farm, of the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. Soil fertilization was applied according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The produced tubers were stored for 90 days at ambient room temperature. Changes in morphological, physiological and molecular parameters were investigated as affected by radiation and storage. The data recorded were:

2.1. Morphological Changes

Plant height (cm), number of branches and leaves per plant, stem diameter, leaf area (cm^2), and tuber diameter

before and after storage (cm), tuber weight before and after storage (g) and tuber weight loss (g).

2.2. Physiological Changes

2.2.1. Physical changes

The physical properties determined were the moisture content of tubers that was determined according to [13]. Total soluble substances (TSS) were determined in a drop of potato juice, using refractometer, before and after storage according to [14].

2.2.2. Chemical Changes

The chemical properties determined were total phenol and mineral ratios. Total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent according to [15]. Methanolic extract (0.5 ml), 0.5 ml of Folin reagent, 10 ml of 7.5% sodium carbonate and deionized water were added to a final volume of 25 ml. After 1 h, the absorbance of the sample was measured at 725 nm against a blank by 02-120 shimadzu spectrophotometer. The results were expressed as mg gallic acid equivalent per gram of the fresh samples. Minerals were measured before storage in the NCRRT on Energy Dispersive X-Ray-Analysis Model: Oxford attached to a scanning electron microscope (JEOL-JSM 5400). Analysis depend on X-ray radiation emitted from each element-when the specimen is bombarded with high energetic electrons. That utilized to determine the kind of elements that exists in the specimen surface and their percentage. The elements estimated were calcium (Ca), chlorine (Cl), potassium (K), magnesium (Mg), sodium (Na) and phosphorus (P). The differences between the means were compared, using Duncan's multiple range tests [16].

2.3. Molecular Characters

DNA was estimated by extraction and purification of sample using DNeasy mini Kit (QIAGEN). Inter simple sequence repeats (ISSRs) procedure (ISSR-PCR) reactions were conducted using five primers. The primer names and sequences are shown in Table 1.

Table 1. List of the primer names and their nucleotide sequences used in the study for ISSR procedure

No	Name	Sequence
1	OP-A14	5`CTC TCT CTC TCT CTC TTG 3`
2	OP-A44	5` CTC TCT CTC TCT CTC TAC 3`
3	OP-A88	5 `CAC ACA CAC ACA AG 3`
4	OP-B88	5`CTC TCT CTC TCT CTC TGC3`
5	OP-B98	5` CAC ACA CAC ACA GT 3`

Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl $MgCl_2$ (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of Primer (10 pmol), 3.0 µl of template DNA (25 ng/ µl), 1 µl of Taq polymerase (1U/ µl) and 12.5 µl of sterile double distilled H_2O [17]. The PCRs were programmed for one cycle at 94ºC for 4 min followed by 45 cycles of 1 min at 94 ºC, 1 min at 57ºC, and 2 min at 72ºC. The reaction mixture was finally stored at 72ºC for 10 min. The PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with the 100bp ladder marker (3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp). The similarity matrices were done using Gel works ID advanced software UVP-England Program.

3.Results

3.1. Morphological Changes

Compared with non-irradiated control samples, the whole period of emergence was prolonged by 12-15 days for tubers treated with the dose 30 Gy of gamma rays. It was observed that 10 Gy dose promoted the emergence. No emergence occurred in Silana variety irradiated by 30 Gy dose. The data obtained in (Table 2) show that, in variety Silana, radiation increased plant height, stem diameter and tuber area but caused an inverse effect on most of parameters measured in Daimont variety.

Table 2. Morphological Characters of two potato varieties Silana (S) and Daimont (D) under study

Variety	γ-irradiation dose (Gy)	Growth parameter									
		Plant height (cm)	No. of leaves	Leaf area (cm^2)	No. of Branches.	Stem diameter (cm)	Tuber diameter before storage (cm)	Tuber diameter after storage (cm)	Tubers weight before storage (g)	Tubers weight after storage (g)	Tubers weight loss (g)
S	Cont.	43.4b	18.4a	18.25b	16.2a	0.94a	27.48c	24.48a	506.12ab	372.18ab	121.54a
	10 Gy	46.8a	13.8b	19.68ab	14.6b	0.90a	43.96a	25.48a	427.19b	355.44b	71.75c
D	Cont.	33.8c	12.6b	23.52a	14.2b	0.70c	32.04b	26.94a	560.26a	480.08a	80.18b
	10 Gy	27.0d	13.4b	9.16c	13.0b	0.88ab	34.28b	28.58a	419.68b	349.12b	70.56c
	30 Gy	10.2e	8.8c	2.78d	9.4c	0.76bc	30.96b	27.46a	331.96c	260.0c	71.96c

The control represents untreated tubers (cont.). The other groups of tubers are treated with different doses of gamma irradiation (10 and 30 Gy). Eachvalue is the mean of ten replicates. In case of variety Silana 30 Gy was lethal hence no data are present. Different letters indicate significant variation.

Gamma irradiation had a pronounced effect on weight lose of tuber after storage. Weights lose decreased (about 50 g in Silana and 10g in Daimont) by the effect of gamma irradiation, as compared with un-irradiated tubers (Table 2). Weight losses were more obvious in un-irradiated Silana than in Daimont variety.

3.2. Physical Changes

Data in Table 3 showed significant change in moisture content before and after storage due to radiation effects. Moisture content before storage increased significantly due to gamma irradiation in the two varieties (Silana and

Daimont), while total soluble solids were not affected by irradiation in variety Salina before and after storage.

Table 3. Physical Changes of Two Potato Varieties Silana (S) and Daimont (D) under study

Variety	γ-irradiation dose (Gy)	Physical changes			
		Moisture content before storage	Moisture content after storage	Total soluble solids (TSS) Before storage	Total soluble solids (TSS) After storage
S	Cont.	79. 95b	79. 38 b	6.50b	7.62a
	10	84.89a	82.02a	6.40b	6.88a
D	Cont.	78.99b	73.37c	6.25b	7.88a
	10	79.62a	72.84c	7.25ab	7.00a
	30	77.9b	67.17d	8.25a	7.00a

The control represents untreated tubers (cont.). The other groups of tubers are treated with different doses of gamma irradiation (10 and 30 Gy). Each value is the mean of ten replicates. In case of variety Silana 30 Gy was lethal hence no data are present. Different letters indicate significant variation.

But, in Daimont variety total soluble solids were increased by gamma irradiation before storage.There was a slight decrease in moisture and total soluble solids in the irradiated and un-irradiated Daimont, whereas an increase in total soluble solids was obtained in irradiated and un-irradiated Salina.

3.3. Chemical Changes

Total phenol content and mineral ratio was determined and shown in Table 4. γ-irradiation had non-significant effect on total phenols in the two varieties before and after storage. Total phenol content was increased in both potato varieties under investigation after storage. Mineral ratio were variable in the two varieties, Silana contained high levels of Ca and Fe. Daimont on the other hand, contained high levels of Cl, K, Mg, Na and P. Gamma irradiation increased Cl, K, Na and P contents and decreased Ca, Fe and Mg contents in variety Silana. In variety Daimont, gamma irradiation increased the contents of Ca, Cl, Fe, K and Na but decreased thos of Mg and P.

3.4. DNA Polymorphism Using Inter Simple Sequence Repeats (ISSRs)

All the five primers used successfully amplified DNA fragments from potato DNA samples. The results indicated occurrence of structural changes in irradiated potato with the five primers: OP-A14, OP-A44, OP-A88, OP-B88 and OP-B98, respectively. A total of 45 fragments were visualized. Banding patterns for the used five random primers were scored as present (1) or absent (0) (Table 5). Genomic DNA polymorphisms due to irradiation are presented in Figure (1) for the five primers. In variety Silana, the percentage of polymorphism was 17.78%. The result of ISSR analysis refers to appearance of one band having a molecular size 700 bp with primer OP-A14, two bands (515 and 345 bp) with primer OP-A44, three bands with primer OP-A88 (730, 600 and 160bp) and one band with primer OP-B88 (240 bp) were appeared under gamma irradiation treatment by 10Gy. Meanwhile, with primer OP-B98, the band with molecular size 160 bp disappeared. In variety Daimont, the percentage of polymorphism in treatment with 10 Gy sample was 15.56% and was 26.67% in application of 30Gy) Gamma irradiation also induced the appearance of a band with primer OP-A14 having a molecular size 700 bp and caused disappearance of two bands (620 and 295 bp) under the effect of 10 and 30Gy. A band (120 bp) disappeared only at dose 30 Gy. In case of the primer OP-A44, a band (515bp) appeared in both irradiation doses. Gamma irradiation induced the disappearance of five bands with primer OP-A88; one of them (240 bp) by 10 Gy and the rest (540, 370, 180 and 120 bp) by 30 Gy. With primer OP-B98, one band (280 bp) disappeared under gamma irradiation and two bands appeared one having the molecular size 160 bp by 10 Gy and the other (630 bp) by 30 Gy.

Table 4. Chemical Changes of Two Potato Varieties Silana (S) and Daimont (D) under study

Variety	γ-irradiation dose (Gy)	Phenol content g/100g		Minerals						
		Before storage	After storage	Ca	Cl	Fe	K	Mg	Na	P
S	Cont.	0.565a	0.573a	2.77a	10.77c	2.31a	50.84d	5.86c	5.4d	2.48e
	10	0.567a	0.573a	0.48c	14.21a	0.51d	69.6a	3.21e	26.77a	4.89c
D	Cont.	0.566a	0.573a	1.29b	11.87b	1.94b	61.34b	6.45b	13.36b	3.76d
	10	0.566a	0.573a	-	7.71d	-	58.93c	11.33a	12.63c	11.21a
	30	0.565a	0.573a	1.40b	11.04c	1.68c	69.56a	4.4d	2.25e	9.67b

The control represents untreated tubers (cont.). The other groups of tubers are treated with different doses of gamma irradiation (10 and 30 Gy). Each value is the mean of ten replicates. In case of variety Silana 30 Gy was lethal hence no data are present. (-) undetected.

4. Discussion

The results obtained referred that potato tubers exposure to gamma irradiation before potato tubers sowing induced in morphological, physiological and molecular changes in two *Solanum tuberosum* L. varieties (Daimont and Silana). Also, it affected the produced tubers at harvest time and after storage. Gamma irradiation and storage effects differed in the two varieties used. Similar conclusions were also obtained by [18,19] who noticed that the type of irradiation, dose rate or dose applied differently affected physiological parameters of different species, varieties, and cultivars. In the present study, gamma irradiation of tubers stimulated some morphological characters of the resulting plants. The exposure to low dose of γ-rays (10Gy) had stimulatory effect on specific morphological parameters and led to increased plants yield. Similar findings were found by [20,21,22]. The biological effect of γ-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals [5]. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry, and physiology of plants, depending on the radiation dose [23].

The physical characters of the two varieties studied in the present work varied in their response to gamma irradiation and storage. All results of physical characters evaluated are in consistent with [24] who observed differences between them in potato cultivars. Also, the

effect of gamma irradiation on storage of the yielded tubers varied in the two varieties and these results are in agreement in with those of [25,26]. They studied the effects of low doses of gamma rays and storage less than 120 days on potato cultivars and noticed insignificant changes on dry matter and phenol contents but weight loss increased with the increase in radiation doses. Radiation did not affect TSS in stored dragon fruits [27] and this concord with the present finding obtained with the two potato varieties. The previous studies indicated significant decrease in total soluble solids of potato tubers, insignificant decrease in total phenols and moisture content of potato tubers that exposed to 2.8 kGy of gamma rays [28]. These effects included changes in the plant cellular structure and metabolism e.g., accumulation of phenolic compounds [4,5,29,30] Phenolic contents increased more with storage time than with gamma irradiation dose [31,32]. The increase in phenolic content might be due to dehydration that led to a higher concentration of solids at the end of the storage period. Additionally, stimulation of the synthesis of both antioxidants and polyphenols is known to occur with stress,which might have increased at the end of the storage period due to dehydration [33]. Storage exerted a much greater influence on carotenoid content and phenolic content than did low-doses of gamma irradiation [31]. Potato tubers contain several minerals that are important in the diet, including phosphorous, calcium, zinc, nitrogen and iron [34]. Gamma irradiation induced changes in the mineral content of the two potato varieties. Molecular markers are important tools for precisely detecting the effects of gamma radiation since they identify genetic polymorphisms at DNA level and have been used to study genetic dissimilarity in many crop species [9,11]. More polymorphism has been detected with the use of ISSRs than with any other assay procedure [35]. In the present work, the polymorphic effect of gamma irradiation was depending on the applied dose and potato variety. In this respect variety Silana is more sensitive to gamma irradiation than variety Diamont. In case of Silana, the percentage of polymorphism was 17.78% on treatment with 10Gy and the 30Gy dose considered a lethal dose, where the tuber cannot germinate. The high dose of γ - irradiation is more effective than low in variety Diamont, the polymorphic percentage at dose 30Gy was 26.67% and 15.56% at 10Gy dose.

Table 5. DNA polymorphism using Inter simple sequence repeats (ISSRs) for two potato varieties Silana (S) and Daimont (D)

primers	Band No.	M.W bp	Varieties &Treatments					Polymorphism
			CS	CD	10S	10D	30D	
OP-A14	**1**	700.0	0	0	1	1	1	Polymorphic
	2	620.0	1	1	1	0	0	Polymorphic
	3	455.0	1	1	1	1	1	monomorphic
	4	335.0	1	1	1	1	1	monomorphic
	5	295.0	1	1	1	0	0	Polymorphic
	6	242.0	1	1	1	1	1	monomorphic
	7	160.0	1	1	1	1	1	monomorphic
	8	140.0	1	1	1	1	1	monomorphic
	9	120.0	1	1	1	1	0	Polymorphic
OP-A44	**1**	720.0	1	1	1	1	1	monomorphic
	2	680.0	1	1	1	1	1	monomorphic
	3	515.0	0	0	1	1	1	Polymorphic
	4	430.0	1	1	1	1	1	monomorphic
	5	375.0	1	1	1	1	1	monomorphic
	6	345.0	0	1	1	1	1	Polymorphic
	7	275.0	1	1	1	1	1	monomorphic
	8	245.0	1	1	1	1	1	monomorphic
	9	190.0	1	1	1	1	1	monomorphic
OP-A88	**1**	730.0	0	1	1	1	1	Polymorphic
	2	600.0	0	1	1	1	1	Polymorphic
	3	480.0	1	1	1	1	1	monomorphic
	4	390.0	1	1	1	1	1	monomorphic
	5	375.0	1	1	1	1	1	monomorphic
	6	320.0	1	1	1	1	1	monomorphic
	7	250.0	1	1	1	1	1	monomorphic
	8	180.0	1	1	1	1	1	monomorphic
	9	160.0	0	1	1	1	1	Polymorphic
OP-B88	**1**	540.0	1	1	1	1	0	Polymorphic
	2	480.0	1	1	1	1	1	monomorphic
	3	370.0	1	1	1	1	0	Polymorphic
	4	330.0	1	1	1	1	1	monomorphic
	5	180.0	1	1	1	1	1	monomorphic
	6	240.0	0	1	1	0	1	Polymorphic
	7	200.0	1	1	1	1	1	monomorphic
	8	180.0	1	1	1	1	0	Polymorphic
	9	120.0	1	1	1	1	0	Polymorphic
OP-B98	**1**	630.0	0	0	0	0	1	Unique
	2	540.0	1	1	1	1	0	Polymorphic
	3	500.0	1	1	1	1	1	monomorphic
	4	430.0	1	1	1	1	1	monomorphic
	5	325.0	1	1	1	1	1	monomorphic
	6	280.0	0	1	0	0	0	Unique
	7	245.0	1	1	1	1	1	Monomorphic
	8	220.0	1	1	1	1	1	Monomorphic
	9	160.0	1	0	0	1	0	Polymorphic

The control represents untreated tubers (CS & CD). The other groups of tubers are treated with different doses of gamma irradiation 10 and 30 Gy (10S, 10D and 30 D). In case of variety Silana 30 Gy was lethal hence no data are present.

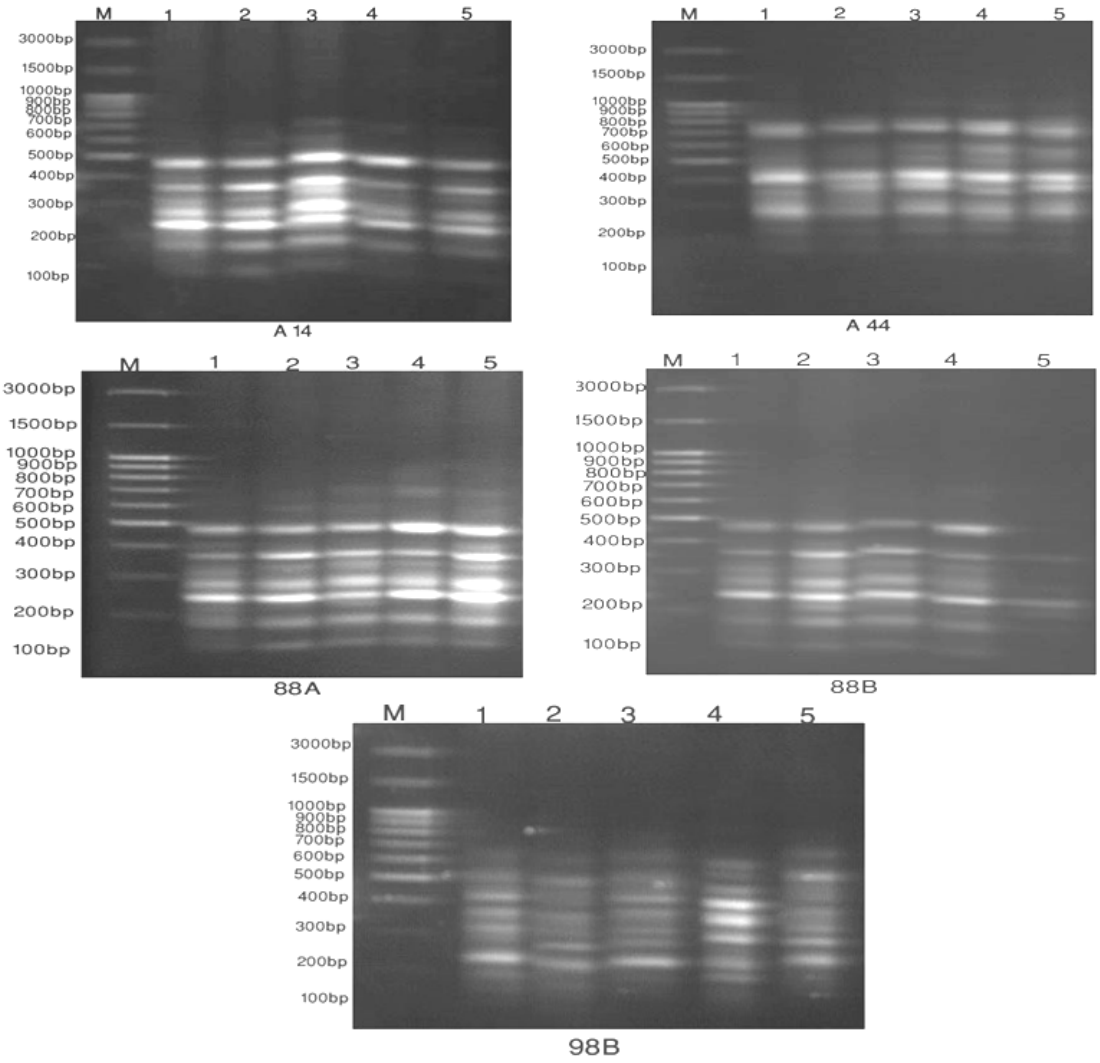

Figure 1. DNA polymorphism using Inter simple sequence (ISSRs) repeats using five primers (A14, A44, A88, B88, B98) for two potato varieties (Silana and Daimont). The control represents untreated tubers of Silana (1) and Diamont (2), where as the other groups of tubers are treated with different doses of gamma irradiation (10and 30 Gy). Silana irradiated by 10 Gy (3) and Diamont irradiated by 10 and 30 Gy(4 & 5)

In variety Diamont, polymorphism percentage at dose 30Gy was 26.67% and at dose 10Gy was 15.56%. Gamma irradiation as a physical mutagen, is potent, inexpensive and easy to apply on the potato plantlets in vitro to create point mutations. The mutant plant variants can be easily selected from potato plants by RAPD-PCR [36]. Nine potato unique markers could be identified among the 45 polymorphic bands, as analyzed by random amplified polymorphic DNA-polymerase chain reaction profiles [37]. They detected one marker for the 5 Gy gamma dose and none for the 10 Gy and the highest number of markers (4) was obtained with the 2.5 Gy dose. Gamma rays induced molecules ionization to make apart free radicals these free radicals attacked to DNA molecule which make breaking one or two chains of DNA [38]. Gamma irradiation resulted in appearance or disappearance of bands that can considered as molecular marker for radiation process [39].

5. Conclusion

Irradiation by low doses of γ-rays (≥ 10Gy) before sowing may be used as a valuable parameter to improve potato tubers yield and their shelf - life. It was observed that variety Silana is more sensitive to gamma irradiation than variety Diamont. The two bands with molecular size 700 and 515 bp at OP-A14 and OP-A44, respectively can be used as positive molecular markers for gamma radiation.

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