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Impact of gamma irradiation pretreatment on biochemical and molecular responses of potato growing under salt stress

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Abstract

Background: Previous literatures revealed that gamma rays have an increasing effect on salt tolerance in different plants. In vitro experiment was conducted to study the effect of gamma rays (20 Gray) on salt tolerance of four potato cultivars (Lady Rosetta, Diamante, Gold, and Santana).

Results: Gamma-treated Santana plantlets were more tolerant to salinity as compared to other cultivars. It showed a significant increment of fresh weight (250% over the untreated). Gamma-treated plantlets of Lady Rosetta, Diamante, and Gold showed higher activity of peroxidase (POD) and polyphenol oxidase (PPO). Isoenzymes analysis showed an absence of POD 3, 4, and 5 in Gold plantlets. The dye of most PODs and PPOs bands were denser (more active) in gamma-treated plantlets of Santana as compared to other cultivars. Both gamma-treated and untreated plantlets showed the absence of PPO1 in Lady Rosetta and Diamante, and PPO 3, 4, and 5 in Gold plantlets. Genetic marker analysis using ISSR with six different primers showed obvious unique negative and positive bands with different base pairs in mutant plantlets as compared to the control, according to primer sequence and potato genotype. The 14A primer was an efficient genetic marker between mutated and unmutated potato genotypes. Santana had a unique fingerprint in the 1430-pb site, which can be a selectable marker for the cultivar. An increment in genetic distance between Gold cultivar and others proved that the mutation was induced because of gamma rays.

Conclusion: We assume that irradiation of potato callus by 20-Gy gamma rays is an effective process for inducing salt resistance. However, this finding should be verified under field conditions.

Keywords: *Solanum tuberosum* L., Callus, ISSR, Peroxidase, Polyphenol oxidase, Isoenzymes

Background

Potato (*Solanum tuberosum* L.), a member of Solanaceae family, has a high economic value in domestic and global markets. Potato tubers are known for their high nutritional value (20–30% starch, 2% protein, vitamins B1, B2,

C, minerals, etc.). Potato cultivars such as Lady Rosetta are used for cooking and in chips industry. Cultivars such as Diamante and Santana with superficial eyes-tubers, creamy pulp and more than 20% of dry matter are used as half-fried [1].

Potato is a moderately salt-tolerant vegetable crop (1.7 dS/m = 1088 ppm). Plant growth and productivity are affected by salinity [2–5]. Salt stress negatively affects tuber sprouting, adventitious root development and biomass production, which significantly reduce yield [6]. Salt-stressed plants induce ionic and osmotic stress signals to re-establish cellular homeostasis and

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detoxification signals to repair stress damages coordinate cell division [7].

Commercial cultivars of potato are tetraploid ($2n=4x=48$) and extremely heterozygous, with simplex inheritance (Aaaa) for different characteristics. Genetic manipulation in potato could be easily conducted through tissue culture technique and conventional breeding methods [8].

Previous reports revealed that radiation was an effective method to increase tolerance on plants against factors such as salt and drought stress [9–12]. Gamma radiation is the most preferred physical mutagen by plant breeders. The impact of radiation is mainly related to the species, cultivar, and plant age, physiology, morphology and genetic organization. Ionizing radiation causes structural and functional changes in DNA molecule, which have role at cellular and systemic levels. The nature of DNA modifications includes base alteration, substitution, and deletion, in addition to chromosomal aberration, as reported by [13]. Afrasiab and Iqbal [14] selected six gamma mutant lines of potato in terms of average shoot height, number of shoot/plant, number of nodes/shoot, average tuber number, size and weight, and number of eyes/tuber. Cheng et al. [15] found that radiation with 20 Gy in Shepody potato improved tuber formation. The average number and diameter of Minitubers (V1) were significantly increased over the control by 71% and 34%, respectively. The higher the radiation dose of 40 and 50 Gy, the lower the emergence percentage, plant height and root length of mini-tuber potato plants with about 67% and 31% for each dose, respectively. Yalcili and Alikamanoğlu [16] obtained salt-tolerant mutants of Marfona potato using gamma irradiation (20 or 30 Gy) and regenerated them on selection medium containing 100 mM NaCl. A polymorphism rate of 89.66% was calculated to the selected primers using Inter simple sequences repeat (ISSR) technique, and the mutants were 27.5% genetically different from the control plants. Molecular markers such as enzymes and protein are considered one of the effective indicators to differentiate between varieties, DNA identification and genetic compatibility, as well as the detection of genetic variability of plants susceptible to mutagenesis by comparing the results of DNA analysis with the origins. The reliability of the used primers is considered as a function of environmental stresses [17]. Inter Simple Sequences Repeat (ISSR) markers provide greater opportunities in determining the exact relationship between a specific DNA sequence and the examined trait, and also in determining a plant's identity using particular genes. ISSR is popular DNA fingerprinting method, because it is inexpensive, highly polymorphic, quick, and easy to perform [18]. The primers are designed using microsatellite sequences to amplify the genomic regions flanked by microsatellite

repeats. It is possible to amplify multiple fragments using one primer of ISSR analysis. The information obtained from ISSR analysis is more reliable than RAPD to supply supplementary data of genetic variations of the mutants from non-overlapping genome regions [18]. Yalcılı and Alikamanoğlu [16] show 89.66% polymorphism rate with six primers among mutant potato plants, which were improved as salt-tolerant using gamma radiation. AL-Hussaini et al. [19] studied the Differential Display Reverse Transcriptase (DDRT) as a genetic marker for salt tolerance in mutant clones of potato calli exposed to 8, 10 and 12 dSm⁻¹ and their parental cultivars (Riviera and Burren) using 10 randomized primers. They stated that RNA concentration of 103.0 and 226.0 ng mmol⁻¹ showed a purity of 1.86 and 2.01, respectively. DDRT with seven primers succeeded to amplify DNA fragments involved in salt tolerance. Cluster analysis separated genotypes in groups with a range of genetic variability among them.

The aim of this research was to study the differential ISSR amplified fragments over four potato cultivars (Lady Rosetta, Diamante, Gold and Santana) and to examine the ability of gamma rays for inducing salt resistant in potato cultivars, then confirm these results using molecular characteristics under field conditions compared with non-irradiated plantlets.

Methods

In vitro propagation of potato cultivars and the application of gamma rays

Plantlets of Lady Rosetta, Diamante, Gold and Santana cultivars were kept at 4 °C, and then stored in dark at 25 °C for 2 weeks until developing 5–6 cm-height sprouts [20]. Samples of 1–2 mm-length sprouts were collected and sterilized with 1% sodium hypochlorite for 3 min., and dipped in absolute ethanol, then washed in sterilized water for 5 minutes before cultivating on half-strength Murashige and Skoog's medium (MS) [21]. Shoot tips (10 mm) were cultivated on callus production medium (containing 1 mg/L of NAA (naphthalene acetic acid) and 0.5 mg/L of BAP (benzyl amino purine). All cultures were stored in a culture room at light/dark photoperiod (16/8 h) and 25 °C. Two weeks later, calluses were initiated, and then 4 segments of callus ($\approx 1 \text{ cm}^2$) from each container were sub-cultured on the same medium at an interval of 2 weeks. Twenty containers having four segments of callus were then treated with 20 Gray (Gy) gamma rays ($\approx 0.653 \text{ rad/Sec}$) for 30 min (137Cs-Gamma Cell-40, Canada) at the Egyptian Atomic Energy Authority, Egypt. Potato callus was irradiated by rotating 360° in a cylindrical radiation field. Radiation was repeated twice at an interval of 7 days. All experiments were represented by 4 replicates in a completely randomized design [22]. Differentiated

calluses on half-MS medium without growth hormones produced complete plantlets. Uniform plantlets (3 cm height) were examined for salt tolerance by adding 2720 ppm of NaCl ($4dSm^{-1}$) to MS media. After 1 month, the fresh weight (FW) of gamma-treated and control plantlets was measured (g). The biochemical and molecular analysis of potato plantlets were also carried out:

Activity of peroxidase (POD) and polyphenol oxidase (PPO)

Enzyme extraction

200 mg of fresh plantlets were homogenized with 10 ml of 0.1 M phosphate buffer (pH 6.8), and then centrifuged (20000 rpm) at 20 °C for 20 min. The clear supernatant was collected for the determination of enzyme activities [23].

Activity of POD (EC 1.11.1.7)

The activity of POD was assayed using a solution containing 5.8 ml of 50 mM phosphate buffer (pH 7.0), 0.2 ml of the enzyme extract, and 2 ml of 20 mM H_2O_2 after the addition of 2 ml of 20 mM pyrogallol. The rate of increase in absorbance as pyrogallol was determined spectrophotometrically using UV-spectrophotometer for 60 second at 470 nm and 25 °C. One unit of enzyme activity was defined as the amount of the enzyme that catalyzed the conversion of one micromole of H_2O_2 per minute at 25 °C. The blank sample was made using buffer instead of enzyme extract according to [24].

Activity of PPO (EC 1.10.3.1)

The activity of PPO was determined using 125 μ mol of phosphate buffer (pH 6.8), 100 μ mol pyrogallol, and 2 ml of enzyme extract. After the incubation period of 5 min at 25 °C, the reaction was ceased with the addition of 5% H_2SO_4 (1 ml). The blank sample was made using a very well boiled enzyme extract, and the developed color was read at 430 nm. Enzyme activity was expressed as the changes in the optical density/g fresh weight/h [25].

Isoenzymes of POD and PPO

Polyacrylamide gel electrophoresis (PAGE) was conducted to identify isoenzyme variations, among the studied gamma-treated plantlets and the control, using peroxidase (PODs) and polyphenol oxidase (PPO) according to [26]. Isoenzymes were extracted by homogenizing 0.5 g fresh samples in 1 ml extraction buffer (10% glycerol), and the extract was centrifuged at 10000 rpm for 5 minutes. The supernatant was used for electrophoretic analysis. A sample of 40 μ l extract was mixed with 20 μ l sucrose and 10 μ l bromophenol blue, and then a volume of 50 μ l of this mixture was applied to each well. Gel run was performed at 150 V until the bromophenol blue dye has reached the separating gel, and then voltage was

increased to 200 V. Electrophoresis apparatus was placed inside a refrigerator during the run.

POD staining and detection

After electrophoresis, the gel was stained with benzidine di-HCl (0.125 gm), glacial acetic acid (2 ml) and the mixture was topped up to 50 ml with distilled water. Gel was placed into this solution, and 5 drops of hydrogen peroxide was added. The gel was incubated at room temperature until bands appear as described by [27].

PPO staining and detection

The gel was stained with 0.1 M of phosphate buffer (pH 6.5), 100 mg of sulfanilic acid, 200 mg catechol in 2 ml acetone. The gel was then placed into this solution and incubated at 30 °C for 30 min until the bands appeared, and then gels were scanned and analyzed using Gel Doc Vilber Lourmat system.

ISSR analysis

Genomic DNA extraction

Genomic DNA was extracted using 500 mg of plantlets with 400 μ l extraction buffer containing 200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA (pH 8.0) and SDS (0.5 %). This mixture was vortexed in Eppendorf tubes for 20 s, and warmed in water bath at 65 °C for 15 min, and 200 μ l sodium acetate (3 M) was added to the solution and centrifuged at 13,000 rpm for 10 min. The supernatant (500 μ l) was transferred to new Eppendorf with 500 μ l cold isopropanol, and shaken for 5 min. then centrifuged at 13,000 rpm for 10 min. The residual was washed with 200 μ l cold ethyl alcohol (70%) then centrifuged at 13,000 rpm for 5 min. This step was repeated twice, and then 50 μ l dH_2O was added to the dry residual and stored at -20 °C overnight, followed by the addition of 1 μ l RNAase (10mg/1ml H_2O) and incubation at 37 °C for 1 h. This mixture was then provided with 150 μ l dH_2O and 200 μ l phenol and shaken well for 2 min., and then centrifuged at 13,000 rpm for 10 min. The supernatant was mixed with 100 μ l phenol, 100 μ l chloroform and shaken for 2 min, followed by centrifugation at 13,000 rpm for 10 min. Dried residual was mixed with 50 μ l dH_2O and stored at -20 °C [28]. The primers names and their nucleotide sequences used for ISSR procedure are listed in Table 1.

Polymerase chain reaction (PCR)

The amplification of DNA was performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94 °C for 4 min, followed by 45 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C. The reaction was finally stored at 72 °C for 10 min. PCR was performed in 30 μ l-volume tubes that contained 3 μ l

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

No.	Name	Sequence	No.	Name	Sequence
1	14A	5' CTC TCT CTC TCT CTC TTG 3'	4	HB-11	5' GTG TGT GTG TGT TGT CC 3'
2	44B	5' CTC TCT CTC TCT CTC TGC 3'	5	HB-13	5' GAG GAG GAG C 3'
3	HB-9	5' CAC CAC CAC GC 3'	6	HB-15	5' GTG GTG GTG GC 3'

Table 2 Fresh weight (g) of potato plantlets (30 days old) as affected by gamma rays after the application of NaCl (4 dSm⁻¹)

Cultivars	Control	Gamma irradiation	% of control
Lady Rosetta	3.2 e	6.9 b	115
Diamante	4.1 d	8.5 a	107
Gold	2.6 f	5.6 c	115
Santana	1.6 g	5.6 c	250

Values followed by the same letter within a column are not significantly different at $P \leq 5\%$ using Duncan's multiple range test (DMRT)

dNTPs (2.5 mM), 3 μ l MgCl₂ (25 mM), 3 μ l Buffer (10 x), 2 μ l primer (10 pmol), 0.2 μ l Taq DNA polymerase (5U/ μ l), 2 μ l Template DNA (25 ng) and 16.8 μ l H₂O (dw), according to [29].

Agarose (1.5%) was warmed with 100 ml of TBE (Tris/borate/EDTA) buffer, and then 5 μ l ethidium bromide was added after the temperature became 55 °C. Samples of DNA amplified product (15 μ l) was loaded in each well. DNA ladder (100 bp) mix was used as standard DNA with molecular weights of 3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad. The Cluster analyses were done using Gel works in ID advanced software UVP-England Program.

[30]. The polymorphism percentage was calculated, according to [31].

Statistical analysis

Data were statistically analyzed using the MSTAT-C statistical analysis package [32] and means were separated using LSD test at $P \leq 0.05$.

Results

Effect of gamma rays on fresh weight

Our results revealed that salt-stressed Diamante potato plantlets treated with gamma rays have the highest significant values of fresh weight (8.5 g) compared to the control (Table 2). Fresh weight of gamma-treated plantlets of Santana potato has increased by 250% over the control.

Effect of gamma rays on antioxidant enzymes

Both peroxidase (POD) and polyphenol oxidase (PPO) had increased in gamma-treated plantlets over the control of four potato cultivars (Fig. 1). The highest increment of POD and PPO activity was seen in gamma-treated plantlets of Lady Rosetta, Diamante and Gold cvs.

Effect of gamma irradiation on isoenzymes of POD

Three isoenzymes of POD (3, 4 and 5) were disappeared as a result of gamma rays application on salt-stressed Gold potato, as shown in Fig. 2 and Table 3. Density of

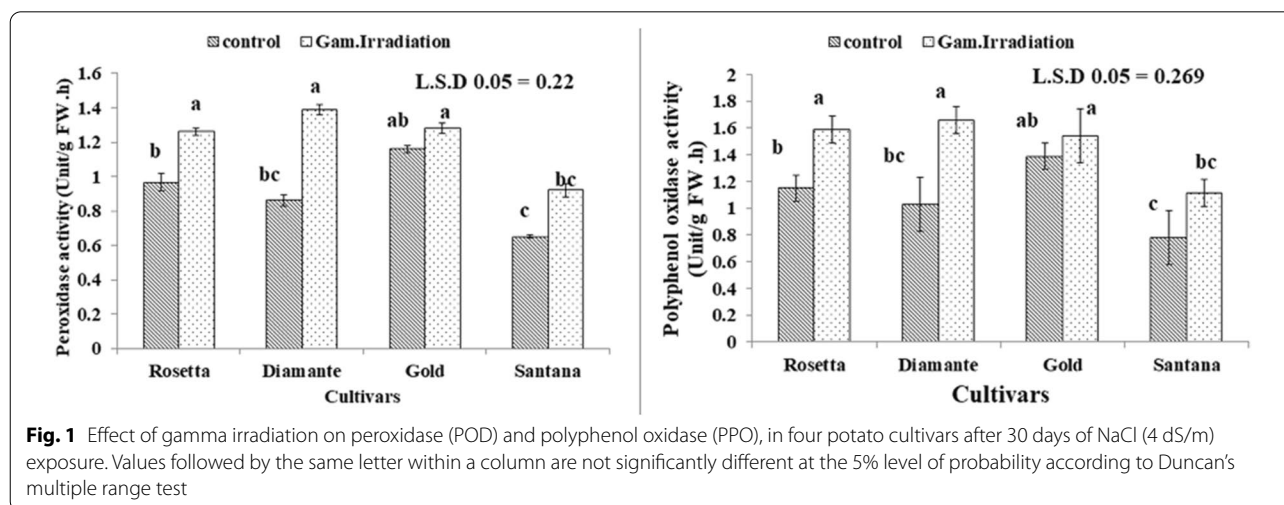


Fig. 1 Effect of gamma irradiation on peroxidase (POD) and polyphenol oxidase (PPO), in four potato cultivars after 30 days of NaCl (4 dS/m) exposure. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test

isoenzymes bands has differed according to cultivar and gamma rays treatment. In this respect, density of all PODs isoenzymes bands was high in salt-tolerant Santana potato.

Effect of gamma irradiation on isoenzymes of PPO

Both gamma-treated and untreated plantlets of Gold and Santana cvs. had shown five isoenzymes of PPO compared to only four isoenzymes in Lady Rosetta and Diamante cvs. [-PPO 1] (Fig. 3 and Table 4). In addition, three bands of PPO 3, 4 and 5 were disappeared in Gold cv. because of gamma rays application. Gamma-treated Plantlets of Santana cv. showed high density of PPO1, 2 and 3, compared to all other cultivars.

Molecular characteristics of six ISSR primers

Six ISSR primers were amplified to detect the genetic changes among mutated and unmutated plants, and display the perceptive polymorphism concerning ISSR amplified fragments in four potato cultivars. As expected, the range of amplified sizes by each primer across different mutated and control plants was noteworthy [100 bp in 44B to1430 bp in HB15] (Table 5). However, the amplified DNA fragments had been categorized into

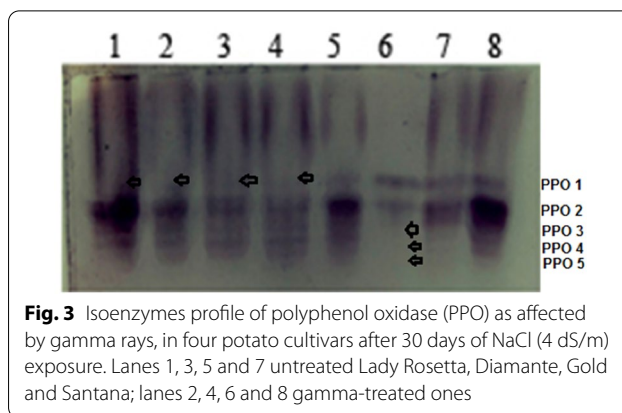


Fig. 3 Isoenzymes profile of polyphenol oxidase (PPO) as affected by gamma rays, in four potato cultivars after 30 days of NaCl (4 dS/m) exposure. Lanes 1, 3, 5 and 7 untreated Lady Rosetta, Diamante, Gold and Santana; lanes 2, 4, 6 and 8 gamma-treated ones

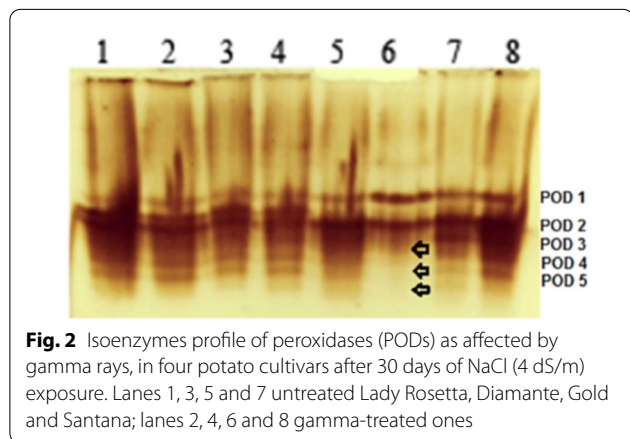


Fig. 2 Isoenzymes profile of peroxidases (PODs) as affected by gamma rays, in four potato cultivars after 30 days of NaCl (4 dS/m) exposure. Lanes 1, 3, 5 and 7 untreated Lady Rosetta, Diamante, Gold and Santana; lanes 2, 4, 6 and 8 gamma-treated ones

polymorphic and monomorphic fragments. The total number of bands produced 34 bands with average of 5.7 bands per primer. The Hb15 primer produced the highest number of bands (8), while the HB13 primer produced the lowest number of bands (4). Moreover, Table 5 reveals that out of 34 bands scored, 22 bands were monomorphic identical for all mutated and control plants. The percentage of polymorphic bands recorded for each primer was not associated to the total number of bands. For example, the total number of bands scored for HB9 primer and Hb15 primer was high (7 and 8, respectively, with 29% and 50% of them being polymorphic). In contrast, 14A primer produced 5 bands and 60% of them were polymorphic.

The genetic variations of the control plants for all four potato cultivars, and their mutated plants have been revealed by six ISSR markers (Fig. 4 and Table 6). Therefore, ISSR profile of mutated plants of Lady Rosetta lost two bands (30 bands) compared to the control plants (32 bands). On the other hand, the mutated plants gained several bands (29 bands and 30 bands) than losing bands, as in Gold and Santana cvs., respectively. Diamante cv. did not show any difference on the number of bands for both mutated and control plants (30 bands). Control plants of Santana cultivar showed negative unique

Table 3 Ideogram of POD isoenzymes as affected by gamma rays in four potatoes cultivars after the exposure to 4 dS/m of NaCl

Peroxidase isoforms	Relative mobility	Cultivars							
		Lady Rosetta		Diamante		Gold		Santana	
		Con	Tre	Con	Tre	Con	Tre	Con	Tre
POD 1	0.55	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	1 ⁺	1 ⁺⁺
POD2	0.60	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	1 ⁺⁺	1 ⁺⁺
POD3	0.65	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	—	1 ⁺	1 ⁺⁺
POD4	0.70	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	—	1 ⁺	1 ⁺⁺
POD5	0.75	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁻	—	1 ⁺	1 ⁺⁺

—low density, + moderate density, ++ high density, _ absent band

Table 4 Ideogram of PPO isoenzymes as affected by gamma rays in four potatoes cultivars after the exposure to 4 s dS/m of NaCl

Polyphenol oxidase isoforms	Relative mobility	Cultivars							
		Lady Rosetta		Diamante		Gold		Santana	
		Con	Tre	Con	Tre	Con	Tre	Con	Tre
PPO 1	0.55	–	–	–	–	1 ⁺	1 ⁺⁺	1 ⁺	1 ⁺⁺
PPO 2	0.60	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺⁺
PPO 3	0.65	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺⁺	–	1 ⁺	1 ⁺⁺
PPO 4	0.70	1 ⁺⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺	–	1 [–]	1 ⁺⁺
PPO 5	0.75	1 ⁺	1 ⁺	1 ⁺	1 ⁺	1 ⁺	–	1 [–]	1 ⁺

– low density, + moderate density, ++ high density, _ absent band

Table 5 Molecular characteristics of six ISSR primers used to analyze mutated and control plants of four potato cultivars

Primers	Band size (bp)	Total bands	Poly-bands	Mono-bands	Polymorphism %
14A	230–680	5	3	2	60
44B	100–415	5	1	4	20
HB9	185–1360	7	2	5	29
HB11	340–1080	5	1	4	20
HB13	265–480	4	1	3	25
HB15	430–1430	8	4	4	50
Over total		34	12	22	34

band with 570 bp corresponding to HB9 primer, while control plants of Gold cultivar revealed negative unique bands with 1080 bp using HB11 primer. Moreover, many constant bands (800 bp and 715 bp of both HB9 and HB15 primers, respectively) appeared in both control and mutated plants of Lady Rosetta and Diamante, but they disappeared in Santana and Gold cvs. They could be genetically related to genotypic of cultivar plants or to the germination and growth processes of the plants. Mutated plants of Diamante and Gold cvs. showed negative unique bands with 415 bp and 965 bp corresponding to 44B and HB15 primers, respectively. Meanwhile, a positive unique band with 820 bp was found in mutated plants of Gold cultivar.

Genetic similarity was calculated for all four potato cultivars and found varied from 0.812 (Santana vs. Diamond) to 0.938 (Rosetta vs. Diamond). The high value of genetic similarity between Rosetta and Diamond could be a sign that a large part of the genome of both cultivars under study is identical.

A dendrogram based on UPGMA analysis with ISSR data (similarity matrix) is illustrated in Fig. 5. The four potato cultivars were divided into two main clusters. The first cluster has included Rosetta and Diamond cultivars that they were closed to each other, while the second cluster has included Gold and Santana cultivars. Genetic similarity was calculated for mutated plants of

four potato cultivars and was varied from 0.788 (Gold vs. Diamante) to 0.875 (Rosetta vs. Diamante) and (Santana vs. Diamante).

A dendrogram based on UPGMA analysis with ISSR data (similarity matrix) is illustrated in Fig. 5. The mutated plants of four potato cultivars were divided into two main clusters. Mutated Gold cultivar separated from the other three cultivars. The second cluster was divided into two groups. Subgroup1 has included Santana and Diamante, while Rosetta was in subgroup 2. However, there were differences in genetic similarity of the control and mutated plants of the studied cultivars. For instance, Gold and Diamante were partly closed in the control plants, but they were far away from each other in the mutated plants (Fig. 6).

Discussion

Gamma treatment had more pronounced effect on the fresh weight of potato plantlets of all cultivars grown under salinity conditions (Table 1). Its effect was different from one potato genotype to another. Vigorous growth under stressful conditions is often required suitable physiological status related to enzymes and other molecules. Results confirmed the increment of the activity of both antioxidant enzymes; peroxidase (POD) and polyphenol oxidase (PPO) in gamma-treated plantlets in comparison to the untreated ones (Fig. 1). In the same

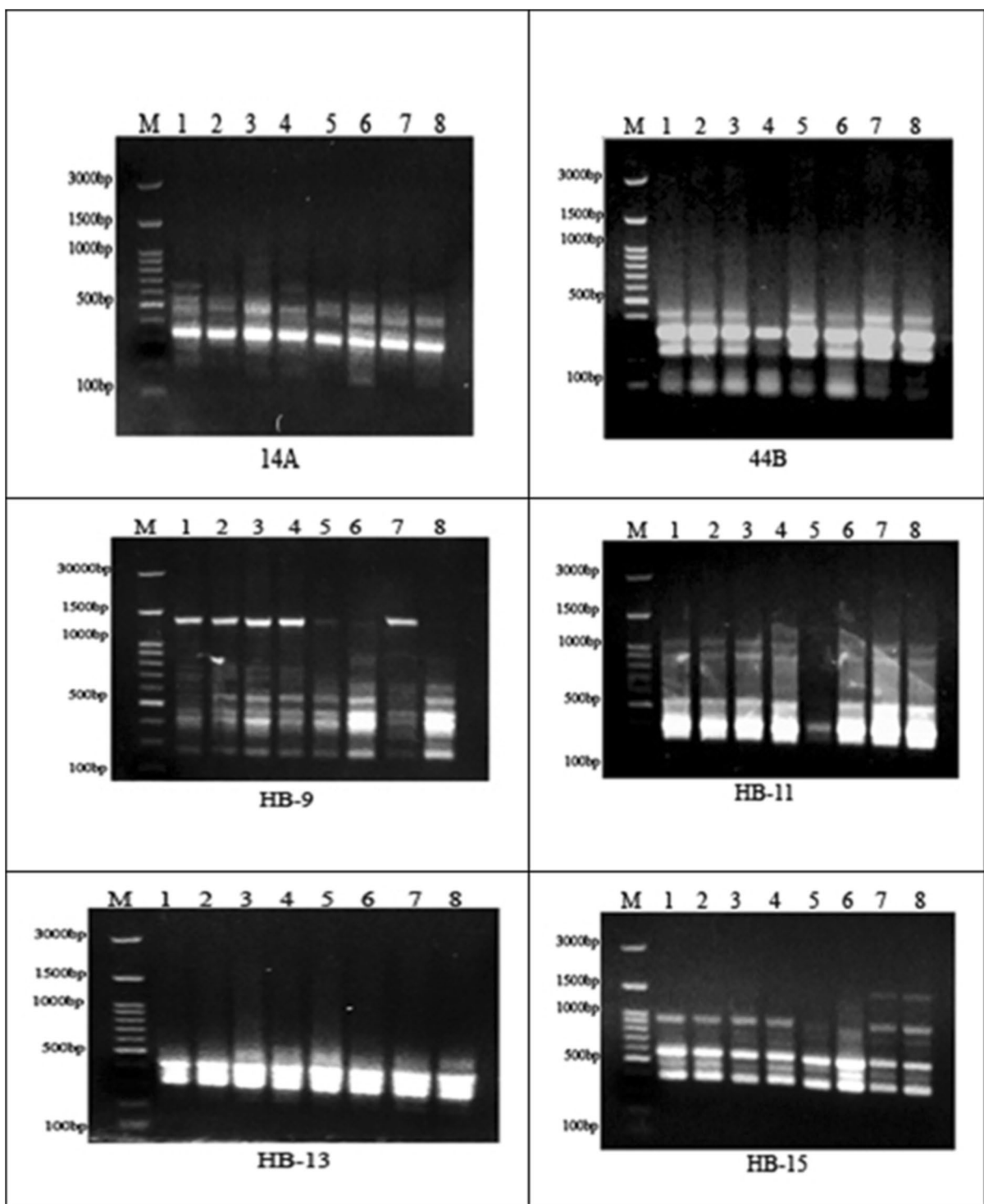


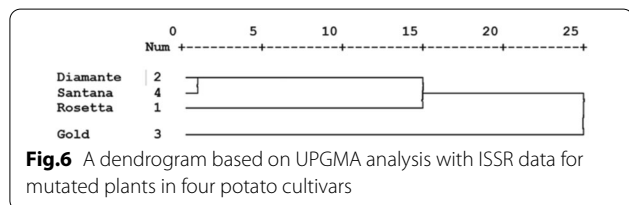
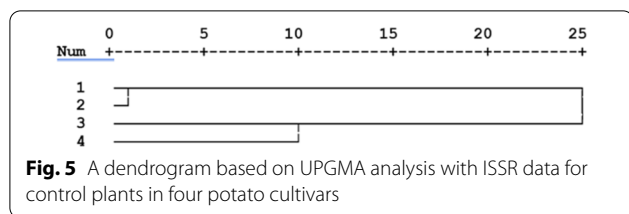
Fig. 4 ISSR amplification profile of potato mutants and four potato parent cultivars produced by 6 different primers as affected by gamma rays. M, marker, lanes 1, 3, 5 and 7 untreated Lady Rosetta, Diamante, Gold and Santana; lanes 2, 4, 6 and 8 gamma-treated ones

Table 6 Variation among treated and control plants of potato cultivars in the number of bands of six ISSR primers

Primer name	Band no.	MW bp	Cultivars							
			Lady Rosetta		Diamante		Gold		Santana	
			Con	Tre	Con	Tre	Con	Tre	Con	Tre
14A	1	680	1	0	0	1	0	1	0	1
	2	570	1	1	0	0	1	1	1	0
	3	490	1	1	1	1	1	1	1	1
	4	365	1	1	1	1	1	1	1	1
	5	230	1	1	1	1	0	1	0	1
	Total		5	4	3	4	3	5	3	4
44B	1	415	1	1	1	0	1	1	1	1
	2	340	1	1	1	1	1	1	1	1
	3	260	1	1	1	1	1	1	1	1
	4	175	1	1	1	1	1	1	1	1
	5	100	1	1	1	1	1	1	1	1
	Total		5	5	5	4	5	5	5	5
HB 9	1	1360	1	1	1	1	1	1	1	1
	2	800	1	1	1	1	0	0	0	0
	3	690	1	1	1	1	1	1	1	1
	4	570	1	1	1	1	1	1	0	1
	5	465	1	1	1	1	1	1	1	1
	6	415	1	1	1	1	1	1	1	1
	7	185	1	1	1	1	1	1	1	1
	Total		7	7	7	7	6	6	5	6
HB 11	1	1080	1	1	1	1	0	1	1	1
	2	910	1	1	1	1	1	1	1	1
	3	530	1	1	1	1	1	1	1	1
	4	410	1	1	1	1	1	1	1	1
	5	340	1	1	1	1	1	1	1	1
	Total		5	5	5	5	4	5	5	5
HB13	1	480	1	0	1	1	1	0	1	1
	2	425	1	1	1	1	1	1	1	1
	3	315	1	1	1	1	1	1	1	1
	4	265	1	1	1	1	1	1	1	1
	Total		4	3	4	4	4	3	4	4
HB 15	1	1430	0	0	0	0	0	0	1	1
	2	965	1	1	1	1	1	0	1	1
	3	820	0	0	0	0	0	1	0	0
	4	715	1	1	1	1	0	0	0	0
	5	630	1	1	1	1	1	1	1	1
	6	600	1	1	1	1	1	1	1	1
	7	490	1	1	1	1	1	1	1	1
	8	430	1	1	1	1	1	1	1	1
	Total		6	6	6	6	5	5	6	6
	Overall bands		32	30	30	30	27	29	28	30

context, salt-tolerant cultivars of various crops, such as rice showed high activity of POD, which quenched reactive oxygen species (ROS) and indirectly increased plant fresh weight [25]. Our results confirm the previous

findings of [33] who found that wild potato (*S. bulbocastanum*) had high activity of antioxidant enzymes (POD, SOD and CAT) under saline conditions in vitro. However, the reduction of salt tolerance capacity was correlated



with the absence of some isoenzymes like PODs and PPOs in salt-sensitive Gold potato after gamma rays application, as shown in Figs. 2 and 3 and Tables 3 and 4. Furthermore, POD and PPO isoenzymes bands were denser (high activity indicator) in salt-tolerant Santana potato than other cultivars. As known, isoperoxidase catalyzes the breakdown of H_2O_2 to H_2O and O_2 in the presence of ascorbic acid in taro (*Colocasia esculenta*) under salinity conditions [34]. In addition, PODs are ubiquitous enzymes in the plant, and often occur as multiple isoforms. For instance, they are encoded with 73 different genes in *Arabidopsis thaliana* [35]. Such abundance of isoforms is consistent with diverse physiological functions in peroxidases family [36]. Results of the current study were in consistency with previous findings that confirm the role of gamma rays improving salt-stressed and non-stressed potato growth [37, 38].

The presence or absence of isoenzymes is mainly related to changes in potato genetic structure. Therefore, genetic marker analysis using ISSR with six different primers was used. According to primer sequence and potato genotype, obvious unique negative and positive bands with different base pairs in mutant plantlets could easily be detected in comparison to the control (Fig. 4 and Table 6). Mutation can induce changes in genome sequence, which affect primer-annealing sites, reduce the number of binding sites for Taq polymerase, and change DNA bands in ISSR profile. One-base difference in genome sequence may hinder the annealing of the primers [39].

The range of amplified sizes by each primer across different mutated and control plants was noteworthy (100 and 1430 bp using 44B and HB15 primers, respectively). The 14 A primer was an efficient genetic marker between mutated and unmutated potato genotypes (Table 5), because it has more CTC codon sequence (Table 1),

which might be an indicator of DNA of potato genotypes that had more GAG codon sequence. In the same context, HB11 primer that had more TGT nucleotides repeats was the lowest effective primer, which means that the genome of all four potato cultivars had low ACA repeats. This difference was detected by loci test. In case of the wide divergent sizes, the actual number of nucleotides in the allele would need to be proven by sequencing. However, the allele size is not only dependent on the number of nucleotides, but also there are several factors affecting the allele size including the mobility of the fragment by electrophoresis, the distance of the allele from the standard used, the type of fluorescent label used, and the use of different instruments with different softwares [40]. Moreover, Table 5 reveals that out of 34 bands scored, there were 22 monomorphic-identical bands for all mutated and control plants. This may indicate that the primer has annealing sites at the same position of all genotypes' genomes and/or may be attributed to the amplification of highly conserved regions in the genome that make amplification bands similar to each other in molecular weight (monomorphic) [41].

The increment of genetic distance between the most salt-sensitive Gold cv. and other genotypes has proven that mutation took place because of gamma rays (Fig. 6). Also, the presence or absence of bands was clearer in Gold cv. than other cultivars. Therefore, bands with 1080 and 820 bp were, respectively, existed in gamma-treated Gold cv. using HB11 and HB15, but bands with 480 and 965 bp were not detected using HB13 and HB15, respectively. On the other hand, salt-tolerant Santana cv. showed the presence of both genetic bands with 230 and 570 bp using 14A and HB9 primers, respectively. Gaining bands may be due to the insertion and loss of bands related to nucleotides deletion. These results may confirm that induced mutagenesis using gamma rays could be conducted in wide aspect, but individual plant reacted different to the induced mutant [42]. In addition, new bands found in the mutated plants of four potato cultivars could be referred to ISSR primers, which have possible linkage changes in non-coding regions that led to specific changes in the genome after gamma-ray treatment [43].

Because the ISSR primers were amplified with numerous sequences, bands were extremely precise to classify the genotype. Therefore, Santana cv. had a unique fingerprint in the 1430-pb site, which can be a selected marker for this cultivar. The negative unique bands could be applied in further investigations, as practical markers to recognize tolerant and sensitive genotypes of the potato cultivars [44]. The high values of genetic similarity (93 and 87% for unmutated and muted plantlets, respectively) between Rosetta and Diamond potatoes (Fig. 5) could

be used as an indication that a large part of the genome of both cultivars is identical. This is primarily due to the lack of parental diversity, because both cultivars may share similar parents in the pedigree [13]. This explanation is might be due to the lack of ISSR primers selection, which makes it difficult to generalize them in control and mutated plants [16].

Conclusion

We conclude that mutation of potato plants induced by gamma radiation application induces salt resistance in the plant, especially in cv Santana. The applied radiations induced new genetic bands that can be expressed as new isoenzymes such as peroxidase or polyphenol oxidase, responsible for quenching reactive oxygen species under salt stress.

Acknowledgements

Not applicable.

Authors' contributions

The authors confirm that they have equal contribution. All authors read and confirm the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 3 April 2021 Accepted: 21 May 2021

Published online: 14 July 2021

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