Evaluation of some Physiological and Molecular Characters of Salt-Tolerant Potato Mutants Induced by Gamma Irradiation

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Abstract: Four Potato cultivars (Lady Rosetta, Diamante, Gold and Santana) were treated with 20 Gray of gamma rays then screened for salt tolerance by application of 2270 ppm of NaCl *in vitro*. Evaluation of tolerant mutants was done by biochemical and molecular analysis. Results showed that, Ch.a and b were increased in gamma treated-plantlets compared to control and the highest values of Ch.a was found in Lady Rosetta, Diamante and Santana cvs, while ch.b as well as Chl. a+b were highest in Diamante and Santana cvs. Phenolics and Carotenoids concentrations were also increased in plantlets as a result of gamma application especially in Santana and Lady Rosetta cvs., respectively. Free amino acids concentration had variable effect between gamma treated and non-treated plantlets, so it increased in Santana and Gold cvs., it decreased in Lady Rosetta and Diamante cvs. Superoxide dismutase and Catalase activity were also increased in all gamma-treated cultivars especially in Lady Rosetta, Diamante and Gold cvs. SDS-PAGE of protein revealed that, gamma-treated and untreated plantlets expressed about 14 to 17 protein bands. Gamma-treated plantlets of Lady Rosetta cv. missing two protein bands with 55 and 6 KDa compared to control, Gamma-treated plantlets of Gold cv. lost three protein bands with 23, 17 and 6 KDa compared to untreated ones while the band with 55 KDa was detected in gamma treated-plantlets. We can conclude that, using of 20-gray gamma rays' dose was more effective tool for induction salt-tolerant mutant plantlets of potato cultivars especially with Santana cv. and such mutants must be re-evaluated on the field experiments.

Keywords: *Solanum tuberosum* L., photosynthetic pigments, phenolics, free amino acids, antioxidant enzymes, protein electrophoresis

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important solanaceous vegetable crops in Egypt for both local consumption and exportation. The total area devoted for production in the year 2017 in Egypt was 163939 ha, with total production of about 4 325478 tons (average 26.4 ton/ha). Worldwide, potato is the fourth most important crop, with an annual production of about 388190674 tons, produced from about 19302642 ha (average 20.1 ton/ha) (FAO state, 2017).

Salinity is a serious problem for commercial agriculture worldwide where about one billion ha are affected by salinity (Christiansen, 1982). Potato is classified as moderately salt sensitive crop, whereas its ECs (Electrical conductivity of salts) threshold is about 1.7dS/m (= 1088ppm) (Katerji *et al.,* 2002). However, more information is needed regarding the tolerance or sensitive genotypes to salt stress, due to the significant variation in salt tolerance among potato genotypes (Khrais *et al.,* 1998). Abiotic stresses, such as high salinity often result in significant losses to the yields of economically important crops such as potato (Ahmed and Rashid, 1990).

Plant cells had two categories of antioxidants, non-enzymatic antioxidants such as phenolics, carotenoids, amino acids,…etc. and enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), polyphenol oxidase (PPO) and catalase (CAT) which quench cells from reactive oxygen species (ROS) as superoxide (O_2^-), hydrogen peroxide (H_2O_2), …etc. during stress. Potluri and Prasad (1993) reported that the proline accumulation under salinity stress was cultivar-dependent. Plants constantly exposed to capricious conditions have adapted at the molecular, cellular, physiological and biochemical levels, enabling them to survive and cope with adverse environmental stresses. However, in another study, the activity of SOD decreased with salinity stress (Zhang *et al.,* 2007). In most cases, antioxidant enzymes activity such as SOD and CAT increased in salt tolerant potato genotypes (Sajid and Faheem, 2014). Also, Asensi-Fabado *et al.* (2014) found increase in total free amino acids with salinity stress. El-Magawry *et al.* (2015) reported that the interaction between potato genotypes and salinity grown under *in vitro* conditions was significant in some biochemicals such as proline and free amino acids as well as the activity of CAT and SOD. In some cases, their results indicated that the tolerant genotypes had less proline content and low activities of SOD and CAT, however, the sensitive genotypes had high proline, free amino acids and the activities of SOD and CAT. In potato, a decreased relative abundance of some proteins involved in protein and amino acid biosynthesis such as an mRNA binding protein and glutamine synthetase (Aghaei *et al.,* 2008). Increase in osmotin and osmotin-like proteins (stress-protective proteins which associated with plant adjustment to an enhanced osmotic stress) has been found in various salt-treated plants ranging from a salt-tolerant cultivar of potato (Aghaei *et al.,* 2008). Plants usually induce biosynthesis of several low-molecular, highly hydrophilic organic compounds as well as high-molecular hydrophilic proteins such as late embryogenesis abundant proteins which do not only decrease intracellular osmotic potential, but also enhance protective properties on other cellular compounds adversely affected by dehydration (Kosová *et al.,* 2010).

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Today, mutagenic radiation applications are used to develop resistant plants to abiotic stresses such as, salinity, drought and diseases. By irradiating the plant explants, some radicals and biochemical compounds can be formed in cells as a result of genetic modifications. If radiation is used at the appropriate dose and time, positive changes in yield, tolerance, quality, earliness and adaptability of plants can be achieved (Hassan, 2011). Gamma ray, which is a physical mutagen, is a widely used method of having diversity on many plant species and creating new variations. In the compilation, different gamma ray practices on multifarious plant species were studied (Ulukapi and Nasircilar, 2015).

Rasheed *et al.* (2001) revealed that gamma rays' doses higher than 20 Gray (Gy) were lethal to micropropagated plants of potato. Also, irradiation of the explants generated mutants with desirable properties, phenolic compound, phytoalexins, pathogenesis related enzymes such as calatalase (CAT), glucanase, peroxidase (POD) or free radicals can generate in cells. Ahmed (2004) found that 20 Gy of gamma dose gave better potato cv. Alpha characteristics such as longer stem length, root length and higher yield of tubers compared to other irradiated and non-irradiated treatments. AI-Safadi and Arabi (2007) found that, salt tolerance was differed according to potato genotypes after expose *in vitro* cultured explants of three potato cvs. Draga, Diamante and Spunta with gamma ray doses of 25, 30, and 35 Gy then screened to salt tolerance using 50 to 200 mM of NaCl. After that surviving plantlets were propagated and re-cultured then acclimatized under greenhouse and irrigated with saline water containing NaCI (50 to 250 mM). Evaluation of mini-tubers weight revealed that Spunta cv. was more tolerant to salt stress followed by Diamante but Draga was more susceptible.

The main purpose of research is to determine the effect of gamma rays (20 Gray) on four potato cultivars namely; Lady Rosetta, Diamante, Gold and Santana to obtain new potato genotypes with enhanced tolerance to salinity *in vitro*. Evaluating the physiological and molecular characters of survival explant of both gamma-irradiated and non-irradiated plantlets under saline conditions.

MATERIALS AND METHODS

Potato cultivars, *in vitro* propagation and gamma ray's irradiation:

Four potato cultivars (Lady Rosetta, Diamante, Gold and Santana) were obtained from the El-Dakahlia Agricultural Development Co., wadi El-Molak, Ismailia, Egypt. The tubers stored at 4°C, then incubated in the dark at room temperature for 2 weeks until 5-6 cm-long shoots appeared (Sharabash, 2001). Bud sprouts (l-2 mm long) were sterilized either with 1% of sodium hypochlorite for 3 minutes, then dipped in absolute ethanol, washed in sterile water for 5 minutes and cultured on half-strength of Murashige and skoog's medium (MS) (1962). About 10 mm segments of shoot tips from *in vitro* grown plants on MS medium were transferred on callus production medium (containing 1mg/l of NAA (naphthalene acetic acid) and 0.5 mg/l of BAP (benzyl amino purine). All cultures were kept in a culture room at a 16/8 light/ dark photoperiod and 25 °C. After two weeks calli were initiated and then 4 segments of calli of approximately 1 cm^2 in each container were sub-cultured on the same medium at an interval of 2 weeks. Twenty containers having 4 segments of callus were then exposed to 20 Gray (Gy) of gamma rays with approximately 0.653 rad/Sec, for 30 min (137Cs-Gamma Cell-40, Canadian). The radiation treatment was repeated twice at an interval of 1 week. All experiments were carried out in 4 replications under a completely randomized design. Differentiated calli on half-MS medium without growth hormones produced complete plantlets (Ahloowalia, 1982). Uniform plantlets (3cm length) were screened to salt tolerance by exposure to 4dS/m of NaCl (2720 ppm). After 30 days potato plantlets were prepared to estimate the following biochemical and molecular analysis:

Determination of photosynthetic pigments:

According to Nagata and Yamashta (1992), chlorophyll a, b and carotenoids (β-Carotene) concentrations were determined. 200 mg of shoots was ground in 10 mL acetone (80%) and centrifuged at 1500 rpm for 5 minutes. The supernatant was transferred to cuvette and absorbance was recorded at 663, 645, 505 and 453 nm by using UV-spectrophotometer (model MA9523-SPEKOL 211, ISKRA, Horjul, Slovenia). Chlorophyll a, b and β-Carotene concentrations were used calculated as:

Chlorophyll a = 0.999 A663 – 0.0989 A645

Chlorophyll b = 0.328 A663 + 1.77 A645

β- Carotene = (0.216 A663 – 1.22 A645) – (0.304 A505 + 0.452 A453).

Determination of free phenolics: -

Fresh potato plantlets (1g) were macerated and then extracted by simple diffusion for 72 hours at 5^{o}C by 70 % Ethanol (ETOH) as described by Abdel-Rahman *et al.* (1975). Samples were re-extracted with 96% ETOH using Bailly-Wolker extractor for 6 hours for complete extraction of native chemical compounds. Ethanolic fractions were filtered through Whatman No. 1 filter paper. A modified Folin-Ciocalteu method (Singleton *et al.,* 1999) was used to determine the free phenolics at 650nm. Concentration of free phenolics was calculated as mg 100g-1 FW of the extracted tissue using a correction factor 0.0042 from catechol standard curve.

Determination of free amino acids:

Total free amino acids were estimated using the method of Rosen (1957) with ninhydrin reagent (4 g ninhydrine +300 ml acetone). For determination, 0.1 ml of ethanolic extract added to 5 ml methanol +1 ml ninhydrin reagent and heated in water bath at 60^{o}C for 20 min. The blue colored samples were measured against blank sample at 570 nm. Concentrations of free amino acids were calculated in different samples as mg 100 g^{-1} FW of the extracted plantlets using correction factor 0.0042 from glycine standard curve.

Activity of superoxide dismutase (SOD) and catalase (CAT):

Enzyme extraction:

200 mg of the fresh plantlets were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 20°C for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant was taken for enzymes activity determination (Mukherjee and Choudhuri, 1983).

Activity of SOD:

SOD activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by Marklund and Marklund (1974). The solution (10 ml) consisted of 3.6 ml of distilled water, 0.1 ml of enzyme, 5.5 ml of 50 mM phosphate buffer (PH 7.8) and 0.8 ml of 3 mM pyrogallol (dissolved in 10 mM HCl). The rate of pyrogallol reduction was measured at 325 nm with UV-spectrophotometer (Jenway). One unit of enzyme activity was defined as amount of the enzyme that resulted in 50% inhibition of the auto-oxidation rate of pyrogallol at 25°C (Kong *et al.,* 1999).

Activity of CAT:

CAT activity was assayed according to the method of Chen *et al.* (2000). The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H2O2 phosphate buffer pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer). Catalase activity was determined by measuring the rate change of H_2O_2 absorbance in 60 second with a UV-Spectrophotometer (Jenway) at 250 nm. The blank sample was made by using buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50% of the H_2O_2 in 60 second at 250C (Kong *et al.,* 1999).

SDS-PAGE of protein:

One dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970) was used to fractionate the soluble proteins of potato plantlets. Twenty milligrams of shoots were dispersed in 1 ml SDS 10% with 100 µl β-mercaptoethanol for 15 min, then centrifuged at 11000 rpm for 10 min. Twenty µl of extraction were mixed with 20 µl of SDS- loading sample buffer (SDS 4%, β-mercaptoethanol 3%, glycerol 20%, Tris HCl 50 mM pH 6.8 and bromophenol blue traces), heated at 96 °C for 3 min and 10 µl aliquot was electrophoresed (10 µl of protein/ lane). The resolving and stacking gels were prepared as in Table (1) according to the standard procedure of Davis (1964). The electrode buffer contained 50 mM TRIS, glycine 0.384 M and SDS 0.1%. The protein bands were developed with Commassie Brilliant Blue R-250 dye (0.2% solution, freshly prepared in 45% methanol, 10% glacial acetic acid and 45% distilled water) at room temperature overnight. The gel was photographed and analysis using standard protein marker Medium range from 5 KDa to 180 KDa (Fermentas.Com).

Table (1): Resolving and stacking gel solutions for SDS- polyacrylamide gel electrophoresis.

Stock solution	Resolving gel (15%)	Stacking gel (4%)
Acrylamide mixture 40%	3.75 ml	0.5 ml
1.5M Tris-buffer (pH 8.8)	2.5 ml	-
0.5M Tris-buffer (pH 6.8)	-	1.25 ml
Distilled water	3.5 ml	3.15 ml
SDS 10%	100 µl	50 µl
APS 10%	100 µl	50 µl
TEMED	10 µl	5 µl
Total volume	10 ml	5ml

Statistical analyses:

All data were statistically analyzed as randomized complete blocks design (Steel *et al.,* 1997); using the MSTAT-C statistical package (M-STAT, 1990) and means were separated by LSD test, $P \leq 0.05$.

RESULTS AND DISCUSSION

In potatoes, which are difficult to breed by conventional methods, such mutant production is an indispensable part of gene discovery for molecular breeding as well as breeding of new cultivars (Fischer *et al.,* 2008). Also, gamma radiation is widely used to induce mutations in breeding researches than chemical mutagens (Pathirana, 2011). Therefore, four Potato cultivars named Lady Rosetta, Diamante, Gold and Santana were irradiated with 20 Gray of gamma rays then screened for salt tolerance by application of 4 dS/m of NaCl *in vitro*. Estimation of tolerant mutant was done by biochemical and molecular analysis as follow:

Effect of gamma rays on photosynthetic pigments:

Fig. (1), revealed that gamma irradiation treatment was increased the concentration of both Chl. a and b (mg/100g FW) in all potato's cultivars under study than control. In this respect, the highest significant concentration of Chl.a was recorded in Lady Rosetta, Diamante and Santana cvs. but Chl.b concentration was high accumulated in Diamante and Santana cvs. more than other cultivars.

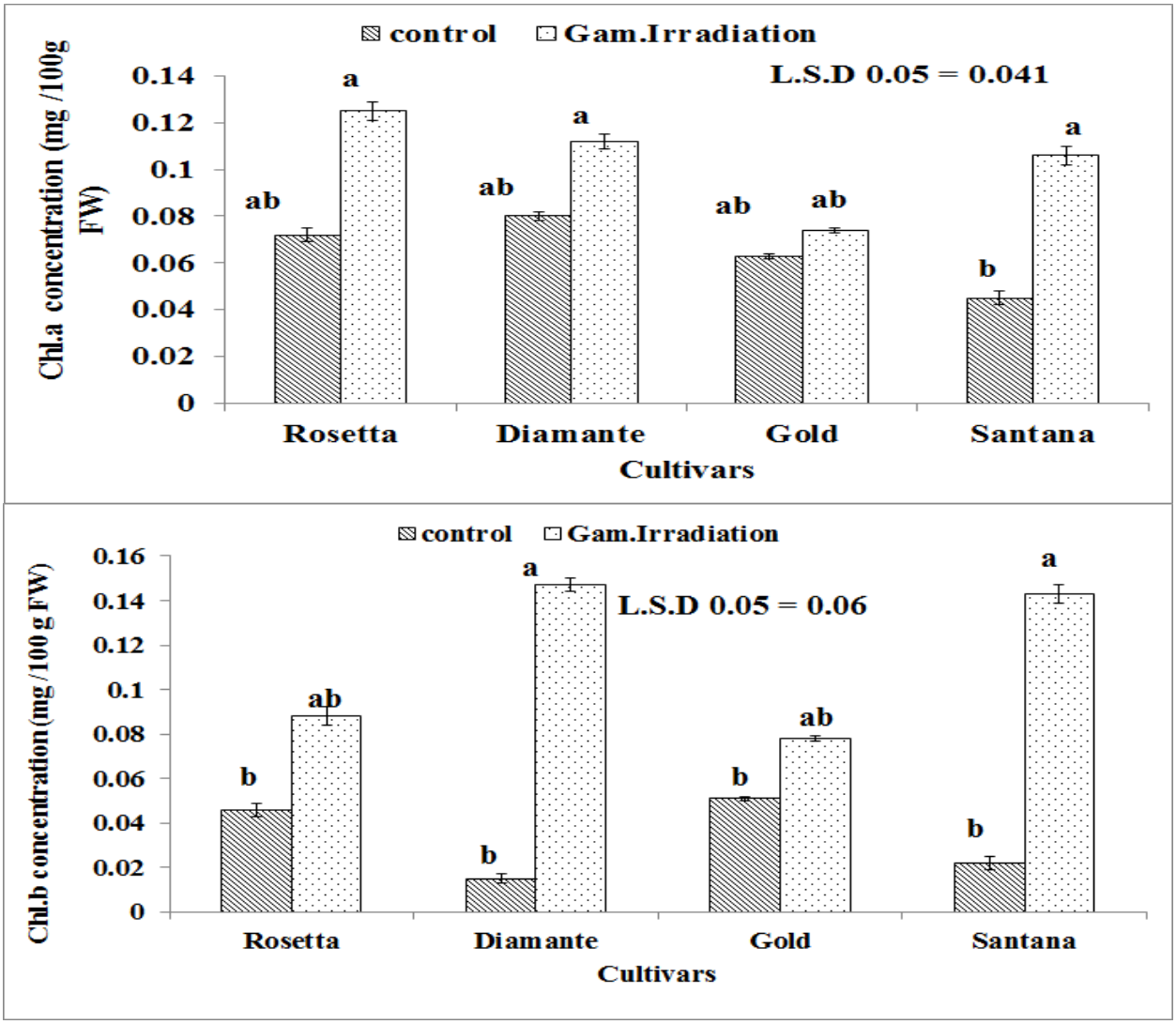

Fig. (1): Effect of gamma irradiation on Chl.a and Chl.b concentration in four potato cultivars after 30 d of NaCl (4 dS/m) exposure. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test

Furthermore, gamma ray's application was enhanced the Chl. a + b in plantlets of all four potato cultivars especially Diamante and Santana cvs as shown in Fig. (2). The reduction of Chl. a/b was done in all gamma treated-plantlets compared to control and the highest reduction was observed in Lady Rosetta and Diamante cvs.

Effect of gamma irradiation on non-enzymatic antioxidants:

As shown in Fig. (3), gamma rays' application was enhanced the concentration of most estimated non-enzymatic antioxidants such as Carotenoids, phenolics and free amino acids in all potato's cultivars under study. The highest significant concentration of Carotenoids was detected in Lady Rosetta cv. plantlets. Santana cv. plantlets showed the highest concentration of phenolics. Plantlets of both Gold and Santana cvs. gave the highest concentration of amino acids. Results reported herein were agreed with Elwan *et al.* (2018) who reported that high content of reducing sugars, phenolics, proline, amino acids, proteins as well as high activity of POD could use as selectable markers for *in vitro* potato tolerance to salinity. In addition, phenolics were protective agents as well as it had strong antioxidant properties that prevent cellular damage from oxidative stress generated by ROSs during salt stress. Furthermore, amino acids were a precursor of different growth regulator compounds in plants (Taiz and Zeiger, 2006). These results were also agreed with Aloni and Rosenshtein (1984) who reported that proline as an important amino acid plays an important role as osmoregulator under salinity and drought conditions, proteins stabilizer, prevention of denaturation of enzymes and conservation of nitrogen and energy for a post-stress period.

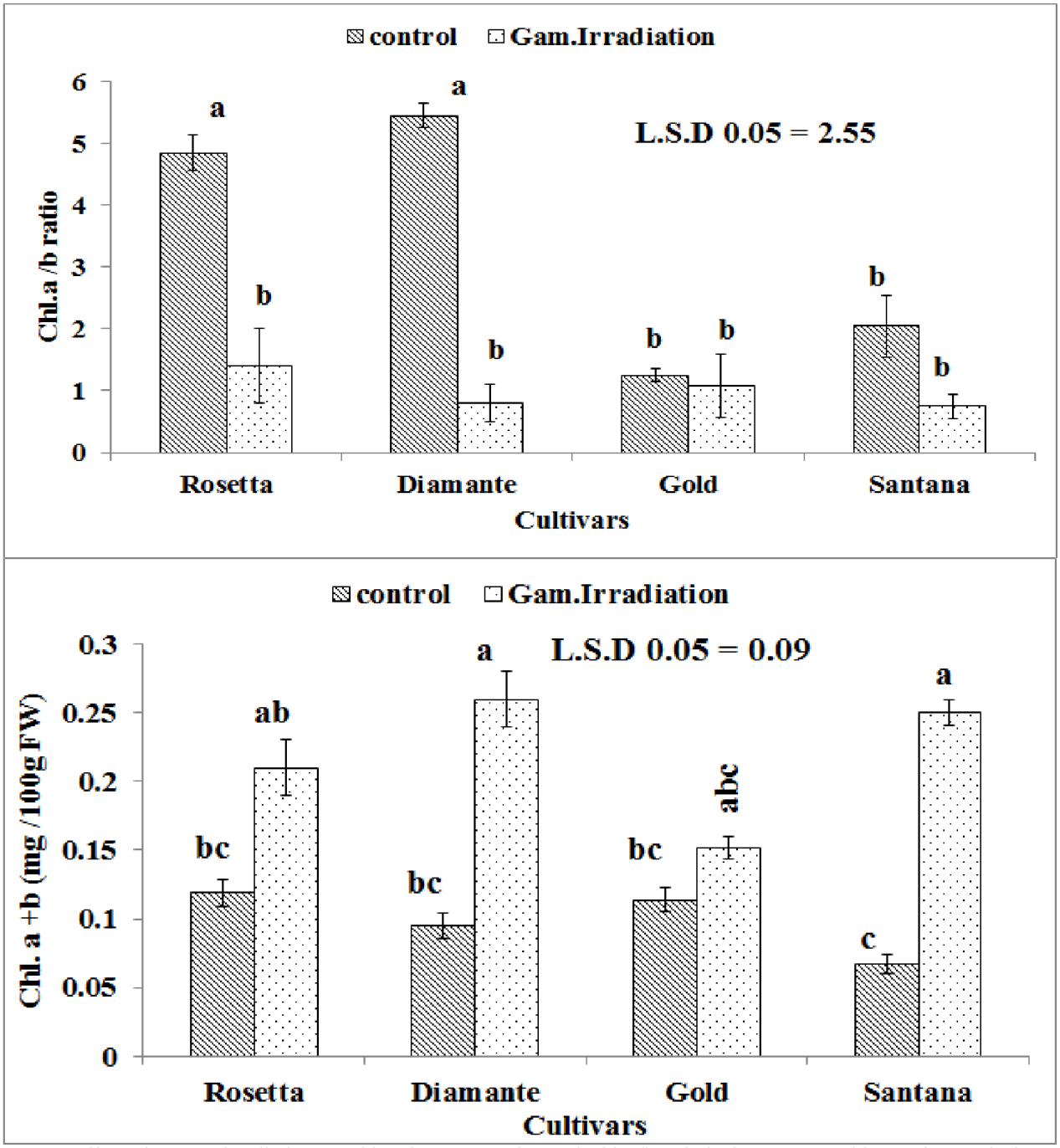

Fig. (2): Effect of gamma irradiation on Chl. a+b concentration and Chl. a/b ratio in four potato cultivars after 30 d of NaCl (4 dS/m) exposure. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test.

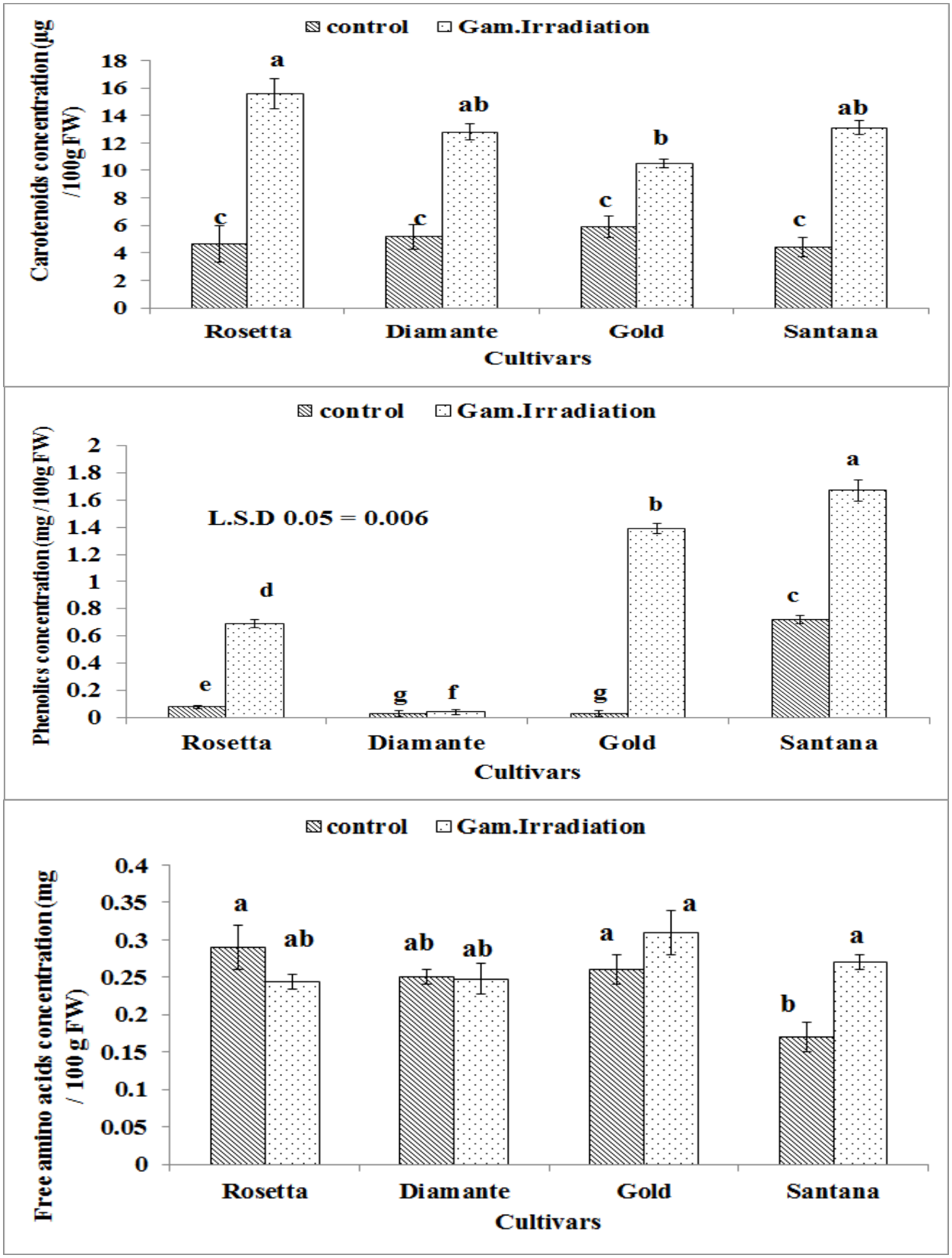

Fig. (3): Effect of gamma irradiation on Carotenoids (a), free phenolics (b) and free amino acids (c) concentration in four potato cultivars after 30 d of NaCl (4 dS/m) exposure. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test

Effect of gamma ray's treatment on enzymatic antioxidants:

Fig. (4) showed that, both enzymatic antioxidants superoxide dismutase (SOD) and Catalase (CAT) were increased in gamma treated-plantlets than untreated ones of four cultivars of potato. The highest increment of SOD and CAT activity was observed in gamma treated plantlets of Lady Rosetta, Diamante and Gold cvs. In salt tolerant cultivars, high activity of SOD led to transform superoxide free radicals (O_2^-) to hydrogen peroxide (H_2O_2) which directly converted to water and molecular oxygen (O_2) led to higher accumulation of photosynthetic pigments such as Ch. a and b as shown previously in Figs. (1 and 2) in plantlets under saline conditions. Our results were coordinated with Daneshmand *et al.* (2009) who obvious that the wild potato (*S. bulbocastanum*) containing high photosynthetic pigments *in Vitro* and antioxidant enzymes activities (SOD and CAT) under salinity stress conditions.

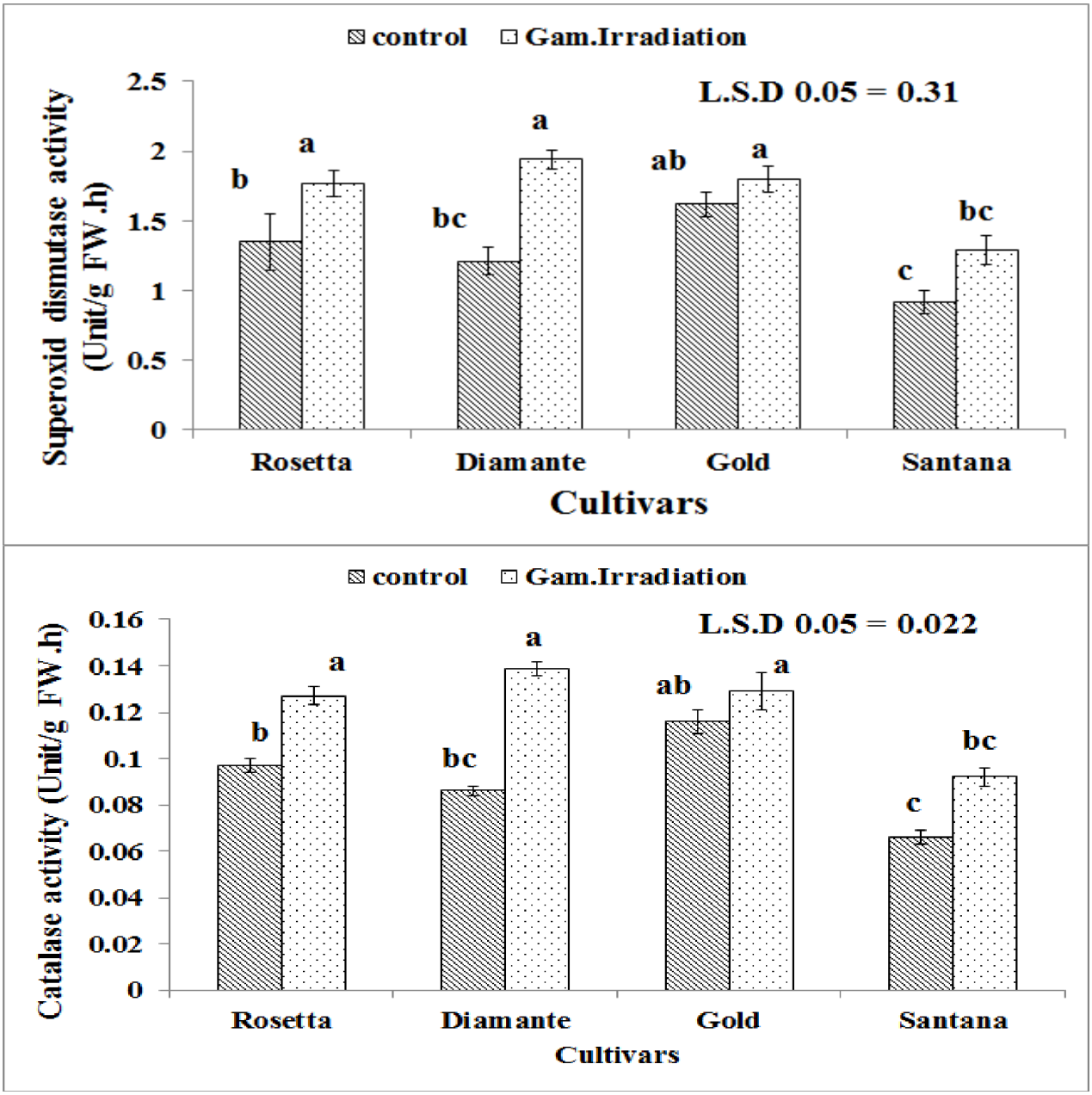

Fig. (4): Effect of gamma irradiation on Superoxide dismutase (SOD) (c) and Catalase activity (d) in four potato cultivars after 30 d of NaCl (4 dS/m) exposure. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test

Effect of gamma irradiation on Protein banding Pattern:

Data in Fig. (5) and Table (2) revealed that plantlets of gamma rays-treated and untreated potatoes expressed 14 to 17 protein bands. Gamma rays–treated plantlets or untreated ones of Diamante or Santana cvs had the same numbers of protein bands (17 and 15, respectively). Plantlets treated with gamma rays of Lady Rosetta cv. lost two protein bands with 55 and 6 KDa compared to control. Also, plantlets treated with gamma rays of Gold cv. lacked three protein bands with 23, 17 and 6 KDa compared to untreated ones. Results were coordinated with Gong *et al.* (2005), who reported that, electrophoretic protein bands and isoenzymes polymorphism used for identification and quantification of the correlation between altered expression of specific genes and changes in the environment. These changes in expression of genes would be involved in adaptation and could be used as molecular markers for salt stress. One-dimensional polyacrylamide gel electrophoresis of proteins has been used extensively for identification and classification at the strain and species level. Also, isoenzymes have been widely used to screen the variability present among population and to select the desirable genotypes.

CONCLSION

Mutation in potato using gamma radiations for salt tolerance is more suitable especially with Santana cv. Mutation with gamma rays appeared new genetic bands which expressed as new protein bands or isoenzymes such as peroxidase or polyphenol oxidase which responsible for quenching reactive oxygen species during salt stress. Gamma ray's application increased the activity of antioxidant enzymes and the concentration of non-enzymatic compounds.

Saving favorable physiological environment in salt-susceptible plantlets led to preserve the macromolecules from oxidation such as photosynthetic pigments and plantlets grow well.

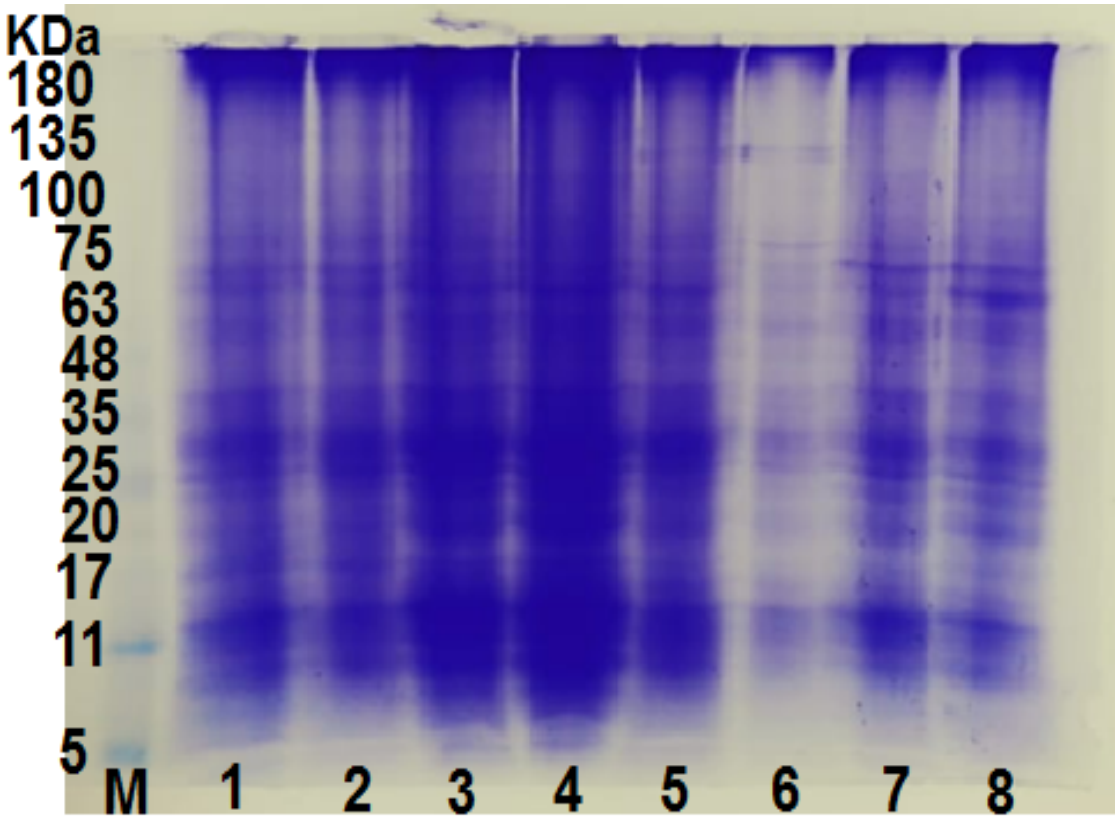

Fig. (5): Effect of gamma irradiation on protein profile in four potato cultivars after 30 d of NaCl (4 dS/m) exposure. M, marker, lanes 1, 3, 5 and 7 untreated Lady Rosetta, Diamante, Gold and Santana; lanes 2, 4, 6 and 8 gamma treated ones.

Table (2): SDS- PAGE of protein bands as affected by gamma ray's application and NaCl (4dS/m)

Band No.	M.W KDa	Cultivars							
		Lady Rosetta		Diamante		Gold		Santana	
		Con.	Tre.	Con.	Tre.	Con.	Tre.	Con.	Tre.
1	**73**	1	1	1	1	1	1	1	1
2	**68**	1	1	1	1	1	1	1	1
3	**66**	1	1	1	1	1	1	1	1
4	**58**	1	1	1	1	1	1	1	1
5	**55**	1	0	1	1	0	1	1	1
6	**38**	1	1	1	1	1	1	1	1
7	**28**	1	1	1	1	1	1	1	1
8	**25**	1	1	1	1	1	1	1	1
9	**23**	1	1	1	1	1	0	1	1
10	**22**	1	1	1	1	1	1	1	1
11	**19**	1	1	1	1	1	1	1	1
12	**17**	1	1	1	1	1	0	0	0
13	**15**	0	0	1	1	1	1	1	1
14	**13**	1	1	1	1	1	1	1	1
15	**11**	1	1	1	1	1	1	1	1
16	**8**	1	1	1	1	1	1	1	1
17	**6**	1	0	1	1	1	0	0	0
Total		**16**	**14**	**17**	**17**	**16**	**14**	**15**	**15**

1, present Band; 0, Absent Band

REFERENCES

Abdel-Rahman, M., T. H. Thomas, G. J. Doss and L. Howell (1975). Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol. Plant., 34(1): 39-43.

Aghaei, K., A. A. Ehsanpour and S. Komatsu (2008). Proteome analysis of potato under salt stress. J. Proteome Res., 7: 4858–4868.

Ahloowalia, B. S. (1982). Plant regeneration from callus culture in potato. Euphytica, 31: 755-759.

Ahmed, K. U. and M. M. Rashid (1990). Eradication of potato virus × (pvx) by thermotherapy and meristem tip culture. Bangladesh J. Plant Pathol., 5: 65-69.

AI-Safadi, B. and M. Arabi (2007). In vitro induction, isolation, and selection of potato mutants tolerant to salinity. Adv. Hort. Sci., 21(3): 127-132.

Aloni, B. and G. Rosenshtein (1984). Proline accumulation: A parameter for evaluation of sensitivity of tomato varieties to drought stress. Physiol. Plant., 61: 231–235.

Asensi-Fabado, M. A., A. Ammon, U. Sonnewald, S. Munne-Bosch and L. M. Voll (2014). Tocopherol deficiency reduces sucrose export from salt stressed potato leaves independently of oxidative stress and symplastic obstruction by callose. J. Exp. Bot., 10(1): 1093.

Chen, Y., X. X. D. Cao, Y. Lu and X. R. Wang (2000). Effect of rare earth metal ions and their EDTA complex on antioxidant enzymes of fish liver Bull. Environ. Contam. Toxicol., 65: 357-365.

Christiansen, M. N. (1982). World environmental limitation to food and fiber culture. In: Breeding Plants for Less Favorable Environment. MN Christiansen and CF Lewis Edition, Wiley, New York. pp. 1-11.

Daneshmand, F., M. J. Arvin and K. M. Kalantari (2009). Effect of acetylsalicylic acid (Aspirin) on salt and osmotic stress tolerance in *Solanum bulbocastanum* in Vitro: enzymatic antioxidants. American-Eurasian J. Agric. & Environ. Sci., 6(1): 92-99.

Davis, B. J. (1964). Disc electrophoresis–II method and application to human serum proteins. Ann. N. Y. Acad. Sci., 121(2): 404-427.

El-Magawry, A. Nahlla, F. H. Mohamed, M. W. M. Elwan, K. E. Abdel-Hamid and M. M. Abdel-Salam (2015). In Vitro screening of different Potato genotypes for salinity tolerance. Hortscience J. Suez Canal University, 3: 35-46.

Elwan, M. W. M., M. A. M. Elhamahmy and F. H. Mohamed (2018). Physiological effect of Potato

genotypes and salicylic acid on plantlets growth and microtuber production under salt stress. Hortscience Journal of Suez Canal University, 7(2): 7-14.

FAO-STAT (2017). Agricultural Data. http:// faostat.fao.org/ (last accessed, 1 June 2013).

Fischer, L., H. Lipavska, J. F. Hausman and Z. Opatrny (2008). Morphological and molecular characterization of a spontaneously tuberizing potato mutant: an insight into the regulatory mechanisms of tuber induction. BMC Plant Biol., 8: 117.

Gong, Z., C. Dong, H. Lee, H. Zhu, J.L. Xiong, D. Gong, B. Stevenson and J.K. Zhu (2005). A DEAD BOX RNA helicase is essential for mRNA export and important for development and stress responses in Arabidopsis. Plant Cell., 17: 256–267.

Hassan, I. O. I. (2011). Biotechnological studies on the irradiated potato (*Solanum tuberosum*) with gamma rays. Master thesis. Department of Genetics Faculty of Agriculture, Cairo University, EGYPT, 2011.

Katerji, N., A. Hamdy, I. W. van Hoorn and M. Mastrorilli (2002). Mediterranean crop responses to water and soil salinity: eco-physiological and agronomic analyses. Bari: CIHEAM/IAMB Options Méditerranéennes: Serie B. Étudeset Recherches, 36: 231-249.

Khrais, T., Y. Leclerc and D. Donnelly (1998). Relative salinity tolerance of potato cultivars assessed by in vitro screening. American J. Potato Res., 75: 207-210.

Kong, F. X., W. Hu, S. Y. Chao, W. L. Sang and L. S. Wang (1999). Physiological responses of mexicana to oxidative stress of SO2. Environ. and Exp. Bot., 42: 201-209.

Kosová, K., P. Vítámvás and I. T. Prášil (2010). The role of dehydrins in plant stress response. In Handbook of Plant and Crop Stress, 3rd ed.; Pessarakli, M., Ed.; CRC Press, Taylor and Francis: Boca Raton, FL, USA, pp. 239–285.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Marklund, S. and G. Marklund (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

M-STAT (1990). A Microcomputer program for the design management and analysis of agronomic research experiments. Michigan State Univ.

Mukherjee, S. P. and M. A. Choudhuri (1983). Implication of water stress-induced changes in the level of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol. Plant., 58: 166-170.

Murashige, T. and T. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-479.

Nagata, M. and I. Yamashta (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Japanese Soc. Food Sci. Technol., 39: 925–928.

Pathirana, R. (2011). Plant mutation breeding in agriculture. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 6: 107–126.

Potluri, S. D. P. and P. V. D. Prasad (1993). Influence of salinity on axillary bud cultures of six lowland tropical varieties of potato (*Solanum tuberosum*). Plant Cell, Tiss. Org.Cult., 32: 185-191.

Rasheed, S. U., M. Asad and Y. Zafar (2001). Use of radiation and in vitro techniques for development of salt tolerant mutants in sugarcane and potato. IAEA Technical Document, 1227: 51-60.

Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys., 67(1): 10-15.

Sajid, Z. A. and A. Faheem (2014). Plant regeneration from in vitro-selected salt tolerant callus cultures of (*Solanum tuberosum* L.). Pak. J. Bot., 46(4): 1507-1514.

Sharabash, M. T. (2001). 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.

Singleton, V. L., R. Orthofer and R. M. Lamuela-Raventos (1999). Analysis of total phenols and other oxidation substances and antioxidants by means of Folin Ciocalteu reagent. Methods in Enzymology, 299: 152-178.

Steel, R. G. D., J. H. Torrie and D. A. Dickey (1997). Principles and Procedures of Statistics: a biometrical approach., 3rd ed. (McGraw-Hill: New York).

Taiz, L. and E. Zeiger (2006). Plant Physiology. Benjamin-Cummings Pub Co, Reading Massachusetts.

Ulukapi, K. and A. G. Nasircilar (2015). Developments of gamma ray application on mutation breeding studies in recent years. International Conference on Advances in Agricultural, Biological & Environmental Sciences. July 22-23, London (UK).

Zhang, R., G. Shang, M. Meng, F. Men, H. Li and J. Guo (2007). Effects of NaCl stress on antioxidant defense system of potato. Chinese Potato J., 21: 11-14.

تقييم بعض الخواص الفسيولوجية والجزيئية لطفرات البطاطس المتحملة للملوحة والمستحدثة عن طريق أشعة جاما

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تم معاملة ٤ أصناف من البطاطس هي روزيتا – دايمونت – جولد- سانتانا بـ ٢٠ جراى من أشعة جاما ثم قيمت من حيث تحملها للملوحة بنموها على بيئة مورشيج وسكوج تحتوى ٢٢٧٠ جزء في المليون من كلوريد الصوديوم. تم التقييم من الناحية الفسيولوجية والجزيئية. أوضحت النتائج زيادة تركيز كلوروفيل أ و ب في النباتات المعاملة بالإشعاع مقارنة بالكنترول وكانت أعلى قيم لكلوروفيل أ في الأصناف روزتا ودايمونت وسانتانا بينما كان أعلى تركيز لكلوروفيل ب ومجموع أ+ب في الأصناف دايمونت وسانتانا. زادت أيضا تركيز الفينولات والكاروتينيدات كمضادات أكسدة غير إنزيمية نتيجة المعاملة بالإشعاع خاصة في الأصناف سانتانا وروزتا. تباين تركيز الأحماض الامينية بين النباتات المعاملة بالإشعاع والغير معاملة حيث زادت في الأصناف سانتانا وجولد بينما انخفضت في روزتا ودايمونت. زاد نشاط كلا من إنزيم السوبراوكسيد ديسميوتز والكتاليز نتيجة المعاملة بالإشعاع خاصة في الأصناف روزتا ودايمونت وجولد. اظهر التفريد الكهربي للبروتين وجود من ١٤ إلى ١٧ حزمة بروتينية. كذلك اختفاء الحزمتين ذات الوزن الجزيئى ٥٥ و ٦ كليودالتون مقارنة بالكنترول في الصنف روزتا. اختفاء ٣ حزم بروتينية ٢٣ و ١٧ و ٦ كيلو دالتون في الصنف جولد وفى نفس الوقت ظهور الحزمة ٥٥ كيلو دالتون نتيجة المعاملة بالإشعاع. يمكن استنتاج أن المعاملة باستخدام ٢٠ جراى من أشعة جاما وسيلة فعالة لإنتاج طفرات متحملة للملوحة في البطاطس خاصة مع الصنف سانتانا مع وجوب إعادة تقييم هذه الطفرات على مستوى الحقل.