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Physiological and Biochemical Effects of γ-Irradiation on Cowpea Plants (*Vigna sinensis*) under Salt Stress

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Abstract

Soil salinity is one of the most severe factors limiting growth and physiological response in cowpea plants. In this study, the possible role of γ-irradiation in alleviating soil salinity stress during plant growth was investigated. Increasing salinity in the soil (25, 50, 100 and 200 mM NaCl) decreased plant growth, photosynthetic pigments content, total carbohydrate content and mineral uptake compared to control, while increased total phenol content, proline, total free amino acids and lipid peroxidation. Seed irradiation with gamma rays significantly increased plant growth, photosynthetic pigments, total carbohydrate, total phenol, proline, total free amino acids and the contents of N, P, K^+, Ca^{+2} and Mg^{+2} compared to non irradiated ones under salinity. On the other hand, irradiation with gamma rays decreased lipid peroxidation, Na^+ and Cl^- contents which may contribute in part to activate processes involved in the alleviation of the harmful effect of salt at all concentrations used (25, 50 and 100 mM) except at the high concentration (200 mM). Electrophoretic studies of α-esterase, β-esterase, polyphenol oxidase, peroxidase and acid phosphatase isozymes showed wide variations in their intensities among all treatments.

Keywords: cowpea, gamma rays, isozyme, lipid peroxidation, salt stress

Introduction

Cowpea is one of the most important food legume crops in the semi-arid tropics and contains high level of protein. Cowpea is a multipurpose crop and is grown as a grain legume mainly for dry beans and green pods and also as forage, green manure and cover crop.

Salinity is one of the most important abiotic stress factors limiting plant growth and productivity (Khan and Panda, 2008; Mohamed and Gomaa, 2012). Salinity is the main environmental factor accountable for decreasing crop productivity in many geographic areas mainly in arid and semi-arid regions (Greenway and Munns, 1980). Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (25 mM annual rainfall) and irrigation with saline water (El-Hendawy *et al*., 2004).

There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002).

Also, salt stress and it osmotic effects have been shown to enhance the production of reactive oxygen species (ROS) in a variety of cells resulting oxidative stress (El-Beltagi *et al.,* 2008; Mittler, 2002). ROS are the byproducts of many degenerative reactions in crop plants, which will affect the regular metabolism by damaging the cellular components (Foyer and Noctor, 2002). Extensive study on oxidative stress has demonstrated that exposure of plants to adverse environmental conditions induces the overproduction of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), H_2O_2 and hydroxyl radical ($HO^{\cdot}$) in plant cells (Wise and Naylor, 1987). In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage (Afify *et al*., 2011; El-Beltagi, 2011; El-Beltagi *et al*., 2011a, Kobeasy *et al*., 2011; Mohamed, 2011; Mohamed *et al*., 2009; O'Kane *et al*., 1996;), particularly when plants are exposed to stress conditions such as organisms, roasting, nematode infection, micro-organisms, lead toxicity, cadmium stress, chilling stress and Fe deficiency.

Lipid peroxidation induced by free radicals, is also important in membrane deterioration (Khan and Panda, 2008). The level of lipid peroxidation, measured as malondialdehyde (MDA) content, has been considered as an indicator of salt induced oxidation in cell membranes and a tool for determining salt tolerance in plants (Hernandez and Almansa, 2002). Lipid peroxidation rate was increased with increase of salt stress especially in sensitive cultivars (Arora *et al*., 2008).

Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic ionic stress caused by high salinity. These mechanisms include osmotic adjustment by accumulation of compatible solutes such as proline and lowering the toxic concentration of ions in the cytoplasm by restriction of Na^+ influx or its sequestration into the vacuole (Binzel *et al.,* 1988; Bohnert *et al.,* 1999). Therefore, salt stress caused disruption of ionic equilibrium, influx of Na^+, dissipates the membrane potential and facilitates the uptake of Cl^- down the chemical gradient. Na^+ is toxic to cell metabolism and has deleterious effect on the functioning of some of the enzymes (Niu *et al.,* 1995). High concentration of Na^+ causes osmotic imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion. High Na^+ levels also lead to reduction in photosynthesis and production of reactive oxygen species (Yeo, 1998).

Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al*., 2008). The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs and Keresztes, 2002). 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 (Ashraf *et al*., 2003). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Ashraf, 2009). The relatively low-doses ionizing irradiation on plants and photosynthetic microorganisms are manifested as accelerated cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (Chakravarty and Sen, 2001; El-Beltagi, 2001, 2004; El-Beltagi *et al.,* 2011b). On the other hand, the irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Aly and El-Beltagi 2010; Hameed *et al*., 2008a).

The present study aims to investigate the effect of irradiation seeds with gamma rays, on improving the salt tolerance of cowpea (*Vigna sinensis*) plants grown under different levels of NaCl.

Materials and methods

Plant materials, experimental design and irradiation treatments

Seeds of cowpea plants (*Vigna sinensis*) were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. The seeds were thoroughly washed with continuous current of tap water for one hour. The washed seeds were sown in pots (25 cm in diameter and 25 cm in depth) containing 3.5 kg of homogeneous loamy clay soil. Ten seeds were sown in each pot. Pots were divided into four groups. The seeds of the first group did not have any treatment to serve as control, while the seeds of the second group were irrigated with NaCl at concentrations (25, 50, 100 and 200 mM) to raise the pots at 80% of soil water holding capacity. The seeds of the third group were irradiated with gamma rays (50 Gy) at the Middle Eastern Regional Radioisotopes center for the Arab countries (Dokki, Cairo) and the fourth group was combination between gamma rays and NaCl. The pots were irrigated with the water holding capacity of the soil (80%) to serve as control and the other groups with NaCl. This experiment was conducted under natural conditions (day length 12- 14 hrs, temperature 25-27°C and humidity 70%). At 60 days from sowing the plants were collected to determine the growth parameters (shoot length, root length, number of lateral roots, number of leaves, number of internodes, number of flowers, fresh and dry weights of shoots and roots and assimilating area). In addition, plants were collected and frozen in liquid nitrogen and stored in the deep freezer for further chemical analysis.

Chemical analysis

Determination of photosynthetic pigments

Chlorophyll a, Chlorophyll b and Carotenoids were determined in cowpea leaves. The spectrophotometric method recommended by Vernon and Seely (1966) was used. The pigment contents were calculated as mg/g fresh weight of leaves.

Determination of total carbohydrates

Total carbohydrates were determined based on the method of phenol sulfuric acid as described by Dubois *et al.* (1956). Pure glucose was used as standard and the amount of total carbohydrates was expressed as mg glucose/100g dry weight.

Determination of total phenols

Levels of soluble phenols in cowpea leaves were determined in accordance with Dihazi *et al.* (2003). The absorbance of the developed blue colour was read at 725 nm. Tannic acid was used as standard and the amount of soluble phenols was expressed as mg tannic acid/g dry weight.

Lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). The absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of malondialdehyde was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of osmoregulators

The proline content was estimated by the method of Bates *et al.* (1973). Total free amino acids (FAA) content was estimated according to Moore and Stein (1954).

Determination of mineral

Na^+, Ca^{+2} and Mg^{+2} were determined in the acid digested of roots and shoots of cowpea plants by atomic absorption spectrometry according to A.O.A.C. (2005). N, P and K were determined according to A.O.A.C. (1995). For chloride determination, Cl^- was determined by the silver ion-titration method according to Bozcuk (1970).

Electrophoretic analysis of isozymes

Isozymes α- and β- esterase, acid phosphatase, peroxidase and polyphenol oxidase were analyzed on 12% polyacrylamide slab gels. Detection of esterase isozymes was carried out by the method described by Scandalios (1964), acid phosphatase isozymes was detected by method of Wendel and Weeden (1989), peroxidase was carried out by the method described by Larsen and Benson (1970) and polyphenol oxidase was done by the method Sato and Hasegawa (1976).

Statistical analysis

The data were statistically analyzed using F-test and LSD at 5% and 1% levels of probability according to SAS-Programme (1982).

Results and discussion

The growth parameters (Tab. 1) of cowpea plants exhibited differential responses to the imposed salt stress and gamma irradiation. The shoot and root lengths were highly significantly increased only at the low concentration of NaCl (25 mM). On the other hand all concentrations of NaCl significantly decreased shoot and root lengths of cowpea plants. This decrease was alleviated by gamma irradiation at all levels of NaCl. The other growth parameters such as number of lateral roots and bacterial nodules showed highly significant increment at all treatments. Although number of leaves, leaves area, fresh weight of shoots and roots and dry weight of shoots and roots were significantly inhibited with increasing concentrations of NaCl expect at the low concentrations of NaCl (25 mM) which showed high significant increase in all the above parameters. Whereas, the irradiated seeds of cowpea plants with gamma rays under salt stress caused highly significantly increment in all the above growth parameters as compared with control (H_2O) except the high concentration of NaCl (200 mM). These results are in accordance with Amirjani (2011) who found that salinity stress significantly affected plant growth components such as shoot and root lengths and fresh (FW) and dry weights (DW) of rice seedlings. Also, Nassar *et al*. (2004) found that, irradiating chamomile seeds with different doses of gamma rays (0, 20, 40, 60, 80 or 100 Gy) before sowing increased significantly the plant height and the number of branches with increasing the dose of gamma irradiation.

As illustrated in Tab. 2, chlorophyll a and b, carotenoids and total photosynthetic pigment contents in leaves of cowpea plants were significantly increased in irradiated and non-irradiated treatment under the low concentrations of NaCl (25 and 50 mM). On the other hand, highly significant decrease in chlorophyll a, b, carotenoids and total photosynthetic pigment contents at the higher salinity levels (100 and 200 mM). Irradiating seeds with gamma rays caused enhancement in photosynthetic pigment contents under salt stress except the high concentration of NaCl (200 mM). These results are in accordance with

Tab. 1. Effect of gamma rays on growth parameters of cowpea plants under salt stress

Treatments	Shoot length (cm)	Root length (cm)	No of lateral roots	No of bacterial nodules	No of leaves	Area of leaves (Cm^2)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
0.0 mM NaCl	16.22±1.5	22.00±2.3	21.06±2.1	2.17±0.2	4.00±0.5	97.4±4.3	4.17±1.0	0.64±0.12	1.34±0.3	0.08±0.02
25 mM NaCl	17.04±1.8^c	24.33±2.5^a	30.2±2.6^a	4.00±0.5^a	4.20±0.6^a	105.3±5.8^a	4.76±0.9^a	0.73±0.15^a	2.01±0.5^a	0.14±0.04^a
50 mM NaCl	14.46±1.3^b	20.33±2.1^b	40.8±3.0^a	2.60±0.3^a	3.80±0.4^b	94.2±4..0^c	3.95±0.7^c	0.60±0.14^b	1.18±0.2^c	0.070.01^c
100 mM NaCl	14.00±1.2^b	18.75±1.8^b	27.00±2.4^a	2.53±0.2^a	3.80±0.4^b	81.4±3.9^b	3.86±0.8^b	0.57±0.16^b	0.99±0.1^b	0.06±0.00^b
200 mM NaCl	11.62±1.0^b	17.30±1.7^b	28.6±2.7^a	3.20±0.4^a	3.40±0.2^b	69.4±3.5^b	3.11±0.6^b	0.45±0.12^b	0.62±0.1^b	0.04±0.01^b
50 Gy	18.50±1.9^a	30.88±2.7^a	24.4±2.6^a	2.60±0.2^a	4.40±0.5^a	107.1±6.1^a	4.48±1.0^a	0.76±0.28^a	1.61±0.4^a	0.10±0.02^a
25 mM NaCl + 50 Gy	19.46±2.1^a	25.43±2.5^a	38.1±2.9^a	4.67±0.5^a	4.33±0.5^a	111.9±6.5^a	5.34±1.3^a	0.83±0.13^a	2.43±0.5^a	0.16±0.03^a
50 mM NaCl + 50 Gy	19.00±2.1^a	24.50±2.2^a	49.7±3.3^a	5.40±0.5^a	4.20±0.5^a	108.6±6.3^a	5.03±1.1^a	0.71±0.12^a	1.64±0.4^a	0.11±0.01^a
100 mM NaCl + 50 Gy	18.33±1.8^a	23.80±2.3^a	32.3±2.8^a	2.83±0.3^a	4.17±0.5^a	107.2±6.0^a	4.43±0.9^a	0.70±0.10^a	1.55±0.3^a	0.10±0.01^a
200 mM NaCl + 50 Gy	13.50±1.2^b	18.17±1.9^b	27.00±2.2^a	4.00±0.4^a	3.62±0.4^b	83.1±3.5^b	3.35±0.8^b	0.58±0.09^b	1.01±0.2^b	0.06±0.01^b
L.S.D at 1%	0.83	1.27	2.66	0.33	0.10	4.40	0.22	0.03	0.16	0.011

Means ± SD (n=10) of measurements on each ten plants. Means followed by a,b are highly significant increase, decrease and c significant increase and decrease different at $p \leq 0.01$, according to least significant difference (LSD) test

Tab. 2. Effect of gamma rays on photosynthetic pigment contents in leaves of cowpea plants under salt stress

Treatments	Chl a mg/g	Chl b mg/g	Chl a+b mg/g	Carotenoids mg/g	Total Pigments mg/g
0.0 mM NaCl	8.73±1.0	2.83±0.3	11.56±1.0	4.95±0.5	16.52±1.9
25 mM NaCl	12.53±1.5[a]	4.67±0.5[a]	17.20±1.5[a]	6.56±0.7[a]	23.76±2.5[a]
50 mM NaCl	10.09±1.3[a]	4.39±0.5[a]	14.48±1.4[a]	5.55±0.6[a]	20.03±1.8[a]
100 mM NaCl	7.78±1.0[b]	2.39±0.4[b]	10.17±1.0[b]	4.53±0.4[b]	14.70±1.2[b]
200 mM NaCl	7.65±0.9[b]	2.22±0.4[b]	9.87±0.9[b]	4.48±0.4[b]	14.35±1.1[b]
50 Gy	11.90±1.1[a]	4.66±0.6[a]	16.56±1.4[a]	6.49±0.7[a]	23.05±1.9[a]
25 mM NaCl + 50 Gy	15.13±1.6[a]	5.25±0.7[a]	20.38±1.7[a]	8.89±0.8[a]	29.27±2.4[a]
50 mM NaCl+ 50 Gy	13.30±1.4[a]	4.99±0.5[a]	18.29±1.6[a]	7.27±0.7[a]	25.56±2.1[a]
100 mM NaCl+ 50 Gy	10.37±1.1[a]	3.69±0.4[a]	14.06±1.5[a]	6.25±0.6[a]	20.30±2.0[a]
200 mM NaCl+ 50 Gy	8.22±0.8[d]	2.46±0.3[b]	10.68±1.2[c]	4.74±0.5[d]	15.42±1.3[c]
L.S.D at 1%	0.79	0.36	1.14	0.44	1.57

Means ± SD (n=3) of measurements on each three plants. Means followed by a,b are highly significant increase, decrease, c significant increase and decrease and d non significant effect different at $p \leq 0.01$, according to least significant difference (LSD) test

the results of Dhanapackiam and Muhammad (2010) who found that the levels of salinization (40 and 50 mM NaCl) induced a significant decrease in the contents of pigment fractions (chlorophyll a and b) and consequently of the total photosynthetic pigment content as compared with control plants. While, the total photosynthetic pigment content of the leaves of *Sesbania grandiflora* seedlings exhibited a little increase when grown at 10 and 20 mM NaCl. In addition, an increase in chlorophyll *a, b* and total photosynthetic pigment levels was observed in *Paulownia tomentosa* plants that were exposed to gamma irradiation as compared to the non-irradiated plants (Alikamanoglu *et al.,* 2007). Modulation in photosynthesis in irradiated plants might partly contribute to increased growth (Wi *et al*., 2007).

The results in Tab. (3) showed that the total carbohydrate content in shoots and roots of cowpea plants was highly significantly increased at the low concentrations of NaCl (25 and 50 mM). On the other hand, the high concentrations of NaCl (100 and 200 mM) caused highly significantly decreases in the same contents as compared with control plants. These results are in accordance with those of Almodares *et al*. (2008) who reported that the amount of soluble carbohydrate decreases in two sweet sorghum cultivars (Keller and Sofra) as salinity increased. Seeds irradiated with gamma rays showed highly significantly increases in total carbohydrate contents in shoots and roots of cowpea plants under all salt stress as compared with the corresponding controls. These results are in accordance with Moussa (2011) who found that gamma irradiation (20 Gy) increased the soluble sugars in drought stressed soybean leaves.

Also, the results presented in Tab. 3 revealed that the total phenol level was significantly increased in root and shoot of cowpea plants grown under all NaCl levels alone or in combination with gamma rays as compared with those of untreated and treated plants. Similarly, Ashraf *et al*. (2010) found that, the phenolic contents of bread wheat (*Triticum aestivum* L.) were affected significantly due to salt stress (150 mM). Also, Aly (2010) reported that γ-irradiation increased the biosynthesis of phenolic

Tab. 3. Effect of gamma rays on total carbohydrates, total phenols contents and lipid peroxidation in cowpea plants under salt stress

Treatments	Total carbohydrates mg/100 g		Total phenol µg/g		Lipid peroxidation n mole/g F.wt	
	Root	Shoot	Root	Shoot	Root	Shoot
0.0 mM NaCl	32.1±2.5	41.2±3.0	7.7±0.9	70.0±2.5	5.1±0.5	9.3±0.4
25 mM NaCl	44.3±3.3[a]	47.6±3.3[a]	31.6±1.9[a]	136.3±3.7[a]	12.9±0.9[a]	13.8±0.6[a]
50 mM NaCl	40.7±2.7[a]	44.4±2.9[a]	25.3±1.5[a]	98.2±2.6[a]	15.4±1.1[a]	20.0±0.9[a]
100 mM NaCl	30.1±2.2[b]	37.4±2.4[b]	24.0±1.4[a]	79.3±2.1[a]	18.7±1.3[a]	23.3±1.0[a]
200 mM NaCl	29.4±2.0[b]	34.4±2.2[b]	10.9±1.0[a]	75.0±2.0[a]	21.5±1.5[a]	35.7±1.2[a]
50 Gy	38.4±2.4[a]	45.4±2.8[a]	32.0±1.8[a]	114.6±2.8[a]	8.3±0.8[a]	12.7±0.5[a]
25 mM NaCl 50 Gy	46.6±3.5[a]	49.3±3.1[a]	34.3±2.0[a]	155.8±4.0[a]	9.4±0.9[a]	12.9±0.6[a]
50 mM NaCl+ 50 Gy	42.2±2.9[a]	46.3±3.5[a]	33.3±2.1[a]	98.8±1.9[a]	11.6±0.8[a]	13.7±0.6[a]
100 mM NaCl+ 50 Gy	37.2±2.4[a]	45.1±3.2[a]	32.3±2.0[a]	83.7±1.7[a]	12.4±0.9[a]	15.1±0.7[a]
200 mM NaCl+ 50 Gy	30.9±2.3[d]	37.4±2.6[b]	21.2±1.6[a]	80.3±1.5[a]	12.9±0.4[a]	15.6±0.7[a]
L.S.D at 1%	1.93	1.54	2.93	8.75	1.49	2.43

Means ± SD (n=3) of measurements on each three plants. Means followed by a and b are highly significant increase and decrease different at $p \leq 0.01$, according to least significant difference (LSD) test compounds in Culantro *(Eryngium foetidum* L.) fresh plantlets.

Tab. 3 showed that all NaCl levels induced intense lipid peroxidation as indicated by the increase in the thiobarbituric acid reactive substances (TBARs) product in root and shoot of cowpea plants. Treatment with gamma irradiation either alone or in combination with different NaCl levels reduced the level of TBARs. The increase in the MDA content at all salinity levels may be due to oxidative damage affecting both organelles (chloroplasts and mitochondria). These results are in accordance with Moussa (2011) who found that gamma irradiation decreased the malondialdehyde concentration of the leaves of soybean plants under drought stress. Also, Hameed *et al*. (2008b) reported that lipid peroxidation, which have an important role in abiotic stresses decreased significantly from 100-200 Gy radiation dose in chickpea. Similarly, Baek *et al.* (2006) found that gamma irradiation lowered the MDA content in rice plants under salt stress.

Salt stress significantly increased proline and total free amino acid contents of roots and shoots of cowpea plants as compared to that of control (Tab. 4). Treatment with gamma irradiation significantly induced additional increases in the same contents as compared to the value of the control under salt stress. These results are in accordance with Mahajan and Tuteja (2005) who found that proline accumulation may contribute to the adjustment at the cellular level which may acts as an enzyme protectant and stabilizing the structure of macro molecules. Proline also acts as a major reservoir of energy and nitrogen for utilization upon exposure to salinity. It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis.

The higher concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Also, proline can confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in nonenzymic free radical detoxifications (Khan *et al.,* 2002). Moreover, free amino acids accumulation in plants under salt stress has been attributed to alteration in biosynthesis and degradation processes of amino acids and proteins (Silva *et al.,* 2008). The increase in amino acids may be attributed to the changes caused by water deficit which promote protein degradation by protease. There was significant increase in the level of amino acids in *Vigna unguiculata* L. plants under water stress (Lobato *et al.,* 2008).

Tab. 4. Effect of gamma rays on proline and total free amino acids contents in cowpea plants under salt stress

Treatments	Proline µg/g		Total free amino acid µg/g	
	Root	Shoot	Root	Shoot
0.0 mM NaCl	2.88±0.2	3.04±0.2	23.0±1.5	26.2±2.0
25 mM NaCl	3.78±0.3^{a}	3.54±0.2^{a}	30.2±2.4^{a}	31.9±2.4^{a}
50 mM NaCl	4.25±0.4^{a}	3.96±0.3^{a}	34.0±2.5^{a}	36.7±2.6^{a}
100 mM NaCl	4.38±0.5^{a}	4.03±0.4^{a}	35.0±2.6^{a}	38.8±2.5^{a}
200 mM NaCl	5.20±0.5^{a}	6.11±0.5^{a}	41.6±2.9^{a}	46.5±2.9^{a}
50 Gy	3.48±0.2^{a}	3.20±0.3^{a}	27.8±1.7^{a}	55.9±3.3^{a}
25 mM NaCl + 50 Gy	4.29±0.3^{a}	3.93±0.3^{a}	34.3±2.0^{a}	59.5±3.5^{a}
50 mM NaCl+ 50 Gy	4.44±0.3^{a}	4.02±0.4^{a}	35.5±2.2^{a}	69.0±3.9^{a}
100 mM NaCl+ 50 Gy	5.03±0.4^{a}	4.59±0.4^{a}	40.2±2.4^{a}	82.6±4.0^{a}
200 mM NaCl+ 50 Gy	5.46±0.5^{a}	6.36±0.5^{a}	43.7±2.6^{a}	107.5±4.5^{a}
L.S.D at 1%	0.25	0.35	1.96	7.80

Means ± SD (n=3) of measurements on each three plants. Means followed by a is highly significant increase different at $p≤0.01$, according to least significant difference (LSD) test

High doses of gamma radiation were reported to induce oxidative stress. To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments. This defense was brought by alteration in the pattern of gene expression. This led to modulation of certain metabolic and defensive pathways. One of the protective mechanisms in the synthesis of osmolytes which is essen-

Tab. 5a. Effect of gamma rays on (N, P, K) contents in cowpea plants under salt stress

Treatments	N g/ 100g		P g/100 g		K g/100 g	
	Root	Shoot	Root	Shoot	Root	Shoot
0.0 mM NaCl	2.51	2.90	0.131	0.159	1.55	1.41
25 mM NaCl	2.22^{b}	2.70^{b}	0.125^{d}	0.143^{b}	1.44^{b}	1.19^{b}
50 mM NaCl	2.12^{b}	2.51^{b}	0.105^{b}	0.120^{b}	1.40^{b}	0.80^{b}
100 mM NaCl	1.93^{b}	2.32^{b}	0.097^{b}	0.113^{b}	1.38^{b}	0.78^{b}
200 mM NaCl	1.74^{b}	2.12^{b}	0.064^{b}	0.112^{b}	1.29^{b}	0.72^{b}
50 Gy	2.62^{c}	3.01^{d}	0.238^{b}	0.262^{a}	1.87^{a}	1.51^{c}
25 mM NaCl + 50 Gy	2.80^{a}	4.00^{a}	0.187^{a}	0.207^{a}	1.73^{a}	1.72^{a}
50 mM NaCl+ 50 Gy	2.72^{a}	3.5^{a}	0.166^{a}	0.186^{a}	1.67^{a}	1.65^{a}
100 mM NaCl+ 50 Gy	2.66^{a}	3.42^{a}	0.141^{d}	0.179^{a}	1.59^{c}	1.60^{a}
200 mM NaCl+ 50 Gy	1.90^{b}	2.55^{b}	0.112^{b}	0.140^{b}	1.40^{b}	1.13^{b}
L.S.D at 1%	0.119	0.181	0.015	0.015	0.056	0.118

Means followed by a,b are highly significant increase, decrease and c significant increase and decrease different at $p≤0.01$, according to least significant difference (LSD) test

Tab. 5b. Effect of gamma rays on (Na, Cl, Ca, Mg) contents in cowpea plants under salt stress

Treatments	Na g/ 100g		Cl g/100 g		Ca g/100 g		Mg g/100 g	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0.0 mM NaCl	0.057	0.137	0.62	0.37	0.431	0.662	0.317	0.374
25 mM NaCl	0.069^{a}	0.171^{a}	0.87^{a}	0.44^{c}	0.397^{c}	0.616^{b}	0.307^{d}	0.351^{c}
50 mM NaCl	0.080^{a}	0.205^{a}	0.94^{a}	0.49^{a}	0.384^{b}	0.593^{b}	0.275^{b}	0.317^{b}
100 mM NaCl	0.092^{a}	0.217^{a}	1.08^{a}	0.54^{a}	0.291^{b}	0.532^{b}	0.263^{b}	0.281^{b}
200 mM NaCl	0.103^{a}	0.228^{a}	1.15^{a}	1.01^{a}	0.268^{b}	0.461^{b}	0.258^{b}	0.265^{b}
50 Gy	0.046^{b}	0.135^{d}	0.51^{b}	0.23^{b}	0.593^{a}	0.686^{c}	0.333^{c}	0.412^{a}
25 mM NaCl + 50 Gy	0.045^{b}	0.127^{c}	0.54^{b}	0.20^{b}	0.523^{a}	0.686^{c}	0.338^{a}	0.392^{c}
50 mM NaCl+ 50 Gy	0.048^{b}	0.130^{d}	0.56^{c}	0.22^{b}	0.577^{a}	0.693^{a}	0.363^{a}	0.458^{a}
100 mM NaCl+ 50 Gy	0.050^{b}	0.132^{d}	0.58^{d}	0.24^{b}	0.602^{a}	0.751^{a}	0.482^{a}	0.529^{a}
200 mM NaCl+ 50 Gy	0.052^{c}	0.135^{d}	0.60^{d}	0.27^{b}	0.315^{b}	0.514^{b}	0.309^{d}	0.284^{b}
L.S.D at 1%	0.006	0.012	0.074	0.075	0.039	0.028	0.019	0.026

Means followed by a,b are highly significant increase, decrease and c significant increase and decrease different at $p \leq 0.01$, according to least significant difference (LSD) test

tial to plant growth was proline synthesis (Esfandiari *et al*., 2008).

It is clear from the results in Tab. 5 that salinity caused highly significantly increased in the Na^+ and Cl^- content and decrease in the contents of N, P, K^+, Mg^{+2} and Ca^{+2} in the roots and shoots of cowpea plants. It is probably that salt stress causes nutrient deficiency due to competition between $Na^+$and other nutrients such as K^+, Mg^{+2} and Ca^{+2}. Other possibility is that reduction in uptake of mineral nutrients under saline conditions may occur due to Na induced blockage or reduced activity of the transporters, resulting in ionic imbalance of K, Ca^{+2} and Mg^{+2} as compared to Na^+. After treating with gamma irradiation, reduction in the Na^+ and Cl^- uptake of plants and/or increased the K^+, N, P, Mg^{+2} and Ca^{+2} uptakes compared to control treatment under salt stress. These results are in accordance with Shereen *et al.* (2009) who found that shoot ions concentration showed an increase in accumulation of Na^+ coupled with decrease in K^+ concentration in rice plants under salt stress.

The present study showed that, the radiation dose of 50 Gy was found to be very effective for improving the ionic balance in roots and shoots of cowpea plants. The reduction in the uptake of toxic ion like Na^+ may have exerted positive role in enhancement of cellular functions like pigment production and other growth attributes, which collectively resulted in better growth of cowpea plants under saline conditions.

Reduction in plant growth due to salinity is a common feature as was observed in cowpea plants. This reduction in growth may be attributed to osmotic effects which the water availability to plants is hindered. There may be salt specific effects. If absorption of excess quantity of toxic ions takes place, the concentration inside the plant may reach to toxic level and causes ionic imbalances at cellular and organ level. These imbalances may cause hindrances in various physiological functions and ultimately the growth and yield is affected (Hameed *et al*., 2008a). One of the most important factors under saline environment may be the overall control mechanism of salt uptake through root and its subsequent distribution to shoot. The other factor is the maintenance of mineral nutrients such as K^+ and Ca^{+2} which are essentially required for the activities of enzymes, proteins synthesis and integrity of cell wall and plasma membrane (Taiz and Zeiger, 2006).

The electrophoretic profiles of five enzymes; α and β esterase, polyphenol oxidase, peroxidase and acid phosphatase of cowpea leaves under different concentrations of NaCl alone or in combination of gamma ray are presented in Tab. 6 and Fig. 1.

α- esterase electrophoretic patterns are illustrated in Tab. 6 and Fig 1. Four bands were exhibited with different intensities among all treatments. Band No. 1 and 4 which have R_f 0.026 and 0.95 were present in all treatments (common bands). The other two bands were present in some treatments and absent in the others (polymorphic). NaCl concentrations (25, 50, 100 and 200 mM) increased the activity of esterase isozymes. The activity of esterase increased at concentrations of NaCl (25, 100 and 200 mM) after treatment with gamma rays. These results are in agreement with Hassanein (1999), who found that salinity increase esterase isozymes and the highest numbers of esterase isozymes were detected under the highest NaCl concentration. Also, El-Sayed *et al.* (2007) who found that salinity and gamma rays caused the appearance and disappearance of bands in two wheat cultivars.

In Tab. 6, two bands with different intensities were observed among β- esterase profiles of all treatments. One band was presented in all treatments (monomorphic bands) at R_f 0.20. The other band was presented in some treatments and absent in the others. The band which has R_f0.59 become very intensified at concentrations of NaCl (25, 100 and 200 mM) after treatment with gamma rays as compared with control treatment. These results are similar to Mohamed (2005) who found that under salt stress, 150

Tab. 6. Effect of gamma rays on different isozymes profiles in cowpea leaves under salt stress

Enzymes	R_f	Treatments									
		Control	50 Gy	25 mM NaCl	25 mM NaCl+50 Gy	50 mM NaCl	50 mM NaCl+50 Gy	100 mM NaCl	100 mM NaCl+50 Gy	200 mM NaCl	200 mM NaCl+50 Gy
α-esterase	0.026	1.21	1.93	3.42	3.99	3.17	2.9	2	2.41	2.88	3.16
	0.57	-	-	-	8.38	6.42	11.7	6.89	9.09	7.06	5.43
	0.68	16.6	-	11.6	14.7	12.2	10.6	18.8	14	10.5	14.3
	0.95	9.77	11.8	8.54	6.69	7.8	5.66	6.56	5.04	11.5	10.3
Total No. of bands		3	2	3	4	4	4	4	4	4	4
β-esterase	0.20	4.07	4.31	5.82	5.69	4.9	4.82	4.71	6.07	5.21	4.69
	0.59	11.7	-	-	12	4.59	5.98	9.54	13.1	5.86	13.3
Total No. of bands		2	1	1	2	2	2	2	2	2	2
Polyphenol oxidase	0.03	-	-	7.23	8.05	4.44	5.77	4.93	5.49	4.73	6.56
	0.09	5	6.29	6.88	8.33	5.23	4.91	-	-	-	-
	0.15	6.98	7.7	6.61	6.21	6.95	7.32	5.19	6.07	7.28	6.93
	0.24	6.95	7.18	8.18	7.75	5.86	5.87	7.4	4.98	6.56	4.88
	0.34	8.52	7	7.9	7.47	-	-	-	-	-	-
	0.40	-	-	10.6	7.33	11.1	11.6	11.5	10.6	9.38	14.9
	0.58	18.3	6.43	2.73	-	-	-	-	-	5.53	-
	0.67	-	3.78	2.56	3.12	6.33	2.47	6.59	4.33	7.91	5.74
	0.72	-	-	-	2	4.04	3.1	3.36	4.52	-	-
	0.87	-	-	-	-	4.27	4.08	5.01	5.14	5.48	4.83
Total No. of bands		5	5	8	8	8	8	7	7	7	6
peroxidase	0.038	4.04	4.65	4.23	3.88	4.56	5.47	5.77	8.14	9.89	9.32
Total No. of bands		1	1	1	1	1	1	1	1	1	1
Acid phosphatase	0.044	12.7	17.4	25.3	22.5	15.1	14.0	13.5	11.9	13.5	12.1
Total No. of bands		1	1	1	1	1	1	1	1	1	1

mM NaCl caused enhancement of the esterase isozyme bands in shoots and roots of maize plants.

Polyphenol oxidase electrophoretic patterns are illustrated in Tab. 6 and Fig. 1. Eight bands with different intensities were observed among the profiles of all treatments. Two bands were presented in all treatments (monomorphic bands) at R_f 0.15 and 0.24. The other bands were presented in some treatments and absent in the others (polymorphic bands). These results are in agreement with El-Sayed *et al*. (2007) who found that salinity and gamma rays caused the appearance and disappearance of bands in two wheat cultivars.

Expression of the peroxidase isozyme was detected in cowpea leaves treated with different concentrations of NaCl alone or in combination with gamma rays. The results in Tab. 6 and Fig. 1 showed that one band was exhibited at R_f 0.038 with different intensities in untreated and salt treated plants. The activity of this band increased with increasing NaCl concentrations alone or in irradiated plants. These results indicated that salt stress increased the accumulation of the peroxidase enzyme and that the encoding gene (s) was accelerated in response to salt stress. These results are in harmony with the findings of El- Baz *et al*. (2003), who used peroxidase isozyme as marker for salt stress tolerance in cucumber plants. This behavior may be due to its ability to tolerate salt stress or due to the effect of salt stress which may cause some shift in gene expression. In addition, The results from the present study are in conformity with the study by Rashid *et al.* (2001) on the detection of genetic variation using molecular techniques among the irradiated and salt stress (200 mM NaCl) calli.

An isozyme profile of acid phosphatase in cowpea leaves grown under NaCl alone or in combination with gamma rays is shown in Tab. 6 and Fig. 1. It is evident that one common band was observed under control as well as salinity and gamma rays treatments. This band becomes much intensified in salinized cowpea leaves alone or in combination with gamma rays at 25 and 50 mM NaCl as compared with control treatment. These results are in agreement with the findings of Mohamed (2005) who reported that the induction of new isozymes and the change in the acid phosphatase isozyme profile is considered to play an important role in the cellular defense against oxidative stress, caused by salt stress.

In conclusion, this study focused on the possibility to alleviate the harmful effect of salt stress by gamma irradiation treatment. Our results provide some evidences to the important functions of γ-irradiation to solve the production problems caused by high salinity which need further investigation in the future. Seed irradiation with 50 Gy significantly increased plant growth, photosynthetic pigments, total carbohydrate, total phenol, proline, total free

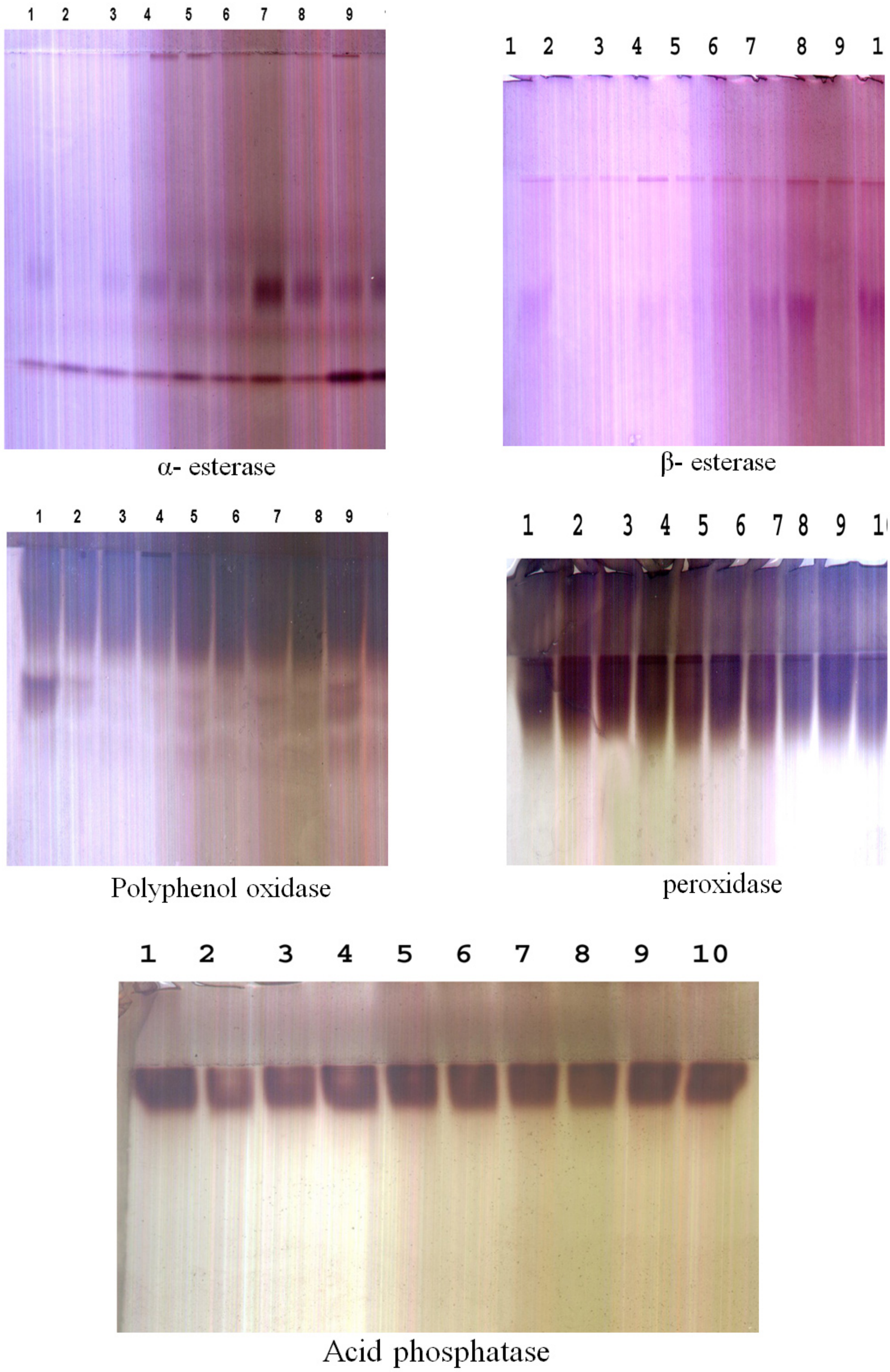

Fig. 1. Electrophoretic patterns of α- and β- esterase, polyphenol oxidase, peroxidase and acid phosphatase isozyme of cowpea leaves in response to treatment with different concentrations of NaCl alone or in combination with gamma ray.

1: Control; 2: 50 Gy; 3: 25 mM NaCl; 4: 25 mM NaCl+50 Gy; 5: 50 mM NaCl; 6: 50 mM NaCl+50 Gy; 7: 100 mM NaCl; 8: 100 mM NaCl+50 Gy; 9: 200 mM NaCl; 10: 200 mM NaCl+50 Gy amino acids and the contents of N, P, K^+, Ca^{+2} and Mg^{+2} compared to non irradiated ones under salinity. On the other hand, irradiation with gamma rays decreased lipid peroxidation, Na^+ and Cl^- contents which may contribute in part to activate processes involved in the alleviation of the harmful effect of salt. Electrophoretic studies of α-esterase, β-esterase, polyphenol oxidase, peroxidase and acid phosphatase isozymes showed wide variations in their intensities among all treatments.

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