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ORIGINAL ARTICLE

Pre-exposure to gamma rays alleviates the harmful effect of salinity on cowpea plants

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Soil salinity is one of the most severe factors limiting growth and physiological response in cowpea plants. In this study, the low concentrations of NaCl (25mM) increased plant growth, photosynthetic pigments content, total soluble protein content, nucleic acids contents (DNA and RNA), lipid peroxidation, non enzymatic antioxidants (anthocyanin, ascorbic acids and α-tocopherol), number of legumes per plant, number of seeds per legume, number of seeds per plants, legume length, fresh and dry weight of legumes and weight of 1000 seeds and total soluble proteins and carbohydrate contents in harvested seeds as compared to control. On the other hand, the high concentrations of NaCl (50, 100 and 200 mM) caused reduction in plant growth, photosynthetic pigments content, total soluble protein content, nucleic acids contents (DNA and RNA), all yield attributes and harvested seeds components but increased lipid peroxidation and non enzymatic antioxidants (anthocyanin, ascorbic acids and α-tocopherol). Electrophoretic studies of proteins showed three types of modifications are observed in the protein patterns of cowpea seeds, some protein bands were disappeared, other proteins were selectively increased and synthesis of new set of protein was induced. Some of these responses were observed under gamma rays and salinity treatments, while others were induced by either gamma rays or salinity. Seeds irradiation with gamma rays alleviates the adverse effect of salt stress compared to non irradiated seeds.

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Key words: lipid peroxidation, non enzymatic antioxidants, nucleic acids, protein electrophoresis, yield.

Salinity in soil or water is one of the major stresses especially in arid and semi-arid regions, can severely limit plant growth and productivity (Mahajan and Tuteja, 2005). According to FAO, more than 800 million hectares of world's agricultural land are seriously affected by salinity (Munns and Tester, 2008).The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Ashraf, 1994).

Salinity is often associated with a reduction in photosynthetic capacity (Parida and Das, 2005).

Photosynthesis may decrease due to stomatal closure or by the direct effect of salt on the photosynthetic apparatus. Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered an indicator of oxidative damage. Salt stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Ben Amor, *et al*., 2007). ROS cause chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity. Chlorophyll loss, lipid peroxidation measured as MDA (malondialdehyde, a product of lipid peroxidation) content, and electrolyte leakage are considered to be indicators of oxidative damage (Wise and Naylor, 1987).

To minimize the effects of oxidative stress, plants must possess efficient antioxidant system. Plant cells have evolved a complex antioxidant system, which is composed of low molecular mass antioxidants (glutathione, ascorbic acids, α-tocopherol, phenolic compounds and carotenoids) as well as ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) (Piotr and Klobus, 2005).

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). 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 (Hameed *et al*., 2008).

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.

The objective of this work was to investigate whether pre-treatment of dry seeds of cowpea (*Vigna sinensis*) plants with a low dose of gamma rays (5 Krad) before planting could act as a protective agent to nullify the influence of salt stress.

MATERIALS AND METHODS

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 (5 Krad) at the Middle Eastern regional radioisotopes center for the Arab countries (Dokki, Cairo) and the fourth group was combination between gamma rays and NaCl treatments. The pots were irrigated with the water holding capacity of the soil (80%) to severe as control and the other groups were irrigated with NaCl concentrations. This experiment was conducted under natural conditions (day length 12-14 hrs, temperature 25-27 °C and humidity 70%). At 130 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 to determination of photosynthetic pigments, total soluble protein, nucleic acids (DNA and RNA), non enzymatic antioxidants (anthocyanin, ascorbic acid, α-tocopherol) and lipid peroxidation. The seeds were collected when the fruits become mature at 160 days to determine morphological criteria in addition to determination of total carbohydrate content, total soluble protein, mineral contents and protein electrophoresis.

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^{-1} fresh weight of leaves.

Determination of 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 $155mM^{-1}$ cm^{-1}.

Quantitative Estimation of Nucleic Acids

The method used for the extraction of total RNA and DNA were determined according to the method described by Morse and Carter (1949). Ribonucleic acid (RNA) was estimated colourmetrically by the orcinol reaction as described by Dische (1953). While, deoxyribonucleic acid (DNA) was estimated by DPA (diphenylamine) colour reaction described by Burton (1956). The nucleic acids were determination by Spekol Spectrocolourimeter VEB Carl Zeiss.

Assay of Non Enzymatic Antioxidant

Shoots of cowpea plants were used for the estimation of anthocyanin contents according to Neff and Chory (1998). Ascorbic acid content was measured in roots and shoots of cowpea plants according to the method described by Mukherjee and Choudhuri (1983). The α-Tocopherols were determined using the method of Philip *et al*. (1954).

Determination of Total Soluble Protein

Root, shoot and seeds of cowpea plants were grinded in sodium phosphate buffer at pH 6.5. The total soluble protein content in the supernatant was determined according to Lowry *et al*. (1951).

Determination of Total Carbohydrates

Total carbohydrates in seeds of cowpea plants were determined using the colorimetric method described by Dubois *et al*. (1956).

Protein Electrophoresis

For SDS-PAGE, seeds of cowpea plants were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (0.5 M Tris-HCl, pH 6.5, 1% SDS, 5% 2-mercaptoethanol, 20% sucrose, 0.4% bromophenol blue), followed by centrifugation at 10.000 ×g at 4 ^{o}C for 15 min. Extracts were stored at -20^{o}C until used. Gel electrophoresis SDS-PAGE was carried out in 12.5% separating gel and 5% stacking gel according to the method of Laemmli (1970). Gels were run at ambient temperature (20 °C) for 6 h at constant power of 130V until the tracking dye reached the end of the gel. After electrophoresis, the gel was stained with Coomassie blue R-250. Relative molecular weight of each protein was determined using a standard protein marker. The gel was scanned using Gel pro-Analyzer.

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

Changes in Growth Parameter

In response to salinity stress, the lowest concentration of NaCl (25 mM) caused significant increase in the shoot and root lengths at fruiting stage. On the other hand, the higher concentrations of NaCl (50, 100 and 200 mM) caused highly significant decrease or significant decrease as being compared with the control. In response to gamma radiation, shoot and root length showed a highly significant increase with different salinity concentrations (25, 50 and 100 mM) as compared with control except (200 mM) which showed a highly significant decrease (Table 1). The other growth parameters such as number of lateral roots showed highly significant increase at all treatments. Number of bacterial nodules of cowpea plants showed variable changes. The low concentrations of NaCl (25 and 50 mM) alone or in combination with gamma ray (5 Krad) caused highly significant increase in the number of bacterial nodules of cowpea plants. High concentrations of NaCl (100 and 200 mM) alone or in combination with gamma rays showed variable effects in the same parameter as compared with control. Although number of leaves, leaf 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.

Changes in Some Metabolites

As illustrated in table (2), chlorophyll a and b, carotenoids and total photosynthetic pigment contents in leaves of cowpea plants showed highly significant increase above the control value in response to treatment with the lower concentrations of NaCl (25 and 50 mM). On the other hand, the other concentrations of NaCl (100 and 200 mM) caused high significant decrease in the same contents except at 100 mM of NaCl which showed high significant increased in chlorophyll b content. Irradiating seeds with gamma rays caused enhancement in photosynthetic pigment contents under salt stress except the high concentration of NaCl (200 mM).

Data showed in table (2) that the thiobarbituric acid reactive substances (TBARS) content increased progressively in shoots and roots of NaCl treated and untreated cowpea plants. Such increase was highly significant in NaCl treated cowpea plants as compared with the untreated controls. In addition, salinized cowpea plants with gamma rays reduced the levels of TBARS in their shoots and roots as compared with salinized plants alone.

The results in table (2) showed that the total soluble protein content in shoots and roots of cowpea plants was highly significantly increase at the lowest concentrations of NaCl (25 mM) but it showed significant increased in roots of cowpea plant and no significant effect in shoots. 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.

Data in table (3) revealed that the lowest concentration of NaCl (25 mM) causes a highly significant increase in the DNA and RNA contents of shoots and roots of cowpea plants. While the other concentrations of NaCl (50, 100 and 200 mM) caused a high significant decrease or significant decrease in the same content in shoots and roots of cowpea plants.

In addition, The effect of gamma radiation is shown to induce a highly significant increase or significant increase in the DNA and RNA contents of shoots and roots of cowpea plant under (25, 50 and 100 mM) of NaCl except 200 mM which induce a highly significant decrease or significant decrease in the same contents of both shoots of cowpea plants as compared with control.

Changes in Non Enzymatic Antioxidants

It is demonstrated in table (3) that salinity at different concentrations (25, 50, 100 and 200 mM) stimulated the accumulation of non enzymatic antioxidants (anthocyanin, ascorbic acids and α-tocopherol) in shoots and roots of cowpea plants as compared with that of the unsalinized control. Generally, gamma radiation seems to induce a highly significant increase in non enzymatic antioxidants compounds in shoots and roots of cowpea plant under all salinity levels (25, 50, 100 and 200 mM). In addition, salinized cowpea plants with gamma rays increased the levels of non enzymatic antioxidants in their shoots and roots as compared with salinized plants alone.

Changes in Yield Components

Results in table (4) revealed that the lowest salinity concentration (25 mM) induce a highly significant increase in number of legumes per plant, number of seeds per legume, number of seeds per plants, legume length, fresh and dry weight of legumes and weight of 1000 seeds. Also, 50 mM of NaCl induce a significant increase or highly significant increase in all the above parameter except number of legumes per plant which showed high significant decreased. In addition, 100 mM of NaCl induce a highly significant decrease in all the above parameter as compared with control plants. Moreover, radiation with 5 Krad induces a highly significant increase with different salinity concentrations (25, 50 and 100 mM) as compared with control in the same parameter.

Changes in Chemical Composition of harvested Seeds

Total carbohydrates and total soluble protein contents in seeds

The lowest concentration of NaCl (25 mM) induces highly significant increase in total carbohydrate and total soluble protein contents in seeds of cowpea plants. On the other hand the high concentrations of NaCl (50 and 100 mM) induce highly significant decrease in the same contents. In addition radiation with gamma ray (5Krad) induces highly significant increase in total carbohydrate and total soluble proteins contents in seeds under different salinity concentrations (25, 50 and 100 mM) compared to those of the corresponding controls (table 4).

Changes in Protein Electrophoresis in Harvested Seeds

In the present work (Table 5 and Figure 1), three types of modifications are observed in the protein patterns of cowpea seeds, some protein bands were disappeared, other proteins were selectively increased and synthesis of new set of protein was induced. Some of these responses were observed under gamma rays and salinity treatments, while others were induced by either gamma rays or salinity.

19 protein bands of molecular weight ranged between 203 and 22 KDa were observed in cowpea seeds. The protein patterns of cowpea seeds under all treatments comprise eight major bands (common bands) having molecular weights of (203, 158, 139, 115, 108, 34, 30 and 27 KDa). One protein band of molecular weight (180 kDa) was de novo synthesized in cowpea seeds irradiated with 100 mM NaCl alone and with combination with gamma rays (5 Krad). Generally the results of SDS-PAGE analysis could reveal two different genetic mechanisms i.e., salt stress resulted in the overexpression of some gene and / or de novo induction of gene expression.

The total number of bands not effected under all treatments but some bands appeared and the other bands disappeared. The total number of protein bands was increased from 18 bands in the untreated (controlled) plants to 19 bands under plants irradiated with gamma rays alone (5 Krad).

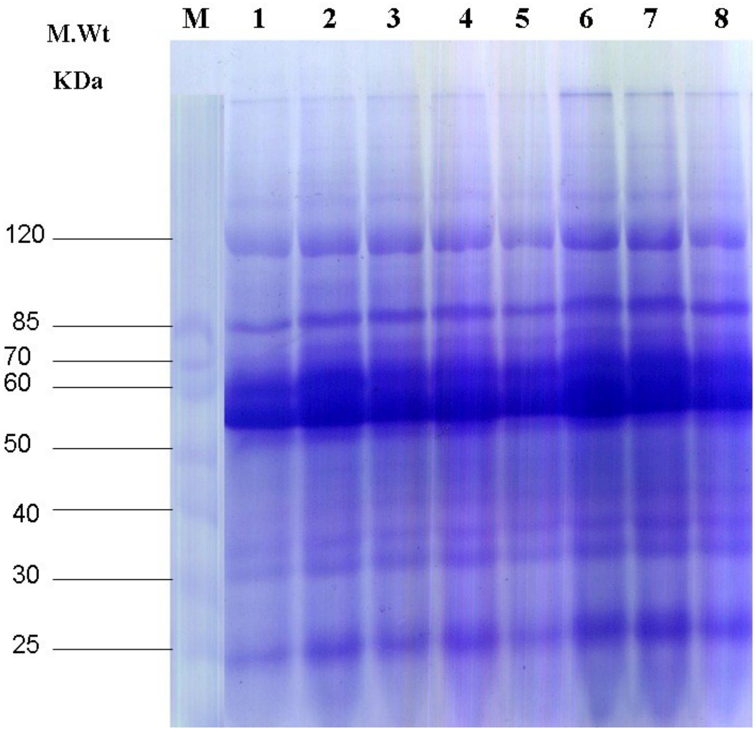

Figure 1. Electrophoretic banding patterns of cowpea seeds in response to treatment with different concentrations of NaCl alone or in combination with gamma rays.
1-Control; 2-5 Krad; 3-25 mM NaCl; 4- 25 mM NaCl+5 Krad; 5- 50 mM NaCl; 6- 50 mM NaCl+5 Krad; 7- 100 mM NaCl; 8- 100 mM NaCl+5 Krad

in in roots and shoots of cowpea

Total soluble protein mg/g	
Roo	Shoot
10.4	26.75
13.3	32.26^{a}
11.	27.84^{c}
7.8	20.41^{b}
7.0	17.84^{b}
12.5	29.9^{a}
16.3	37.08^{a}
14.3	33.8^{a}
12.6	30.24^{a}
8.04	22.9^{b}
0.65	1.30
0.94	1.87

Table 1. Effect of gamma rays on growth parameters of cowpea plants under salt stress. Each value is the mean of ten replicate

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	25.3	26.6	15.20	4.4	8.0	151.8	7.91	1.06	2.22	0.28
25 mM NaCl	35.5^{a}	30.5^{a}	37.20^{a}	8.8^{a}	9.0^{a}	198.0^{a}	11.31^{a}	1.87^{a}	3.30^{a}	0.33^{a}
50 mM NaCl	21.84^{b}	25.3^{b}	34.80^{a}	5.8^{a}	7.0^{b}	122.6^{b}	6.98^{b}	0.95^{-s}	1.98^{b}	0.22^{b}
100 mM NaCl	19.4^{b}	24.5^{b}	26.40^{a}	3.6^{b}	6.2^{b}	114.3^{b}	6.81^{b}	0.88^{b}	1.90^{b}	0.20^{b}
200 mM NaCl	15.6^{b}	21.6^{b}	18.40^{a}	2.8^{b}	5.2^{b}	103.7^{b}	4.35^{b}	0.74^{b}	1.72^{b}	0.17^{b}
5 Krad	38.4^{a}	30.9^{a}	32.00^{a}	6.0^{a}	8.6^{a}	232.1^{a}	12.92^{a}	1.92^{a}	3.26^{a}	0.39^{a}
25 mM NaCl + 5 Krad	41.^{a}	35.7^{a}	23.40^{a}	6.2^{a}	11.8^{a}	255.8^{a}	13.35^{a}	1.99^{a}	3.71^{a}	0.37^{a}
50 mM NaCl + 5 Krad	34.28^{a}	31.8^{a}	49.20^{a}	10.6^{a}	9.2^{a}	208.6^{a}	11.38^{a}	1.76^{a}	2.78^{a}	0.34^{a}
100 mM NaCl + 5 Krad	29.2^{a}	29.1^{a}	22.40^{a}	7.8^{a}	8.9^{a}	205.7^{a}	10.34^{a}	1.59^{a}	2.66^{a}	0.33^{a}
200 mM NaCl + 5 Krad	20.2^{b}	23.04^{b}	20.40^{a}	4.0^{c}	6.7^{b}	126.5^{b}	6.71^{b}	0.80^{b}	1.90^{b}	0.21^{b}
L.S.D at 5%	1.92	0.95	2.22	0.53	0.40	11.67	0.65	0.11	0.15	0.017
1%	2.77	1.37	3.19	0.76	0.58	16.79	0.94	0.16	0.21	0.025

a high significant increase b high significant decrease c non significant effect

Table 2. Effect of gamma rays on photosynthetic pigment contents in leaves, lipid peroxidation and total soluble protein in roots and shoots of cowpea plants under salt stress. Each value is the mean of three replicate

Treatments	Chl a mg/g	Chl b mg/g	Carotenoids mg/g	Total Pigments mg/g	Lipid peroxidation n mole/g F.wt		Total soluble protein mg/g	
					Root	Shoot	Root	Shoot
0.0 mM NaCl	4.48	1.45	3.31	9.24	10.0	17.5	10.41	26.75
25 mM NaCl	8.59^{a}	2.64^{a}	6.50^{a}	17.73^{a}	18.2^{a}	22.5^{a}	13.34^{a}	32.26^{a}
50 mM NaCl	5.60^{a}	2.43^{a}	4.83^{a}	12.86^{a}	19.9^{a}	28.3^{Hs}	11.2^{a}	27.84^{c}
100 mM NaCl	3.16^{b}	1.86^{a}	2.31^{b}	7.33^{-Hs}	23.9^{a}	34.5^{a}	7.84^{b}	20.41^{b}
200 mM NaCl	2.45^{b}	0.99^{b}	1.55^{b}	4.99^{b}	25.5^{a}	40.0^{a}	7.01^{b}	17.84^{b}
5 Krad	7.74^{a}	3.01^{a}	6.19^{a}	16.94^{a}	12.3^{a}	16.1^{c}	12.53^{a}	29.9^{a}
25 mM NaCl + 5 Krad	9.65^{a}	3.85^{a}	7.43^{a}	20.93^{a}	14.0^{a}	20.2^{a}	16.31^{a}	37.08^{a}
50 mM NaCl + 5 Krad	6.82^{a}	3.17^{a}	6.00^{a}	15.99^{a}	15.7^{a}	20.8^{a}	14.34^{a}	33.8^{a}
100 mM NaCl + 5 Krad	5.98^{a}	2.73^{a}	5.26^{a}	13.97^{a}	16.7^{a}	22.4^{a}	12.68^{a}	30.24^{a}
200 mM NaCl + 5 Krad	3.46^{b}	1.09^{b}	2.19^{b}	6.74^{b}	17.1^{a}	24.4^{a}	8.04^{b}	22.9^{b}
L.S.D at 5%	0.52	0.20	0.44	1.15	1.04	1.62	0.65	1.30
1%	0.75	0.29	0.64	1.65	1.49	2.33	0.94	1.87

a high significant increase b high significant decrease c non significant effect

value is the mean of ten replicate

Total carbohydrates mg/100g	Total soluble protein g/ 100g
3.26	13.28
4.06^{a}	14.04^{a}
3.03^{b}	12.67^{b}
2.69^{b}	12.48^{b}
3.43^{a}	14.04^{a}
4.58^{a}	16.89^{Hs}
4.41^{a}	15.61^{a}
3.98^{a}	14.28^{a}
0.128	0.281
0.184	0.404

Table 3. Effect of gamma rays on nucleic acids contents and non enzymatic antioxidants compounds in roots and shoots of cowpea plants under salt stress. Each value is the mean of three replicate

Treatments	DNA µg/g		RNA µg/g		Anthocyanin g/100 ml	Ascorbic acid µg/g		Tocopherol µg/g	
	Root	Shoot	Root	Shoot	Shoot	Root	Shoot	Root	Shoot
0.0 mM NaCl	58.5	139.5	866	826	42.38	4.54	7.58	11.87	12.05
25 mM NaCl	85.5^{a}	153.3^{a}	954^{a}	933^{a}	65.13^{a}	5.59^{a}	8.74^{a}	15.52^{a}	17.41^{a}
50 mM NaCl	47.5^{b}	101.9^{b}	780^{b}	787b^{-S}	55.55^{a}	5.65^{a}	9.80^{a}	15.72^{a}	20.04^{a}
100 mM NaCl	40.3^{b}	75.7^{b}	699^{b}	629^{b}	58.24^{a}	6.01^{a}	10.75^{a}	16.15^{a}	23.03^{a}
200 mM NaCl	26.4^{b}	49.9^{b}	610^{b}	563^{b}	51.09^{a}	7.25^{a}	11.82^{a}	17.90^{a}	25.94^{a}
5 Krad	75.2^{a}	156.4^{a}	906^{a}	932^{a}	69.13^{a}	7.68^{a}	9.55^{a}	18.39^{a}	24.61^{a}
25 mM NaCl + 5 Krad	139.4^{a}	179.2^{a}	1214^{a}	967^{a}	82.73^{a}	8.05^{a}	13.27^{a}	20.07^{a}	26.66^{a}
50 mM NaCl + 5 Krad	100.4^{a}	159.6^{a}	1042^{a}	933^{a}	69.02^{a}	8.82^{Hs}	13.67^{a}	20.61^{a}	32.39^{a}
100 mM NaCl + 5 Krad	89.4^{a}	151.7^{a}	947^{a}	883^{a}	53.94^{a}	9.54^{a}	14.13^{a}	21.12^{a}	38.88^{a}
200 mM NaCl + 5 Krad	49.0^{b}	83.7^{b}	656^{b}	665^{b}	48.33^{a}	12.08^{a}	16.18^{a}	21.55^{a}	45.12^{a}
L.S.D at 5%	7.23	9.36	39.98	31.21	2.99	0.48	0.58	0.66	2.12
1%	10.39	13.46	57.49	44.88	4.31	0.69	0.84	0.95	3.05

a high significant increase b high significant decrease c non significant effect

Table 5. Effect of gamma rays on the protein patterns separated by SDS- PAGE in seeds of cowpea plants under salt stress

		Treatments						
NO.	M.Wt KDa	Control	5 Krad	25 mM NaCl	25 mM NaCl+5 Krad	50 mM NaCl	50 mM NaCl+5 Krad	100 mM NaCl
1	203	+	+	+	+	+	+	+
2	180	—	—	—	—	—	—	+
3	175	+	+	+	+	+	+	—
4	158	+	+	+	+	+	+	+
5	139	+	+	+	+	+	+	+
6	115	+	+	+	+	+	+	+
7	108	+	+	+	+	+	+	+
8	102	—	+	+	+	+	+	+
9	93	+	—	+	+	+	+	+
10	85	+	+	—	—	—	+	+
11	76	+	+	+	+	+	—	—
12	67	—	—	+	—	+	+	+
13	62	+	+	—	+	—	—	—
14	57	—	+	+	+	+	+	+
15	54	+	+	—	—	—	—	—
16	49	—	+	+	+	+	+	+
17	44	+	—	—	—	+	+	+
18	41	+	+	+	+	—	—	—
19	37	—	+	+	+	+	+	+
20	34	+	+	+	+	+	+	+
21	30	+	+	+	+	+	+	+
22	27	+	+	+	+	+	+	+
23	24	+	—	—	—	—	+	—
24	22	+	+	+	+	+	—	+
Total No. of bands		18	19	18	18	18	18	18

DISCUSSION

Salinity stress caused reduction in cowpea plant growth at the high concentrations (50, 100 and 200 mM). These results are similar to Dolatabadian *et al*. (2011) who found that salinity stress was significantly decreased shoot and root weight either fresh weight or dry weight, in addition, total plant weight, plant height and leaf number were decreased due to salinity stress. Interestingly, leaf area was not affected by salinity stress. It has been reported that the plants had the reduction in their fresh weights because of the proportional increase in Na^+ concentration, which could imply that an ionic effect was being manifested. It is also assumed that in addition to toxic effects of NaCl, higher concentration of salt reduces the water potential in the medium which hinders water absorption and thus reduces plant growth.

Table 5. Effect of gamma rays on the protein patterns separated by SDS- PAGE in seeds of cowpea plants under salt stress

		Treatments						
NO.	M.Wt KDa	Control	5 Krad	25 mM NaCl	25 mM NaCl+5 Krad	50 mM NaCl	50 mM NaCl+5 Krad	100 mM NaCl
1	203	+	+	+	+	+	+	+
2	180	—	—	—	—	—	—	+
3	175	+	+	+	+	+	+	—
4	158	+	+	+	+	+	+	+
5	139	+	+	+	+	+	+	+
6	115	+	+	+	+	+	+	+
7	108	+	+	+	+	+	+	+
8	102	—	+	+	+	+	+	+
9	93	+	—	+	+	+	+	+
10	85	+	+	—	—	—	+	+
11	76	+	+	+	+	+	—	—
12	67	—	—	+	—	+	+	+
13	62	+	+	—	+	—	—	—
14	57	—	+	+	+	+	+	+
15	54	+	+	—	—	—	—	—
16	49	—	+	+	+	+	+	+
17	44	+	—	—	—	+	+	+
18	41	+	+	+	+	—	—	—
19	37	—	+	+	+	+	+	+
20	34	+	+	+	+	+	+	+
21	30	+	+	+	+	+	+	+
22	27	+	+	+	+	+	+	+
23	24	+	—	—	—	—	+	—
24	22	+	+	+	+	+	—	+
Total No. of bands		18	19	18	18	18	18	18

DISCUSSION

Salinity stress caused reduction in cowpea plant growth at the high concentrations (50, 100 and 200 mM). These results are similar to Dolatabadian *et al*. (2011) who found that salinity stress was significantly decreased shoot and root weight either fresh weight or dry weight, in addition, total plant weight, plant height and leaf number were decreased due to salinity stress. Interestingly, leaf area was not affected by salinity stress. It has been reported that the plants had the reduction in their fresh weights because of the proportional increase in Na^+ concentration, which could imply that an ionic effect was being manifested. It is also assumed that in addition to toxic effects of NaCl, higher concentration of salt reduces the water potential in the medium which hinders water absorption and thus reduces plant growth. Reduction in growth attributes especially plant height may be due to changes in plant-water relationships under salt stress, which suppress meristem activity as well as cell elongation

(Dorgham, 1991).

Stimulating effect of low salinity level on plant growth may be resulted from the beneficial effect of low concentration of chloride on many physiological processes such as osmoregulators, photosynthesis and enzyme activities, chloride acts a cofactor of an NH_2OH sensitive, Mn-containing, O_2-evolving enzyme. Moreover, Critchley (1983) suggested that chloride facilitates electron transport by reversible ionic binding to the O_2^- evolving complex or to the thylakoid membranes. In addition, some enzymes are known to be stimulated by Cl^- such as ATPase and alpha-amylase hydrolysize starch to sugars requires chloride for activation. On the other hand, salinity stress can affect seed germination through reduction of water uptake leading to moisture stress (osmotic effect), by ion toxicity and/or ionic imbalance, or by the accumulation of Na^+ and Cl^- ions and inhibition of the uptake of several essential nutrients such as K^+ causing nutritional imbalance in the plants or accumulation of these factors (Taamalli *et al*., 2004) and/or decreasing the activity of certain enzymes by either decreasing the rate of transcription or translation, which lead to decreasing both cell division and cell elongation (Dodd and Donovan, 1999).

The irradiated seeds of cowpea plants with gamma rays under salt stress caused highly significantly increase in all the above growth parameters as compared with control (H_2O) except the high concentration of NaCl (200 mM). Irradiation with gamma rays will induce the growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition (Wi *et al*., 2007).

The severe reduction in photosynthetic pigments in cowpea leaves in response to salinity treatment might be ascribed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and / or due to damage of the chloroplast thylakoid. The observed severe reduction in Mg^{+2} ions in salt treated plants which are essential for chlorophyll biosynthesis reinforced the view that salinity decreased chlorophyll biosynthesis. These results are similar to Helaly and El-Hosieny (2011) who found that the interaction treatments (salinity and gamma) indicated that irradiation counteracted the inhibiting effects of salinity on chlorophylls concentration in Citrus limon even at the highest level of salinity. The effect of irradiation on increasing chlorophylls may be due to its effects on stimulation chlorophyll its biosynthesis and/or delaying of its degradation.

Gamma rays caused reduction in lipid peroxidation in cowpea plants. Similar results obtained by Moussa (2011) who found that gamma irradiation decreased the malondialdehyde concentration of the leaves of soybean plants under drought stress. Also, Hameed *et al.* (2008) 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.

The total soluble protein contents in cowpea plants increased at lowest concentrations of salinity but decrease at the high concentrations of salinity. These results are in accordance with Astorga and Meléndez (2010) who found that protein content increase at the lowest concentration of NaCl (20 mM) but decreased at the high concentrations (40, 60 and 80 mM) of NaCl in Paulownia imperialis plants. One characteristic of saline stress is the removal of potassium ions by plant roots, which causes physiological imbalance because potassium is necessary for protein synthesis. Potassium loss causes diminished plant growth and development (Chen *et al*., 2007). If the stress is prolonged, it could affect protein synthesis and eventually cause it to decline (Caplan *et al*., 1990). A decrease in the protein level in salt-stressed plants may be attributed to a decrease in protein synthesis; the decreased availability of amino acid and the denaturation of enzyme involved in amino acid and protein synthesis.

Treatment with gamma rays caused highly significantly increase under all salinity treatments (25, 50 and 100 mM) except at 200 mM of NaCl which caused high significant decrease in the same content. These results are in accordance with Moussa (2011) who stated the total soluble protein content of soybean plants pre-exposed to a low dose of gamma rays (20 Gy) was 11% higher than that of plants subjected to drought stress. Owing to altered gene expression under gamma stress, qualitative and quantitative changes in total soluble protein content were observed (Corthals *et al*., 2000). These proteins might play a role in signal transduction, anti-oxidative defense, anti-freezing, heat shock, metal binding, anti-pathogenesis or osmolyte synthesis, which are essential to a plant's function and growth (Gygi, *et al*., 1999). Also, Ling *et al*. (2008) reported that a low dose of gamma irradiation (30 Gy) enhanced protein synthesis in *Citrus sinensis*.

Nucleic acids content was in parallel with growth parameters. These results are in accordance with Zeid (2009 a) who found that leaf content of DNA and RNA of maize seedlings decreased under salinity stress. Growth which is the integral of cell division and cell elongation was negatively affected by salinity which reduced the cellular water content and decreased content of nucleic acids required for anabolic processes. Also, Zeid (2009 b) suggested that the decrease in nucleic acids content in bean concurrently with the increase in RNase activity caused by the increase in salinity level might be involved in inhibiting nucleic acids biosynthsis and/or stimulating their degradation. In addition, Kabanov and Aziyashvili (1976) attributed the depression of nucleic acid metabolism to the cation imbalance (in particular to the abundance of Na^+ cations in the cell) and also to intensify the activity of cytoplasmic RNase or to the reduction of phosphorus incorporation into nucleic acids.

The effect of gamma radiation is shown to induce a highly significant increase or significant increase in the DNA and RNA contents of shoots and roots of cowpea plant under (25, 50 and 100 mM) of NaCl except 200 mM which induce a highly significant decrease or significant decrease in the same contents of both shoots of cowpea plants as compared with control. These results are accordance with Khodary (2004) who found that treatment with gamma rays increased nucleic acids contents in salinized lupine plants. The mitigating effect of γ - rays on the nucleic acids level in those plants treated with NaCl was also reported by Mostafa *et al*. (2000), who observed a stimulation in RNA synthesis of embryos of castor oil plants and maize grains irradiated with γ - doses that activated plant growth and development.

Salinity caused accumulation in non enzymatic antioxidants in cowpea plants. Thus, these results seem to support the hypothesis that tolerance to oxidative stress plays an important role in adapting plants to adverse environmental conditions (Smirnoff 1998). The same trend also was reported by YU and LIU (2003) in wild soybean. The increased ascorbic acid (AsA) content is a stress-protecting mechanism of plants under salinity conditions (Shalata *et al*., 2001). A high level of endogenous AsA is essential for maintaining the non-enzymatic scavenging system that protects plants from oxidative damage due to salinity stress (Shigeoka *et al*., 2002). In addition, α- tocopherols are groups of compounds that play different rols in plant metabolism and can play important roles in amelioration of biotic and abiotic stress (Hussein *et al*., 2007).

The high concentrations of NaCl (200 mM) affect on yield of cowpea plants and don't produce legumes. Enhancement of seed yield by using gamma rays was recorded by (Sundaravadivelu *et al*., 2006) in cotton, (El Sherif *et al*., 2011) in *Hibiscus Sabdariffa*.

The magnitude of salt-induced yield losses is dependent on different physiological and biochemical factors governed at different developmental stages of plants. 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 which are essentially required for the activities of enzymes, proteins synthesis and integrity of cell wall and plasma membrane (Taiz and Zeiger, 2006).

Gamma rays and salinity stress induced a considerable variation in the protein patterns of cowpea seeds. This variation has been manifested as the novel expression of some polypeptide, the absence of others and over expression of a third class polypeptides.

The protein bands which have molecular weights (102, 57, 49 and 37 kDa) were de novo synthesized in salinized cowpea seeds alone or in combination with gamma rays. It has been suggested that these proteins have an osmoprotection function or protected cellular structures. In this respect, Eman *et al*. (1992) showed the appearance of excess bands in leaf samples of *Zea mays* under salt stress. These findings indicated that these bands were salt inducible and could be involved in plant adaptation for growth under stress condition. Also, salt stress lead to difference in gene expressions where alterations in protein could be due to alteration in regulation of transcription, mRNA processing or due to altered rates of protein degradation. Also, Changes in protein synthesis under salinity and gamma rays treatments may be due to changes in the efficiency of mRNA translation or the regulation of RNA transcription transport and stability

The high concentrations of NaCl (100 mM) caused disappearance of some bands in cowpea seeds. These bands have molecular weights of 175, 67, 62, 54 and 41 kDa. One possible explanation for complete disappearance of some proteins under salt stress is that the gene (S) responsible for certain proteins might be completely suppressed as a result of stress. Therefore, the developed tissues had lost their ability to synthesize these proteins. It is also possible that the gene (S) had not been completely suppressed, but inhibited as the result of stress, and complete recovery of the inhibition was not achieved. This may apply to the protein that stained less densely under stress (Elobeidy *et al*., 2001).

Beltagi *et al*. (2006) studied the effect of gamma rays (2 and 32 Krad) on SDS PAGE proteins in salinized common bean plants. They found that, the low dose (2 Krad) of gamma radiation induced stability in the total number of protein bands of common bean and synthesis of certain polypeptides under the highest level (3000 ppm) of NaCl.

CONCLUSION

Soil salinity is an enormous production problem for vegetable crops as saline conditions are known to suppress plant growth under irrigation. Salt stress affects all the major processes such as growth, yields, photosynthesis, protein synthesis and lipid metabolism. To conclude the present study, gamma irradiation can use to alleviate the harmful effect of salt stress. Gamma irradiation can solve the production problems caused by high salinity.

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