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The Use of γ-Irradiation in Counteracting the Effect of Salinity for Cultivation of Barley and Pea Plants

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ABSTRAC

The biochemical changes induced by salinity in two economic plants (Barley and Pea) and the probable counteraction of gamma irradiation for enhancement of growth were studied. The data obtained revealed that the reduction in pigments content due to salinity treatment was more pronounced in pea plants than barley. However, gamma irradiation caused a significant increase in pigment content of both plants. The interaction effect of salinity and radiation varied from an increase in case of barley to a reduction in peas. In both plants, soluble sugars content increased due to salinity and/ or γ -radiation. Moreover, total carbohydrates increased due to the combined treatment. A matched increase in free proline content was recorded with increase of salinity. While, γ - irradiation showed a different trend. Protein and nucleic acids contents were proportionally decreased with increase of salinity levels, whereas gamma radiation induced an increase in both protein and nucleic acids content. A progressive reduction in the yield by increasing salinity was observed, while γ- irradiation increased the yield of both plants. $^{14}CO_2$ fixation was reduced by salinity treatment while γ- radiation increased it. Contrary to $^{14}CO_2$ fixation, salinity enhanced respiration, while radiation retarded it.

Key Words: Salinity / γ-radiation / Barley / Pea / Biochemical analysis.

INTRODUCTION

Salinity limits the growth of agricultural soil in large areas of the world, especially in countries like Egypt. Soils from Nile delta have different chemical property [1] of different degrees of salinity, and hence such soils are unfavorable for normal growth of plants. Thus, the restriction of plant growth and productivity caused by salinity is especially acute in arid and semi arid regions [2]. Because of the high cost of soil reclamation, research has been carried out for the development of plants that are salt tolerant representing the adapted strategies to minimize the impact of drought and salinity on rural community and on the stability of land resources [3]. The use of gamma radiation for the induction of such plants was reported by Abrifarine &Neil Rutager [4].

The aim of this work was to investigate the possible existence of biochemical changes induced by salinity in two economic plants (Barley v. Giza 124 and Pea v. lincoln) and the probable counteraction of γ-irradiation for the enhancement of growth.

MATERIALS AND METHODS

Plant material and condition for growth:

Plant: Grains of barley (*Hordeum vulgaris,var. Giza124*) and seeds of pea (*Pisum sativum,var,linclon)* were purchased from the Crop Institute Agriculture Research Center, Giza, Egypt.

Salt solution: Salt treatments were made according to the method described by Palmer [5]. The NaCl concentration was adjusted to give 1.55 bar, which is almost similar to that found in Sidi Salem region

(Kafer El-Sheikh Governorate, Egypt). Another two solutions were prepared by changing only NaCl cocentration to obtain 0.50 and 2.50 bar.

Gamma irradiation:

Irradiation of either seeds or grains was carried out at the Middle Eastern Regional Radioisotope Center, Dokki, Egypt, using ^{60}Co source with a dose rate 6.18 rad/sec. (3.7 Gy/ min).

Pots experiment:

Depending on the results of a preliminary laboratory experiment, plants were cultivated in pots under the effect of selected levels of salinity and/or selected doses of gamma radiation (10&50 Gy for barley and 10 Gy for pea). Four pots (Ø 25 cm) for each treatment were used, each pot contained a plastic bag filled with 7 Kg clay soil. The pots were irrigated depending on the water capacity with different saline solution to maintain the required levels of soil salinity [6].Grains of barley (10) and seeds of pea (10) were sown in each pot, after germination seedlings thinned to four plants. The irrigation was carried out according to Al-Akied, [7] maintaining the water holding capacity of the soil at 70%.

Sampling, yield and biochemical measurement:

Measurements were carried out at the onset of flowering. After sampling, all plants were dried at 80 ^{o}C for the biochemical determination except for yield and pigment determination where fresh samples were taken. For the biochemical analysis, sampling of plants was carried out at the onset of flowering, whereas some plants yield (plant productivity) was calculated depending on the number of spikes or fruit/ plant, number of grains or seeds/spike or fruit and weight of grains or seeds/plant.

Biochemical analysis:

The method of Metzznur et al [8] was used for the quantitative determination of pigments of plant leaves (chlorophyll" a", chlorophyll"b "and carotenoids. Soluble sugar was determined according to the method of Yemme and Willis [9]. The non-soluble sugars were determined according to the method of Abdel-Ghany [10]. Free proline was estimated according to the method of Singh etal [11] where protein determination was performed according to Naguib [12] and Lawery et al [13]. Nucleic acids determination was carried out according to Ashwell [14].

Carbon dioxide fixation, photosynthesis and respiration:

The plants were cultivated in plastic pots (Ø 7cm) under the effect of 1.55 bar salinity and/or γ-irradiation (50 Gy in case of barley and 10 Gy in case of pea) and the interaction of 1.55 bar and either 10 or 50 Gy. After 30 days, the plants were used to compare their activity for $^{14}CO_2$ assimilation using sodium bicarbonate (NaH $^{14}CO_3$) of original specific activity 23.2 MBq/mg, purchased from Radiochemical Laboratory, Amersham, England.

RESULTS AND DISCUSSION

The results presented in table (1) show that, chlorophyll"a",chlorophyll"b" and total pigments detected at different levels of salinity were reduced below those of the control plants. The reduction was observed to increase with increase in salinity levels up to 2.5 bar, the reduction in pigments content was more pronounced in pea plants where a salinity level of 2.5 bar led to incomplete growth of the plant. Contrary to the effect of salinity, gamma irradiation caused a significant increase in pigment content of both plants. Regarding the interaction of salinity and radiation, it was observed that the results varied from an increase in the case of barley to a reduction in peas. It has been found that salinity induced some increase in the concentration of chlorophyll and other photosynthetic pigments/unit area in barley grown in nutrient solution [15]. On the other hand, chlorophyll floresence, an analytical technique that monitors the function of the photosynthetic apparatus, has been suggested to change in response to water stress and salinity [16,17,18].

Table (1): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on pigments (mg/g f.wt.) of barley and pea plants.

Treatment		Barley							
		Chl."a"	%	Chl."b"	%	Carot.	%	Total	%
Control		0.78± 0.01	100	0.50± 0.00	100	0.29± 0.01	100	1.57	100
Salinity (bar)	0.5	0.71± 0.02	91.0	0.46± 0.02	92.0	0.28± 0.01	96.6	1.45	92.4
	1.55	0.60± 0.01	76.9	0.42± 0.01	84.0	0.27± 0.01	93.1	1.29	82.2
	2.5	0.52± 0.01	66.7	0.37± 0.11	74.0	0.13± 0.01	44.8	1.02	64.9
Radiation (Gy)	10	0.80± 0.06	102.6	0.51± 0.01	102.0	0.34± 0.03	117.2	1.65	105.1
	50	0.85± 0.06	108.9	0.58± 0.66	116.0	0.39± 0.01	134.5	1.82	115.9
Salinity + Radiation	0.5+10	0.81± 0.01	103.8	0.54± 0.01	108.0	0.31± 0.01	106.9	1.66	105.7
	1.55+50	0.75± 0.01	96.2	0.52± 0.02	104.0	0.31± 0.01	106.9	1.58	100.6
	2.5+50	0.70± 0.03	89.7	0.50± 0.05	100.0	0.29± 0.01	100.0	1.49	94.9
Treatment		Peas							
		Chl."a"	%	Chl."b"	%	Carot.	%	Total	%
Control		0.89+0.00	100	0.68+0.00	100	0.24+0.00	100	1.81	100
Salinity (bar)	0.5	0.80+0.02	89.9	0.61+0.03	89.7	0.21+0.00	87.5	1.62	89.5
	1.55	0.65+0.00	73.0	0.54+0.02	79.4	0.13+0.00	54.2	1.32	72.9
	2.5	Ig	Ig	Ig	Ig	Ig	Ig	Ig	Ig
Radiation (Gy)	10	1.03+0.05	115.7	0.80+0.03	117.6	0.30+0.04	125.0	2.13	117.7
Salinity + Radiation	0.5+10	0.88+0.03	98.9	0.62+0.00	91.2	0.23+0.01	95.8	1.73	95.6
	1.55+10	0.80+0.01	89.9	0.63+0.02	92.6	0.20+0.00	83.3	1.63	90.1
	2.5+10	Ig	Ig	Ig	Ig	Ig	Ig	Ig	Ig

±. Standard error.
Data are mean of three replicates.
Ig incomplete growth.

The data presented in table (2) illustrate that salinity decreased the total carbohydrates in barley plants, which might be due to the reduction in polysaccharides. On the other hand, soluble sugars fraction was found to increase by the increase in salinity levels up to 2.5 bar in case of barley. However, the same salinity level (2.5 bar) led to incomplete growth in peas. The effect of gamma irradiation showed an increase in soluble fraction from 10 to50 Gy, but the total carbohydrate values were lower than those of the corresponding controls. On the other hand, 50 Gy significantly increased the polysaccharides. The increment in total carbohydrates caused by radiation treatment was due to the net effect on both soluble sugars & polysaccharides. All combined treatments led to an increase in total carbohydrates, which was more obvious as the salinity and gamma dose increased.

In case of peas, salinity caused a significant increase in soluble sugars & a reduction in polysaccharides and total carbohydrates contents, the increment of radiation being parallel to salinity level (table2). A gamma dose of 10 Gy caused a remarkable increase in soluble sugar whereas polysaccharides were constantly reduced during the three growth stages. The combined effect of 10 Gy with either 0.5 or 1.55 bar salinity increased the total carbohydrates due to a significant increase in soluble sugars, while (2.5 bar + 10Gy) led to incomplete growth. In this connection, Turner etal [19] have suggested that the accumulation of soluble sugars may produce significant change in the osmotic pressure and the accumulation of soluble sugars could be a result of greater degree of conversion of starch into soluble sugar and/or to low sugar utilization. Yenkatesworh etal [20] proposed that high soluble sugars content may lower the soluble (solute) potential of ground nut nodule cells which could help them maintaing turgidity in a stress environment.

Table (2): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on carbohydrates content (mg/g. d.wt.) of barley and pea plants.

Treatment		Barley					
		Soluble.	%	Poly.	%	Total	%
Control		97.71± 1.40	100	113.21± 0.40	100	192.92	100
Salinity (bar)	0.5	88.35± 0.48	110.8	79.50± 0.33	70.2	185.85	96.3
	1.55	96.85± 0.44	121.5	85.09± 0.37	75.2	181.94	94.3
	2.5	110.60± 0.54	138.8	64.92± 0.28	57.3	175.52	90.9
Radiation (Gy)	10	82.33± 1.23	103.3	64.14± 0.99	56.7	146.49	75.9
	25	91.06± 0.97	114.2	66.23± 1.35	58.5	157.29	81.5
Salinity + Radiation	0.5+10	96.71± 0.96	121.3	89.94± 0.51	79.4	186.55	96.7
	1.55+10	101.50± 0.10	127.3	91.59± 0.39	80.9	193.09	100.1
	2.5+50	104.30± 0.39	130.8	94.59± 0.36	83.6	198.89	103.1
Treatment		Pea					
		Soluble.	%	Poly.	%	Total	%
Control		32.36±0.46	100	63.96±0.47	100	96.32	100
Salinity (bar)	0.5	35.47±0.06	109.6	49.92±0.15	78.0	85.39	88.7
	1.55	41.09±0.06	126.9	41.38±0.26	64.7	82.47	85.6
	2.5	Ig	-	Ig	-	Ig	-
Radiation (Gy)	10	48.31±0.23	149.3	48.55±1.21	75.9	96.86	100.6
Salinity + Radiation	0.5+10	41.16±0.08	127.7	56.77±0.52	88.4	97.93	101.7
	1.55+10	50.16±0.15	155.0	43.24±0.11	67.6	93.40	96.9
	2.5+10	Ig	-	Ig	-	-	-

Data are mean of three replicates.

± Standard error.

Ig Incomplete growth.

Table (3) illustrates the effect of salinity and/or gamma doses on free proline content in barley and pea plants. Under he effect of different salinity levels there was a parallel increase in proline content with the increase in salinity. The influence of gamma radiation showed a different trend, in case of pea plants, whereas an increase of 122% was recorded in case of pea plants (10 Gy).

In barley, treatment of 0.5 bar+10 Gy, 1.55 bar+ 50 Gy and 2.5 bar+ 50 Gy decreased proline content if compared with the effect of salinity, but the values were still higher than the corresponding control values. In peas, a combination of 10 Gy radiation dose with salinity of either 0.5 or 1.55 bar resulted also in a non- significant increase of proline than the control. However, the values were still lower than those obtained with salinity of either 0.50 or1.55 bar alone.

Table (3): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on proline content (μMol/g. f.wt.) of barley and pea plants.

Treatment		Barley			Treatment		Pea	
		Proline	%				Proline	%
Control		144±18.03	100		Control		90±2.00	100
Salinity (bar)	0.5	151±9.59	104.0		Salinity (bar)	0.5	95±0.88	105.0
	1.55	286±4.05	198.0			1.55	130±10.53	144.0
	2.5	345±9.45	239.0			2.5	Ig	-
Radiation (Gy)	10	137±11.10	95.0		Radiation (Gy)	10	110±2.26	122.0
	25	142±15.34	98.0					
Salinity + Radiation	0.5+10	144±6.50	100.0		Salinity + Radiation	0.5+10	91±3.88	101.0
	1.55+10	242±2.91	168.0			1.55+10	99±2.40	110.0
	2.5+50	275±12.88	190.0			2.5+10	Ig	-

Data are mean of three replicates.
± Standard error.
Ig Incomplete growth.

The results obtained seem to agree with those of Iqbal & Abobaker [21,22], who worked on Cucumber and Zea respectively. Total sugar and proline accumulation could provide the plant an osmotic pressure to prevent excessive water loss. In addition, proline concentration can also protect cell metabolism by avoiding protein denaturation and/or by controlling the cell pH [23]. Several possible functions have been ascribed to such imino acid accumulation induced by water shortage such as, osmoregulation,a sink for energy and nitrogen and an indicator of drought resistances [23].
Venekamp et al [24], related drought increase proline synthesis in *Vicia faba* with organic acid. They suggested that the sharp increase in leaf free proline accumulation did concide with the decline in soluble protein.

Data obtained in table (4) show that protein content was proportionally decreased with increase in salinity levels in both plant species.

Table (4): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on protein content (mg/g. d.wt.) of barley and pea plants.

Treatment		Barley			Treatment		Pea	
		Protein	%				Protein	%
Control		21.9±0.02	100		Control		27.7±0.03	100
Salinity (bar)	0.5	17.4±0.05	79.0		Salinity (bar)	0.5	24.3±0.01	89.0
	1.55	14.4±0.02	66.0			1.55	18.7±0.03	69.0
	2.5	11.8±0.02	54.0			2.5	Ig	-
Radiation (Gy)	10	22.9±0.03	104.0		Radiation (Gy)	10	29.5±0.03	106.5
	25	24.0+0.01	110.0					
Salinity + Radiation	0.5+10	19.4+0.07	89.0		Salinity + Radiation	0.5+10	24.9±0.09	89.9
	1.55+10	20.1±0.02	92.0			1.55+10	24.1±0.03	87.0
	2.5+50	15.3±0.02	70.0			2.5+10	Ig	-

Data are mean of three replicates.
± Standard error.
Ig Incomplete growth.

The 10 Gy dose used with barley did not cause any measurable increase while it recorded the highest value with peas. Yankulov etal [26], Chauban etal [27] and Farg [28] reported similar observations. The interaction effect of gamma radiation & salinity on protein content showed a remarkable increase when compared with the effect of different salinity levels alone, and a decrease when compared with the control samples.

Table (5) demonstrates the effect of salinity and/or γ radiation on the nucleic acids of barley & pea. Progressive reduction in DNA, RNA and total nucleic acids was observed with increasing salinity levels where the highest reduction was at 2.5 bar in barley and 1.55 bar in peas. On the other hand, a noticeable increase in DNA, RNA and total nucleic acids was related to gamma radiation.

Table (5): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on nucleic acids content (mg/g. d.wt.) of barley and pea plants.

Treatment		Barley					
		RNA	%	DNA	%	Total	%
Control		17.07± 0.26	100	1.35±0.06	100	18.42	100
Salinity (bar)	0.5	17.01± 0.08	99.6	1.09± 0.10	80.7	18.10	98.3
	1.55	14.92± 0.05	87.4	0.97± 0.04	71.9	15.89	86.2
	2.5	14.55± 0.12	85.2	0.82± 0.06	60.7	15.37	83.4
Radiation (Gy)	10	18.77± 0.03	109.9	1.41± 0.03	104.4	20.18	109.6
	25	19.43± 0.03	113.8	1.45± 0.05	107.4	20.87	1.3
Salinity + Radiation	0.5+10	17.31± 0.012	101.4	1.31± 0.06	97.0	18.62	101.1
	1.55+10	17.25± 0.02	101.1	1.16± 0.04	85.9	18.41	99.9
	2.5+50	15.35± 0.03	89.9	1.05± 0.04	77.8	16.40	89.0
Treatment		Peas					
		RNA	%	DNA	%	Total	%
Control		15.63±0.07	100	3.07±0.08	100	18.69	100
Salinity (bar)	0.5	13.92±0.26	89.1	2.89±0.02	94.1	16.82	89.9
	1.55	11.78±0.24	75.4	2.59±0.10	84.4	14.38	76.9
	2.5	Ig	-	Ig	-	Ig	-
Radiation (Gy)	10	19.04±0.03	121.8	3.18±0.03	103.6	22.22	118.9
Salinity + Radiation	0.5+10	14.87±0.27	95.1	3.07±0.05	100.0	17.94	95.9
	1.55+10	14.89±0.08	85.2	3.15±0.07	102..6	18.04	96.5
	2.5+10	Ig	Ig	Ig	-	Ig	-

Data are mean of three replicates.
± Standard error.
Ig Incomplete growth.

The combined treatment of salinity with radiation caused different degrees of reduction in DNA, RNA and total nucleic acids of both plants when compared with their controls. However, the values were higher when compared with the effect of corresponding salinity levels alone. Earlier results obtained in the literature showed some pattern of increase in nucleic acids of different plant species as affected by X- ray or γ irradiation [30&31].

The effect of different levels of salinity (bar) and/or different doses of γ irradiation (Gy) on the yield (g/plant) of barley and peas is demonstrated in tables (6). It can be seen that there was a progressive reduction in the yield by increasing salinity, which may be attributed to the reduction in photosynthetic capacity or altered metabolism of all cell expansion [32&33].

Table (6): The effect of different levels of salinity (bar) and/or different doses of gamma radiation (Gy) on the yield (g/plant) of barley (A) and pea (B) plants.

(A)

Yield	Control	Salinity (bar)			Radiation (Gy)		Salinity + Radiation		
		0.5	1.55	2.5	10	50	0.5+10	1.55+50	2.5+50
g/plant	9.81 ±1.54	8.67 ±1.10	7.55 ±1.56	5.24 ±0.95	11.69 ±1.32	12.71 ±1.71	10.69 ±0.93	11.49 ±1.01	8.95 ±0.95
%	100	88.4	76.9	53.4	119.2	129.6	108.9	117.1	91.2

(B)

Yield	Control	Salinity (bar)			Radiation (Gy)	Salinity + Radiation		
		0.5	1.55	2.5	10	0.5+10	1.55+10	2.5+10
g/plant	8.83 ±1.23	5.64 ±0.91	2.92 ±0.09	Ig	12.20 ±1.52	8.91 ±0.91	9.12 ±1.01	Ig
%	100	63.9	33.1	-	138.2	100.9	103.0	-

Data are mean of 12 replicates
± standard error
Ig Incomplete growth

The results of $^{14}CO_2$ fixation(table 7) show that the capacity of pea plants for $^{14}CO_2$ fixation was higher (46.9%) than that of barley. Salinity treatment resulted in a reduction of $^{14}CO_2$ fixed by both plants. However, 1.55 bar was more suppressive on pea plants by (39.4%) of the controls than on barley (19.5%). On the other hand, gamma radiation treatment increased the capacity of both plants fore$^{14}Co_2$ fixation, the increment of 14.9% and 11.2% was recorded in case of pea and barley plants respectively.

Table (7) the effect of soil salinity (1.5 bar) and/or gamma radiation (Gy) on the photosynthetic activity as indicated by$^{14}CO_2$fixation for 30 min. (dpm/mg f.wt) of barley and pea plants.

Plants	Control	Salinity(1.5 bar)	*Radiation (Gy)	Salinity + radiation
Barley	1144000	932000	1272000	1177000
Pea	1680000	1018000	1930000	1782000

*50 Gy in barley & 10Gy in pea

The interaction effect of salinity and radiation showed a recovery of the inhibitory effect of salinity as indicated by a slight increase than the control values. The results are in agreement with those obtained by [36 & 37]. The lesser suppressive effect of salinity on barley plants may be attributed to their relative tolerance to grow in saline soils.

The rate of respiration under salinity of 1.55 bar was much higher than all other treatment. No noticeable effect was observed between radiation and combined treatment of radiation and salinity although the rate of respiration was slightly inhibited than control by both treatments. It can be concluded that an increase in respiration rate was decreased in this study due to γ -irradiation of barley and pea plants. It is worth to mention that, potato [39] apple [40] and cucumber [41] showed an increase of photosynthetic capacity when the plants were exposed to different levels of irradiation.

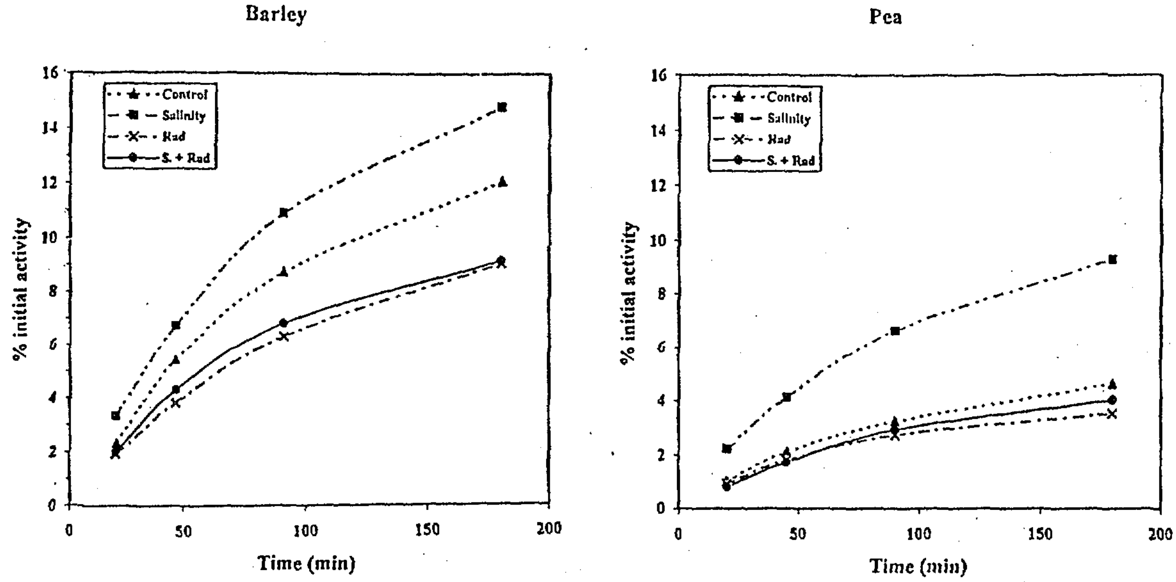

Fig. ( ): Cumulative % $^{14}CO_2$ evolution of initial activity.

In summary it can be seen that γ- irradiation can, to some extent, counteract the inhibitory effect of salinity on cultivation and productivity of barley and pea plants and this treatment can be adopted for cultivation of vast regions in Egypt.

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