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BOTANICAL STUDIES ON *PISUM SATIVUM* L. UNDER THE EFFECT OF γ-IRRADIATED SEEDS AND IRRIGATION WITH DIFFERENT SEA WATER SALINITY

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ABSTRACT

Two pot experiments were conducted on pea plants cv. Master B during the two successive winter growing seasons of 2008 and 2009 to evaluate the efficiency of pre-sowing seed exposure to different γ-irradiation doses; different sea water salinity levels beside tap water as a control (for irrigation) and their interactions effect on plant morphology, plant physiology as well as leaf anatomy.

Increasing γ-irradiation dose up to 3 K-rad significantly increased plant height, number of leaves/plant, dry weight of plant, leaf tissue contents of chlorophyll (a and b), carotenoids and total chlorophyll. Also, blade thickness, palisade tissue, spongy tissue and midvein bundle of leaves were gradually increased under irradiation levels up to 3 k-rad. Further increases in γ-ray dose up to 7 K-rad decreased all the above-mentioned characters. Proline content and catalase activity in leaf tissues were linearly increased with increasing irradiation up to the highest tested dose of 7 K-rad.

Gradual decreases in plant morphology and leaf anatomy characters were recorded with increasing salinity concentration up to the highest tested level of 6000 ppm. On contrary, proline percentage and catalase activity in leaf tissues exhibited opposite trend with increasing salinity level up to 6000 ppm. The best beneficial interaction treatment was pre-sowing treating pea seeds by 3 K-rad γ-irradiation × irrigation by tap water (control).

Keywords: Pea (*Pisum sativum* L.), γ-irradiation, sea water salinity, morphology, physiology, anatomy.

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INTRODUCTION

Pea (*Pisum sativum* L.) is popular vegetable and one of the most important legume crops for local consumption in Egypt. It is widely used in human diets due to its high content of protein, ascorbic acid, carbohydrates, balanced amino acids and good digestibility. Generally, Pea seeds contain 18-20% dry matter, 10-12% carbohydrate and 5-8% protein (Vural *et al.*, 2000). Pea is used as a fresh vegetable, frozen or canned. According to FAO (2004), about 12.2 million ton of pea production were achieved from 6.3 million hectare (ha) with an average yield of 1930 kg ha^{-1}.

Irrigation water is one of the major resources that limits agricultural development especially at arid and semi-arid zones including Egypt.

Water salinity is a major problem that negatively impacts agricultural activities especially at arid and semi-arid regions. According to Munns (2002), increasing irrigation water salinity inhibited plant growth through its osmotic stress, nutritional imbalance, and/or specific ion toxicity.

Salinity greatly suppressed plant growth parameters expressed as height, number of leaves and stems/plant as well as dry matter accumulation (Khedr *et al.*, 2005; Tantawy, 2007 and Tantawy *et al.*, 2009). This is due to specific toxic effect of the accumulated Na and Cl, nutrition imbalance and hyper osmotic effect which lead to sever dehydration within plant tissues (Liu and Zhu, 1998).

Gamma irradiation has been widely applied in medicine and biology at low doses as a stimulator or at high-doses as an inhibitor (Charbaji and Nabulsi, 1999). On *Scilla indica* Chakravarty and Sen (2001) found that, low-doses of ionizing irradiation accelerated cell proliferation, cell growth, enzyme activity and increased stress resistance. Also, pre-sown seed irradiation with gamma ray at 1000 R significantly increased tomato plant height, shoot fresh weight, total yield/plant, average fruit weight and average fruit number/ plant, while increasing radiation dose over than 1000 R resulted in significant reduction in the above mentioned traits (Badr *et al.*, 1997). In addition, gamma ray at different doses has been reported to induce morphological, anatomical, biochemical and physiological behavior in plant

tissues (Wi *et al.*, 2005). Also, changes in carbohydrates metabolism were noticed in banana tissues under the effect of gamma irradiation (El-Fiki *et al.*, 2003).

The aim of this study was to investigate the effect of pre-sown seed irradiation with γ-ray, irrigation with sea water at different dilution levels and their interactions on pea plant growth as well as its morphological, physiological and anatomical characteristics.

MATERIALS AND METHODS

The present work was carried out during two successive winter growing seasons of 2008 and 2009 under wire-house of the Agric. Botany and Plant Path. Dept., Fac. Agric., Zagazig University, Egypt.

Seed source: Seeds of pea cv. Master B were obtained from Hort. Res. Inst., Agric. Res. Center, Ministry Agric., Giza, Egypt.

Seed irradiation: Pea Seeds were exposed to 0.0, 0.5, 1.0, 3.0, 5.0, and 7.0 K-rad using Gamma Cell 220, Model G.C.220 Type B. Atomic energy of Cobalt 60 at dose rate of 17.25 rad/second at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Sea water: Sea water (51.56 dSm^{-1} EC) was obtained from Suez Canal, Pilagat road, near 25 January City, Ismailia governorate, Egypt. Tap water (500 ppm) was used as control and to dilute sea water to be contains 1500, 2000, 4000 and 6000 ppm salinity levels.

Experimental Design and Treatments

A factorial (6 X 5) experiment in randomized complete block design with three replicates was used. So, the experiment was included 30 treatments, which were the combination between five γ-irradiation doses (0.5, 1.0, 3.0, 5.0 and 7.0 K-rad beside un-irradiated seeds as control) and four levels of irrigation sea water (1500, 2000, 4000 and 6000 ppm salinity beside tap water which containing 500 ppm salinity as a control). Each replicate contained three pots.

Plastic pots 30 cm diameter (40 cm height) contain 10 kg air-dried clay loam soil were used in this experiment. The physical and chemical analysis of used soil during the two tested seasons were clay loam in texture, with 7.5 and 7.7 pH, 1.80 and 1.86 dSm^{-1} (EC), 0.04% and 0.04% total N, 0.004 and 0.005 % total P, 0.033 and

0.022% total K as well as 30.0 and 30.2 (F.C.) during the 1st and 2nd seasons, respectively.

Ten pea seeds were sown per pot on 20th October in both experimental seasons. All treatments were irrigated immediately after sowing with tap water (non saline water). Then, irrigation with the above mentioned design sea water treatments was done when it was necessary to kept the soil moisture at 50% of field capacity. Twenty days after sowing, germinated seedlings were thinned to remain three uniform seedlings/pot.

All treatments received the recommended NPK fertilizer rates as forms of: urea (46.5% N) at a rate of 40 kg/fed (0.8 g/pot), calcium superphosphate (15.5% P_2O_2) at a rate of 200 kg/fed (4 g/pot) and potassium sulphate (48% K_2O) at a rate of 50 kg/fed (1 g/pot). All phosphorus quantity, half quantity of K and one third quantity of N fertilizers were added during soil preparation just before sowing, whereas, the rest amount of K fertilizer was added 45 days after sowing and the other two thirds of N were divided into two equal portions and added at 25 and 50 days after sowing. The other normal agricultural practices of pea plants were carried out as commonly followed in the district.

Chemical analyses of irrigation water were: 1.42 and 51.56 dSm^{-1} (EC), 7.8 and 8.36 pH of the used tap water and sea water, respectively.

Data Recorded

Sixty days after sowing for the two experimental seasons (on 20th December) random samples were taken for each treatment to record all studied measurements which included the following morphological, physiological and anatomical characters:

Morphological Characters

1. Plant height (cm) and number of leaves/plant.
2. Dry weight of the various plant organs (g) was estimated according to the method of AOAC (1970). Then dried leaf samples were finely ground and kept for proline determination.

Physiological Characters

Photosynthetic pigments

Samples were taken from the third upper leaf of pea plants for each treatment, and extracted according to Fadeel's Method (Fadeel, 1962). Determinations were done using "Spectronic-20"

spectrophotometer at 662, 644 and 440.5 nm for chlorophylls a, b and carotenoids, respectively. Then obtained data were calculated according to Wettsteins formula (Wettestein, 1957).

Proline content

Leaf tissue content of proline was determined in dried finely ground leaf samples according to the method described by Bates *et al.* (1973) using ninhydrin and glacial acetic acid at 520 nm by Spectrophotometer.

Catalase activity

The method described by Feinstein (1949) was used for determination catalase activity in the upper second fresh leaf of pea plants.

Anatomical Studies

Anatomical studies were carried out only in the second season to follow changes occurred in pea leaves resulted from the studied treatments.

Anatomical leaf samples were taken from the third node of the main stem, then were cleaned using tap water, dried, cut into small pieces, killed, fixed in F.A.A. solution, dehydrated in different concentrations of ethyl alcohol, clearing in different concentrations of ethyl alcohol + xylene, infiltrated and embedded in pure paraffin wax (M.P. 58-60°C) as mentioned by Johansen, (1940). Then, leaves sectioning at thickness of 20μ. was performed using rotary microtome. Paraffin ribbons were mounted on slides and sections stained with safranin and fast green (Corgen and Widmayer, 1971). Then sections were mounted in canda balsam and examined microscopically and microphotographed as follows: Midvein bundle thickness, palisade tissue thickness, spongy tissue thickness and blade thickness.

Statistical Analysis

Obtained data were statistically analyzed according to Snedecor and Cochran (1982), and means separation was done according to L.S.D. at 5% level of significance.

RESULTS AND DISCUSSION

Morphological Characters

Plant height and number of leaves/plant

Data in Table 1 showed that, there were significant increases in both plant height and number of leaves / plant due to pre-sowing

Table 1. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on morphological characters at 60 days after sowing at two seasons 2008 and 2009

Characters	Plant height (cm)							Number of leaves/plant						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
							1st season (2008)							
Tap water	39.33	42.33	42.67	42.00	38.00	36.67	40.17	15.33	17.00	20.00	21.00	12.67	11.67	16.28
1500	38.00	38.67	40.33	41.33	37.33	36.33	38.67	15.33	16.67	19.00	20.00	12.33	11.00	15.72
2000	36.33	36.67	39.33	41.00	34.33	31.33	36.50	14.33	14.33	18.00	18.33	12.00	10.33	14.56
4000	30.00	30.00	38.33	37.33	27.00	25.33	31.33	12.67	13.33	15.00	16.33	11.00	9.67	13.00
6000	21.33	21.33	23.00	22.67	20.33	19.33	21.33	11.00	12.00	12.67	14.00	9.33	9.00	11.33
Mean	33.00	33.80	36.73	36.87	31.40	29.80	----	13.73	14.67	16.93	17.93	11.47	10.33	----
LSD. for	(I) : 0.90		(S): 0.82		(I) × (S): 2.01			(I) : 0.71		(S): 0.65		(I) × (S): 1.58		
							2nd season (2009)							
Tap water	44.67	46.00	46.67	47.00	37.67	35.67	42.94	16.00	16.67	20.00	22.00	14.33	13.00	17.00
1500	41.33	42.00	42.33	42.33	36.33	35.33	39.94	15.67	16.33	19.67	21.00	13.67	12.67	16.50
2000	37.67	38.33	40.00	40.33	34.00	33.00	37.22	14.67	15.67	18.67	20.00	12.67	11.33	15.50
4000	30.33	31.33	31.33	32.00	28.33	26.00	29.89	12.67	14.33	17.00	18.00	11.00	9.00	13.67
6000	21.67	22.67	23.67	24.67	19.33	19.33	21.89	12.00	13.00	14.33	15.67	9.33	8.67	12.17
Mean	35.13	36.07	36.80	37.27	31.13	29.87	----	14.20	15.20	17.93	19.33	12.20	10.93	----
LSD. for	(I) : 0.92		(S): 0.84		(I) × (S): 2.05			(I) : 0.60		(S): 0.55		(I) × (S): N.S		

N.S.: Non significant at 0.05 levels of significance.

treatment of pea seeds with γ-irradiation up to 3 K-rad, while further increase in irradiation dose (5 or 7 K-rad) resulted opposite effects. These results were confirmed during the two study seasons. In addition, no significant difference was found neither between 1 and 3 K-rad in the two seasons nor between the control and 0.5 K-rad in the first season on plant height. Similar stimulation effect was previously reported by Badr *et al.* (1978 and 1997) on tomato plant under the effect of low γ-irradiation doses. These stimulation effects might be attributed to the increased rate of cell division and cell enlargement (Holsten *et al.*, 1965). Al-Safadi and Simon (1990) stated that, cell size of carrot plant increased at all γ-ray doses between 0.5 to 4.0 K-rad. On contrary, Bhat *et al.* (2006) on *vicia faba*, mentioned that plant height decreased with increasing rates of γ-irradiation. Also, Shukla and Datta (1993) recorded reductions in plant height, branches and leaves number/ plant when root cuttings of chrysanthemum were irradiated with gamma rays at 1.5, 2 or 2.5 k-rad.

Presented data in Table 1 cleared that, increasing salinity level in irrigation water significantly decreased pea plant growth represented as height and number of leaves/ plant in the two seasons. Water salinity level at 1500 ppm had no significant comparing to control treatment respecting leaves number/plant. Similar inhibition effect under salinity conditions on tomato plant growth characters were previously reported by Khedr *et al.* (2005); Hajer *et al.* (2006); Tantawy (2007) and Tantawy *et al.* (2009). Also, Sarg (1998) on spinach (cv.Salonikey) found that number of leaves per plant and plant height were decreased as salinity of irrigation water was increased. As suggested by Bernstein (1963) and Cusido *et al.* (1987), plant growth suppression under saline conditions may be due to osmotic reduction in water availability or to excessive accumulation of Na and Cl in plant tissues.

The interaction between γ-irradiation rates and water salinity levels (Table, 1) showed significant effect on plant height in both seasons of study. The interaction treatments of 0.5, 1 and 3 K-rad × control water irrigation recorded the maximum with no significant differences within them, whereas the minimum ones were obtained by the interaction between 7 K-rad × 6000 ppm

irrigation water salinity values of plant height and number of leaves/plant. However, low irradiation doses of 1 or 3 K-rad tended to overcoming salinity deleterious effects since, under the same salinity level, exposed seeds to 1 or 3 K-rad gamma doses, generally, resulted plants with more height and leaves number/plant comparing to plants resulted from seeds exposed to lower or higher gamma doses. This was true during the two seasons.

Roots, stems, leaves and whole plant dry weight

Treated pea seeds before sowing with γ-irradiation at rates of 0.5, 1 and 3 K-rad significantly increased roots, stems, leaves and whole plant dry weight comparing to control treatment (Tables 2 and 3). These results hold true in both seasons of study. Results clearly indicated also that, the highest dry weight of different plant organs were recorded with 3 K-rad, while further increase in irradiation rates (5 and 7 K-rad) significantly decreased dry weights. Present results can be accompanied with results of Naheed *et al.* (2010) on *Vigna radiata* L. and Moussa and Jaleel (2010) on *Trigonella foenum-graecum*, they noticed increases in shoot fresh weight of winged bean at low irradiation doses, while decreases at high doses. Similarly, Kon *et al.* (2007) recorded a decline in shoot dry weight of beans under exposition of high γ-irradiation doses.

For salinity effects, dry weight of roots, stems, leaves and total dry weight/pea plant were significantly decreased by increasing irrigation water salinity levels up to the highest tested level of 6000 ppm. The low salinity concentration of 1500 ppm had no significant effects on plant dry weights comparing to control treatment in most cases. In general, maximum values of plant dry weight were recorded under control treatment followed by 1500 ppm salinity level (Tables 2 and 3).

Nour *et al.* (2010) reported that, increasing salinity level from 0 up to 100 mM significantly decreased tomato plant dry weight. These results agree with those reported by Hajer *et al.* (2006) on tomato cultivars, Eraslan *et al.* (2007) on lettuce and Jamil *et al.* (2007) on sugar beet. They concluded that shoots and roots fresh and dry weights were decreased as irrigation water salinity was increased.

Dry weight of roots, stems, leaves/plant (Tables 2 and 3) were significantly affected by the interaction between pre-sowing

Table 2. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on morphological characters at 60 days after sowing at two seasons 2008 and 2009

Characters	Roots dry weight/ plant (g)							Stems dry weight/ plant (g)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
						1st season (2008)								
Tap water	0.506	0.607	0.639	0.641	0.508	0.403	0.551	1.057	1.113	1.187	1.190	1.020	0.903	1.078
1500	0.466	0.521	0.556	0.580	0.452	0.369	0.491	1.013	1.077	1.170	1.160	0.990	0.890	1.050
2000	0.418	0.451	0.530	0.571	0.424	0.334	0.455	0.973	1.023	1.057	1.090	0.957	0.853	0.992
4000	0.286	0.297	0.427	0.508	0.245	0.207	0.328	0.913	0.930	0.973	1.023	0.883	0.763	0.914
6000	0.243	0.277	0.284	0.356	0.203	0.185	0.258	0.850	0.880	0.903	0.963	0.760	0.683	0.840
Mean	0.384	0.431	0.487	0.531	0.366	0.300	----	0.961	1.005	1.058	1.085	0.922	0.819	----
LSD. for	(I) : 0.02		(S): 0.01		(I) × (S): 0.04			(I) : 0.01		(S): 0.01		(I) × (S): 0.03		
						2nd season (2009)								
Tap water	0.606	0.670	0.681	0.723	0.558	0.387	0.604	1.130	1.170	1.183	1.220	1.113	1.027	1.141
1500	0.604	0.650	0.661	0.707	0.550	0.376	0.591	1.117	1.160	1.173	1.207	1.103	1.007	1.128
2000	0.584	0.610	0.633	0.673	0.514	0.370	0.564	1.097	1.117	1.147	1.183	1.090	0.977	1.102
4000	0.417	0.463	0.507	0.625	0.567	0.301	0.480	0.973	1.003	1.050	1.150	1.000	0.897	1.012
6000	0.338	0.388	0.442	0.450	0.330	0.229	0.363	0.900	0.930	0.983	1.027	0.860	0.727	0.904
Mean	0.510	0.556	0.585	0.635	0.504	0.333	----	1.043	1.076	1.107	1.157	1.033	0.927	----
LSD. for	(I) : 0.04		(S): 0.03		(I) × (S): 0.08			(I) : 0.01		(S): 0.01		(I) × (S): 0.03		

N.S.: Non significant at 0.05 levels of significance.

Table 3. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on morphological characters at 60 days after sowing at two seasons 2008 and 2009

Characters	Leaves dry weight/ plant (g)							Total dry weight/ plant (g)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
							1st season (2008)							
Tap water	2.013	2.043	2.127	2.120	1.803	1.763	1.978	3.57	3.76	3.95	3.95	3.33	3.07	3.61
1500	2.003	2.027	2.117	2.117	1.783	1.747	1.966	3.48	3.65	3.84	3.86	2.89	3.01	3.46
2000	1.913	1.960	2.013	2.047	1.723	1.690	1.891	3.33	3.43	3.60	3.72	3.10	2.88	3.34
4000	1.823	1.837	1.900	1.930	1.633	1.537	1.777	3.02	3.06	3.30	3.46	2.76	2.51	3.02
6000	1.633	1.693	1.773	1.787	1.577	1.430	1.649	2.73	2.84	2.96	3.10	2.54	2.30	2.74
Mean	1.877	1.912	1.986	2.000	1.704	1.633	----	3.23	3.35	3.53	3.62	2.92	2.75	----
LSD. for	(I) : 0.02		(S): 0.02		(I) × (S): 0.04			(I) : 0.08		(S): 0.07		(I) × (S): N.S		
							2nd season (2009)							
Tap water	1.977	1.997	2.420	2.470	1.597	1.460	1.987	3.71	3.84	4.28	4.74	3.27	2.88	3.79
1500	2.013	2.007	2.290	2.463	1.587	1.453	1.969	3.73	3.82	4.16	4.37	3.24	2.84	3.75
2000	1.940	1.993	2.237	2.390	1.507	1.407	1.912	3.62	3.72	4.08	4.25	3.11	2.75	3.59
4000	1.903	1.927	2.247	2.273	1.567	1.283	1.867	3.30	3.39	3.80	3.72	2.80	2.48	3.25
6000	1.563	1.757	1.943	2.097	1.220	1.160	1.623	2.80	3.07	3.37	3.57	2.41	2.12	2.89
Mean	1.879	1.936	2.227	2.339	1.495	1.353	----	3.43	3.57	3.94	4.13	2.96	2.68	----
LSD. for	(I) : 0.05		(S): 0.05		(I) × (S): N.S			(I) : 0.13		(S): 0.12		(I) × (S): N.S		

N.S.: Non significant at 0.05 levels of significance.

seed irradiation rates and water irrigation salinity levels in both tested seasons, except leaves dry weight in the second one. While, total plant dry weight did not significantly affect by such interaction in the two seasons of study. The most favorable beneficial interaction treatment regarding dry weight of pea plant, roots, stems, leaves as well as total plant dry weight was pre-sowing treating pea seeds by 3 K-rad γ-irradiation × irrigation by tap water (control). On the contrary, the least dry weight values of pea plants were recorded under the combination treatment of high γ-irradiation dose 7 K-rad × high irrigation salinity level (6000 ppm). Generally, low irradiation doses (1 or 3 K-rad) exhibited an enhancing effect on plant growth, under salinity conditions, determined as dry weights of different plant organs.

Physiological Characters

Photosynthetic pigments

Results presented in Tables 4 and 5 reflected a significant favorable effect on chlorophyll a, b, (a+ b) and carotenoids in pea leaf tissues due to pre-sowing seed treatment with γ-irradiation at low doses of 0.5, 1 or 3 K-rad. The highest scores for the tested leaf pigments content, *i.e.* chlorophyll a, b, (a+ b) and carotenoids were recorded by exposure pea seeds to 3 K-rad γ-irradiation as compare to control or the higher doses of 5 or 7 K-rad in both seasons. The obtained results are in harmony with those reported by Naguib *et al.* (2007) on *Tagetes erecta* and Khalaf (2008) on *Amaranthus caudatus*. They concluded that, gamma irradiation increased photosynthetic pigments in leaves tissue.

Respecting salinity effects, presented data in Tables 4 and 5 show that photosynthetic pigments, *i.e.* chlorophyll a, b, (a + b) and carotenoids content in pea leaf tissues were significantly and adversely affected by different diluted sea water application containing more than 1500 ppm salinity. Data indicated that 1500 ppm water salinity level had no significant in this respect comparing to control treatment in most cases. These results agree with those reported by Hajer *et al.* (2006) on tomato cultivars, Najafi *et al.* (2006) on pea and Sun Jin *et al.* (2008) on two spinach cultivars (Helan number.3 and Yuanye). They found that photosynthetic pigments of leaf tissue reduced with increasing sea water salinity levels.

Table 4. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on chlorophyll a & chlorophyll b content at 60 days after sowing at two seasons 2008 and 2009

Characters	Chlorophyll a (mg/g. Fresh weight)							Chlorophyll b (mg/g. Fresh weight)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
							1st season (2008)							
Tap water	3.08	3.11	3.27	3.37	3.07	2.98	3.15	1.88	1.89	1.99	2.07	1.88	1.73	1.90
1500	3.04	3.09	3.24	3.36	3.04	2.84	3.10	1.91	1.88	1.99	2.07	1.87	1.72	1.90
2000	2.97	2.97	3.07	3.24	2.97	2.76	3.00	1.80	1.82	1.92	1.94	1.76	1.72	1.83
4000	2.61	2.66	2.73	2.73	2.55	2.28	2.59	1.75	1.75	1.77	1.82	1.70	1.65	1.74
6000	2.19	2.20	2.37	2.38	2.11	2.01	2.21	1.71	1.74	1.76	1.80	1.64	1.56	1.70
Mean	2.77	2.81	2.93	3.02	2.75	2.57	-----	1.81	1.82	1.89	1.91	1.77	1.67	----
LSD. for	(I) : 0.02		(S): 0.02		(I) × (S): 0.04			(I) : 0.02		(S): 0.02		(I) × (S): 0.04		
							2nd season (2009)							
Tap water	3.27	3.34	3.85	3.88	3.07	2.91	3.39	1.99	2.00	2.09	2.11	1.96	1.90	2.01
1500	3.26	3.32	3.71	3.73	3.01	3.19	3.37	1.97	1.98	2.05	2.07	1.96	1.90	1.99
2000	3.00	3.13	3.40	3.64	2.93	2.83	3.16	1.87	1.93	1.97	1.98	1.87	1.82	1.90
4000	2.80	2.80	2.86	2.87	2.73	2.46	2.75	1.75	1.76	1.83	1.88	1.81	1.72	1.79
6000	2.25	2.31	2.50	2.65	2.15	2.01	2.31	1.65	1.68	1.75	1.82	1.65	1.55	1.68
Mean	2.92	2.98	3.26	3.35	2.78	2.68	----	1.85	1.87	1.94	1.97	1.85	1.77	----
LSD. for	(I) : 0.09		(S): 0.08		(I) × (S): 0.21			(I) : 0.02		(S): 0.02		(I) × (S): N.S		

N.S.: Non significant at 0.05 levels of significance.

Table 5. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on chlorophyll a + b & carotenoids content at 60 days after sowing at two seasons 2008 and 2009

Characters	Chlorophyll a + b (mg/g. Fresh weight)							Carotenoids (mg/g. Fresh weight)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
							1st season (2008)							
Tap water	4.96	5.00	5.26	5.38	4.95	4.71	5.04	3.15	3.17	3.26	3.26	3.14	3.10	3.18
1500	4.95	4.98	5.23	5.37	4.92	4.56	5.00	3.14	3.15	3.20	3.23	3.13	3.10	3.16
2000	4.77	4.79	4.99	5.17	4.73	4.48	4.82	2.80	2.80	2.87	2.92	2.72	2.63	2.79
4000	4.35	4.41	4.51	4.55	4.25	4.33	4.40	2.62	2.63	2.76	2.78	2.48	2.46	2.62
6000	3.90	3.93	4.13	4.51	3.76	3.57	3.97	2.31	2.31	2.46	2.53	2.21	2.16	2.33
Mean	4.59	4.62	4.82	5.00	4.25	4.33	---	2.80	2.81	2.91	2.94	2.74	2.69	---
LSD. for	(I) : 0.11		(S): 0.10		(I) × (S): N.S			(I) : 0.02		(S): 0.01		(I) × (S): 0.04		
							2nd season (2009)							
Tap water	5.26	5.34	5.74	5.99	5.03	4.81	5.36	3.20	3.21	3.36	3.37	3.14	3.11	3.23
1500	5.23	5.29	5.76	5.80	5.31	4.76	5.36	3.20	3.20	3.36	3.53	3.13	3.11	3.25
2000	4.87	5.06	5.52	5.62	4.80	4.65	5.09	2.88	2.89	2.95	2.97	2.84	2.79	2.89
4000	4.56	4.56	4.69	4.75	4.51	4.32	4.56	2.57	2.58	2.68	2.81	2.46	2.40	2.58
6000	3.90	3.99	4.25	4.47	3.81	4.58	4.00	2.38	2.38	2.39	2.43	2.31	2.25	2.35
Mean	4.76	4.85	5.19	5.32	4.69	4.42	---	2.85	2.85	2.95	3.02	2.78	2.73	---
LSD. for	(I) : 0.10		(S): 0.09		(I) × (S): 0.22			(I) : 0.04		(S): 0.04		(I) × (S): 0.09		

N.S.: Non significant at 0.05 levels of significance.

Interaction between irradiation doses and sea water salinity levels significantly affected pigments content *i.e.* chlorophyll a, b, (a+ b) and carotenoids in leaf tissues in most cases in the two seasons of study (Tables 4 and 5). In general, the highest values in this respect were recorded in leaf tissues of treated plants with 0.5, 1 or 3 K-rad γ-ray X tap water irrigation during the two seasons. However, γ-irradiation at low doses (0.5, 1 or 3 K-rad) exhibited an enhancing effect on leaf pigments synthesis under salinity stress, while high gamma doses recorded opposite trend. This result was confirmed during the two tested seasons.

Proline content and catalase activity

From data in Table 6 it is clear that as tested γ-irradiation dose increased, proline content and catalase activity in pea leaf tissues increased up to the highest dose of 7 K-rad during the two seasons. Maximum values of proline content (16.12 and 14.41 µm/mg dw) and catalase activity (248 and 250 µm/g/min) in leaves (in first and second seasons, respectively) were recorded in leaves of plants resulted from seeds treated with the high γ-ray dose of 7 K-rad. These results are in agree with those reported by Ahmed (1997) who found that exposing rice to gamma rays significantly increased proline content in plants tissue. Also, Aly (2000) on potato and Moussa (2008) on faba bean recorded increases in catalase activity under the effect of increasing γ-rays doses.

Data in Table 6 showed that, irrigation pea plants with different salinity levels significantly increased proline content as well as catalase activity in pea leaves. The highest values of proline content and catalase activities were obtained with 6000 ppm salinity level in both seasons of study. According to Chowdhury *et al.* (1993), changes in proline content within plant leaves have been correlated with their ability to tolerate or adapt to saline conditions. Obtained results are in agreement with those reported by Li-Huizhen *et al.* (2006) on potato and Eraslan *et al.* (2008) on spinach. They found that salinity increased proline concentration in plants. Lechno *et al.* (1997) on cucumber; Panda and Khan (2003) on rice concluded that activity of catalase was increased in leaves of plants subjects to salinity stress.

Interaction between γ-irradiation doses and sea water salinity levels

Table 6. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on physiological characters at 60 days after sowing at two seasons 2008 and 2009

Characters	Proline content (µm/mg/Dry weight)							Catalase (µm/g/min)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
							1st season (2008)							
Tap water	9.16	9.15	9.56	9.58	13.17	15.06	10.95	199	201	217	218	228	230	215
1500	9.29	9.25	9.66	9.95	13.80	15.22	11.19	202	213	220	221	230	230	219
2000	10.25	10.26	10.62	11.02	14.15	15.97	12.05	202	213	221	221	231	241	222
4000	14.83	14.80	14.88	14.91	15.71	16.89	15.34	229	229	239	240	255	261	242
6000	16.08	16.84	16.98	17.06	17.05	17.68	17.07	253	256	258	258	269	279	262
Mean	12.07	12.06	12.34	12.50	14.78	16.12	----	217	223	231	231	242	248	----
LSD. for	(I) : 0.20		(S): 0.19		(I) × (S): 0.46			(I) : 1.68		(S): 1.53		(I) × (S): 3.75		
							2nd season (2009)							
Tap water	8.81	8.79	9.13	9.35	10.17	11.77	9.67	197	197	205	212	225	233	211
1500	9.01	8.99	9.45	9.58	10.94	11.99	9.99	199	203	209	214	225	241	215
2000	9.64	9.60	9.74	9.74	11.01	12.08	10.30	198	204	218	220	226	241	218
4000	15.00	14.97	15.00	15.05	16.08	18.06	15.69	239	246	247	248	258	261	250
6000	16.29	16.26	16.56	16.67	17.95	18.13	16.98	247	249	258	259	273	275	260
Mean	11.75	11.72	11.98	12.08	13.23	14.41	----	216	220	227	230	241	250	----
LSD. for	(I) : 0.22		(S): 0.20		(I) × (S): 0.50			(I) : 1.30		(S): 1.18		(I) × (S): 2.90		

N.S.: Non significant at 0.05 levels of significance

significantly affected proline content as well as catalase activity in pea leaves (Table, 6).

It is obvious that increasing the interacted salinity level under the same irradiation level or increasing the interacted gamma ray dose under the same salinity level increased proline content and catalase activity in leaf tissues. Interaction treatment of 7 K-rad γ-irradiation × 6000 ppm sea water salinity gave the highest values of proline content and catalase activity in the two tested seasons of study.

Anatomical Characters

It is clear from data in Table 7 and Fig. 1 that the thickness of blade, palisade tissue, spongy tissue and midvein bundle were increased by pre-sowing gamma irradiation treatments up to 3 K-rad. Increasing the dose of γ-irradiation to 5 or 7 K-rad decreased all studied leaf anatomy traits. These results are in agreement with those reported by Harb *et al.* (2005) who found that, exposure propagated banana to gamma irradiation (10 and 20 Gy) had simulative effects on anatomical parameters (leaf blade and midrib thickness, number of midrib vascular bundles, vascular cylinder diameter, and number of vessels). On the other hand, higher doses of irradiation (30 and 40 Gy) adversely affected all the tested parameters. Also, Ali and Abdel Hady (2006) on wheat (*Triticum aestivum* L.) induced variability on thickness of flage leaves vascular bundles components with 350 and 450 Gy. On tomato plant, Mahna and Singh (1976) found that γ-irradiation caused an increase in epidermal cell size in tomato leaves.

Regarding the effect of salinity levels on the above mentioned leaf anatomy characters (Table, 7 and Fig. 1) it could be concluded that, generally, increasing salinity level more than 1500 ppm decreased blade thickness, palisade tissue, spongy tissue and midvein bundle of pea leaves. The best treatments in this respect were irrigation plants with tap water or with water containing 1500 ppm salinity levels. Irrigation plants with 2000, 4000 or 6000 ppm salinity levels decreased all the above mentioned leaf anatomy traits. Osman (2005) found that after salinity treatment (8000-10000 ppm), thickness of spongy cells tissues and depth of palisade layers were increased in tolerant olive variety. Picual variety was adapted trough change in number

Table 7. Effect of pre-sowing gamma irradiation treatment of pea seeds, sea water levels and their interactions on leaflet anatomy of the third compound leaf developed on the main stem of pea plants at 60 days after sowing at the season 2009

Characters	Blade thickness (µm)							Spongy tissue thickness (µm)						
	Gamma irradiation (K-rad)													
	0	0.5	1	3	5	7	Mean	0	0.5	1	3	5	7	Mean
Sea water levels (ppm)														
Tap water	82.64	79.20	89.53	103.3	68.87	68.87	82.07	41.32	41.32	41.32	34.43	30.99	30.99	36.73
1500	82.64	79.20	86.08	92.97	68.87	68.87	79.77	41.32	37.88	34.43	34.43	30.99	30.99	35.01
2000	75.75	75.75	75.75	82.64	65.42	65.42	73.46	27.55	30.99	30.99	41.32	27.55	27.55	30.99
4000	65.42	65.42	72.31	72.31	61.98	61.98	66.57	24.10	24.10	30.99	34.43	27.55	24.10	27.55
6000	68.87	65.42	65.42	68.87	61.98	61.98	65.42	20.66	20.66	24.10	20.66	20.66	20.66	21.23
Mean	75.06	73.00	77.82	84.02	65.42	65.42	----	30.99	30.99	32.37	33.06	27.55	26.86	--
LSD. for	(I) : 3.19		(S): 2.91		(I) × (S): 7.14			(I) : 2.88		(S): 2.63		(I) × (S): 6.45		
	Midvein bundle thickness (µm)							**Palisade tissue thickness (µm)**						
Tap water	48.21	41.32	58.54	61.98	51.65	51.56	52.22	29.27	29.27	30.99	41.32	24.10	24.10	29.84
1500	48.21	41.32	58.54	61.98	51.65	48.21	51.65	25.82	27.55	29.27	37.88	24.10	24.10	28.12
2000	58.54	58.54	58.54	61.98	48.21	48.21	55.67	25.82	25.82	25.82	30.99	24.10	22.38	25.82
4000	55.09	58.54	61.98	61.98	44.76	48.21	55.08	22.38	22.38	24.10	24.10	20.66	18.94	22.09
6000	51.65	51.65	58.54	61.98	44.73	48.15	52.78	18.94	20.66	20.66	24.10	13.77	12.05	18.36
Mean	52.34	50.27	59.22	61.98	48.20	48.87	----	24.45	25.14	26.17	31.68	21.35	20.31	---
LSD. for	(I) : 3.75		(S): N.S		(I) × (S): 8.39			(I) : 3.09		(S): 2.82		(I) × (S): 6.91		

N.S.: Non significant at 0.05 levels of significance.

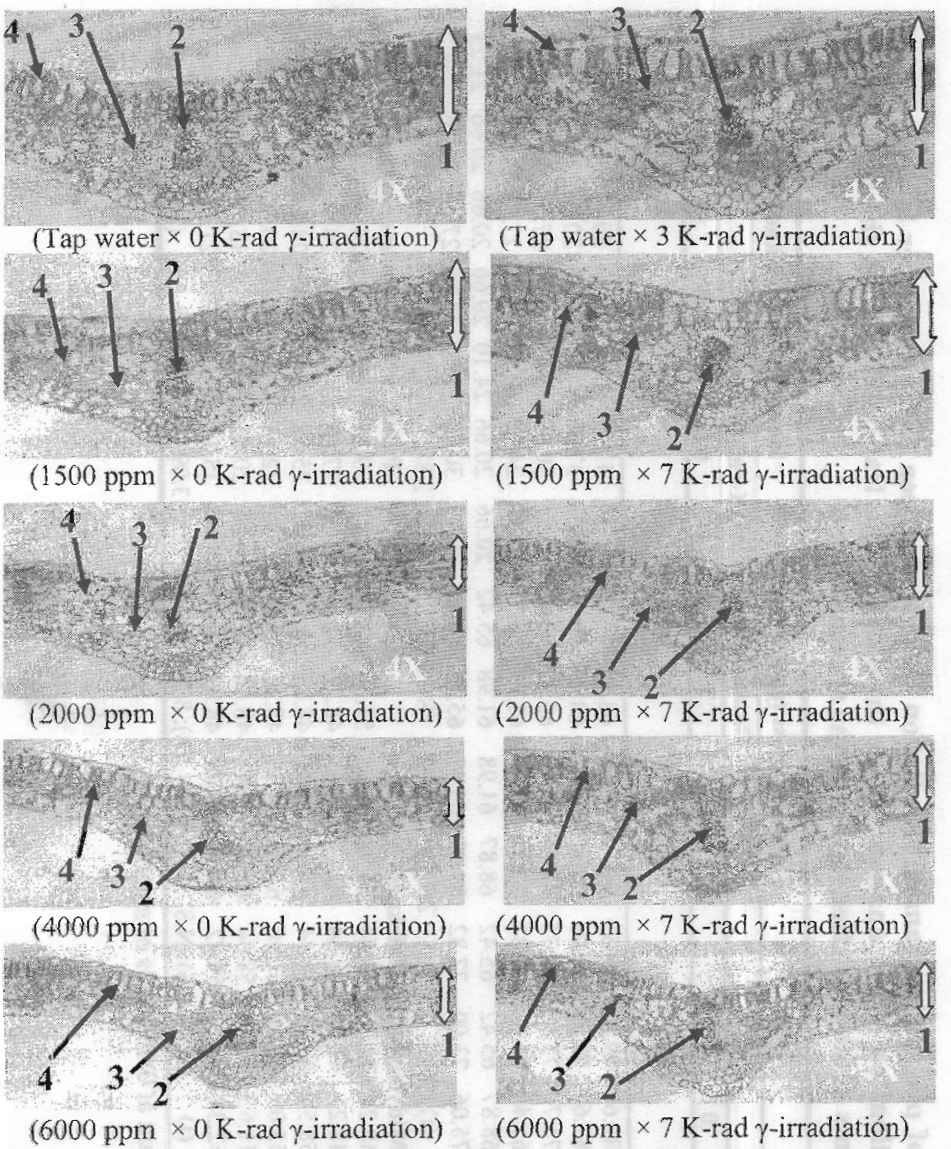

Fig. 1. Effect of pre-sowing γ-irradiation treatment of pea seeds, sea water levels and their interactions on leaflet anatomy at 60 days after sowing at the season of 2009

1: Blade thickness; 2: Midvein bundle; 3: Spongy tissue; 4: Palisade tissue

of palisade cell layers from 8 to 3 layers, both of spongy cells and air space among spongy cell has been decreased. Also, Boghdady (2009) stated that salinity at 3000 or 4000 ppm reduced the thickness of midvein; lamina, upper and lower epidermis and palisade and spongy tissues as well as dimensions of midvein bundle, number of vessels per midvein bundle and vessel diameter. In addition, Strogonov (1964) on tomato, cotton and bean, Meiri and Poljakoff-Mayber (1967) on bean plants and Wignarajahk *et al.* (1975) on *Phaseolus vulgaris* observed inhibition in differentiation and change in diameter and number of xylem vessels, reduction in thickness of lamina and mesophyll due to treating the studied plants with salinity.

Also, data in Table 7 and Fig. 1 show that, the interaction between γ-ray doses and salinity levels was significant regarding leaf anatomy characters. The most favourable interaction treatment for increasing the thickness of blade and palisade tissue was pre-sowing seed treatment with 3 k-rad tap water. While, the best interaction treatment for spongy tissue thickness was 3 K-rad γ-irradiation × irrigation with 2000 ppm salinity level. In addition, the best interaction treatment for midvein bundle thickness were treating pea seeds with 3 k-rad γ-irradiation × irrigation of pea plants with different salinity levels (1500, 2000, 4000 and 6000) beside irrigation with tap water.

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دراسات نباتية على نباتات البسلة تحت تأثير معاملة البذور بأشعة جاما والري بمستويات مختلفة من ملوحة ماء البحر

فتحى محمد السعدونى - حسن محمد المسلمى

السـيد محمود مقبيل- محمد نبيل عطية سعيد

قسم النبات الزراعي وأمراض النبات - كلية الزراعة - جامعة الزقازيق - مصر

أجريت دراسات نباتية فى تجربتين أصص خلال فصل الشتاء للموسمين ٢٠٠٨، ٢٠٠٩ فى الصوبة السلكية لقسم النبات الزراعى وأمراض النبات، كلية الزراعة، جامعة الزقازيق، لدراسة تأثير معاملة بذور البسلة صنف ماستر ب قبل الزراعة بأشعة جاما، الرى بمستويات مختلفة من ماء البحر والتفاعل بينهما على الصفات المورفولوجية، محتوى الأوراق من الكلوروفيلات، النشاط الإنزيمى والتركيب التشريحى لأوراق النباتات.

وأظهرت النتائج أن زيادة الجرعة المستخدمة من أشعة جاما حتي ٣ كيلو راد المستخدمة في معاملة بذور البسلة قبل زراعتها أدت إلي زيادة ارتفاع النبات، عدد الأوراق، الوزن الجاف لمختلف أجزاء النبات (الجذر، الساق، الأوراق)، ومحتوي الأوراق من الكلوروفيل (أ، ب)، الكاروتينويدات، الكلوروفيل الكلي. بالإضافة إلي زيادة نسبة البرولين ونشاط إنزيم الكتاليز وكذلك سمك نصل الورقة ، النسيج العمادي، النسيج الإسفنجي وسمك الحزمة الوعائية. بينما أدت زيادة الجرعة المستخدمة إلي خمسة أو سبعة كيلو راد إلى انخفاض معنوي في كل الصفات سابقة الذكر، باستثناء محتوي الأوراق من البرولين ونشاط إنزيم الكتاليز في أوراق البسلة.

ظهر انخفاضًا تدريجيًا في كل الصفات سابقة الذكر مع زيادة الملوحة حتي أعلي مستوي (٦٠٠٠ جزء في المليون)، وعلي العكس ازدادت نسبة البرولين ونشاط إنزيم الكتاليز في الأوراق بزيادة مستوي الملوحة المستخدم حتي ٦٠٠٠ جزء في المليون. بينما لم يكن هناك إختلافًا معنويًا بين النباتات التي تم ريها بماء الصنبور (الكنترول) والتركيز ١٥٠٠ جزء في المليون في معظم الصفات المدروسة في كلا موسمي الدراسة. كانت أفضل معاملات التفاعل هي معاملة بذور البسلة بالجرعة ٣ كيلو راد أشعة جاما × الري بمياه الصنبور (الكنترول).