

SOME BIOCHEMICAL CHANGES IN TWO EGYPTIAN BREAD WHEAT CULTIVARS IN RESPONSE TO GAMMA IRRADIATION AND SALT STRESS

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Abstract

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Wheat plays an important role in every day's life of the world's population. The present study discusses the morphological traits and some biochemical changes of two Egyptian bread wheat cultivars irradiated at 0.0, 100, 200 and 300 Gy dose levels and cultivated in presence of different sodium chloride concentrations (0.0, 60 and 120 mM). The data indicated that all treatments increased the morphological traits except the No. of spikes/plant. The treatment 100 Gy combined with the lower concentration of NaCl (60 mM) caused enhancement in photosynthetic pigment contents. Also, with increasing irradiation dose level and sodium chloride concentration, proline content was increased and the highest content was observed in 300 Gy dose combined with 120 mM NaCl concentration as compared to control plants in both cultivars Sids-1 and Sakha-93 (0.530 and 0.451 mg/g FW, respectively). Percentage of nitrogen content was found to be decreased by salt stress in all concentration but when salt stress combined with gamma irradiation, all dose levels increased the percentage of nitrogen content and reached to the maximum increase (2.61 and 2.48) in the treatment (100 Gy combined with 60 mM NaCl) in Sids-1 and Sakha-93 respectively. Salinity increased the Na⁺ content and decreased K⁺ and Ca⁺² contents in two cultivars grains. Gamma irradiation could be used successfully to develop mutants in wheat and abiotic stress tolerant.

Key words: wheat; morphological traits; biochemical changes; irradiation; sodium chloride

Introduction

Wheat (*Triticum aestivum* L.) is the major human consumable commodity in most areas of the world including Egypt. Wheat is a major cereal crop in many parts of the world and it is commonly known as the king of cereals. It is a moderately salt tolerant crop and its yield is substantially reduced as the soil salinity level rises to 100 mM NaCl (Munns et al., 2006).

Salinity is a severe problem, affecting more than 800 million hectares of land worldwide that accounts for more than 6% of the global land mass (Munns and Tester, 2008). The effect of salinity on plants may cause disturbances in plant metabolism (El-Tayeb, 2005). Salt tolerance in higher plants

is regulated by a number of different physiological and biochemical processes. There is evidence that high levels of salt cause an unbalance of the cellular ions leading to both ion toxicity and osmotic stress (Ashraf and Harris, 2004). Salinity significantly reduces the total chlorophyll content and the degree of reduction in total chlorophyll depending on the salt tolerance of plant species and salt concentrations. In salt-tolerant species, chlorophyll content increased, while in salt-sensitive species it was decreased (Ghogdi et al., 2012). The ability of plants to tolerate and flourish in saline soils is of great importance in agriculture because it indicates that the affected plants have genetic potential for salt tolerance, which is a highly desirable trait (Mensah et al., 2006).

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There are two ways to increase local production of wheat; the first way is through vertical expansion, i.e., increasing wheat production per unit area through the development of new cultivars of high yielding ability, early maturity and resistance to biotic and abiotic stresses. The second way is through horizontal expansion, i.e., by increasing the area cultivated with wheat. But this depends on the availability of irrigation water.

Gamma radiation can be useful for changing physiological characteristics (Kiong et al., 2008). The biological effect of gamma radiation is due to the interaction between atoms or molecules in the cell, especially the H_2O (Kovács and Keresztes, 2002).

These radicals can damage or modify important components of plant cells and thus affect the morphological and chemical composition of plant depending on the level of radiation dose (Ashraf et al., 2003).

Induced mutagenesis and its breeding strategies are potential tools for improving both quantitative and qualitative traits in crops within a much shorter period of time than conventional breeding. Because of its relative simplicity and low cost, mutagenic treatment of seeds and other parts of the plant remains a useful tool for isolating the desired variants and developing resistance to biotic and abiotic stresses in various crops (Oladosu et al., 2014). The reduction in plant growth in saline environments could be due to either adverse water relations or the toxic effects of Na^+ and Cl^- ions on metabolism (Yeo and Flowers, 1983). It has been shown that under stress conditions the photosynthetic capacity is affected by salinity, which causes its reduction (Parida and Das, 2005).

Irradiation of seeds may cause genetic, variability that enables plant breeders to select new genotypes with improved characteristics such as salinity tolerance, grain yield and quality (Ashraf et al., 2003). Due to limited genetic variability among the existing plant genotypes opened a good era for crop improvement and now mutation induction has become an established tool in plant breeding that can improve cultivars in certain specific traits as well (Oladosu et al., 2016).

Considering the effects of radiation on plants, the present study was conducted to determine the impact of gamma irradiation combined with sodium chloride on morphologi-

cal traits as well as the content of photosynthetic pigments, proline, mineral, sugars and phenolic content.

Materials and Methods

Plant material

Grains of two bread wheat cultivars (*Triticum aestivum* L.), Sids-1, Sakha-93 used in the present study were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. The names, pedigree and origin of these cultivars are presented in Table 1.

Two cultivars were irradiated with gamma rays at dose levels (0.0, 100, 200 and 300 Gy with a dose rate of 1.9 kGy/h). The source of irradiation installed at the National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. Irradiated and unirradiated grains were sown in first of December 2013 at the experimental farm belonging to Natural Products Department, National Center for Radiation Research and Technology to get M_1 of the grains. Soil mechanical and chemical analysis of the experimental site is shown in Table 2.

Table 2
Mechanical and chemical properties of the experimental soil

Soil properties	Experimental year	
	2013	2014
pH(1:1)	7.32	7.31
EC (1:1) dS/m	2.2	1.8
Soluble anions (meq/l)		
$CO_3^{=}$	1.5	1.3
HCO_3^-	4.5	3.6
Cl^-	8.0	5.9
$SO_4^{=}$	11.7	9.8
Soluble cations (meq/l)		
Ca^{++}	10.5	9
Mg^{++}	3.5	2
K^+	0.50	0.4
Na^+	11.2	9.2
Sand (%)	57.3	58.4
Silt (%)	21.2	18.2
Clay (%)	21.5	23.4
Texture class	Sandy clay loam	Sandy clay loam

Table 1
Pedigree and the most important traits of the studied wheat cultivars

Cultivars	Designation	Pedigree	Origin
Sids-1	Sd-1	HD2172/PAVON"s"/1158-57/MAGA74"s"	ARC – Egypt
Sakha-93	Sk-93	Sakha 92/TR810328S8871-1S-2S-1S-0S	ARC – Egypt

ARC = Agricultural Research Center

To rise M_2 generation, random grain samples main spikes were taken from M_1 generation grains for each irradiation dose level. These samples were sown in December 2014 and irrigated with NaCl at concentrations (0.0, 60 and 120 mM NaCl/l). A complete randomized blocks design with three replicates was used. After 45 days from sown fresh leaves were taken from five plants per treatment for proline determination and chlorophyll content in M_2 . At harvest, fifteen individual plants per treatment were used for estimating the plant height, spikes length, number of spikes/plant and 100-grain weight in M_1 and M_2 respectively. Wheat grains for M_2 were harvested manually at maturity. The grains were dehydrated under sunlight and were ground to pass through a 1.0 mm screen. The ground samples were stored in plastic bags at 4°C for subsequent chemical analyses.

Determination of photosynthetic pigments

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

Proline content

Proline content was estimated by the method of Bates et al. (1973). The results were expressed as mg/g of proline equivalent of the fresh weight of the samples.

Total nitrogen

Total nitrogen was determined in the dried samples using the micro Kjeldahl method as described in AOAC (1995).

Mineral content

Sodium and calcium were determined in the acid digested of grins by atomic absorption spectrometry according to AOAC (2005). Potassium was estimated according to AOAC (1995).

Total sugars

Total sugars were determined by Phenol – Sulfuric acid method at 488 nm against blank according to Dubois et al. (1956). The results were expressed as mg/g of glucose equivalent of the dry weight of the samples.

Total phenolic contents

Total phenolic contents were determined according to the method of Shahidi and Naczk (1995) using the Folin–Denis

reagent. The results were expressed as mg/g of GA equivalent of the dry weight of the samples.

Statistical analysis

The data were presented as the mean $\pm$ SD. All the statistical analyses were performed using an ANOVA, and we applied Duncan's multiple range tests (Duncan, 1955) to compare the results of the experiments ($P \leq 0.05$).

Results and Discussion

Morphological traits

Effect of gamma rays on morphological traits of wheat cultivars in M_1 are shown in Tables 3 and 4. Plant height, 100-grain weight and spikes length increased in all irradiation doses as compared to un-irradiated samples. Maximum increase was observed at 100 Gy dose level but a number of spikes/plant did not affect by increasing the dose level and the best dose of all results was 100 Gy. Whereas, the effect of gamma rays and salinity on morphological traits of wheat cultivars in M_2 shown in Tables 5 and 6, data revealed that increasing salinity level reduced all growth parameter (plant height, spikes length, number of spikes/plant and 100-grain weight). The same results were obtained by Amira (2009) who stated that morphological traits appeared to be decreased with salinity in wheat on the other hand, gamma rays increase this parameter. These results agreed with those obtained by Sheldon et al. (2004) who reported that the growth of both wheat and chickpea was reduced by using salt stress due to salinity has a dual effect on plant growth via an osmotic effect on plant water uptake, and specific ion toxicities. By decreasing the osmotic potential of the soil solution, plant access to soil water is decreased, because of the decrease in total soil water potential. As the soil dries, the concentration of salt in the soil solution increases, further decreasing the osmotic potential.

Also, Rahman et al. (2008) concluded that the increase in salt concentration decreased the shoot and root biomass of wheat cultivars through the presence of enough salt in the medium which decreases the osmotic potential to such point

Table 3
Effect of gamma irradiation on morphological traits in M_1 of Sids-1 Egyptian wheat cultivar

Dose (Gy)	Plant height (cm)	100 grain Weight	Spikes length (cm)	No. of Spikes/plant
0	60.50 d	3.25 b	14.30 bc	2.75 b
100	100.00 a	4.00 a	15.78 a	3.00 a
200	87.50 b	4.00 a	15.00 b	2.75 b
300	66.10 c	3.47 b	14.00 c	2.75 b
LSD	2.018	0.4819	0.7044	0.2008

The means with different letters were used for comparing between means in the column and means with the different letters indicate statistically significant differences at $P \leq 0.05$

Table 4**Effect of gamma irradiation on morphological traits in M₁ of Sakha-93 Egyptian wheat cultivar**

Dose (Gy)	Plant height (cm)	100 grain Weight	Spikes length (cm)	No. of Spikes/plant
0	71.50 d	3.50 ab	12.66 c	2.75 b
100	115.20 a	3.85 a	14.20 a	3.00 a
200	96.00 b	3.70 ab	13.40 b	2.75 b
300	78.12 c	3.40 b	12.30 c	2.75 b
LSD	2.150	0.4162	0.6321	0.1787

The means with different letters were used for comparing between means in the column and means with the different letters indicate statistically significant differences at $P \leq 0.05$

Table 5**Effect of gamma irradiation and salt stress on morphological traits in M₂ of Sids-1 Egyptian wheat cultivar**

Dose (Gy)	Plant height (cm)			100 grain Weight			Spikes length (cm)			No. of Spikes/plant		
	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl
0	67.20 h	64.00 i	61.75 j	3.77 b	2.26 de	1.91 f	15.00 c	14.50 cd	13.00 ef	2.75 b	1.50 cd	1.40 d
100	112.40 a	92.20 c	81.13 d	4.21 a	3.72 b	2.35 cd	16.90 a	14.63 c	13.75 de	3.00 a	1.60 c	1.50 cd
200	94.00 b	78.20 e	67.13 h	4.19 a	2.58 c	2.04 ef	15.88 b	14.50 cd	12.80 f	2.75 b	1.60 c	1.40 d
300	74.20 f	70.63 g	62.63 j	3.87 b	2.32 d	1.91 f	14.80 c	12.25 fg	11.50 g	2.75 b	1.50 cd	1.40 d
LSD for Irradiation x Salinity	1.309			0.2383			0.8619			0.1780		

The means with different letters were used for comparing between means in the column and means with the different letters indicate statistically significant differences at $P \leq 0.05$

Table 6**Effect of gamma irradiation and salt stress on morphological traits in M₂ of Sakha-93 Egyptian wheat cultivar**

Dose (Gy)	Plant height (cm)			100 grain Weight			Spikes length (cm)			No. of Spikes/plant		
	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl	0 mM NaCl	60 mM NaCl	120 mM NaCl
0	73.30 h	65.00 i	62.54 j	3.61 ab	2.41 c	1.66 d	13.83 bc	11.64 d	10.74 e	2.75 b	1.60 d	1.40 d
100	120.33 a	93.20 c	84.58 d	3.94 a	2.68 c	1.70 d	15.07 a	13.10 c	11.82 d	3.20 a	2.00 c	1.60 d
200	99.20 b	79.70 f	74.26 gh	3.91 a	2.47 c	1.65 d	14.30 ab	12.20 d	11.70 d	2.75 b	1.60 d	1.40 d
300	82.50 e	75.33 g	63.33 ij	3.30 b	1.80 d	1.44 d	13.20 c	10.40 ef	9.70 f	2.75 b	1.40 d	1.33 d
LSD for Irradiation x Salinity	1.0909			0.4390			0.7739			0.3520		

The means with different letters were used for comparing between means in the column and means with the different letters indicate statistically significant differences at $P \leq 0.05$

which retards or prevent the uptake of water necessary for mobilization of nutrient required for growth. Valdez-Aguilar et al. (2009) concluded that, increasing EC from 2 to 3 ds m^{-1} induced 83-78% decrease in growth of ranunculus.

Photosynthetic pigments

As illustrated in Figures 1 and 2, Chlorophyll a, b, total chlorophyll and carotenoids contents in the leaves of two Egyptian wheat cultivars Sids-1 and Sakha-93 showed a highly significant increase in comparison to the control value in the treatment 60 mM NaCl. On the other hand, the other

concentration of NaCl caused a significant decrease in the same contents. Irradiating plants with gamma rays at dose levels 100 and 200 Gy caused enhancement in photosynthetic pigment contents under salt stress and the maximum increase was observed in total chlorophyll for the treatment 100 Gy combined with the lower concentration of NaCl (60 mM) 1.82 and 2.67 mg/g fresh weight of Sids-1 and Sakha-93, respectively. These results are in accordance with Helaly and El-Hosieny (2011) found that the interaction between salinity and gamma rays batter the inhibiting effects of salinity on chlorophylls concentration in *Citrus limon* even at the high-

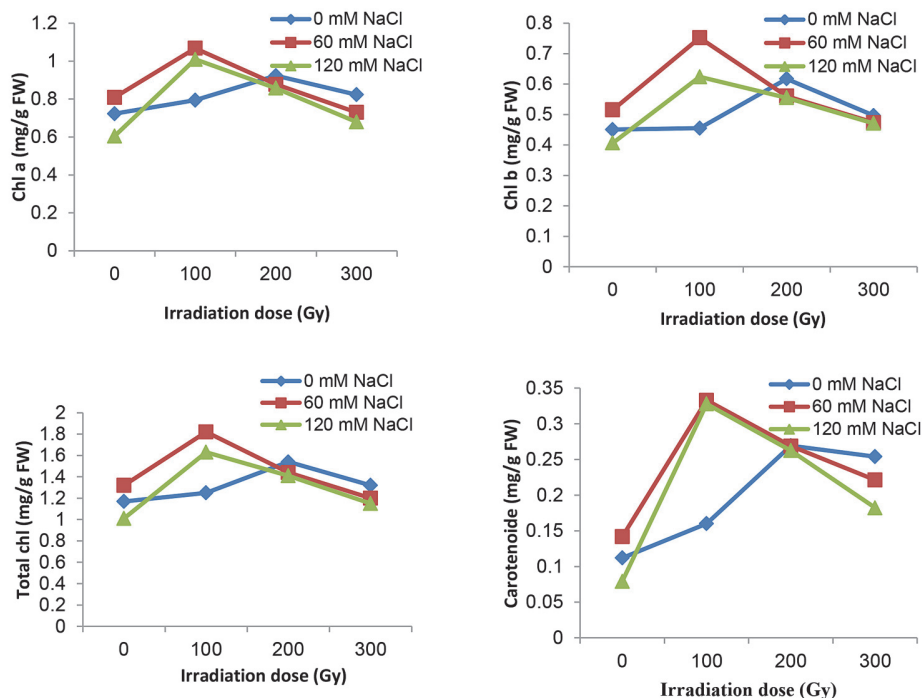


Fig.1. Changes in photosynthetic pigments (mg/g FW) of Sids-1 Egyptian wheat cultivar grown under gamma irradiation and sodium chloride stress

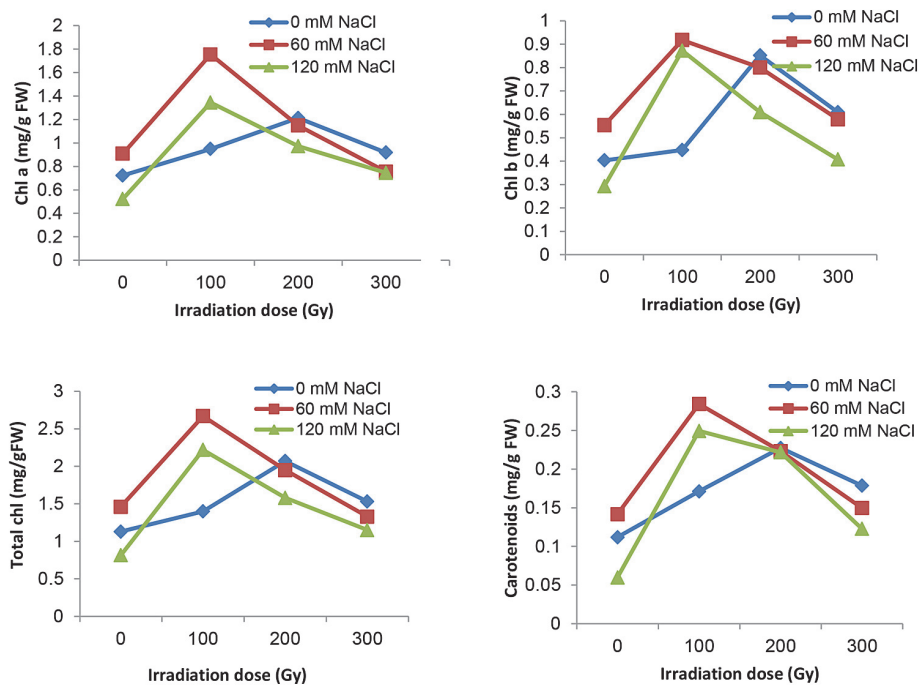


Fig. 2. Changes in photosynthetic pigments (mg/g FW) of Sakha-93 Egyptian wheat cultivar grown under gamma irradiation and sodium chloride stress

est level of salinity. Modulation in photosynthesis in irradiated plants might partly contribute to increase growth (Wi et al., 2007). Increased chlorophylls by irradiation may be due to stimulation biosynthesis of chlorophyll and/or delaying of its degradation.

Proline content

The accumulation of proline is widely used as a selection criterion for stress. Exposure to gamma radiation, alone or in combination with salt stress, significantly increased proline accumulation (Figure 3). Proline content was increased in samples exposed to different levels of gamma irradiation as compared with the un-irradiated sample (control). However, with increasing irradiation dose level and salt concentration the proline content was increased and the highest content of proline was observed in 300 Gy combined with 120 mM NaCl concentration as compared to control plants in both cultivars Sids-1 and Sakha-93 (0.561 and 0.451 mg/g FW,

respectively). One of the protective mechanisms in the synthesis of osmolytes which is essential to plant growth was proline synthesis (Esfandiari et al., 2008). The results of this study revealed that increase in proline content was observed in irradiated and salt-stressed plants. There was a persuasive proof which showed that the osmolyte synthesis such as proline involved in protective mechanisms was altered with several environmental stresses, including gamma irradiation (Al-Rumaih and Al-Rumaih, 2008). It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. Accumulation of proline may be involved in adaptive at the cellular level which may acts as an enzyme protectant and stabilizing the structure of macro molecules (Mahajan and Tuteja, 2005). Also, proline can confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in nonenzymic free radical detoxifications (Khan et al., 2002).

Minerals content

Nitrogen

Percentage of nitrogen content (Figures 4 and 5) was found to be decreased by salt stress in all treatments but when salt stress combined with gamma irradiation all dose levels caused an increase in the nitrogen content and reached to the maximum increase (2.61 and 2.48%) in the treatment (100 Gy + 60 mM NaCl) in both cultivars (Sids-1 and Sakha-93), respectively. Khodary (2004) reported a similar inhibition in the uptake of NO_3 in lupine plants. It is likely that salinity induced stomatal closure and reduced transpiration rate. But Application of γ -rays to the stressed lupine plants significantly stimulated the absorption of NO_3 as compared with those received NaCl only. This increase might be due to the effect of such rays on accelerating the transpiration rays through increasing the stomatal aperture.

Sodium

In the absence of salt, the grains of the two cultivars presented a very low Na^+ content but in the presence of salt, Na^+ accumulation was increased in grains of the Sids-1 and Sakha-93. Sodium accumulation was more pronounced in Sakha-93 cultivar than Sids-1 as shown in Figures 4 and 5.

Potassium

Potassium content in grains of both cultivars is presented in Figures 4 and 5. Without the treatments of salt and radiation, the K^+ content was found to be significantly higher in Sakha-93 cultivar than in Sids-1. In the present study due to salinity stress, the two genotypes showed decreasing in K^+ content. The reduction in K^+ was due to the existence of excessive Na^+ in the growth medium since the high external Na^+ content is recognized to have an aggressive effect on

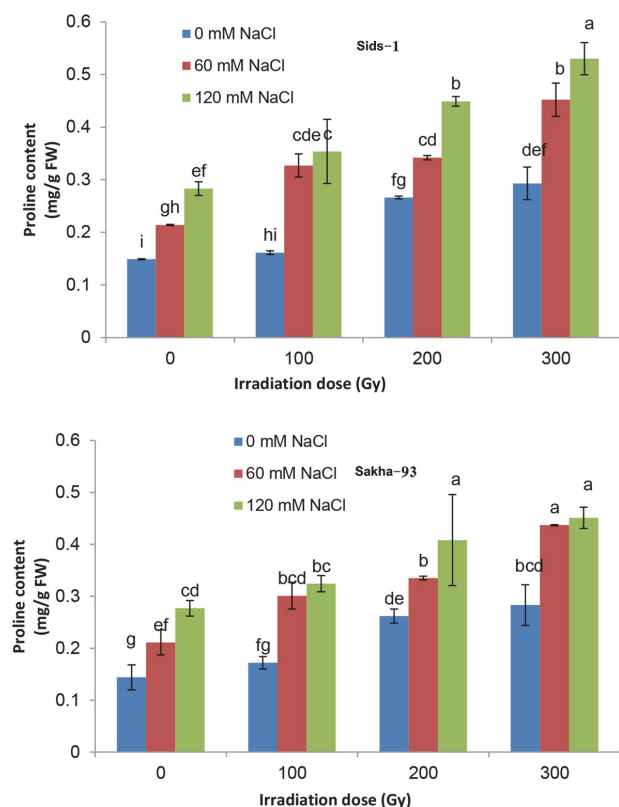


Fig. 3. Changes in proline content (mg/g FW) of Sids-1 and Sakha-93 Egyptian wheat cultivars grown under gamma irradiation and sodium chloride stress

Vertical bars show standard deviation ($n = 3$); Different letters indicate statistically significant differences at $P \leq 0.05$

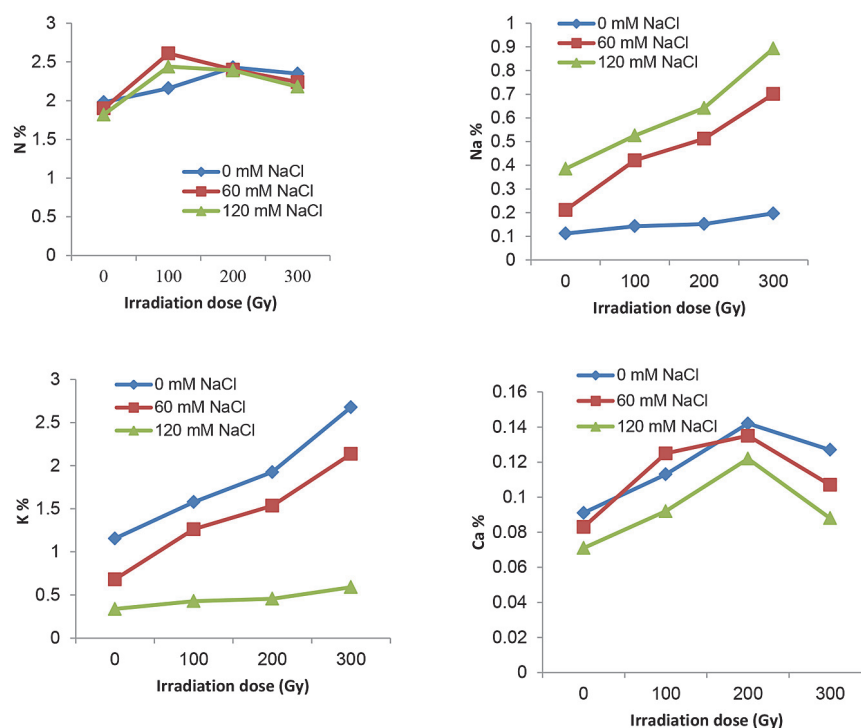


Fig. 4. Changes in nitrogen, sodium, potassium and calcium contents of Sids-1 Egyptian wheat cultivar grown under gamma irradiation and sodium chloride stress

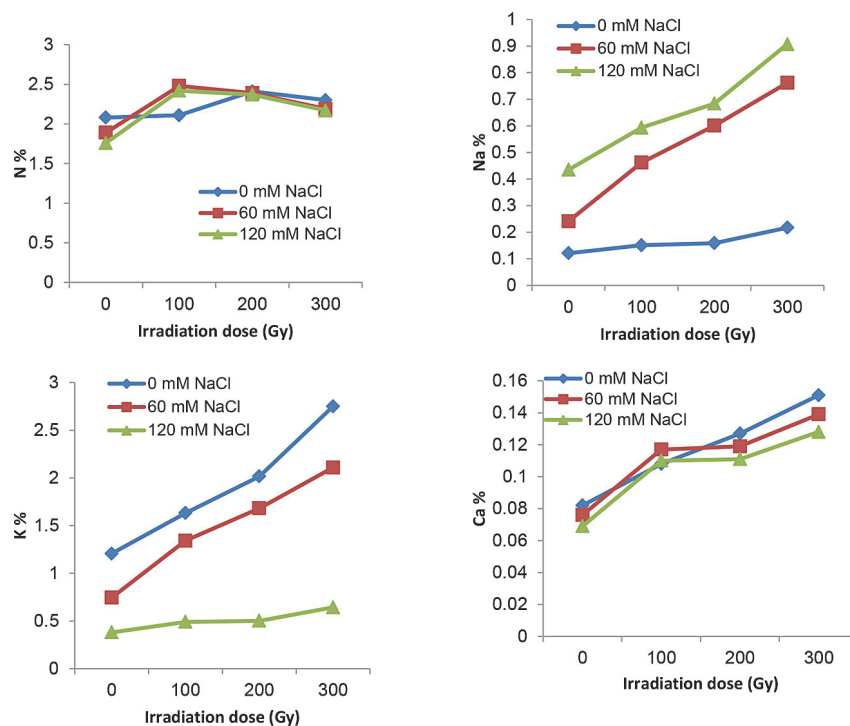


Fig. 5. Changes in nitrogen, sodium, potassium and calcium contents of Sakha-93 Egyptian wheat cultivar grown under gamma irradiation and sodium chloride stress

K⁺ uptake in a plant (Sarwar and Ashraf, 2003). Regulation of K⁺ uptake and avoidance of Na⁺ entrance, efflux of Na⁺ from a cell are the strategies normally used by plants to uphold desirable K⁺ and Na⁺ ratio in the cytosol. In the present study, cultivar Sakha-93 is relatively higher in accumulating more K⁺ than Sids-1 cultivar. Potassium and sodium ratio is the criterion which is recognized by the scientist and genetically accepted for salt tolerance. Consequently, the varieties maintaining higher K⁺ and Na⁺ ratio are the salt tolerant and showing a positive correlation between grain yield and K⁺ and Na⁺ ratio.

Gamma rays application at low doses has been reported to potentiate the biosynthesis of endogenous phytohormones (e.g. indole acetic acid, gibberellic acid, cytokines) and nitrogenous compounds (Khodary, 2004).

Calcium

The data presented in Figures 4 and 5 showed that the Ca²⁺ accumulation decreased with the salinity increasing but with gamma irradiation, all dose levels increased Ca²⁺. The stimulative effect of γ -rays on Ca²⁺ absorption was observed. Calcium is essential for the maintenance of cell membrane integrity. Calcium plays an important role in the synthesis of new walls in the cell, particularly the middle lamellae that separate newly divided cells (Hakim et al., 2014). The membrane was damaged and enhanced permeability due to the

displacement of Ca²⁺ and increasing Na⁺ from the binding sites of phospholipids of membranes. In the present study, calcium ion decreased with the increase in salinity levels.

These results are in accordance with Shereen et al. (2009) who reported that the salinity lead to increase Na⁺ accumulation and decrease the concentration of K⁺ in rice plants. The present study showed that the irradiation dose level of 100 Gy was very effective for improving the ionic balance in grains of wheat. The reduction in the uptake of toxic ion such as Na⁺ has played a positive role in promoting cellular functions such as pigment production and other growth characteristic, which collectively resulted in better growth of wheat plants under saline condition.

Total sugars and total phenolic content

Effect of different doses of gamma irradiation and salt stress on total sugars and total phenolic content is shown in Figure 6. All the treatments showed enhanced total sugars and total phenolic content compared to control. One and a half fold increase in total phenolic content was observed at treatment (300 Gy + 60 mM NaCl) in Sids-1. There was an increase in total phenolic content with an increased dose of radiation and salinity, the highest being in 300 Gy which is two-fold higher compared to control (Sakha-93).

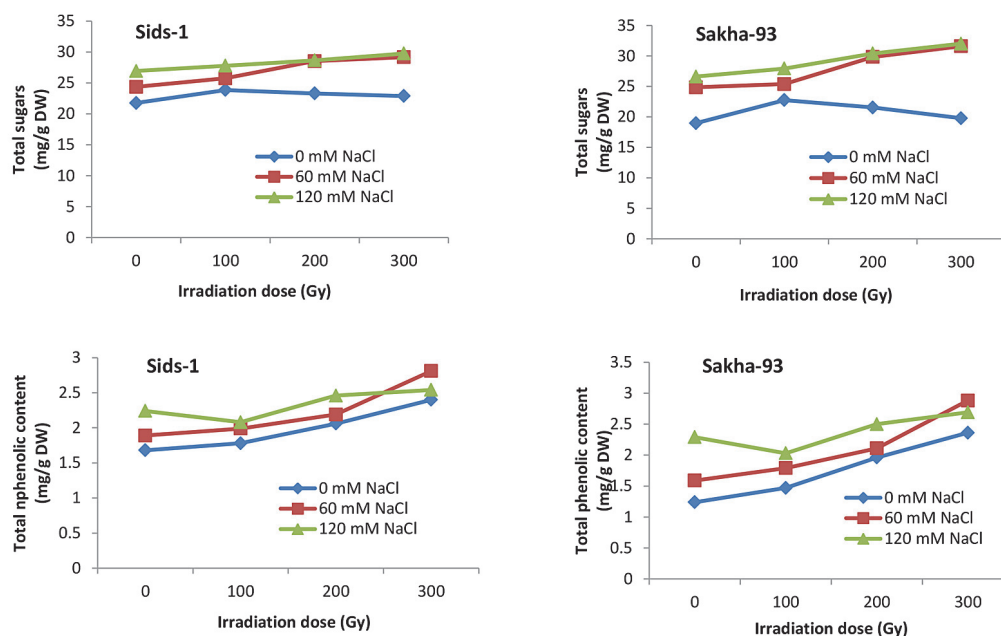


Fig. 6. Effect of gamma irradiation and sodium chloride stress on total sugars and phenolic contents (mg/g DW) of Sids-1 and Sakha-93 Egyptian wheat cultivars

These results are in accordance with Akshatha and Chandrashekar (2013) reported that the application of gamma irradiation at a dose of 25 Gy and 200 Gy significantly increased the phenolic content compared to control ones in *Pterocarpus santalinus*. Also, EL-Hefny (2012) found that total soluble phenols increased significantly by increasing NaCl concentration and gamma irradiation dose levels in *Ambrosia maritima*. The same observation was obtained by Ashraf et al. (2010) who reported that the salinity (150 mM) effected significantly on the phenolic contents of bread wheat (*Triticum aestivum* L.). El-Beltagi et al. (2013) reported that seed irradiation with gamma rays significantly increased plant growth, photosynthetic pigments, total carbohydrate, total phenol, proline, total free amino acids and the contents of N, P, K⁺, Ca⁺² and Mg⁺² compared to nonirradiated ones under salinity.

Simple organic molecules such as sugars, free amino acids and total soluble phenols may act as on osmoticum for the regulation of plants osmosis under saline soil conditions. Phenolic compounds accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals that formed during stress conditions and this free radical scavenger could be oxidized for preventing subcellular damages (Tekam et al., 2014).

Conclusions

The results of this study showed that different doses of gamma rays have different effects on the morphological and biochemical characteristics of plant, such as stimulation of germination and seedling growth and increasing of proline content. It is clear that gamma rays can be used to the produce mutations resistant to environmental stress such as salinity.

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