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EFFECTS OF GAMMA IRRADIATION AND SALT STRESS ON AMINO ACIDS AND PROTEIN FRACTIONS OF TWO EGYPTIAN BREAD WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

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Abstract

Effects of gamma rays (0.0, 100, 200 and 300 Gy) and NaCl (0.0, 60 and 120 mM) on the amino acids composition, protein fractions and electrophoretic protein patterns of two Egyptian wheat cultivars (Sids-1 and Sakha-93) were investigated. The results showed that content of amino acids increased by salinity and irradiation doses compared to the control. Concerning, protein fractions of albumin + globulin percentage increased by increasing salinity only and reached the maximum increase by treatment of 120 mM NaCl in the two cultivars. But for glutenin, the highest increase was observed in treatment 100 Gy + 60 mM NaCl. The changes in protein patterns profile were found to be dependent on irradiation dose and salinity level in wheat grains. Total number of protein bands decreased from 20 bands (untreated plants) to 13 bands (irradiated plants) due to treatment of 200 Gy and 60 mM NaCl in Sids-1 cultivar.

Introduction

Wheat plays an important role in everyday's life of the world's population and provides over 21% of the food calories and 20% of the protein to more than 4.5 billion people, thereby playing a fundamental role in food security (Braun *et al*. 2010). The gap between wheat consumption and production in Egypt is continuously increased due to steady increases in the human population with limited cultivated area. Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, and ultimately inhibits crop production (Wani *et al.* 2016). Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species, differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture (Rahnama *et al*. 2010). The response of plants to accumulation of proteins under salinity depends on plant species and cultivars. The degradation of protein content with salinity increase might be the result of breakdown of protein molecules, which are used as substrate for biosynthesis of proline (Mohamed and Ismail 2011). The content of total protein in bread wheat ranged from 10.87 to 13.04% and most of the physiologically active proteins are influenced by the processing and rheological properties of wheat flour (Žilić *et al.* 2011c). The gluten proteins consist of monomeric gliadins and polymeric glutenins. Glutenins and gliadins are recognized as the major wheat storage proteins, constituting about 75 - 85% of the total grain proteins with a ratio of about 1 : 1 in common or bread wheat (Belderok *et al.* 2000). Not only genotype but also environmental conditions such as temperature, nutrient availability and water availability are reported to influence the properties of gluten proteins (Koga *et al*. 2016). Ionizing radiation affects cellular components; thus, potentially inducing physiological changes in plants. Evaluation of these biological changes is very important as they occur through such physicochemical changes (Çelik *et al.* 2014).

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Thus this experiment was carried out to evaluate amino acids composition, protein fractions, and electrophoretic protein patterns of two Egyptian bread wheat (Sids-1 and Sakha-93) cultivars as affected by salt stress and gamma irradiation.

Materials and Methods

Grains of two bread wheat (*Triticum aestivum* L.), cultivars Sids-1 and Sakha-93 were used in the present experiment (Aly *et al.* 2018). The grains were irradiated with gamma rays (0.0, 100, 200 and 300 Gy with a dose rate of: 1.9 kGy/h). All grains were sown at the experimental farm belonging to Natural Products Department, National Center for Radiation Research and Technology to get M_1 seeds. Physical and chemical analyses of the soils of experimental sites were previously mentioned by Aly *et al.* (2018). To raise M_2, random grain samples of main spikes were taken from bulked M_l grains for each irradiation dose. These samples were sown and irrigated with NaCl at concentration levels of 0.0, 60 and 120 mM. A complete randomized blocks design with three replicates was used. Wheat grains from M_2 were harvested manually at maturity and dehydrated under sunlight then ground to pass through a 1.0 mm sieve. The ground seeds were stored in plastic bags at 4ºC for estimating the following parameters in M_2.

Total nitrogen was determined using micro Kjeldahl method as described in AOAC (1990). The crud protein content was calculated as %N × 5.7. Total free amino acids were determined by ninhydrin test, using the method outlined by Jayeraman (1985) and total soluble protein was estimated according to Bradford (1976).

Amino acids profile of samples was analyzed by Baxter (1996) using a high-performance amino acid analyzer. While, the protein fractions procedure was the original Osborne-Mendel method as described by Sharobeem *et al.* (1986).

Electrophoretic protein patterns using sodium dodecylsulphate polyacrylamide gel electrophoresis was performed on vertical slab using Biogene Limited apparatus according to the method of Laemmli (1970).

All the statistical analyses were performed using an ANOVA and DMRT (Duncan 1955) were applied to compare the treatment variations of the experiments ($p \leq 0.05$).

Results and Discussion

The data presented in Figs. 1 and 2 showed nitrogen, crude protein, total free amino acids and total soluble protein contents in Sids-1 and Sakha-93, respectively. Nitrogen content was found to be decreased by salinity in all treatments but when salinity combined with gamma irradiation all doses caused an increase in the nitrogen content and reached the maximum increase, 2.61 and 2.48%, in the treatment (100 Gy + 60 mM NaCl) for Sids-1 and Sakha-93, respectively. The same trend was observed for crude protein and maximum increase was 14.88 and 14.14% in Sids-1 and Sakha-93, respectively, in the same treatment. But for total free amino acids and total soluble protein were different, results showed an increase in all treatments as compared to the control. These results agreed formally with the findings of Khan *et al.* (2008) who showed that salinity increased protein content of wheat grains of salt tolerant cultivars and it was higher than that of salt sensitive ones. These proteins might have played a role in signal transduction, anti-oxidative defense, anti-freezing, heat shock, metal binding, anti-pathogenesis and osmolyte synthesis, which are essential to a plant's function and growth (Qureshi *et al.* 2007). Also, Shuhua *et al.* (2002) indicated that the protein content of wheat cultivars increased markedly with the improvement of soil salinity. Because of acceleration in crop maturity and reduction in photosynthate transfer period under saline conditions, they are likely to reduce the amount of starch and consequently,

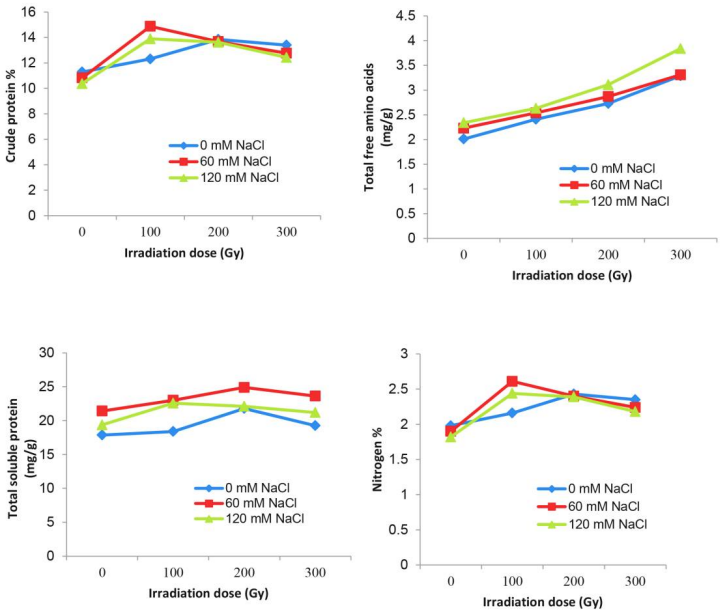

Fig. 1. Changes in crude protein, total free amino acids, total soluble protein and nitrogen in Sids-1 wheat cultivar under gamma irradiation and sodium chloride stress.

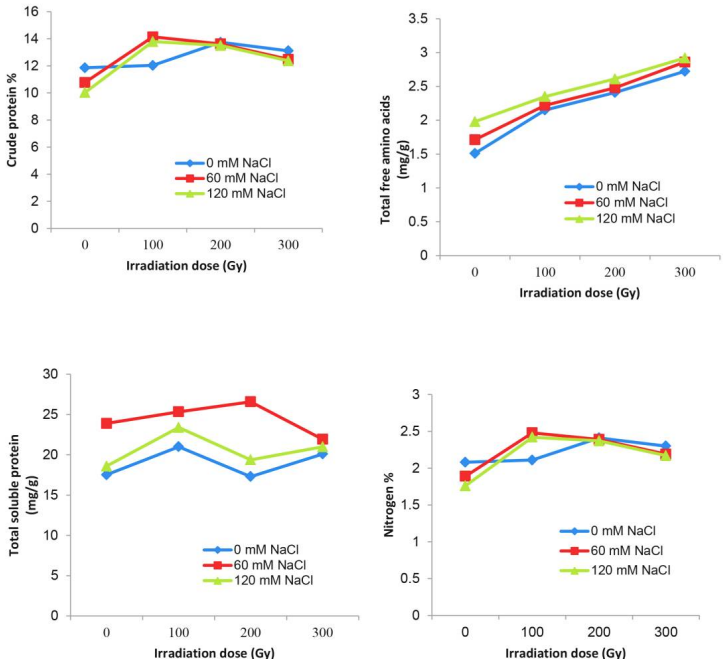

Fig. 2. Changes in crude protein, total free amino acids, total soluble protein and nitrogen in Sakha-93 wheat cultivar under gamma irradiation and sodium chloride stress.

Table 1. Effect of gamma irradiation on amino acids composition of Sids-1 cultivar grown under salt stress.

(a) Indispensable amino acids

Indispensable amino acids	Conc. mg/gm											
	0 Gy + 0.0 mM NaCl	0 Gy + 60 mM NaCl	0 Gy + 120 mM NaCl	100 Gy + 0.0 mM NaCl	100 Gy + 60 mM NaCl	100 Gy + 120 mM NaCl	200 Gy + 0.0 mM NaCl	200 Gy + 60 mM NaCl	200 Gy + 120 mM NaCl	300 Gy + 0.0 mM NaCl	300 Gy + 60 mM NaCl	300 Gy + 120 mM NaCl
Arginine	5.40	6.30	5.85	5.40	6.30	6.30	4.95	4.95	5.40	5.85	6.30	6.30
Histidine	6.83	6.75	6.30	5.85	6.75	6.30	4.95	5.40	5.85	6.75	6.30	5.80
Isoleucine	5.30	5.40	4.05	3.60	4.50	4.95	3.60	4.05	4.95	5.85	4.95	4.25
Leucine	15.20	16.55	19.80	17.55	20.10	22.20	21.50	19.80	18.00	17.80	18.00	17.20
Lysine	5.40	5.40	4.95	4.95	5.40	5.40	4.05	4.50	4.95	4.05	5.40	5.15
Methionine	1.35	0.90	0.60	1.35	1.45	1.35	2.25	2.25	1.35	1.35	1.20	0.95
Phenylalanine	6.80	7.20	6.75	5.85	6.75	6.75	5.40	5.85	6.75	5.85	5.63	5.50
Therionine	4.05	4.50	4.95	4.50	4.00	4.50	4.00	4.05	4.00	4.00	4.05	4.50
Valine	3.05	3.60	3.50	3.95	4.50	4.95	4.05	4.05	4.00	3.52	3.23	3.15
Total amino acids (mg/gm)	53.38	56.60	56.75	53.00	59.75	62.70	54.75	54.90	55.25	55.02	55.06	52.80

(b) Dispensable amino acids

Dispensable amino acids	Conc. mg/gm											
	0 Gy + 0.0 mM NaCl	0 Gy + 60 mM NaCl	0 Gy + 120 mM NaCl	100 Gy +0.0 mM NaCl	100 Gy + 60 mM NaCl	100 Gy + 120 mM NaCl	200 Gy + 0.0 mM NaCl	200 Gy + 60 mM NaCl	200 Gy + 120 mM NaCl	300 Gy + 0.0 mM NaCl	300 Gy + 60 mM NaCl	300 Gy + 120 mM NaCl
Alanine	3.15	2.70	2.50	3.15	3.18	3.00	2.60	2.25	2.25	2.70	2.21	2.21
Aspartic	3.60	4.05	4.50	4.05	4.05	4.50	4.00	3.60	4.05	4.05	4.05	3.60
Cystine	3.70	4.50	3.15	5.85	5.85	5.40	4.70	4.95	4.05	5.85	5.85	4.50
Glutamic	6.75	8.55	8.55	8.20	7.65	8.10	7.10	7.20	7.65	7.50	7.65	7.45
Glycine	3.60	4.00	3.15	4.00	3.60	3.60	4.05	3.15	3.00	2.98	2.53	1.35
Proline	0.94	1.50	1.80	2.25	2.40	2.75	2.83	3.15	3.60	4.05	4.95	5.60
Serine	3.50	4.50	4.50	4.50	3.60	4.95	4.05	4.05	3.60	3.60	3.60	4.50
Tyrosine	3.40	4.50	4.05	4.05	4.95	4.95	4.85	3.60	4.95	5.40	4.95	4.50
Total amino acids (mg/gm)	28.64	34.30	32.20	36.05	35.28	37.25	34.18	31.95	33.15	36.13	35.79	33.71

Table 2. Effect of gamma irradiation on amino acids composition of Sakha-93 cultivar grown under salt stress.

(a) Indispensable amino acids

Indispensable amino acids	Conc. mg/gm											
	0 Gy + 0.0 mM NaCl	0 Gy + 60 mM NaCl	0 Gy + 120 mM NaCl	100 Gy + 0.0 mM NaCl	100 Gy + 60 mM NaCl	100 Gy + 120 mM NaCl	200 Gy + 0.0 mM NaCl	200 Gy + 60 mM NaCl	200 Gy + 120 mM NaCl	300 Gy + 0.0 mM NaCl	300 Gy + 60 mM NaCl	300 Gy + 120 mM NaCl
Arginine	5.70	8.10	7.20	7.20	9.10	9.00	9.00	9.00	8.10	7.20	9.90	9.90
Histidine	8.60	8.10	7.20	7.20	8.90	8.50	8.00	7.90	8.20	8.30	8.00	7.20
Isoleucine	5.40	6.20	5.30	5.30	6.20	7.10	5.30	6.20	6.20	7.10	6.20	5.30
Leucine	19.20	21.10	23.00	22.10	24.60	26.50	25.98	25.60	22.50	22.10	23.00	21.50
Lysine	7.20	6.30	5.70	6.30	8.10	9.90	7.20	7.20	9.00	7.20	9.90	8.10
Methionine	2.50	2.50	1.60	1.20	2.50	3.20	2.40	2.40	2.50	2.50	2.70	2.50
Phenylalanine	7.20	7.50	6.60	6.60	7.80	7.80	6.50	6.60	6.60	6.60	6.40	6.00
Therionine	4.05	4.50	5.70	5.60	5.30	5.60	5.40	5.50	5.40	5.40	5.50	5.60
Valine	3.00	3.40	3.10	3.50	5.20	5.40	5.10	5.10	4.20	3.30	3.10	3.05
Total amino acids (mg/gm)	62.85	67.70	65.40	65.00	77.70	83.00	74.88	75.50	72.70	69.70	74.70	69.15

(b) Dispensable amino acids

Dispensable amino acids	Conc. mg/gm											
	0 Gy + 0.0 mM NaCl	0 Gy + 60 mM NaCl	0 Gy + 120 mM NaCl	100 Gy + 0.0 mM NaCl	100 Gy + 60 mM NaCl	100 Gy + 120 mM NaCl	200 Gy + 0.0 mM NaCl	200 Gy + 60 mM NaCl	200 Gy + 120 mM NaCl	300 Gy + 0.0 mM NaCl	300 Gy + 60 mM NaCl	300 Gy + 120 mM NaCl
Alanine	4.20	3.70	3.50	4.50	4.80	4.00	3.50	3.40	3.40	3.70	3.40	3.40
Aspartic	4.00	5.50	5.70	5.50	5.50	5.90	5.35	5.05	5.60	5.60	5.40	4.20
Cystine	3.80	4.50	3.60	5.40	5.50	5.40	5.25	5.40	4.50	5.50	5.40	4.60
Glutamic	7.00	8.10	8.20	7.95	7.50	8.00	7.30	7.35	7.60	7.50	7.60	7.40
Glycine	4.50	5.40	4.20	5.45	4.50	4.55	5.00	4.40	4.40	4.10	4.00	3.70
Proline	0.90	1.80	1.95	2.20	2.90	3.00	3.90	4.30	4.80	5.70	6.50	7.50
Serine	6.30	6.30	6.30	6.30	6.00	6.70	6.50	6.50	6.00	6.00	6.30	6.30
Tyrosine	6.30	6.60	6.5	6.45	7.10	7.10	6.80	6.50	7.00	7.00	7.10	6.50
Total amino acids (mg/gm)	37.00	41.90	39.95	43.75	43.8	44.65	43.60	42.90	43.30	45.10	45.7	43.60

increase percentage of nitrogen and protein in grains. The free amino acids change as a result of salt stress varied between genotypes and plant organs (Neto *et al*. 2009). Also, it increased by increasing gamma irradiation doses (Maity *et al.* 2009).

Results in Tables 1 and 2 showed the effect of gamma rays and salinity on indispensable and dispensable amino acids of the two wheat cultivars. It is evident that amino acids pool increased by salinity as compared to the control. The results indicated that leucine followed by phenylalanine and glutamic are the predominant amino acids in grains of the two cultivars. Also, concerning amino acids content as affected by gamma irradiation, it was observed that all doses increased amino acids pool. All treatments significantly increased proline content as compared to the control. These results are in agreement with findings of El-Beltagi *et al.* (2013) who reported that gamma irradiation and salinity significantly increased proline and total free amino acid contents of roots and shoots of cowpea plants as compared to that of control. Moreover, free amino acids accumulation in plants under salinity has been attributed to alteration in biosynthesis and degradation processes of amino acids and proteins (Silva *et al*. 2008).

Nutritionally, the albumins and globulins have a very good amino acid balance, many of these proteins are enzymes involved in metabolic activity (Žilić *et al*. 2011c). The data in Tables 3 and 4 indicated that albumin + globulin was increased by increasing salinity for the both cultivars but decreased in the samples exposed to gamma irradiation or for combined treatment with salinity and irradiation. But for gliadin percentage there was an increase and reached the maximum in the treatment, 100 Gy + 60 mM NaCl for Sids-1 and Sakha-93 cultivars (2.05 and 2.21%), respectively. Also, glutenin percentage increased by salinity and irradiation doses (Tables 3 and 4). Shen *et al.* (2007) indicated that in wheat grains the ratios of albumin, globulin and glutenin contents to protein decreased and the ratio of gliadin to protein increased with increasing salinity. In this respect, Zhang *et al*. (2016) revealed that genotype, environment, and their interaction significantly affected most of quality traits and amount of protein fractions in wheat grains. These results are in agreement with the results reported by Manjaya *et al.* (2007) who cited that irradiation of globular protein causes formation of protein aggregates which effect on its solubility. Meanwhile, Khan *et al.* (2008) found that gluten content of salt tolerant wheat varieties was higher than that of salt sensitive ones. Zhang *et al.* (2016) observed that salt stress increased the contents of amino acids, high molecular weight glutenin subunits (HMW-GS) and glutenin macropolymers (GMP) in grains and improved the fraction of large GMP.

The effect of salinity on the protein profiles of wheat cultivars in the absence or presence of gamma irradiation are shown in Tables 5 and 6 as well in Fig. 3. Gamma rays and salinity induced a considerable variation in the protein patterns of the two cultivars of wheat. This variation has been manifested as the novel expression of some polypeptide, the absence of others and over expression of third-class polypeptides. The soluble protein profiles of the two wheat cultivars comprised of six common major bands and a number of minor bands. The main polypeptide bands are located between 25 and 60 kDa. The total number of bands not effected under all treatments, but some bands appeared and other bands disappeared. The total number of protein bands decreased from 20 (control) to 14 under treatment, 200 Gy combined with 60 mM NaCl and 300 Gy combined with 60 mM NaCl. Also, the band with molecular weight 71.188 kDa appeared in control and 300 Gy treatments and disappeared in other treatments in Sids-1 cultivar. But it was different in Sakha-93, the bands ranged from 11 (control) to 18 (treatments 300 Gy). Moreover, the band with molecular weight 47.155 kDa disappeared in control and appeared in the other treatments. The most crucial function of plant cell is to respond to gamma stress by developing defense mechanisms. This defense was brought by alteration in the pattern of gene expression altered under gamma stress, qualitative and a quantitative change in total soluble protein content

Table 3. Effect of gamma irradiation and salt stress on percentage of protein fractions in Sids-1 wheat cultivar.

Dose (Gy)	Albumin + globulin %			Gliadin %			Glutenin %			Gliadin/glutenin		
	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	3.17 c	3.26 b	3.34 a	1.34 g	1.45 fg	1.54 ef	2.52 f	2.62 ef	2.66 def	0.579 c	0.556 d	0.532 e
100	3.11 d	2.74 i	2.89 h	1.62 e	2.05 a	1.98 ab	2.77 de	3.25 a	2.16 g	0.585 c	0.631 a	0.627 ab
200	2.93 gh	2.96f g	2.96 fg	1.93 bc	1.86 cd	1.86 cd	3.06 b	3.03 b	2.98 bc	0.631 a	0.614 b	0.624 ab
300	3.01 ef	3.05 e	3.05 e	1.77 d	1.77 d	1.60 e	2.82 cd	2.79 d	2.73 de	0.628 ab	0.634 a	0.586 c
LSD for interaction		0.05355			0.1197			0.1693			0.01693	

Different letters indicate statistically significant differences at $p \leq 0.05$.

Table 4. Effect of gamma irradiation and salt stress on percentage of protein fractions in Sakha-93 wheat cultivar.

Dose (Gy)	Albumin + globulin %			Gliadin %			Glutenin %			Gliadin/glutenin		
	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	3.51 c	3.66 b	3.82 a	1.29 h	1.34 h	1.54 fg	2.77 g	2.94 f	3.01ef	0.512 e	0.456 g	0.466f g
100	3.24 d	2.61 k	2.70 j	1.61 f	2.21 a	2.15ab	3.10 cdef	3.36 a	3.30 ab	0.519 e	0.658 a	0.652 a
200	2.77 i	2.85 h	2.94 g	2.04 bc	1.96 cd	1.89 d	3.24 abc	3.19 bcd	3.16 bcde	0.630 b	0.614b c	0.598 c
300	3.01 f	3.10 e	3.13 e	1.75 e	1.59 f	1.46 g	3.13 cde	3.06 def	3.05def	0.559 d	0.520 e	0.479 f
LSD for interaction		0.05355			0.1197			0.1693			0.01693	

Different letters indicate statistically significant differences at $p \leq 0.05$.

Table 5. Effect of gamma irradiation on distribution of protein patterns in the Sids-1 cultivar under sodium chloride stress.

Band No.	M.W (kDa)	1	2	3	4	5	6	7	8	9	10	11	12
1	87.550	1	1	1	1	1	1	1	1	1	1	1	1
2	79.302	1	1	1	1	1	1	1	1	1	1	1	1
3	74.463	1	1	1	1	1	1	1	1	1	1	1	1
4	71.188	1	0	0	0	0	0	0	0	0	1	0	0
5	70.550	0	1	1	1	1	1	0	0	0	0	0	0
6	68.827	1	1	1	1	0	1	0	0	0	0	0	1
7	66.844	1	1	1	1	1	1	1	1	1	1	1	1
8	64.627	1	1	0	0	1	0	0	0	0	0	0	0
9	60.275	1	1	1	1	0	1	1	1	0	1	0	0
10	55.588	1	1	1	1	1	1	0	1	0	1	0	1
11	52.549	1	1	1	0	0	1	0	0	0	1	0	0
12	48.028	1	1	1	1	1	1	1	1	1	1	1	1
13	46.331	1	1	1	1	1	1	1	1	1	1	1	1
14	42.728	1	1	1	1	1	1	1	1	1	1	1	1
15	39.052	1	1	1	1	1	1	1	1	1	1	1	1
16	35.057	1	1	1	1	1	1	1	1	1	1	1	1
17	34.123	1	1	1	1	1	1	1	1	1	1	1	1
18	32.041	1	1	1	1	1	1	1	1	1	1	1	1
19	26.826	1	1	1	1	1	1	1	1	1	1	1	1
20	25.416	1	1	1	1	1	1	1	1	1	1	1	1
21	24.081	1	1	1	1	1	1	1	1	1	1	1	1

1 = Presence of band, 0 = Absence of band.

Table 6. Effect of gamma irradiation on distribution of protein patterns in the Sakha-93 cultivar under sodium chloride stress.

Band No.	M.W (kDa)	1	2	3	4	5	6	7	8	9	10	11	12
1	121.711	0	0	0	0	0	0	0	0	1	0	0	0
2	119.621	0	0	0	0	0	0	0	0	0	1	0	0
3	112.830	0	1	1	1	1	1	1	1	1	1	0	0
4	99.301	1	1	1	1	1	1	1	1	1	1	1	1
5	84.785	1	1	1	1	1	1	1	1	1	1	1	1
6	77.921	1	1	1	1	1	1	1	1	1	1	1	1
7	73.497	0	1	1	1	1	1	1	1	1	1	0	1
8	69.324	0	1	1	1	1	1	1	1	1	1	0	1
9	59.576	1	1	1	1	1	1	1	1	1	1	1	1
10	54.516	0	0	1	1	1	1	1	0	0	0	0	0
11	54.163	0	0	0	0	0	0	0	1	1	1	1	1
12	52.660	0	0	1	1	1	1	1	1	1	1	1	1
13	50.867	1	1	1	1	1	1	1	1	1	1	1	1
14	47.155	0	1	1	1	1	1	1	1	1	1	1	1
15	41.411	1	1	1	1	1	1	1	1	1	1	1	1
16	36.053	1	1	1	1	1	1	1	1	1	1	1	1
17	33.786	1	1	1	1	1	1	1	1	1	1	1	1
18	30.518	1	1	1	1	1	1	1	1	1	1	1	1
19	25.832	1	1	1	1	1	1	1	1	1	1	1	1
20	25.115	1	1	1	1	1	1	1	1	1	1	1	1

1 = Presence of band, 0 = Absence of band.

was obvious (Corthals *et al*. 2000). In this respect, Beltagi *et al*. (2006) found variations in number, intensity and/or density of SDS electrophoretic bands of proteins from common bean after gamma irradiation and salt stress.

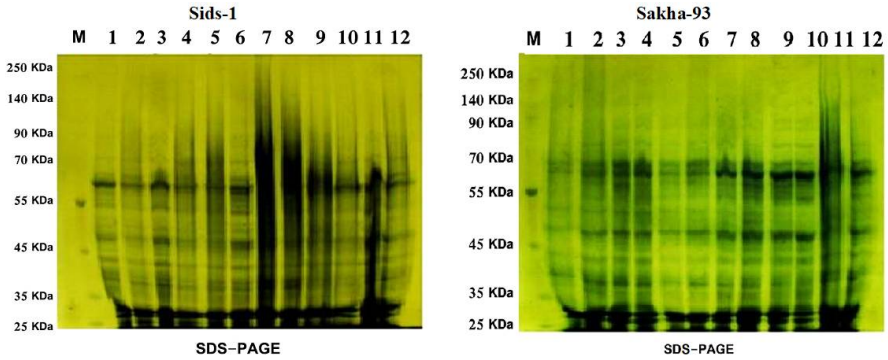

Fig. 3. Electrophoretic banding patterns of total soluble protein from Sids-1 and Sakha-93 wheat cultivars under gamma irradiation and salt stress; M= marker, lane 1 = (control), lane 2 = 60 mM NaCl, lane 3 = 120 mM NaCl, lane 4 =100 Gy, lane 5 = 100 Gy +60 mM NaCl, lane 6 = 100 Gy + 120 mM NaCl, lane 7 = 200 Gy, lane 8 = 200 Gy + 60 mM NaCl, lane 9 = 200 Gy + 120 mM NaCl, lane 10 = 300 Gy, lane 11 = 300 Gy + 60 mM NaCl and lane 12 = 300 Gy +120 mM NaCl.

The results showed that different doses of gamma radiation have different effects on biochemical characteristics, such as the amino acids and different proteins. The changes in protein patterns profile depended even on irradiation dose and the salt stress level in wheat grains. It is clear that this technique can be used for production of a mutant with ability for environmental stress tolerance. This behavior may be due to its ability to tolerate salt stress or because of salt stress which may cause some shift in gene expression. Further studies regarding other molecular aspects are being performed in order to clarify and provide additional information for the complex response of perennial bread wheat to salinity stress.

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References

Aly AA, Maraei RW and Ayadi S 2018. Some biochemical changes in two Egyptian bread wheat cultivars in response to gamma irradiation and salt stress. Bulg. J. Agric. Sci. **24**(1): 50-59.

AOAC 1990. Official methods of analysis of the association of official analytical chemists 15th ed. Published by the association of official analytical chemists, INC suite 400, 2200 Wilson Boulevard. Arlington, Virginia. 22201 USA.

Baxter JH 1996. Amino acids. *In:* Handbook of Food Analysis. Vol.1 edited by Leo M. L. Nollet.Marcel Dekker, Inc., New York. pp. 179-228.

Belderok B, Mesdag J and Donner DA 2000. Bread-making quality of wheat: A Century of Breeding in Europe; Kluwer Academic Publisher: Dordrecht, The Netherlands, 30-31.

Beltagi MS, Ismail MA and Mohamed FH 2006. Induced salt tolerance in common bean (*Phaseolas vulgaris* L.) by gamma radiation. Pak. J. Plant Sci. **9**:1143-1148.

Bradford MM 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Analy Biochem. **72**: 248-254.

Braun HZ, Atlin G and Payne T 2010. Multi- location testing as a tool to identify plant response to global climate change, In: Rrynolds, CRP.(ed). Climate Change and Crop Production, CABI, London, UK.

Çelik Ö, Atak Ç and Suludere Z 2014. Response of soybean plants to gamma radiation: Biochemical analyses and expression patterns of trichome development. Plant Omics J. **7**(5): 382-391.

Corthals G, Gygi S, Abersold R and Patterson SD 2000. Identification of proteins by mass spectrometry. Proteome Res. **2**(1): 286-290.

El-Beltagi HS, Mohamed HI, Mohammed AMA, Zaki LM and Mogazy AM 2013. Physiological and biochemical effects of γ-irradiation on cowpea plants (*Vigna sinensis*) under salt stress, Not. Bot. Hort. Agrobo. Cluj. **41**(1): 104-114.

Jayeraman J 1985. Laboratory manual in biochemistry, Willy Eastern Limited, New Delhi.

Khan NAM and Shambhoo P 2008. Biochemical changes in wheat plants in response to salinity. Int. J. Plant Sci. **3**: 11-15.

Koga S, Bcker U, Moldestad A, Tosi P, Shewry PR, Mosleth EF and Uhlen AK 2016. Influence of temperature during grain filling on gluten viscoelastic properties and gluten protein composition. J. Sci. Food Agric. **96**: 122-130.

Laemmli UK 1970. Cleavage of structural proteins during the assembly of the head Bacteriophage T4. Nature **227**: 680-686.

Maity JP, Chakraborty S, Ka S, Panja S, Jean JS, Samal AC, Chakraborty A and Santra SC 2009. Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. Food Chem. **114**:1237-1244.

Manjaya JG, Suseelan KN, Gopalakrishna T, Pawar SE and Bapat VA 2007. Radiation induced variability of seed storage proteins in soybean [*Glycine max* (L.) Merrill]. Food Chem. **100**:1324-1327.

Mohamed AN and Ismail MR 2011. Changes in organic and inorganic solutes of *in vitro* tomato cultivars under NaCl stress. Aust. J. Crop Sci. **5**(8): 939-944.

Neto ADA, Prisco JT and Gomes-Filho E 2009. Changes in soluble amino-N, soluble proteins and free amino acids in leaves and roots of saltstressed maize genotypes. J. Plant Interac. **4**(2): 137-144.

Qureshi MI, Qadir S and Zolla L 2007. Proteomics-based dissection of stress-responsive pathways in plants. J. Plant Physiol. **164**(10): 1239-1260.

Rahnama A, James RA, Poustini K and Munns R 2010. Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. Funct. Plant Biol. **37**(3): 255-263.

Sharobeem SF, Hidvegi M, Simonne SL, Lasztity R and Salgo A 1986. A kukorica mint fehérje- és aminósavforrás, Élelmezési Ipar. **40**(8): 287-292.

Shen Y, Guo W, Zhou Y, Zhu X, Feng C and Peng Y 2007. Effects of salinity stress on the dynamic changes in the accumulation of grain protein and its components in wheat. J. Triti. Crops **26**(6):100-103.

Shuhua L, Xing X, Hongxia L, Min Q, Jun H and Haili M 2002. Effect of salinity on seed protein and yield characters of wheat. Agric. Res. Arid Areas **2**:38-41.

Silva EC, Nogueira RJM, Araujo FP, Melo NF and Azevedo Neto AD 2008. Physiological responses to salt stress in young umbu plants. Environ/ Experi. Botany **63**:147-157.

Wani S H, Kumar V, Shriram V and Sah SK 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. The Crop J. **4**:162-176.

Zhang X, Shi Z, Tian Y, Zhou Q, Cai J, Dai T, Cao W, Pu H and Jiang D 2016. Salt stress increases content and size of glutenin macropolymers in wheat grain. Food Chem. **197**: 516-521.

Žilić S, Barać M, Pešić M, Dodig D and Ignjatović-Micić D 2011c. Characterization of proteins from grain of different bread and durum wheat genotypes. Int. J. Molec. Sci. **12**(9): 5878-5894.

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