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The Study of Changes in Quaternary Ammonium Compounds and Amino Acids as Biochemical Indicators for Salt Tolerance in Wheat under Wadi Sudr Conditions

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Abstract: Two field experiments were carried out during 2006/2007 and 2007/2008 seasons at Ras Sudr Agricultural Experimental Station of Desert Research Center to study the induced resistance in two wheat cultivars (Sakha94 and Gimeza9) subjected to salinity stress (8614 ppm), by using some chemical materials individually.i.e. glycine (20, 40 and 60 ppm), proline (20, 40 and 60 ppm), choline chloride (250, 500 and 1000 ppm) and glycinebetaine (10, 15 and 20 μM) on quaternary ammonium compounds (QAC), amino acids, proteins patterns and grain yield. The obtained results could be summarized as follows: Generally, foliar application treatments may correct the metabolic disturbance under saline stress condition of Ras Sudr. Water soluble quaternary ammonium compounds (QAC) was significantly increased after treatment with proline, choline chloride and glycinebetaine at high rates during two growth stages. Also, choline content took the same trend when choline chloride and glycinebetaine applied at high rates during two growth stages. In this regard, Sakha94 significantly exceeded Gimeza 9 in such contents. This gave also a good indication of its salt tolerance. While, the effect of foliar application of glycine is meagre or insignificant for two wheat cultivars at two growth stages. Amino acids composition of two wheat cultivars hydrolysate indicated the presence of 16 amino acid including the most essential amino acids. Glutamic acid is the most abundant amino acid followed by aspartic, leucine and proline. While methionine is presented in minute quantities. Also, the obtained results showed that glutamic and aspartic acids were increased in shoots of two wheat cultivars at high rates of proline. While, glutamic acid accumulated in shoots of Sakha 94, when choline chloride and glycinebetaine applied. This depended markedly on the sensitivity degree of cultivars and kind of foliar application treatments. These findings were in harmony with those obtained from grain yield and its attributes with respect to the effect of foliar application on wheat cultivars studied. Generally, the content of glutamic and aspartic in both studied cultivars were mostly higher than other amino acids, which is possibly due to their being precursors for synthesis of most amino acids. Also, the results suggested that these two amino acids could be used as an important biochemical marker to differentiate among cultivars different in salt tolerance. Separation of proteins by SDS-PAGE showed that Sakha94 proteins were resolved into 28 bands, while in Gimeza 9 were resolved into 25 bands. The molecular weight of protein sub units ranged between16 and 88 kDa. Moreover, intensity of polypeptide bands was differentially affected by foliar applications among wheat cultivars which were decreased in Gimeza9 and increased in Sakha94 under saline conditions. Results proved that, Sakha94 was more tolerant to salt stress than Gimeza 9 at Ras Sudr conditions, which appeared clearly from the biochemical content of plant and grain yield.

Key words: *Triticum aestivum* L. · Wheat · Salt tolerance · Salinity · Quaternary ammonium compounds (QAC) · Amino acids

INTRODUCTION

Salinity is known by its depressive effects on metabolic pathways and energy generating processes. These metabolic shifts are due to mineral deficiency or excess of non-nutrient ions, poor development of vascular tissues and hormonal imbalance. Any treatment which decreases the depressive effects of salinity, leads to improvement of yield and serves as a viable means for sustaining agriculture under saline conditions.

Some plants resistant to salinity accumulate a large quantity of organic osmoprotectant solutes. These

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include amino acids and fully N-methyl amino acids generically known as betaines. Proline accumulation is a common metabolic response of higher plants to salinity stress[1,2]. In this respect, spraying wheat plants with proline decreased the adverse effect of different salts on the dry matter yield[3]. Also, El-Bassiouny and Bekheta [4] in their study on wheat and Hassan [5] on barley showed that the predominant amino acids were glutamic and proline under saline conditions.

In plants, glycinebetaine is made in two steps, with glycinebetaine aldehyde as intermediate. These solutes protect plant processes against salinity stress and play a pivotal role in plant cytoplasmic osmotic adjustment in response to osmotic stresses. Also, salinity causes irregularities in nitrogen metabolism and that certain quaternary ammonium compounds (QAC), for example glycinebetaine, are often accumulated in plant shoots. In this connection, glycinebetaine application increased net photosynthesis of salt stressed plants. This was mostly due to increased stomatal conductance and thus favored higher CO_2 fixation rate. Moreover, glycinebetaine application resulted in a significant decrease in photorespiration in salt stressed plants[6,7]. Also, Jagendorf and Takabe [8] showed that glycinebetaine is an osmoprotectant accumulated by barely plants in response to high levels of NaCl.

The quaternary ammonium compounds are derived from amino acids precursors. These compounds share the property of being uncharged at neutral pH, and are of high solubility in water[9]. These compounds include glycinebetaine (N,N,N-trimethylglycine), prolinebetaine (N,N,-Dimethylglycine), hydroxylprolinebetaine, B-alaninebetaine and Choline-O-Sulfate. The quaternary ammonium compounds possess a fully methyl substituted nitrogen atom (creating a permanent positive charge on the N moiety) and a negatively charged carboxyl group (in the case of betaines) or sulphate group (in the case of Choline-O-Sulfate).

Therefore, this investigation was conducted to study the induced resistance in two wheat cultivars subjected to salinity stress under Wadi Sudr conditions, by using some chemical materials which are safe on human health and environment.

MATERIALS AND METHODS

Two field experiments were carried out under calcareous soil of Ras Sudr Agricultural Experiment Station of Desert Research Center, South Sinai Governorate, Egypt during two successive winter seasons of 2006/2007 and 2007/2008 to study changes in quaternary ammonium compounds and amino acids as biochemical indicator for salt tolerance in wheat under salinity stress.

Plant Materials: Grains of the two wheat cultivars (Sakha94 and Gimeza9) were obtained from the Field Crop Institute, Agricultural Research Center, Giza, Egypt.

Planting: Planting was carried out on November 16th during the both seasons at rate of 60 Kg/feddan. The plot area was 6m^2 (2x3 m^2) containing 10 rows. Organic manure and calcium superphosphate fertilizers were added during soil preparation at rates of 20 m^3 and 30 Kg P_2O_5/feddan, respectively. Nitrogen fertilizer as a form of ammonium nitrate (33.5 % N) was addedat rate 70 Kg N/ feddan. The chemical analysis of underground irrigation water and soil are presented in Table 1.

Application of Treatments: The experiment included twenty-six treatments; i.e. four different chemical materials (each treatment was sprayed with three levels as compared with the control (Tap water) and two wheat cultivars. Foliar application treatments and doses were applied as follows:

a- Tap water (control).
b- Glycine (20, 40 and 60 ppm).
c- Proline (20, 40 and 60 ppm).
d- Choline chloride (250, 500 and 1000 ppm).
e- Glycinebetaine (10, 15 and 20 μM).

Each treatment was sprayed on plants at rate of 400 liter/feddan after 35 and 65 days from sowing.

Sampling: Two plant samples were taken randomly from each treatment at 45 and 75 days from sowing. Fresh

Table 1: Water and soil chemical analysis

			Cations (meq L^{-1})				Anions (meq L^{-1})			
EC (dS m^{-1})	ppm	pH	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO^{=}$	HCO^{-}	Cl^{-}	$SO4^{=}$
					Water analysis					
13.46	8614	7.3	32.41	16.35	79.11	0.415	----	3.62	69.13	55.03
					Soil analysis					
14.71	9414	7.6	44.25	25.30	91.40	0.92	----	9.88	96.17	55.51

samples were tested for banding patterns of proteins. Then, dried till constant weight representing dry weight. Dry samples were grounded to fine powder and tested for amino acids, quaternary ammonium compounds and Choline.

Harvesting: Plants were harvested after 145 days from sowing; the grain yield (g m^{-2}) was recorded.

Chemical Analysis

Determination of Total Water Soluble Quaternary Ammonium Compounds (QAC) and Choline: Quaternary ammonium compounds and Choline were determined according to Grieve and Grattan [10]. The shoot dry mass (half g) was added to 20 ml of deionized water and left for 24 hours on a shaker. The plant extract was then diluted with sulphuric acid (2N) with the ratio 1:1.

Quaternary Ammonium Compounds Determination: Half ml of the mixture was then cooled in ice water for 1hour. Cold KI-I_2 reagent (0.2 ml) , prepared by dissolving 15.7 g of iodine and 20 g of KI in 100 ml water , was added and the reactants were gently stirred with a vortex mixer. The tubes were stored at 0-4°C for 16 hours and then centrifuged at 10.000 rpm for 15 min at 00°C. The supernatant was carefully aspirated with a fine tipped glass tube. Due to the solubility of the complexes in the acid reaction mixture increases markedly with temperature, it is important that the tubes were kept cold until the periodide complex is separated from the acid media. The periodide crystals were dissolved in 9 ml of 1,2dichloroethane. Vigorous vortex mixing was frequently required to effect complete solution in the developing solvent. After 2-2.5 hours, the absorbance was measured at 365 nm. Reference standards of glycinebetaine were prepared in 1 N H_2SO_4.

Choline Determination: Thawed sample extracts were diluted 1:1 with KPi buffer (0.2 M, pH 6.8). The periodides were precipitated and analyzed as described for quaternary ammonium compounds.

Qualitative and Quantitative Determination of Amino Acids: Amino acids composition for 75 days old wheat shoots hydrolysate was determined by amino acid analyzer apparatus model (Eppendrof LC 3000). Hydrolysis was carried out according to the method of Block *et al.* [11]. In this method a known weight of wheat shoots was transferred into a tube containing 6 N hydrochloric acid, the sealed and hydrolysis were continued for a period of 24 hours in an oven at 110°C. At the end of this period, hydrolysate was transferred quantitatively and the hydrochloric acid was then evaporated to dryness. Distilled water was added to the hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid. The samples were kept in the refrigerator until separation of the amino acids by amino acid analyzer. The peak area and percentage of each amino acid were calculated by computer software AXXIOM CHROMATOGRAPHY-727.

SDS-PAGE Electrophoresis of Proteins: Sodium dodocyl sulphate polyacrylamide gel electrophoresis was performed according to the method of laemmli [12], as modified by Studier[13]. Leaf samples were treated with liquid nitrogen and ground with water soluble extraction buffer, using mortar and pestle. Samples were centrifuged for 20 min at 12.000 rpm at 4°C. Supernatants containing water soluble protein fraction were then kept under -80°C until used and subjected for further analysis by SDS electrophoresis. The relative mobilities of the proteins were calculated by dividing the migration distance of each protein by that of the bromophenol blue front. All measurements should be done in the middle of the dye bands. By applying proteins of Known sub-unit molecular weight (Markers from Pharmacia) the molecular weight of the samples was calculated.

Statistical Analysis: Data were analyzed statistically according to the procedure outline Snedecor and Cochran [14]. Means followed by the same alphabetical letter (s) are not statistically different at the 0.05 level of significance according to Duncan's multiple test [15].

RESULTS AND DISCUSSION

Effect of Foliar Application and Wheat Cultivars on Quaternary Ammonium Compounds and Choline: Data presented in Table 2 show the effect of foliar application of glycine on quaternary ammonium compounds and choline in shoots of wheat cultivars at two growth stages. Results indicated that QAC was significantly increased by spraying doses of 20, 40 and 60 ppm glycine at 1st growth stage as compared with the control. This was true by foliar application of 60 ppm at 2nd growth stage. Moreover, Sakha94 significantly exceeded Gimeza9 in QAC at 2nd growth stage. Considering the interaction effect between foliar application and wheat cultivars, data indicated that spraying of glycine significantly increased QAC in shoots of Gimeza 9 at 1st growth stage as

Table 2: Effect of foliar application and wheat cultivars on total water soluble quaternary ammonium compounds and Choline in shoots at 45 and 75 days after sowing

Foliar application	*Water soluble quaternary ammonium (QAC) compounds (μ mol/g dry weight)						Choline (μ mol/g dry weight)					
	At 45 days after sowing			At 75 days after sowing			At 45 days after sowing			At 75 days after sowing		
Glycine (ppm)	Sakha94	Gimeza9	Mean	Sakha94	Gimeza9	Mean	Sakha94	Gimeza9	Mean	Sakha94	Gimeza9	Mean
00	68.12ab	60.86c	64.49C	77.66c	65.66d	71.66B	17.88a	16.33a	17.10A	19.71ab	17.23b	18.47B
20	65.14bc	70.91a	68.03B	79.28b	66.70d	72.99AB	18.27a	16.43a	17.35A	17.14b	19.81ab	18.47B
40	68.02ab	69.08ab	68.55B	77.67c	65.34d	71.50B	19.11a	18.32a	18.72A	17.11b	18.97ab	18.04B
60	69.24ab	71.81a	70.52A	81.70a	66.21d	73.95A	18.78a	18.42a	18.60A	18.66ab	21.93a	20.29A
Mean	67.63A	68.17A		79.07A	65.98B		18.51A	17.38A		18.15A	19.48A	
Proline (ppm)												
00	68.12bc	60.86d	64.49B	77.66b	65.66d	71.66C	17.88a	16.33ab	17.10A	19.71ab	17.23bc	18.47A
20	68.24bc	60.41d	64.32B	78.17b	68.91cd	73.54B	14.12b	16.95a	15.53B	18.34abc	16.68c	17.51A
40	74.46a	63.21d	68.83A	86.84a	66.42cd	76.63A	17.19a	16.88a	17.03A	18.11abc	17.28bc	17.69A
60	72.40ab	64.98cd	68.69A	84.96a	70.21c	77.58A	14.13b	17.91a	16.02AB	19.88a	17.11c	18.49A
Mean	70.80A	62.365B		81.90A	67.80B		15.83A	17.02A		19.01A	17.08B	
Choline Chloride (ppm)												
00	68.12e	60.86g	64.49D	77.66e	65.66g	71.66D	17.88b	16.33b	17.10B	19.71c	17.23d	18.47C
250	67.84e	65.52f	66.68C	80.72d	76.82e	78.77C	18.11b	17.34b	17.73B	19.98c	22.71b	21.34B
500	92.02a	71.96c	81.99A	94.22b	67.53f	80.87B	18.28b	23.87a	21.08A	24.92ab	23.95ab	24.44A
1000	84.31b	70.18d	77.24B	97.31a	84.08c	90.69A	19.89b	23.93a	21.91A	24.84ab	25.81a	25.33A
Mean	78.07A	67.13B		87.47A	73.52B		18.54A	20.37A		22.36A	22.42A	
Glycinebetaine (μM)												
00	68.12c	60.86e	64.49C	77.66c	65.66e	71.66B	17.88bcde	16.33e	17.10B	19.71b	17.23d	18.47B
10	68.50c	62.33e	65.42BC	78.23c	69.08d	73.65B	20.16ab	16.66de	18.41B	20.14b	17.98cd	19.06AB
15	69.10c	64.52d	66.81B	78.62c	67.88de	73.25B	19.03bcd	17.72cde	18.38B	20.37b	17.17d	18.77AB
20	88.82a	74.45b	81.64A	98.41a	83.91b	91.1A	22.31a	19.10bc	20.7A	23.99a	18.48c	21.24A
Mean	73.64A	65.54B		83.23A	71.63B		19.85A	17.45B		21.05A	17.72B	

-Values followed by the same letter in columns are not different at $p< 0.05$ by Duncan's multiple range test. *Compared to glycinebetaine standard.
-45 days after sowing = 1st growth stage, -75 days after sowing = 2nd growth stage

compared with the control. Such content in shoots of Sakha94 was increased by spraying of 20 and 60 ppm at 2nd growth stage. Concerning choline content, it was significantly increased with spraying dose of 60 ppm glycine at 2nd growth stage as compared with untreated plants. Regarding the interaction between foliar application and wheat cultivars on choline content, data in the same table show that, choline content in shoots of Gimeza9 increased significantly, when glycine applied at rate 60ppm at 2nd growth stage. Mostafa [16] found that accumulation of glycinebetaine in zea mays under salt stress was higher in Giza2 (salt tolerance) than Trihybrid 321 (salt sensitive). It could be, therefore proposed that glycinebetaine may be correlated with salt tolerance in maize. Various reports indicate that salt tolerance species/cultivars have greater capacity for glycinebetaine accumulation than sensitive ones[7-20]. Also, Chen *et al.* [21] found that hyperaccumulation of known major compatible solutes in barley does not appear to play a major role in salt tolerance, but rather may be a symptom of salt susceptibility.

The effect of foliar application of proline on QAC and choline can be deduced from tabulated data in Table 2. The results show that the spraying of proline tended to significant increase in QAC at 2nd growth stage. This was true after treatment with 40 and 60ppm at 1st growth stage. Also, QAC in shoots of Sakha 94 was significantly increased more than Gimeza9 at two growth stages. As shown from data in the same table, the interaction between foliar application and wheat cultivars showed that QAC was significantly increased in plants when proline applied at rates 40 ppm and (40 and 60ppm) for Sakha 94 at 1st and 2nd growth stage, respectively as compared with the control. Also, Gimeza 9 took the same trend when proline applied at rate 60ppm at 2nd growth stage. While, choline content was significantly decreased by adding proline foliarly at rate 20 ppm at 1st growth stage as compared with the control. In this regard, Sakha94 significantly exceeded the Gimeza 9 in choline content at 2nd growth stage. As to the effect of interaction between foliar application of proline and wheat cultivars, plants sprayed with 20 and 60 ppm showed significant decrease in choline content for Sakha 94 at 1st growth stage. In this respect, Marcum and Murdoch [22] showed that glycinebetaine accumulated in salt stressed seedling (grass) of both cultivars was high enough to be sufficient

for cytoplasmic osmotic adjustment. Mansour [23] reported that high concentration of glycinebetaine is not required for plasma membrane protection of onion, and thus high concentration is essentially involved in osmotic adjustment.

Regarding the effect of foliar application of choline chloride, it was found that plants sprayed with choline chloride showed a significantly increased of QAC at two growth stages as compared with the control. Also, Sakha94 produced the highest value of QAC at two growth stages. Regarding the interaction effect, QAC in shoots of Sakha94 was significantly increased with spraying of 500 and 1000 ppm at 1st growth stage. In this respect, the spraying of choline chloride tended to increase such content in shoots of Gimeza9, when applied at 1st growth stage as compared with the control. This was true for two wheat cultivars at 2nd growth stage. Concerning the choline content, it was significantly increased after treatment with 500 and 1000 ppm at 1st growth stage. This was true at 2nd growth stage, when plants treated with Choline chloride. While, the differences between wheat cultivars are not significant at two growth stages. Data in the same table illustrate the interaction between foliar application of choline chloride and wheat cultivars. Plants sprayed with 500 and 1000 ppm showed a significantly increased of choline content in shoots of Gimeza9 and Sakha94 at 1st and 2nd growth stage respectively, as compared with the control plants. In this connection, data indicated accumulation of such content in shoots of Gimeza9 when plants sprayed with choline chloride at 2nd growth stage. Grieve and Grattan [10] found that QAC in wheat plants increased from 46.9 μ mol/g dry weight before stress to 93.8 μ mol/g dry weight after salinity stress. Also, choline content increased from 17.3 μ mol/g dry weight to 22 μ mol/g dry weight after treatment with the same concentration of salinity.

The effect of foliar application of glycinebetaine on QAC after 45 and 75 days from sowing is presented in Table 2. The data show that QAC significantly increased after treatment with (15 and 20μM) and 20 μM at 1st and 2nd growth stage respectively, as compared with the control. Results indicated that Sakha 94 significantly exceeded the Gimeza9 in such content at two growth stages. The interaction effect between foliar application of glycinebetaine and wheat cultivars in the same table showed that spraying dose of 20μM significantly increased such content in shoots of Sakha94 at two growth stages. Also, Gimeza9 showed a significantly increased in QAC when glycinebetaine applied at rates 15 and 20 μM at 1st growth stage. This was true for the same cultivar after treatment with glycinebetaine at rates 10 and 20 μM at 2nd growth stage. Concerning choline content, it was significantly increased by spraying of 20 μM glycinebetaine at two growth stages. It is quit clear from these results that Sakha 94 significantly exceeded the Gimeza 9 in choline content at two growth stages. Data in the same table show the interaction between glycinebetaine and both cultivars on choline content. The results revealed clearly that, there was significantly increased in such content for two cultivars, when glycinebetaine applied at rate 20μM as compared with the control at two growth stages.

In this respect, Makela *et al.* [24] indicated that wheat plants are able to translocated foliar applied glycinebetaine from their leaves to other organs Also, glycinebetaine is a relatively inert end product in the plant cells, being mainly phloem mobile. Moreover, environmental conditions are shown to affect the uptake and translocation rates of foliar applied glycinebetaine. Also, Makela *et al.* [25] showed that glycinebetaine applied to the foliage was readily absorbed, but the concentrations in leaves of tomato made a negligible contribution to the total leaf sap osmotic potential. Applied glycinebetaine was involved in the regulation of stomatal conductance but not via ABA metabolism or water relations. Glycinebetaine may have been accumulated in specific cells or cellular compartments, with consequences for stomatal functions. Also, Agboma *et al.* [26] showed that residual tissue glycinebetaine levels remained high 3 weeks after application to the crops (maize, sorghum and wheat). It was suggested that the positive effects of glycinebetaine treatment appear to be linked to its physiological role as a plant osmoticum that improves drought tolerance. Makela *et al.* [27] found that foliar application of glycinebetaine promoted accumulation of betaines similar to that of halophytes under stress conditions. Diaz-Zortia *et al.* [28] found that no significant differences were found in yield components, physiological processes, or endogenous levels in glycinebetaine treated wheat. Significant differences were found endogenous levels of glycinebetaine in response to water deficit stress.

Effect of Foliar Application and Wheat Cultivars on Amino Acids Composition of Proteins: The amino acids composition of shoots samples in the present study were quantitatively determined by amino acid analyzer. Data in Table 3 indicated the presence of 16 amino acid including the most essential amino acids.

Acidic Amino Acids Group: Glutamic acid is the most abundant amino acid in all samples of wheat cultivars followed by aspartic acid. Plants sprayed with glycine at rate 60 ppm showed an increase of glutamic acid in shoots of Gimeza9 as compared with the control. Also, the same amino acid was increased in shoots of Sakha 94 with spraying doses of proline at rates 40 and 60 ppm. Such content in shoots of Gimeza9 was increased with increasing the dose of proline. Regarding, the effect of foliar application of choline chloride on glutamic acid in shoots of wheat cultivars, it was increased in shoots of Sakha94 after treatment with choline chloride as compared with the control. In this respect, the spraying of glycinebetaine tended to increase the glutamic acid in shoots of Sakha 94, when applied at rates 15 and 20 μM as compared with the control. Gimeza9 took the same trend when glycinebetaine applied at rate 20 μM.

Concerning aspartic acid content, it was increased in shoots of Gimeza9 after treatment with 60 ppm glycine as compared with the control. The same trend was true for two wheat cultivars when plants sprayed with proline at rates 40 and 60 ppm. Also, data indicated accumulation of aspartic acid in shoots of Sakha 94 with spraying dose of 250 and 1000ppm choline chloride. In this regard, such content for Gimeza9 was increased, when glycinebetaine applied at rate 20μM as compared with the control.

The content of acidic amino acids (glutamic and aspartic) in shoots of both studied cultivars was mostly higher than other amino acids, possibly due to their being precursors for synthesis of most amino acids[29]. Accordingly, the results suggested that these two amino acids could be used as an important biochemical marker to differentiate among cultivars different in salt tolerance. Also, El-Bassiouny and Bekheta [4] in their study on wheat (Gimeza 9) and Hassan [5] on barley showed that the predominant amino acids were glutamic and proline under saline conditions.

Basic Amino Acids Group: This group included three amino acids: arginine, lysine and histidine. The spraying of glycine tended to decrease arginine acid in shoots of Gimeza9 as compared with the control. This was true for Sakha 94 after treatment with 20 and 60 ppm under the same foliar application. In this respect, such content for two wheat cultivars were decreased by foliar application of proline at rate 20 ppm. Also, spraying of proline decreased the arginine acid content, when applied at rates 40 ppm and 60 ppm for Sakha 94 and Gimeza 9 ,respectively, as compared with the control. In this regard, spraying dose of 250 and 500 ppm choline chloride decreased such content in shoots of Sakha 94. Also, such amino acid in shoots of Gimeza9 took the same trend with spraying dose of choline chloride at rate 1000 ppm as compared with the control. Plants sprayed with glycinebetaine showed a decrease of arginine in shoots of Sakha 94. This decrease was true for Gimeza 9, when glycinebetaine applied at rates 10 and 20 μM as compared with the control.

This is possibly due to transformation to order nitrogenous compounds such as synthesis of putrescine from arginine reported by Mifilin [30] to be enhanced at limited level of K produced at high concentration of Na. Arginine was also reported to be degraded to proline, through synthesis of ornothine which may be reversibly converted to glutamic semialdehyde considered to produce proline as a result of higher activity of proline 5 carboxlic enzyme under NaCl stress[31].

The obtained results showed that lysine content for Sakha 94 was decreased by adding glycine, proline and choline chloride foliarly at rates 40 ppm, 20 ppm and 250ppm respectively, as compared with the control. Also, such content in shoots of Sakha 94 was decreased after treatment with glycinebetaine. It is quite clear from these results that, there was decreased in lysine content for Gimeza9 due to foliar application with glycine as compared with the control. This was true in shoots of the same cultivar when plants sprayed with proline at rate 20 ppm. Also, spraying dose of 250 and 1000 ppm choline chloride decreased such content in shoots of Gimeza 9 as compared with the control. In this connection, plants sprayed with 10 and 15 μM glycinebetaine showed a decrease of lysine content in shoots of Gimeza9 as compared with the untreated plants.

Reponses of lysine could be a resultant of hazardous effects of salinity on aspartic acid known to be required for the condensation of aspartate semialdehyde with pyruvate to biosynthesize the indicated amino acid. Other possibility could be the conversion of lysine to pipecolic acid under salinity conditions[30].

Generally, histidine content in shoots of Sakha 94 decreased by application of all foliar treatments as compared with the control. On the other hand, the reverse was true in such content for Gimeza9 under the same conditions.

Histidine being possibly a potential precursor for glucose [32] which was pointed out by Muralitharan *et al.* [33] to be significantly increased with NaCl salinity. Variations between the two studied cultivars could be attributed to contribution of the concerned amino acids for the synthesis of some protein types responsible to

Table 3: Effect of foliar application and wheat cultivars on Amino acids content (mg/g dry weight) in shoots at 75 days after sowing

Treatments			Amino acids content (mg/g dry weight)															
			Acidic		Basic			Neutral								Aromatic and imine		
Foliar application		Wheat Cultivars	Aspartic	Glutamic	Histidine	Lysine	Arginine	Glycine	Alanine	Valine	Isoleucine	Leucine	Threonine	Serine	Methionine	Tyrosine	Phenyl alanine	Proline
Control		Sakha 94	3.92	5.64	4.32	2.69	2.46	2.38	3.58	2.96	2.48	4.17	2.42	1.98	0.51	1.93	3.13	2.99
		Gimeza 9	4.22	5.56	0.33	2.55	1.86	2.23	3.84	2.58	2.02	3.57	2.14	1.80	0.32	1.36	1.84	4.21
Glycine	20	Sakha 94	3.34	5.44	0.038	2.73	1.34	2.01	3.61	2.15	1.19	3.26	2.26	1.82	0.01	1.74	1.38	4.65
(ppm)		Gimeza 9	4.11	5.04	2.06	2.30	1.61	1.91	3.51	2.50	1.63	3.15	1.88	1.67	0.38	0.96	1.90	4.12
	40	Sakha 94	3.48	5.58	3.85	2.01	2.8	2.74	3.41	2.32	2.73	4.29	2.31	1.06	0.29	1.57	2.64	2.95
		Gimeza 9	3.49	4.84	0.38	2.07	1.55	1.82	3.29	1.80	1.61	2.93	1.84	1.54	0.18	0.96	1.71	3.70
	60	Sakha 94	3.44	4.69	2.59	2.87	1.42	2.13	3.37	2.34	1.92	3.79	1.96	1.68	0.15	1.55	2.32	1.38
		Gimeza 9	4.45	5.76	2.36	2.52	1.83	2.31	4.05	3.06	2.01	3.58	2.36	1.99	0.30	1.17	2.01	4.18
Proline	20	Sakha 94	3.33	4.00	1.65	1.78	1.15	1.70	3.40	2.31	1.42	2.51	1.56	1.40	0.24	0.73	1.45	3.18
(ppm)		Gimeza 9	3.94	6.16	0.57	2.34	1.68	2.01	3.46	2.78	1.76	3.13	2.10	1.74	0.58	1.04	1.57	4.07
	40	Sakha 94	4.46	6.81	3.24	3.03	2.41	2.68	4.05	3.22	2.60	4.69	2.68	2.19	0.49	2.05	3.32	3.31
		Gimeza 9	4.71	6.28	0.62	2.77	2.12	2.40	4.27	3.19	2.16	3.93	2.55	2.14	0.42	1.44	2.40	4.62
	60	Sakha 94	4.90	6.19	3.58	3.14	2.81	2.83	4.40	3.52	2.90	5.09	2.96	2.50	0.54	2.28	3.68	3.80
		Gimeza 9	4.30	6.46	0.48	2.58	1.79	2.16	3.79	2.83	1.89	3.46	2.22	1.86	0.28	0.98	2.08	4.64
Choline	250	Sakha 94	4.55	7.09	3.22	2.86	2.42	2.56	4.05	3.18	2.51	4.52	2.51	2.04	0.51	1.94	3.19	3.09
chloride		Gimeza 9	3.48	5.55	0.35	2.19	3.48	1.77	3.14	1.80	1.52	2.71	1.78	1.46	0.11	0.92	1.49	3.79
(ppm)	500	Sakha 94	3.85	6.14	2.52	2.49	2.44	2.29	3.51	2.90	2.13	3.90	2.19	1.75	0.39	1.68	2.77	2.57
		Gimeza 9	3.15	5.52	1.20	9.13	3.94	2.12	3.27	2.72	2.04	5.00	2.14	1.82	1.08	1.91	2.01	3.08
	1000	Sakha 94	4.50	6.85	3.56	3.18	2.70	2.75	4.12	3.40	2.74	4.82	2.77	2.27	0.58	2.19	3.58	4.02
		Gimeza 9	3.36	4.72	0.34	1.85	1.76	1.61	3.04	2.15	1.37	2.49	1.61	1.37	0.35	1.05	1.39	3.81
Glycine-	10	Sakha 94	3.92	4.99	2.08	2.33	1.65	2.150	3.78	2.91	1.79	3.26	2.13	1.82	0.26	1.02	1.90	3.79
betaine		Gimeza 9	3.68	4.41	1.89	2.02	1.60	1.91	3.55	2.57	1.67	2.99	1.83	1.57	0.56	0.91	1.69	3.45
(µM)	15	Sakha 94	3.77	5.89	3.10	2.27	1.96	2.25	3.47	2.67	2.08	3.83	2.10	1.71	0.36	1.60	2.54	2.43
		Gimeza 9	3.45	4.92	0.56	2.49	2.01	2.06	3.24	2.48	1.94	3.62	2.07	1.77	0.36	1.23	1.74	2.38
	20	Sakha 94	4.72	6.46	2.96	2.62	2.24	2.50	3.82	3.00	2.35	4.31	2.37	1.90	0.50	1.93	2.99	2.75
		Gimeza 9	4.11	7.11	0.49	2.61	1.79	2.18	3.72	2.31	1.91	3.40	2.26	1.85	0.30	1.29	2.19	4.62

cope with salinity stress according to differences in the genetic back ground in each cultivar[29].

Neutral Amino Acids Group: It is apparent from data in Table 3 that glycine acid was increased in shoots of Sakha 94 after treatment with glycine at rate 40 ppm as compared with the control. This was true for Gimeza 9 when glycine applied at rate 60 ppm. Also, such content for Sakha 94 increased by adding proline foliarly at rates 40 and 60 ppm as compared with the control. This was true for Gimeza9 when proline applied at rate 40ppm. As to the effect of foliar application of choline chloride, plants sprayed with 250 and 1000 ppm showed an increase of glycine content in shoots of Sakha94 as compared with the control. Also, the content of glycine in shoots of Sakha 94 was increased after spraying of glycinebetaine at rate 20 µM as compared with the control.

In fact, the increase of glycine may be attributed to the activation increase of glycolate oxidase enzyme by application of NaCl[34]; such enzyme catalyzes the oxidation of glycolic acid to glycoxalate which is converted to glycine. As regards serine, it is formed from two molecules of glycine through an oxidation process in the presence of three molecules of ATP [30] whose synthesis is known to be promoted in the presence of sodium ions [35]. Accordingly, promoting effect on glycine may be reflected on the synthesis of serine.

According to Umbarger [36] promtive effect of salinity on alanine, Valine and Leucine may be due to the formation of pyruvic acid from glucose through Embden-Meyerhof BParnas (EMP) reaction pathway[37], glucose being reported by Muralitharan *et al.* [33].

Other neutral amino acids appeared to be decreased or increased depending on the concerned amino acid; response being also dependent on studied cultivar interacted with foliar application treatments.

Aromatic and Imine Amino Acids Group: Data illustrated in Table 3 reveal the effect of foliar application of glycine on proline content in shoots of two wheat cultivars. It was increased in shoots of Sakha94 with spraying dose of 20 ppm as compared with the control. Data in the same table showed an increase in such content for Sakha 94 with increasing the dose of proline. Also, Gimeza9 took the same trend when proline applied at rates 40 and 60 ppm. In this connection, Bandurska [38] found that application of exogenous proline increased the leaf proline content in barely. Also, showed that a possible role of proline in the protection of the enzyme protein against dehydration and osmotic stress. In addition, Ragab *et al.* [39] showed that spraying of 50ppm proline increased the proline content in tomato plants. In this regard, spraying doses of 250 and 1000 ppm choline chloride increased proline content in shoots of Sakha 94.While, such content

Table 4: SDS-PAGE patterns of soluble proteins extracted from Sakha 94 at 75 days from sowing

		Band intensity												
		Treatments												
	Molecular		Glycine (ppm)			Proline (ppm)			Cholinchloride (ppm)			Glycinebetaine (µM)		
Band number	weight (kDa)	Control	20	40	60	20	40	60	250	500	1000	10	15	20
1	88	1	1	1	1	1	1	1	1	1	1	1	1	1**
2	85	1	1	1	1	1	1	1	1	1	1	1	1	1**
3	77	1	2	2	2	2	2	2	2	2	2	2	2	2**
4	72	1	1	1	1	1	1	1	1	1	1	1	1	1**
5	71	2	2	2	2	2	2	2	2	2	2	2	2	2**
6	65	2	2	2	2	2	2	2	2	2	2	2	2	2**
7	62	1	1	1	1	1	1	1	1	1	1	1	1	1**
8	61	1	1	1	1	1	1	1	1	1	1	1	1	1**
9	57	4	5	5	5	5	5	5	5	5	5	5	5	5
10	52	1	1	1	1	1	1	1	1	2	2	2	2	2
11	51	1	1	1	1	2	1	1	1	2	3	3	3	3
12	50	1	1	1	1	1	1	1	1	2	1	1	1	1
13	49	1	1	1	1	1	1	1	1	2	1	1	1	1
14	45	2	2	2	3	3	2	2	2	3	2	2	2	2
15	42	2	2	2	3	3	2	2	2	3	2	2	2	2
16	39	2	2	2	3	3	3	2	2	3	3	3	3	3
17	37	1	1	1	1	1	1	1	1	1	1	1	1	1**
18	36	2	2	2	2	2	2	2	2	2	2	2	2	2**
19	32	1	1	1	2	2	2	1	1	2	2	2	2	1
20	29	1	1	1	1	1	1	1	1	1	1	1	1	1**
21	28	1	1	1	1	1	1	1	1	1	1	1	1	1**
22	25	1	1	1	1	1	1	1	1	1	1	1	1	1**
23	24	1	1	1	1	1	1	1	1	1	1	1	1	1**
24	23	2	2	2	2	2	2	2	2	2	2	2	2	2**
25	22	2	2	2	2	2	2	2	2	2	2	2	2	2**
26	20	1	1	1	1	1	1	1	1	1	1	1	1	1**
27	18	1	2	3	3	3	3	2	2	3	3	3	3	2
28	16	1	2	3	3	3	3	2	2	3	3	3	3	2

5= very deep intensity, 4= deep intensity, 3= moderate deep intensity, 2=moderate intensity, 1= pale intensity and 0= no bands. ** Homologous bands

was decreased in plants, when glycinebetaine applied at rate 10 µM and 20 µM for Gimeza9 and Sakha94 respectively, as compared with the control plants. This was true for both cultivars when glycinebetaine applied at rate 15 µM. In this connection, Ibrahim [40] showed that betaines application reduced proline accumulation in sorghum sap under salinity treatments.

Proline synthesis is implicated as a mechanism of alleviating cytoplasmic k acidosis, and may maintain $NADP^+$/NADPH ratios at values compatible with metabolism [41]. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress-induced damage[41,42]. Also, Greenway and Munns [43] pointed out that many plants species especially the tolerant ones produce different amino acids and carbohydrates to mitigate or prevent the loss of several enzymes activity. Proline suggested to be produced in leaf is transported to the root of the stressed plants, thereby, helping the plant to regulate the osmotic potential of root cells under salinity[44]. In this respect, Ashraf and Foolad [45] added that proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions.

Tyrosine and phenylalanine acids appeared to be decreased or increased depending on the concerned amino acid; response being also dependent on studied cultivar interacted with foliar application treatments.

Effect of Foliar Application and Wheat Cultivars on the Band Patterns of Proteins: SDS-polyacrylamide gel electrophoregrams of soluble proteins for the different samples of Sakha94 and Gimeza9 after treatments by different chemical materials e.g. glycine, proline, choline chloride and glycinebetaine are shown in Fig. 1 and 2.

From data in Table 4 and 5, it is clear the homology in band intensity between the electrophoregrams of the wheat samples. The homologous bands are labeled by double stars. Also, the molecular weight of protein sub units ranged between 16 to 88 kDa. The more intensive

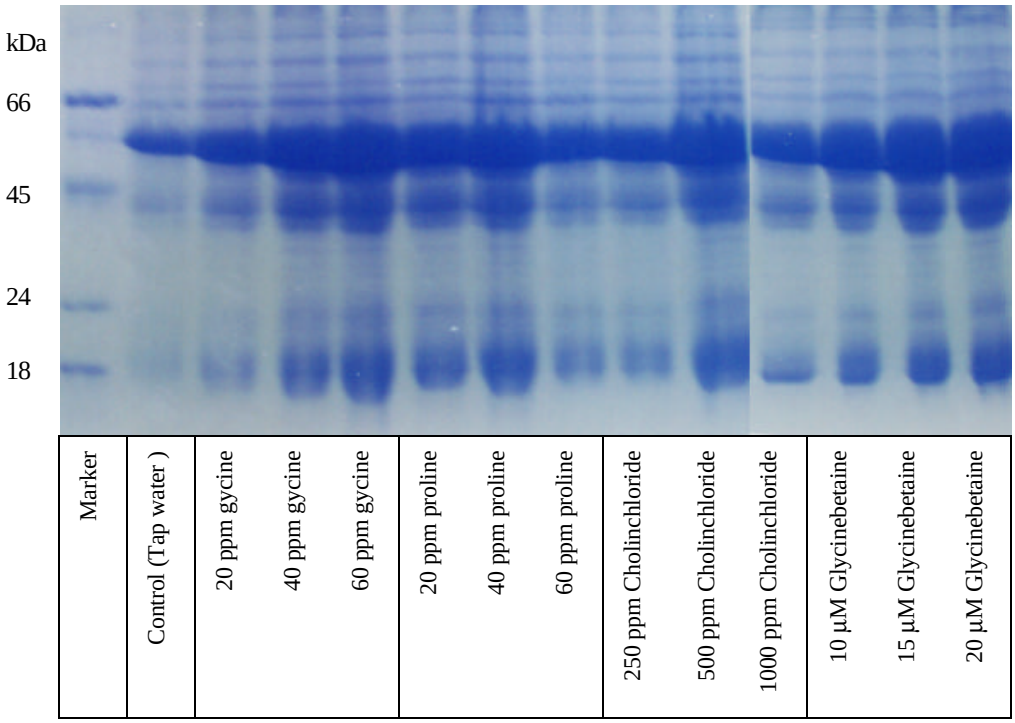

Fig 1: SDS-PAGE profiles of soluble proteins extracted from Sakha 94 at 75 days from sowing

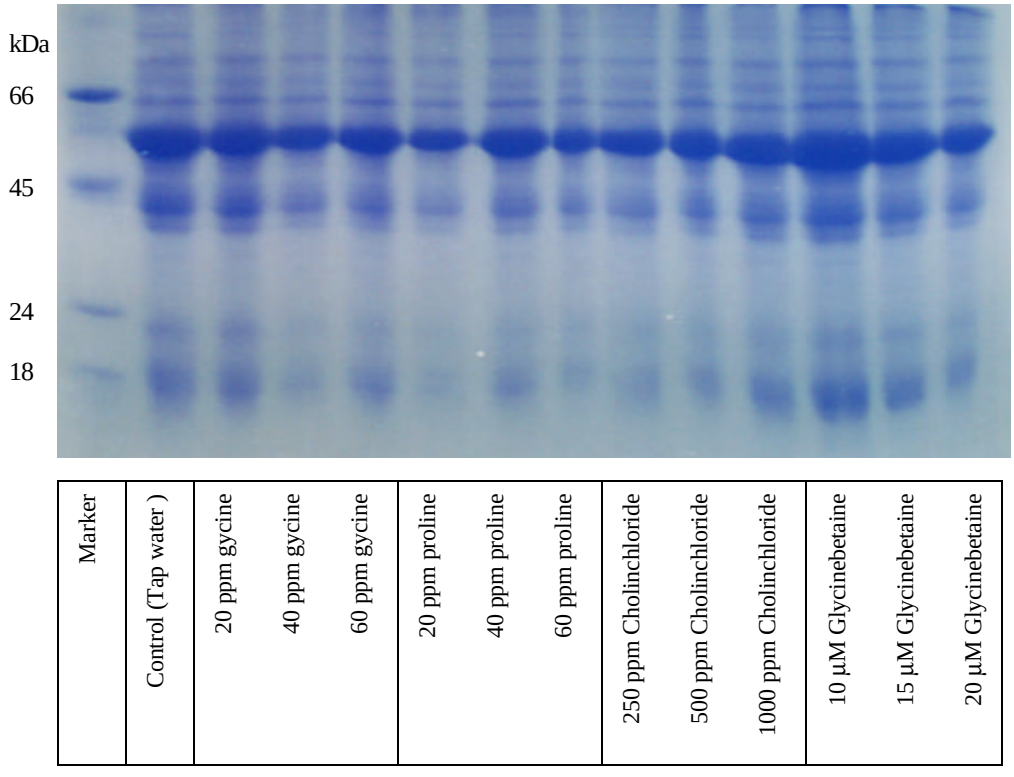

Fig 2: SDS-PAGE profiles of soluble proteins extracted from Gimeza9 at 75 days from sowing

Table 5: SDS-PAGE patterns of soluble proteins extracted from Gimeza9 at 75 days from sowing

		Band intensity												
		Treatments												
	Molecular		Glycine (ppm)			Proline (ppm)			Cholinchloride (ppm)			Glycinebetaine (µM)		
Band number	weight (kDa)	Control	20	40	60	20	40	60	250	500	1000	10	15	20
1	88	1	1	1	1	1	1	1	1	1	1	1	1	1**
2	85	1	1	1	1	1	1	1	1	1	1	1	1	1**
3	77	2	2	2	2	2	2	2	2	2	2	2	2	2**
4	72	1	1	1	1	1	1	1	1	1	1	1	1	1**
5	71	1	1	1	1	1	1	1	1	1	1	1	1	1**
6	65	2	2	2	2	2	2	2	2	2	2	2	2	2**
7	62	1	1	1	1	1	1	1	1	1	1	1	1	1**
8	61	1	1	1	1	1	1	1	1	1	1	1	1	1**
9	57	5	5	5	5	4	5	4	5	4	5	5	5	4
10	52	2	2	1	1	1	1	1	1	1	2	2	2	1
11	51	2	2	1	1	1	1	1	1	1	2	2	2	1
12	50	1	1	1	1	1	1	1	1	1	2	1	1	1
13	49	1	1	1	1	1	1	1	1	1	2	1	1	1
14	45	1	1	1	1	1	1	1	1	1	1	1	1	1**
15	42	2	2	1	1	1	1	1	2	2	2	2	2	1
16	39	3	3	2	2	2	2	2	2	2	2	3	3	2
17	37	1	1	1	1	1	1	1	1	1	1	1	1	1**
18	36	2	2	1	1	1	1	1	1	1	2	2	2	2
19	32	1	1	1	1	1	1	1	1	1	1	1	1	1**
20	29	1	1	1	1	1	1	1	1	1	1	1	1	1**
21	28	1	1	1	1	1	1	1	1	1	1	1	1	1**
22	25	0	0	0	0	0	0	0	0	0	0	0	0	0
23	24	0	0	0	0	0	0	0	0	0	0	0	0	0
24	23	1	1	1	1	1	1	1	1	1	1	1	1	1**
25	22	2	2	1	1	1	1	1	1	1	1	2	2	1
26	20	0	0	0	0	0	0	0	0	0	0	0	0	0
27	18	2	2	1	2	1	2	1	1	1	2	3	3	2
28	16	2	2	1	2	1	2	1	1	1	2	3	3	2

5= very deep intensity, 4= deep intensity, 3= moderate deep intensity, 2= moderate intensity, 1= pale intensity and 0= no bands. ** Homologous bands

band is presented at molecular mass 57 kDa. In this regard, bands of molecular weight 25, 24 and 20 kDa are not presented in the samples of Gimeza9.

As to the effect of foliar application treatments on band patterns of proteins in shoots of wheat cultivars, it is was found that plants sprayed with glycine showed an increase of band intensities at 57, 18 and 16 kDa for Sakha 94 as compared with the control plants. Also, the same cultivar showed increased in band intensities at 45, 42, 39 and 32 kDa by spraying of glycine at rate 60 ppm. Concerning Gimeza9 under the same conditions, the spraying of glycine tended to decrease the band intensities at 52, 51, 42, 39, 36 and 22 kDa when applied at rates 40 and 60 ppm as compared with the control. Also, the last finding was true for the same cultivar at 18 and 16 kDa when glycine applied at rate 40 ppm.

Concerning the effect of proline on band intensities in shoots of Sakha 94, it was increased at molecular mass 57, 18 and 16 kDa with application of proline as compared with the control. Also, this was true at 51, 45 and 42 kDa for the same cultivar after treatment with 20 ppm. In this respect, spraying dose of 20 and 40 ppm proline for the same cultivar increased band intensities at 39 and 32 kDa as compared with the control. In this regard, the spraying of proline levels tended to decrease the band intensities in shoot of Gimeza9 at molecular mass 52, 51, 42, 39, 36 and 22 kDa. Such decrement for the same cultivar was true at 57, 18 and 16 kDa by spraying of 20 and 60 ppm under saline conditions. In this regard, Miteva *et al.* [46] found that salinity induced marked quantitative and qualitative changes in polypeptide profiles of soluble barley leaf proteins. Enhanced levels of 76, 60, 47, 43 and 30 KDa polypeptides and reduced levels of 55 and 15 KDa polypeptides were observed in NaCl-treated plants. While, El-Shintinawy and El-Saourbagy [47] indicated that the content of 26 KDa protein increased in NaCl treated wheat plants. Al-Bana [48] found that salt stress induced a dectable change in number of band intensities in canola plants grown under saline conditions.

The obtained results showed that there was an increase in band intensities for Sakha 94 at 57, 18 and 16 kDa with application of choline chloride as compared with the control. This was true for the same cultivar at molecular mass 52, 51, 39 and 32 kDa when choline

chloride applied at rates 500 and 1000 ppm. Plants sprayed with 500ppm choline chloride show an increase in band intensities for Sakha 94 at 50, 49, 45 and 42 kDa as compared with the control. In this connection, the decrease in band intensities was at 39 and 22 kDa for Gimeza9 after treatment with choline chloride levels. The same trend was true at 52, 51, 36, 18 and 16 kDa for Gimeza9 when plants sprayed with choline chloride at rates 250 and 500 ppm as compared with the control. Also, the last finding was true for the same cultivar at 57 kDa, when applied choline chloride at rate 500 ppm.

The effect of glycinebetaine on protein patterns for wheat plants could be followed from data presented in the same tables. The data indicated that spraying of glycinebetaine tended to increase in band intensities for Sakha94 at molecular mass 57, 52, 51, 39, 18 and 16 kDa as compared with the control. This was true for the same cultivar at 32 kDa, when glycinebetaine applied at rates 10 and 15 µM. While, Gimeza9 showed a decreased in band intensities at 57, 52, 51, 42, 39 and 22 kDa by spraying of 20 µM as compared with the control plants under Ras Sudr conditions. In his connection, El-Saied and Afiah [49] showed that the induction of protein varied for salt stress as well as from one canola genotype to another under the same treatment of salinity. In addition, salt stress lead to different in gene expressions where alterations in protein patterns could be due to altered regulation of transcription, mRNA processing or due to altered rates of protein degradation. A better understanding of the role that altered protein patterns can be achieved when the genes or mRNAs coding for salt related or induced proteins are identified and characterized [50,51].

Effect of Foliar Application and Wheat Cultivars on Grain Yield: Values in Table 6 show the effect of foliar application of glycine on grain yield. It was significantly increased after treatment with 40 and 60 ppm as compared with the control. On the other hand, the differences between wheat cultivars are not significant. Also, the interaction between foliar application of glycine and two wheat cultivars took the same trend.

The results given in the same table show that grain yield was significantly increased by spraying doses of proline at rates 40 and 60 ppm as compared with the control. Also, Sakha94 significantly exceeded Gimeza9 in this parameter under the same conditions. The interaction effect showed that grain yield for two wheat cultivars was significantly increased by adding proline foliarly at rates 40 and 60 ppm as compared with the control. In this connection, Bandurska [38] found that a possible role of proline in the protection of the enzyme protein against dehydration and osmotic stress in barely. Also, Amer [52] showed that growth of barely was decreased by salinity, but some amino acid (arginine or proline+glutamic) treatments ameliorated the effects. In this regard, Ragab *et al.* [39] showed that spraying of proline reduced the harmful effects of salinity on tomato plants. In the same direction, Awaad *et al.* [3] found that spraying wheat plants with proline decreased the adverse effect of different salts on the dry matter yield. Also, Hussein [53] reported that sprayed wheat plants with Nervatin-Vit (containing amino acids) increased grain yield comparing with control plants.

Table 6: Effect of foliar application and wheat cultivars on grain yield (g m^{-2})

Foliar application	Grain yield (g m^{-2})		
Glycine (ppm)	Sakha94	Gimeza9	Mean
00	212.5ab	197.5b	205.0B
20	207.5ab	197.5b	202.5B
40	232.5ab	212.5ab	222.5A
60	237.5a	232.5ab	235.0A
Mean	222.5A	210.0A	
Proline (ppm)			
00	212.5c	197.5c	205.0C
20	212.5c	201.8c	207.2C
40	301.2a	250.0b	275.6B
60	300 a	297.5a	298.8A
Mean	256.5A	236.7B	
Choline chloride (ppm)			
00	212.5cd	197.5d	205.0B
250	217.0cd	200.0d	208.5B
500	312.5a	232.5c	272.5A
1000	310.0a	277.5b	293.8A
Mean	263.0A	226.9B	
Glycinebetaine (µM)			
00	212.5c	197.5c	205C
10	210c	200c	205C
15	252b	210c	231B
20	325a	310a	317.5A
Mean	249.9A	229.4B	

-Values followed by the same letter in columns are not different at $p< 0.05$ by Duncan's multiple range test

Results in the same table indicate that the relationship between foliar application of CC and grain yield was directly proportional. In addition, the highest value was produced from Sakha94. Concerning the interaction effect, plants sprayed with 500 and 1000 ppm showed a significant increase of grain yield for two wheat cultivars as compared with the untreated plants. In this respect, Liang *et al.* [54] reported that choline chloride has protective effect on membrane lipids and increases the level of protection for stress tolerance.

Data presented in Table 6 shows that the grain yield was significantly increased by spraying doses of glycinebetaine at rates 15 and 20 µ M as compared with

the control. Also, Sakha94 significantly exceeded Gimeza9 in grain yield. Regarding the interaction effect between foliar application of glycinebetaine and wheat cultivars, data show that grain yield was significantly increased when glycinebetaine applied at rates (15 and 20 μ M) and 20 μ M for Sakha 94 and Gimeza9 respectively as compared with the control. The increase of yield as a result of glycinebetaine is in agreement with those recorded by Jokinen *et al.* [55] on tomatoes and Diaz-Zortia *et al.* [28] on wheat. In addition, Lopez *et al.* [56] found that glycinebetaine can be used as an alternative treatment to reduce the effects of salt sensitive kidney bean plants. Also, Karjalainen *et al.* [57] showed that exogenous applied glycinebetaine may be used to increase the levels of protective compounds (several phenolic compounds) in strawberries.

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