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Biochemical Changes Associated with Induction of Salt Tolerance in Wheat

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Abstract: Saline stress negatively affects on the biochemical processes of wheat plants as a result of imbalance in catabolism and anabolism operations of biomolecules. Therefore, this study was conducted to evaluate biochemical changes induced by foliar application with amino acids e.g., glycine and proline, as well as, quaternary ammonium compounds e.g., choline and glycinebetaine on wheat plants (Sakha 93 and Gimmeza7) under saline conditions at Ras Sudr, South Sinai, Egypt. Under foliar application treatments, sharp changes in some biochemical indicators for salt tolerance were occurred, which related to the biochemical counteraction of saline stress injury, as well as, increasing of grain yield. Sakha 93 was the best cultivar under saline conditions compared with Gimmeza7. These findings associated with increasing of photosynthetic pigments (chlorophyll a and b), endogenous hormones (gibberellic acid and indole acetic acid), quaternary ammonium compounds (glycinebetaine and choline) and decreased in malondialdehyde content and growth inhibitor (abscisic acid). In light of the results, amino acids composition indicated the presence of 16 acids in wheat plants and these amino acids based on the structural features of side chain were divided into two groups: i) acyclic amino acids represent the largest proportion (ranged between 75.78 to 81.22%) of the total amino acids and ii) cyclic amino acids represent the lowest proportion (ranged between 18.77 to 24.21%) of the total amino acids in two wheat cultivars. Also, foliar application treatments led to increase of total cyclic amino acids content and decrease of total acyclic amino acids content in wheat plants compared with the control. This refers to the role of cyclic amino acids in pushing wheat plants to salt tolerance at Ras Sudr conditions. The highest values of K, Ca and Ca/Na and K/Na and Zn were produced by spraying Sakha 93 plants with proline, choline and glycinebetaine, respectively. Also, the maximum values of Mg and Mn contents were obtained from Gimmeza 7 after treatment with proline and choline, respectively. On the other side, the lowest value of Na content was noticed in Gimmeza 7 after treatment with glycine and glycinebetaine. It is possible to arrange the best treatments on the basis of biochemical indicators and grain yield as follows: glycinebetaine followed by choline, proline and glycine in a descending order. We can benefit from current study in alleviate the adverse effects of saline stress on wheat plants under Ras Sudr conditions at South Sinai, by activating the role of induced resistance using some amino acids e.g., glycine and proline, as well as quaternary ammonium compounds e.g., choline and glycinebetaine, which had a positive effect on most of the biochemical components and wheat grain yield.

Key words: Biochemical changes · Salt tolerance · Induced resistance · Amino acids · Quaternary ammonium compounds · Wheat

INTRODUCTION

Wheat is the world's major cereal crop and it is the staple food for more than 35% of world population [1]. Increasing grain yield of wheat is an important national target to face the continuous increasing food needs of Egyptian population and fill the gap between wheat consumption and production through the expansion of the cultivated wheat area in the newly reclaimed lands outside the Nile Valley (which represent about 3 to 4% of the total area of Egypt). The most new reclaimed lands in Egypt are subject to saline stress like Wadi Sudr in south of Sinai and the soil in this region showed to be saline and highly calcareous, also irrigation depends mostly on

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underground water (saline water). Salt stress causes a reduction in plant growth, development and yield [2, 3], also salt stress reduce the capacity of water absorption by the seeds, which directly influence the germination and seedling development. One of the biochemical changes occurring in plants subjected to saline stress conditions is the production of reactive oxygen species (ROS). In this regard, salinity causes the generation of oxidative stress in plants by producing a variety of ROS like superoxide radical, hydrogen peroxide and hydroxyl radical [4]. Reactive oxygen species (ROS) attack pigments, proteins, lipids and nucleic acids, as well as, the degree of damage depends on the balance between formation of ROS and its removal by the antioxidative scavenging systems [5, 6]. Therefore, plants protect themselves from the oxidative damage by developing an efficient defense system (enzymatic and nonenzymatic). Also, Sharaf [7] observed increases in activities of superoxide dismutase, peroxidase and catalase and glutathione reductase in wheat shoots of salt stressed plants. Under saline stress conditions, the plants accumulate some of compatible organic osmolytes like sugars, amino acids (proline) and quaternary ammonium compounds (choline and glycinebetaine) [8, 9].

Foliar applications of amino acids and quaternary ammonium compounds are one of the most important ways to reduce the adverse effect of saline stress on wheat plants. Confirming the role of these components in the salt stress tolerance, Talat *et al*. [10] showed that salt stress negatively affects on growth, morphology and physiology of wheat but the exogenous application of proline significantly ameliorates the harmful effects of saline stress. Also, Claussen [11] and Ali *et al*. [12] found that exogenous application of proline is known to induce abiotic stress tolerance in plants. In addition, there are many researches used glycinebetaine to protect wheat plants from the harmful effects of saline stress [13], also there are studies showed that glycinebetaine was effective in alleviating the adverse effects of saline stress on other plants [14, 15]. The aim of the present study was to evaluate biochemical changes induced by foliar applied amino acids and quaternary ammonium compounds in wheat plants under Ras Sudr conditions.

MATERIALS AND METHODS

Field Experiment: Two field trials were performed in Ras Sudr Agricultural Research Station, Desert Research Center during two successive growing seasons 2011/2012 and 2012/2013. The experiments were performed to study the biochemical changes associated with induction of salt tolerance in wheat. Chemical analyses of soil and irrigation water are presented in Table 1. Wheat grains were sown in second week of November in both seasons at a rate of 60 kg/ faddan. The experimental unit area was 6 m^2 (2m x 3m) and recommended fertilization for this type of soil was applied according to Desert Research Center. The experiment included ten treatments, four different chemical materials as foliar application (glycine at 60 ppm, proline at 60 ppm, choline chloride at 1000 ppm and glycinebetaine at 20 mM) compared with the control and two wheat cultivars (Sakha93 and Gimmeza 7). Foliar applications were applied twice after 35 and 65 days from sowing. The spray volume was 400 liter/faddan using Tween 20 as a wetting agent. Two plant samples were taken randomly from each treatment at 45 days after planting (1st growth stage) and 75 days after planting (2nd growth stage) from sowing. Fresh samples were tested for photosynthetic pigments, endogenous plant hormones, malondialdehyde content and free proline content. Then, dried till constant weight representing dry weight. Dry samples were grounded to fine powder and tested for quaternary ammonium compounds, choline, amino acids, protein and minerals. All measurements were conducted after the second growth stage (75 days), except malondialdehyde, free proline, glycinebetaine and choline at two growth stages (45 and 75 days). Plants were harvested after 145 days after planting, the grain yield (g /m^2) and protein yield (g /m^2) were recorded.

Chemical Analysis

Photosynthetic Pigments: The amounts of photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted and estimated according to A.O.A.C. [16] and Von Wettstein [17].

Endogenous Plant Hormones: The method used for extraction was that originally described by Shindy and Smith [18]. Phytohormones were determined for all treatments and the control using HPLC according to the method of Lee *et al*. [19] and Crocier and Moritz [20].

Quaternary Ammonium Compounds (Glycinebetaine and Choline): Quaternary ammonium compounds and choline were determined according to Grieve and Grattan [21].

Malondialdehyde Content (MDA): Malondialdehyde content was determined as outlined by Zhao *et al*. [22].

Free Proline Content: Free proline concentration was measured colorimetrically in the extraction of fresh materials according to Bates *et al*. [23].

Table 1: Chemical analysis of the experimental soil and irrigation water

			Cations (meq/l)				Anions (meq/l)			
EC dS/m	ppm	pH	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}
			Chemical analysis of the experimental soil							
10.04	6425	7.46	40.12	14.67	70.22	0.96	-	3.09	68.41	54.47
			Chemical analysis of irrigation water							
7.33	4691	7.18	12.97	9.01	46.88	0.29	---	2.59	38.17	28.39

Amino Acids: Amino acids composition was determined by amino acid analyzer apparatus model ″Eppendrof-Geramany LC 3000″. Hydrolysis was carried out according to the method of Pellet and Young [24].

Minerals: The amounts of sodium, potassium and calcium were determined using a flame photometer (Jenway PEP.7) according to Brown and Lilleland [25]. Also, magnesium, zinc and manganese were estimated by atomic absorption (Unicam 929 AA) according to Cook [26].

Crude Protein: The total protein was determined in wheat grain using micro Kjeldahl method described by A.A.C.C. [27].

Statistical Analysis: Data were analyzed statistically according to the procedure outlined by Snedecor and Cochran [28]. Combined analysis over growing seasons was done when the homogeneity test was insignificant according to Gomez and Gomez [29]. Duncan's multiple range test was used for the comparison between means [30].

RESULTS AND DISCUSSION

Photosynthetic Pigments: Data presented in Fig. 1 showed the effect of foliar applications on photosynthetic pigments in leaves of two wheat cultivars under saline conditions at Ras Sudr region. Chlorophyll (Chl) a, b and carotenoids are the main photosynthetic pigments and they play an important role in photosynthesis. It was interesting to note that the concentration of Chl a was higher than Chl b and carotenoids in two wheat cultivars. Results indicated that all foliar applications had a positive effect on increasing photosynthetic pigments compared with the control. By comparing changes in Chl a in different treatments, the highest value was produced from Sakha 93 by adding glycinebetaine foliarly followed by Gimmeza 7 with choline. Also, the maximum values of Chl b, Chl (a+b) and total pigments were obtained from Sakha 93 after treatments with glycinebetaine and proline compared with the control. However, Sakha 93 treated with choline recorded the maximum value of carotenoids followed by Gimmeza 7 with glycine. The decrease in photosynthetic pigments under saline conditions was reported by many authors [10, 15, 31]. Concerning of carotenoids, they protect plants during oxidative stress, also they contribute to stability of lipid membranes [32].

In this regard, Kausar *et al*. [15] showed that exogenous application of glycinebetaine ameliorates the harmful effects of salt stress on Chl a, b and carotenoids. Also, Luts [33] found that exogenous application of glycinebetaine increased the chlorophyll and carotenoids contents by repairing the chloroplast structure. In the same direction, Akhter *et al*. [13] showed that the highest value of Chl a was recorded after treatment with glycinebetaine (seeds soaking) under saline conditions. There are studies showed that application of glycinebetaine had a positive effect on increasing the content of chlorophyll in plants [34, 35]. In this regard, amino acids help to increase chlorophyll in the plants [36, 37]. Furthermore, Talat *et al.* [10] noticed that application of proline showed an improvement in Chl b content under salt stress. In another study, Gadallah [38] showed that exogenous application of proline increased leaf chlorophyll content, leaf relative water content and overall plant growth. On the other side, Nazarbeygi *et al*. [39] suggested that salt stress leads to more activity of proline synthesis which caused less existing glutamate in biosynthesizing chlorophyll (glutamate is subscriber precursor of chlorophyll and proline biosynthesis). The decrease in chlorophyll content under saline stress may be due to different reasons: 1) The inhibitory effect of the accumulated ions [40]. 2) The disruption of chloroplasts by oxidative stress which cause decreased the photosynthetic reactions [41]. 3) Salinity damages the structure and function of thylakoid membrane, electron transport, gaseous exchange and enzymes [42]. 4) Increased osmotic potential and Na access into organelles create damage in respiratory and photosynthetic electron transport [43], thus the plants can not effectively absorb light energy under stress and reducing photosynthetic pigments [44].

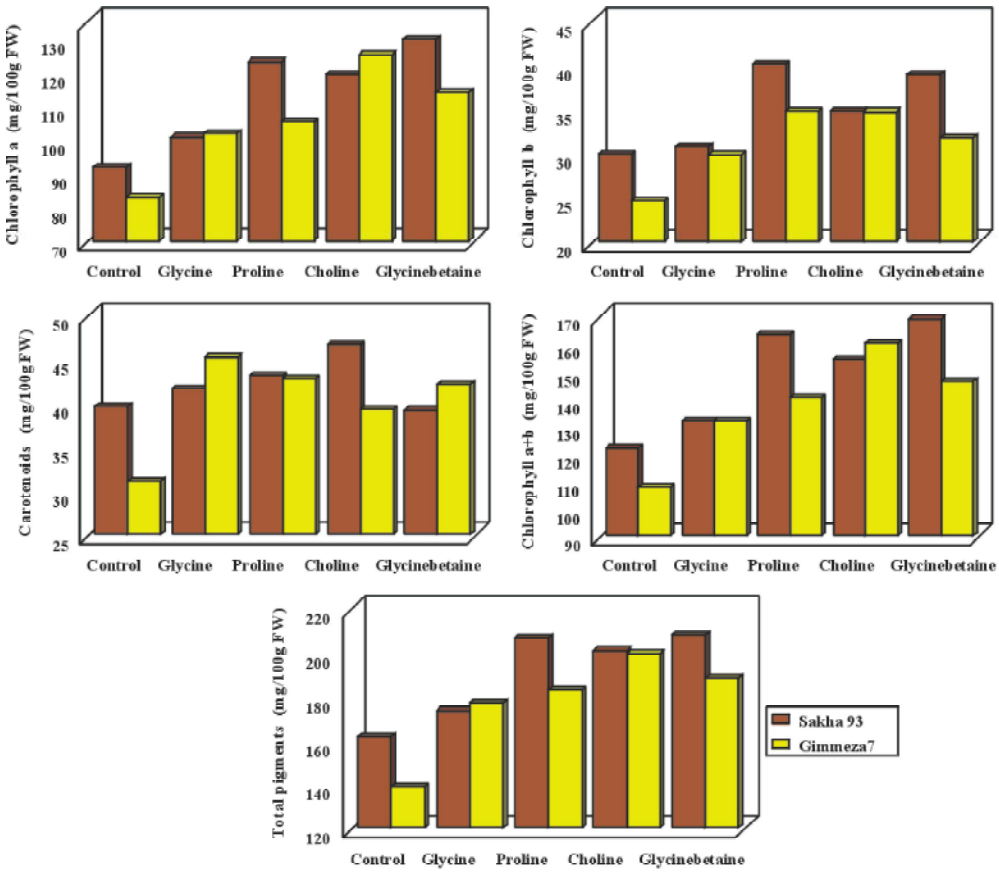

Fig. 1: Effect of glycine, proline, choline and glycinebetaine on photosynthetic pigments (mg / 100 g FW) in leaves of wheat plants under saline conditions at Ras Sudr

Endogenous Hormones: Under environmental stresses conditions such as salinity sharp change in the balance of plant hormones was noticed, which leads to the accumulation of growth inhibitor and decline of growth promoters [45, 46], which in turn activates various stress-associated genes that are thought to function in the accumulation of osmoprotectants. In the present study, the effect of glycine, proline, choline and glycinebetaine on some endogenous hormones in shoots of wheat plants under saline conditions is presented in Fig. 2. Foliar application treatments appeared to be effective on the accumulation of growth promoters (gibberellic acid, GA_3 and indole acetic acid, IAA) in shoots of both cultivars compared with the control. Sakha 93 plants treated with glycinebetaine recorded the maximum value of GA_3 followed by proline treatment with the same cultivar. However, the maximum value of IAA was obtained from Sakha93 after treatment with choline followed by glycinebetaine in the same cultivar.

The increases in these growth promoters (GA_3 and IAA) after treatment with these foliar applications may be due to they play an important role in the metabolism of plants by increasing the activity of enzymes responsible for biosynthesis of growth promoters under saline stress. Foliar application treatments had a positive role on decreasing abscisic acid (ABA) content in shoots of both wheat cultivars compared with the corresponding untreated plants. In this connection, Sakha 93 and Gimmeza 7 gave the lowest values of this content when plants treated with glycinebetaine. Salt stress led to sharp decrease in the levels of IAA and GA_3, while ABA level greatly increased in maize and wheat shoots [7, 31]. Concerning the negative effect of saline stress on IAA content in plants, Hassanein *et al*. [47] showed that the reduction in auxin content in response to salinity treatments might be due to the conversion of auxin to an inactive compound by some biochemical processes e.g., oxidation and/or increase in the activity of IAA-oxidase enzyme. In the same direction, Kim *et al*. [48] found that the reduction in the level of GA_3 under stress conditions could be due to the inhibition of biosynthetic oxidation of ent-kaurene to ent-kaurenic acid.

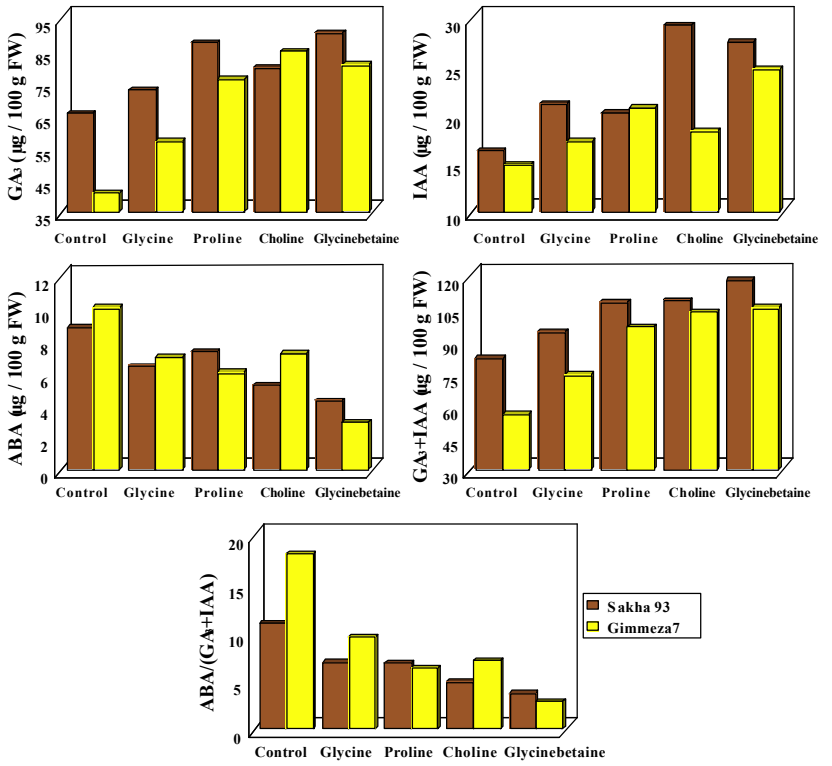

Fig. 2: Effect of glycine, proline, choline and glycinebetaine on some endogenous hormones (µg/100 g FW) in shoots of wheat plants under saline conditions at Ras Sudr

Quaternary Ammonium Compounds (Glycinebetaine and Choline): Data illustrated in Tables 2 and 3 revealed that the effect of foliar applications on glycinebetaine, choline, QAC and (CH/GB) in shoots of two wheat cultivars at two growth stages. Treatments with amino acids and quaternary ammonium compounds had a positive effect on the accumulation of quaternary ammonium compounds during the two growth stages. The highest value was obtained after treatment with choline followed by glycinebetaine compared with the control. Comparison between the two cultivars, Gimmeza 7 significantly exceeded Sakha 93 in contents of glycinebetaine and QAC, however choline and CH/GB in shoots of Sakha 93 were increased more than Gimmeza7 at two growth stages. Concerning the interaction effect between foliar applications and wheat cultivars, data indicated that the highest values of (glycinebetaine and QAC) and (choline and CH/GB) were achieved by Gimmeza 7 and Sakha 93, respectively after treatment with glycinebetaine at the first growth stage. This was true after treatment with choline at the second growth stage. The accumulation of glycinebetaine in salt stressed plants has been proposed to play an important role in salt tolerance [49]. In light of previous findings, there are studies that show the positive effect of glycinebetaine when applied on wheat under saline stress [13, 50]. Also, Raza *et al*. [51] showed that exogenous application of glycinebetaine improved tolerance of wheat under drought stress. In this regard, glycinebetaine was effective in alleviating the adverse effects of saline stress on other plants [14, 15]. Also, Rao *et al*. [52] showed that wheat plants alleviate the deleterious effect of salt stress by increasing production of proline and betaine.

The role of glycinebetaine in alleviating salt stress on plants may be due to: 1) It stabilizes both the quaternary structure of proteins and membranes [53] also stabilizing the structure of key proteins such as Rubisco [54]. 2) CO_2 assimilation rate increased [55], helpful in stabilizing pigments concentration [56] and protecting the photosynthetic apparatus [57]. 3) It ameliorates the harmful effects on gaseous exchange parameters [15]. 4) It serves as compatible osmolytes, protectants of macromolecules and also as scavengers of ROS [58] and preserving the osmotic balance [59]. 5) It is related to the elevated SOD, CAT and APX activity and alleviation of cell membrane damage by reducing oxidation of membrane lipid and improving the ion homeostasis [60].

Table 2: Effect of glycine, proline, choline and glycinebetaine on quaternary ammonium compounds in shoots of wheat plants under saline conditions at Ras Sudr

		1st growth stage					
		Glycinebetaine		Choline			
Treatments		µmol/g (DW)	INC %	µ mol/g (DW)	INC %	QAC (µ mol/g DW)	CH/GB
Foliar applications (FA)							
Control		53.13 c	Con	17.68 cd	Con	70.82 c	33.39 ab
Glycine		56.15 bc	5.68	16.22 d	Red	72.37 bc	29.13 c
Proline		59.56 ab	12.10	18.65 bc	5.48	78.20 ab	31.65 b
Choline		64.75 a	21.87	20.29 ab	14.76	85.04 a	31.73 b
Glycinebetaine		63.03 a	18.63	21.77a	23.13	84.81 a	35.44 a
Wheat cultivars (C)							
Sakha 93		54.96 b	--	20.64 a	--	75.60 b	37.52 a
Gimmeza7		63.68 a	--	17.20 b	--	80.89 a	27.01 b
Interaction (FA x C)							
Control	Sakha 93	52.11 c	Con	20.22 bc	Con	72.33 cd	38.80 b
	Gimmeza 7	54.15 c	Con	15.15 e	Con	69.30 d	27.98 d
Glycine	Sakha 93	52.24 c	0.25	17.10 de	Red	69.34 d	32.73 c
	Gimmeza 7	60.07 bc	10.93	15.33 e	1.19	75.40 cd	25.52 d
Proline	Sakha 93	55.30 c	6.12	20.12 bc	Red	75.42 cd	36.38 b
	Gimmeza 7	63.81 ab	17.84	17.17 de	13.33	80.98 a-c	26.91 d
Choline	Sakha 93	60.08 bc	15.29	22.32 ab	10.39	82.40 a-c	37.15 b
	Gimmeza 7	69.41 a	28.18	18.26 cd	20.53	87.67 ab	26.31 d
Glycinebetaine	Sakha 93	55.09 c	5.72	23.44 a	15.92	78.53 b-d	42.55 a
	Gimmeza 7	70.98 a	31.08	20.11 bc	32.74	91.09 a	28.33 d

-Values followed by the same letter in columns are not different at $p < 0.05$ by Duncan's multiple range test.
-1st growth stage = 45 days after sowing, DW= Dry weight, CH= Choline, GB= Glycinebetaine.
-INC% = Increase, Red= Reduction of GB and CH content compared with the control and Con = Control
-QAC= Quaternary ammonium compounds (CH+GB) and CH/GB= Ratio of choline to glycinebetaine x 100.

Table 3: Effect of glycine, proline, choline and glycinebetaine on quaternary ammonium compounds in shoots of wheat plants under saline conditions at Ras Sudr

		2nd growth stage					
		Glycinebetaine		Choline			
Treatments		µmol/g (DW)	INC %	µ mol/g (DW)	INC %	QAC (µ mol/g DW)	CH/GB
Foliar applications (FA)							
Control		64.56 c	Con	18.60 c	Con	83.17 c	28.99 a
Glycine		75.03 b	16.22	19.11 c	2.74	94.14 b	25.58 b
Proline		76.29 b	18.17	20.49 b	10.16	96.78 b	26.85 b
Choline		85.18 a	31.94	24.80 a	33.33	110.00 a	29.36 a
Glycinebetaine		82.64 a	28.00	24.26 a	30.43	106.90 a	29.40 a
Wheat cultivars (C)							
Sakha 93		72.56 b	--	21.78 a	--	94.34 b	29.95 a
Gimmeza7		80.92 a	--	21.13 a	--	102.04 a	26.12 b
Interaction (FA x C)							
Control	Sakha 93	60.31 e	Con	19.14 c-e	Con	79.46 e	31.73 ab
	Gimmeza7	68.81 d	Con	18.07 e	Con	86.88 de	26.26 cd
Glycine	Sakha 93	68.05 d	12.83	18.22 de	Red	86.27 de	26.77 cd
	Gimmeza7	82.01 bc	19.18	20.01 c-e	10.74	102.02 bc	24.39 d
Proline	Sakha 93	74.44 cd	23.43	20.21 cd	5.59	94.65 cd	27.14 cd
	Gimmeza7	78.15 bc	13.57	20.77 c	14.94	98.92 bc	26.57 cd
Choline	Sakha 93	79.81 bc	32.33	26.68 a	39.39	106.49 ab	33.42 a
	Gimmeza7	90.55 a	31.59	22.91 b	26.78	113.46 a	25.30 cd
Glycinebetaine	Sakha 93	80.21 bc	32.99	24.64 b	28.74	104.85 ab	30.71 b
	Gimmeza7	85.07 ab	23.63	23.89 b	32.21	108.96 ab	28.08 c

- Values followed by the same letter in columns are not different at $p < 0.05$ by Duncan's multiple range test.
- 2nd growth stage = 75 days after sowing, DW= Dry weight, CH= Choline, GB= Glycinebetaine
- INC% = Increase, Red= Reduction of GB and CH content compared with the control and Con = Control
- QAC= Quaternary ammonium compounds (CH+GB) and CH/GB= Ratio of choline to glycinebetaine x 100.

Table 4: Effect of glycine, proline, choline and glycinebetaine on malondialdehyde content (ηmol / g FW) and free proline (μmol / g FW) in leaves of wheat plants under saline conditions at Ras Sudr

		Malondialdehyde content (η mol/g FW)		Free proline (μmol/g FW)	
Treatments		1st growth stage	2nd growth stage	1st growth stage	2nd growth stage
Foliar applications (FA)					
Control		81.18 a	94.86 a	2.01 d	2.27 b
Glycine		66.12 b	83.38 b	1.86 e	1.87 c
Proline		67.93 b	80.57 b	2.46 b	2.36 b
Choline		57.78 c	72.72 c	2.65 a	2.58 a
Glycinebetaine		56.38 c	72.26 c	2.19 c	2.32 b
Wheat cultivars (C)					
Sakha 93		59.61 b	75.48 b	2.48 a	2.63 a
Gimmeza 7		72.15 a	86.04 a	1.98 b	1.93 b
Interaction (FA x C)					
Control	Sakha 93	77.11 ab	90.54 b	2.58 b	2.98 a
	Gimmeza 7	85.24 a	99.18 a	1.45 f	1.57 f
Glycine	Sakha 93	60.04 cd	82.32 cd	1.91 de	2.01 de
	Gimmeza 7	72.2 b	84.45 bc	1.81 e	1.74 ef
Proline	Sakha 93	55.14 cd	69.12 ef	2.92 a	2.48 bc
	Gimmeza 7	80.71 ab	92.02 ab	2.01 de	2.25 cd
Choline	Sakha 93	55.42 cd	65.34 f	2.76 ab	3.04 a
	Gimmeza 7	60.15 cd	80.11 cd	2.54 b	2.12 d
Glycinebetaine	Sakha 93	50.32 d	70.08 ef	2.26 c	2.66 b
	Gimmeza 7	62.45 c	74.44 de	2.12 cd	1.99 de

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

Malondialdehyde and Free Proline Contents: Malondialdehyde content (MDA) and free proline in leaves of wheat cultivars as affected by foliar applications under saline stress are presented in Table 4. One of the biochemical changes possibly occurring when wheat plants are subjected to harmful saline stress conditions is the production of malondialdehyde. In the present study, MDA was decreased after foliar application treatments compared with the control at the two growth stages. However, foliar application of glycinebetaine gave the minimum value of MDA. It is clear from the data that foliar applications had a positive effect on the accumulation of proline content under saline stress (except glycine treatment), where the maximum value was produced after treatment with choline. Regarding wheat cultivars, Sakha 93 recorded the lower MDA level than Gimmeza 7, thus was indicating an increase in lipid peroxidation of Gimmeza 7 under saline stress. The reverse was true in proline content. In this regard, Borzouei *et al*. [61] indicated that malondialdehyde and proline differ according to the cultivars ability in coping oxidative stress caused by salinity, also malondialdehyde content was higher in salt sensitive cultivar than salt tolerant cultivar as well as proline content took the opposite trend. Also, proline accumulation might be used as an indicator in selection for withstanding saline stress through the participation in osmoregulation [62].

As to the effect of interaction between foliar applications and wheat cultivars, data exhibited that the minimum values of MDA were achieved by Sakha93 when glycinebetaine and choline applied at 1st and 2nd growth stages, respectively compared with the control. Concerning proline content, it was increased in leaves of Gimmeza 7 after foliar application treatments at two growth stages. Also, it was increased in samples of Sakha 93 when proline and choline applied at the first growth stage, while choline treatment only had a positive effect on the accumulation of proline in Sakha 93 at 2nd growth stage, compared with the control. There are studies showed that the exogenous application of glycinebetaine had a positive effect on reducing the content of MDA in plants under saline stress [35, 63]. MDA is a lipid peroxidation product, has been consider an indicator of salt-induced oxidation in cell membranes and a tool for determining salt tolerance in plants. In this regard, Don *et al*. [64] showed that the increase in MDA content of salt stressed plants may be caused by ROS generated in the presence of oxidative stress and inadequate activities of antioxidant

enzymes. MDA is an effective means of assessing oxidative stress induced membrane damage [65] and cell membrane stability has been used an efficient criterion to discriminate among cultivars with respect to degree of salt tolerance [66]. In addition, glycinebetaine induced antioxidant responses that protect the plant from oxidative damage [63].

In plants, proline content increases more than other amino acids under water or salt stress and this effect has been used as a biochemical marker to select varieties [67]. In another study, Cha-um and Kirdmanee [68] found that the foliar spraying of salt sensitive cultivar with glycinebetaine was an effective way to stimulate proline accumulation when plants were exposed to salt stress. On the other hand, Nawaz *et al.* [69] showed that foliar applied glycinebetaine did not change the leaf proline concentration in the salt stressed plants. In the same direction, Raza *et al*. [50] noticed that salt stress significantly increased proline accumulation in shoots of sensitive cultivar, while application of glycinebetaine had a non significant effect on accumulation of proline in wheat leaves. Also, Abou El Yazied [34] found reduction of free proline due to the application of glycinebetaine. The vital role of proline accumulation within the plant cell under saline stress conditions due to: 1) Proline as a non toxic and protective osmolyte under saline conditions [70]. 2) Proline is an osmolyte accumulated under stress in almost all the plant species [71]. 3) Proline protecting the photosynthetic apparatus [72]. 4) Proline plays a regulatory role in activity and function of the antioxidant enzymes in plant cells [73]. 5) It plays a vital role in protein protection against denaturation [74], as well as, in scavenging reactive oxygen species, ROS [75].

Amino Acids Composition: Data in Tables 5 and 6 showed the effect of foliar applications on amino acids in wheat plants under saline stress. Amino acids composition indicated the presence of 16 acids and these amino acids based on the structural features of side chain were divided into two groups. Also, cystine, cysteine and tryptophan were not detected in two cultivars under investigation.

Acyclic Amino Acids: In this study, acyclic amino acids represent the largest proportion (ranged between 75.78 to 81.22%) of the total amino acids, which demonstrates the important role to the plants under saline stress. That contributes in the composition of vital proteins in plant cell and salt stress tolerance. Acyclic amino acids contain: aliphatic unsubstituted amino acids such as (glycine, alanine, valine, leucine and isoleucine) and aliphatic substituted such as hydroxy (serine, threonine), thio (methionine), carboxy (aspartic and glutamic), diamino (lysine) and guanidino (arginine). Results indicated that glutamic acid is the most abundant acyclic amino acid followed by aspartic, alanine and leucine in a descending order. Glutamic and aspartic acids in two wheat cultivars were increased due to all exogenous foliar applications (except glycine with Gimmeza 7) compared with the control. In this regard, Gimmeza 7 gave the maximum values of glutamic and aspartic acids after treatment with choline. Also, all treatments promotive the biosynthesis of leucine in wheat plants. The maximum value was produced by glycinebetaine in Sakha93 plants. Data exhibited that the highest values of alanine were achieved by Gimmeza7 and Sakha93 when choline and glycinebetaine applied respectively, compared with the control plants. On the other hand, methionine is presented in minute quantities and ranged between 0.17 mg/g (Gimmeza7 with control) to 0.47 mg/g (Sakha93 with glycinebetaine). In addition, other identified acyclic amino acids different from cultivar to another and this depending on the interaction between foliar application treatments and wheat cultivars under saline conditions.

In addition, Mansour [76] found that amino acids (alanine, arginine, glycine, serine, leucine and valine) were accumulated in plants subjected to salt stress. The increase of amino acids content in plants under salt stress due to the conversion of saccharides into amino acids and proteins which may increase the osmotic potential and increase the osmotic tolerant of wheat plants [77]. Also, Abd Elhamid *et al*. [78] showed that amino acids play an essential and decisive role in partially alleviating the adverse effect of salt stress in wheat cultivars. In plants, Rawia *et al.* [79] showed that aspartate is the precursor to several amino acids.

Amino acids are required by plant for synthesis of most important enzymes and hormones which is needed for completing metabolic activities. Most of protein syntheses are due to amino acids that are known as building blocks of plant proteins. In this regard, Eid *et al.* [79] found that aspartate is the precursor to several amino acids, including methionine, threonine, isoleucine in plants. In addition, Amer [80] showed that carboxy amino acids (glutamic and aspartic) were higher than other amino acids possibly due to their being precursors for synthesis of most amino acids. Also, the accumulation of amino acids in plants exposed to stress probably attributed to the disturbance in amino acid metabolism.

Table 5: Effect of glycine, proline, choline and glycinebetaine on amino acids (mg/g DW) in shoots of wheat plants under saline conditions at Ras Sudr

				Treatments									
				Control		Glycine		Proline		Choline		Glycinebetaine	
Amino acids mg/g (DW)				Sakha 93	Gimmeza7	Sakha 93	Gimmeza7	Sakha 93	Gimmeza7	Sakha 93	Gimmeza 7	Sakha 93	Gimmeza7
Acyclic amino acids	Aliphatic unsubstituted		Gly	1.93	2.09	2.09	2.18	2.34	2.04	2.27	2.67	2.86	2.22
			Ala	3.68	4.31	4.68	3.89	5.07	4.17	4.27	6.35	6.26	4.34
			Val	2.31	1.86	2.04	2.88	2.9	2.71	3.04	3.59	3.51	2.75
			Isol	1.60	1.58	1.65	1.70	2.02	1.64	1.82	2.03	2.62	1.87
			Leu	2.70	2.98	3.33	3.41	3.15	3.27	3.17	3.64	5.14	3.44
	Aliphatic substituted	Hydroxy	Thr	1.80	2.10	2.23	2.21	2.32	2.13	2.68	3.10	3.02	2.21
			Ser	1.36	1.50	1.51	1.51	1.93	1.73	1.87	2.70	2.32	1.70
		Thio	Met	0.18	0.17	0.28	nd	0.33	nd	0.35	0.42	0.47	0.26
		Carboxy	Asp	3.89	4.19	4.80	3.87	5.28	4.99	5.13	5.78	5.72	5.75
			Glu	4.69	4.51	6.71	4.54	5.81	5.09	6.91	9.26	8.62	6.67
		Diamino	Lys	1.72	2.31	2.59	1.89	2.54	2.66	2.53	3.50	3.22	2.53
		Guanidino	Arg	1.89	1.68	2.34	1.87	2.32	1.88	2.51	2.66	3.07	1.87
Cyclic amino acids	Aromatic		Tyr	1.04	0.91	1.61	1.33	1.81	1.28	2.01	1.75	2.51	1.47
			Phen	1.69	2.08	2.35	2.11	2.55	2.53	2.50	3.24	3.37	2.92
	Heterocyclic		Hist	2.19	1.60	2.65	2.44	3.17	2.26	3.00	3.99	3.82	2.73
	Imine		Pro	2.03	2.18	2.87	2.88	2.60	3.07	4.17	3.46	3.54	3.57
	TAA			34.7	36.05	43.73	38.71	46.14	41.45	48.23	58.14	60.07	46.3

Where:

Gly = Glycine, Ala = Alanine, Val = Valine, Leu = Leucine, Isol = Isoleucine, Ser = Serine, Thr = Threonine, Met = Methionine, Asp = Aspartic, Glu = Glutamic, Lys = Lysine, Arg= Arginine, Phen = Phenylalanine, Tyr = Tyrosine, His = Histidine, Pro = Proline, TAA= Total amino acids and DW= Dry weight

Table 6: Effect of glycine, proline, choline and glycinebetaine on ratio of amino acids in shoots of wheat plants under saline conditions at Ras Sudr.

		Amino acids						
Treatments		Acyclic amino acids (ACAA)						Cyclic amino acids
Foliar applications	Wheat cultivars	AUAA (mg/g)	ASAA (mg/g)	Total ACAA (mg/g)	AUAA/ ASAA	AUAA/ ACAA	ASAA/ ACAA	(CAA) (mg/g)
Control	Sakha 93	12.22	15.53	27.75	78.68	44.03	55.96	6.95
	Gimmeza 7	12.82	16.46	29.28	77.88	43.78	56.21	6.77
Glycine	Sakha 93	13.79	20.46	34.25	67.39	40.26	59.73	9.48
	Gimmeza 7	14.06	15.89	29.95	88.48	46.94	53.05	8.76
Proline	Sakha 93	15.48	20.53	36.01	75.40	42.98	57.01	10.13
	Gimmeza 7	13.83	18.48	32.31	74.83	42.80	57.19	9.14
Choline	Sakha 93	14.57	21.98	36.55	66.28	39.86	60.13	11.68
	Gimmeza 7	18.28	27.42	45.7	66.66	40.00	60.00	12.44
Glycinebetaine	Sakha 93	20.39	26.44	46.83	77.11	43.54	56.45	13.24
	Gimmeza 7	14.62	20.99	35.61	69.65	41.05	58.94	10.69

ACAA= Acyclic amino acids, AUAA = Aliphatic unsubstituted amino acids, ASAA= Aliphatic substituted amino acids, Total ACAA= Total acyclic amino acids, CAA= Cyclic amino acids and TAA= Total amino acids.

- AUAA/ASAA = Ratio of aliphatic unsubstituted amino acids to aliphatic substituted amino acids x 100.
- AUAA/ ACAA = Ratio of aliphatic unsubstituted amino acids to acyclic amino acids x 100.
- ASAA/ACAA = Ratio of aliphatic substituted amino acids to acyclic amino acids x 100.
- CAA/ACAA = Ratio of cyclic amino acids to acyclic amino acids x 100.
- ACAA/ TAA = Ratio of acyclic amino acids to total amino acids x 100.
- CAA/TAA = Ratio of cyclic amino acids to total amino acids x 100.

Cyclic Amino Acids: Cyclic amino acids represent the lowest proportion (ranged between 18.77 to 24.21%) of total amino acids. In spite of this, they had an important role to salt stress tolerance. Cyclic amino acids contain: aromatic (phenylalanine and tyrosine), heterocyclic (histidine) and imine acid (proline). It is apparent from data that foliar applications of glycine, proline, choline and glycinebetaine had a positive effect on accumulation of cyclic amino acids compared with the control. Foliar application of glycinebetaine treatment gave the maximum values of phenylalanine and tyrosine in samples of Sakha 93. Also, Sakha 93 and Gimmeza 7 were recorded the highest values of proline and histidine, respectively after treatment with choline compared with the control.

Table 6: Continued

Foliar applications	Wheat cultivars	TAA (mg/g)	CAA/ACAA	ACAA/ TAA	CAA/ TAA
Control	Sakha 93	34.70	25.04	79.97	20.02
	Gimmeza 7	36.05	23.12	81.22	18.77
Glycine	Sakha 93	43.73	27.67	78.32	21.67
	Gimmeza 7	38.71	29.24	77.37	22.62
Proline	Sakha93	46.14	28.13	78.04	21.95
	Gimmeza 7	41.45	28.28	77.94	22.05
Choline	Sakha93	48.23	31.95	75.78	24.21
	Gimmeza 7	58.14	27.22	78.60	21.39
Glycinebetaine	Sakha93	60.07	28.27	77.95	22.04
	Gimmeza 7	46.30	30.01	76.91	23.08

ACAA= Acyclic amino acids, AUAA= Aliphatic unsubstituted amino acids, ASAA= Aliphatic substituted amino acids, Total ACAA= Total acyclic amino acids, CAA= Cyclic amino acids and TAA= Total amino acids.

- AUAA/ASAA = Ratio of aliphatic unsubstituted amino acids to aliphatic substituted amino acids x 100.
- AUAA/ ACAA = Ratio of aliphatic unsubstituted amino acids to acyclic amino acids x 100.
- ASAA/ACAA = Ratio of aliphatic substituted amino acids to acyclic amino acids x 100.
- CAA/ACAA = Ratio of cyclic amino acids to acyclic amino acids x 100.
- ACAA/ TAA = Ratio of acyclic amino acids to total amino acids x 100.
- CAA/TAA = Ratio of cyclic amino acids to total amino acids x 100.

Table 7: Effect of glycine, proline, choline and glycinebetaine on minerals content in shoot of wheat plants under saline conditions at Ras Sudr.

		Minerals content							
		Macronutrients						Micronutrients	
Treatments		Na mg/g (DW)	K mg/g (DW)	K/Na Ratio	Ca mg/g (DW)	Ca/ Na ratio	Mg mg/g (DW)	Zn µg/ g (DW)	Mn µg/ g (DW)
Foliar applications (FA)									
Control		14.73 a	21.18 d	1.44 c	5.12 c	0.35 d	0.25 d	28.58 c	36.17 bc
Glycine		11.64 c	26.67 c	2.30 b	5.51 c	0.47 c	0.29 c	32.37 b	38.69 ab
Proline		13.44 b	28.91 b	2.13 b	6.86 ab	0.51 bc	0.49 a	33.82 b	33.79 c
Choline		13.20 b	29.13 ab	2.24 b	6.63 b	0.52 b	0.47 a	32.17 b	41.74 a
Glycinebetaine		11.62 c	31.13 a	2.68 a	7.34 a	0.63 a	0.42 b	38.67 a	40.00 a
Wheat cultivars (C)									
Sakha 93		12.82 a	28.23 a	2.22 a	6.41 a	0.50 a	0.37 a	34.26 a	39.09 a
Gimmeza 7		13.04 a	26.57 b	2.10 a	6.17 a	0.49 a	0.40 a	31.98 b	37.06 a
Interaction (FA x C)									
Control	Sakha 93	14.15 a	23.05 c	1.62 d	6.04 cd	0.42 c	0.21 g	30.11 cd	37.32 cd
	Gimmeza 7	15.32 a	19.31 d	1.26 e	4.21 e	0.27 e	0.30 ef	27.05 d	35.02 de
Glycine	Sakha 93	12.22 b	24.12 c	1.97 c	4.47 e	0.36 d	0.25 fg	30.41 cd	43.25 ab
	Gimmeza 7	11.07 b	29.23 b	2.64 a	6.55 bc	0.59 b	0.34 e	34.33 bc	34.12 de
Proline	Sakha 93	14.45 a	33.47 a	2.31 b	6.62 bc	0.45 c	0.43 d	37.88 ab	36.44 d
	Gimmeza 7	12.44 b	24.34 c	1.95 c	7.11 ab	0.57 b	0.56 a	29.75 cd	31.14 e
Choline	Sakha 93	11.08 b	27.71 b	2.50 ab	7.72 a	0.69 a	0.45 cd	33.22 c	36.32 d
	Gimmeza 7	15.32 a	30.54 ab	1.99 c	5.54 d	0.36 d	0.50 bc	31.12 cd	47.15 a
Glycinebetaine	Sakha 93	12.21 b	32.81 a	2.69 a	7.24 ab	0.59 b	0.53 ab	39.69 a	42.11 bc
	Gimmeza 7	11.03 b	29.45 b	2.67 a	7.45 ab	0.67 a	0.31 e	37.65 ab	37.88 cd

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

The vital role of proline accumulation within the plant cell under saline stress conditions was discussed in part of free proline and malondialdehyde which has been previously clarified in Table 1. In this regard, Abraham *et al*. [81] showed that proline accumulates in larger amounts than other amino acids under saline stress.

The results in Table 6 indicated that acyclic amino acids in two wheat cultivars were higher than cyclic amino acids. Also, foliar application treatments led to increase of CAA/TAA in wheat plants compared with the control. On the other side, all foliar applications led to a clear reduction of ACAA/TAA in wheat plants. This refers to

the role of cyclic amino acids in inducing wheat plants to salt tolerance at Ras Sudr conditions. The effect of salinity on the amino acids content in wheat plants had studied by many researchers [62, 77, 78]. In addition, Chen *et al.* [82] showed that salt-sensitive genotype showed an increase in leaf total amino acids content compared with a slight increment for salt tolerant genotype.

Minerals Content: Minerals play a vital role for stress tolerance in plants subjected to environmental stress factors, where they had an important role in plant growth and metabolism, also contribute greatly to the survival of plants under stress conditions. Data listed in Table 7 showed that, foliar application treatments enhanced minerals content (except Na) in wheat plants compared with the control. Treatment of glycinebetaine gave the highest values of K, K/Na, Ca, Ca/Na and Zn. Moreover, Mg and Mn recorded the highest value due to proline and choline treatment, respectively compared with the control. On the other hand, the lowest value of Na content was produced after treatment with glycinebetaine and glycine. Comparison between the two wheat cultivars, Sakha 93 had a higher K and Zn contents than Gimmeza7. On contrary, Gimmeza 7 exceeded Sakha 93 in Na and Mg contents. Concerning the effect of interaction between foliar applications and wheat cultivars, data showed that the highest values of K, Ca and Ca/Na and K/Na and Zn were produced by spraying Sakha 93 with proline, choline and glycinebetaine, respectively. Also, the maximum value of Mg and Mn contents were obtained from Gimmeza 7 after treatment with proline and choline, respectively. On the other side, the lowest value of Na content was noticed by Gimmeza7 after treatment with glycine and glycinebetaine compared with the control. In this connection, many investigators pointed out that Na content was increased in wheat plants under salt stress, while K, Ca, Mg and K/Na decreased in response to salt stress [3, 71, 78]. In another study, Rahman *et al*. [83] found that glycinebetaine application reduced the accumulation of Na and promotes the accumulation of K in the cells in rice plants. In this respect, this antagonism could be due to the direct competition between K and Na at the site of ion uptake at plasma lemma.

In addition, Nawaz *et al.* [69] showed that glycinebetaine significantly reduced Na accumulation while K increased in maize roots and leaves, so this led to increased K/Na and Ca/Na ratios under saline conditions. Also, Raza *et al*. [84] found that exogenous application of glycinebetaine enhanced level of glycinebetaine and K in wheat and this phenomenon led to a better maintenance of K/Na and Ca/Na ratios in the shoots. In another study, Akhter *et al.* [13] showed that the concentration of Na and Cl were high in wheat (salt sensitive) and low in salt tolerant after treatment with glycinebetaine under saline conditions. In this respect, Hu *et al*. [35] noticed that application of glycinebetaine suppressed Na accumulation, whereas the K content was significantly increased in Perennial Ryegrass, which led to a higher K/Na ratio under saline conditions. The beneficial effect of glycinebetaine in salt stressed plants may have been due to: 1) The protective effect of glycinebetaine on the integrity of plasma membrane [85]. 2) Providing stabilization of biological membranes and macromolecules (proteins, PS II and transporters) which resulted in Na discrimination against K and Ca (improved K/Na and Ca/Na ratio) rather than simply providing protection against osmotic stress [69], highlighting the crucial role of glycinebetaine for plant performance under saline conditions. Regarding the effect of foliar application of proline on minerals, Ali *et al*. [86] found that exogenous application of proline counteracted the adverse effects of water stress on nutrient uptake because it promoted the uptake of K and Ca in maize cultivars.

Grain and Protein Yield: Data in Fig. 3 showed the effect of foliar application treatments on protein (%), grain yield (g/m^2) and protein yield (g/m^2) in two wheat cultivars under saline conditions. It is obvious that all foliar applications enhanced protein percentage (except glycine treatment with Sakha 93), grain yield and protein yield compared with the control. However, all parameter measurements were differed from cultivar to another, where Sakha93 exceeded Gimmeza7 in these parameters. In the same direction, Borzouei *et al*. [61] observed a greater decline in the growth parameters and grain yield under salt stress conditions in salt sensitive cultivar than salt tolerant. Moreover, the application of proline with Sakha 93 gave the highest value of protein percentage compared with the untreated plants. Also, Sakha93 recorded the maximum values of grain and protein yield after treatment with glycinebetaine. The positive effect of these treatments on grain and protein yield due to they had a clear effect on the accumulation of growth promoters (Figure 2), they also had a positive role on the accumulation of quaternary ammonium compounds (Tables 2 and 3), in addition they had a vital role on accumulation of some amino acids (Tables 5 and 6) which contribute in tolerance of wheat plants against saline stress conditions at Ras Sudr. In respect of the negative

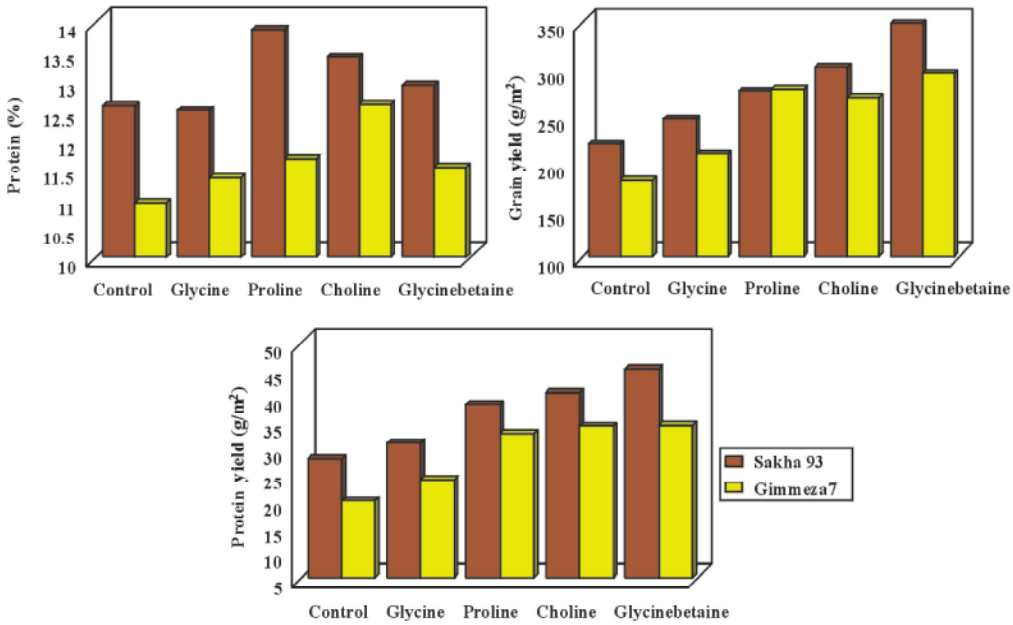

Fig. 3: Effect of glycine, proline, choline and glycinebetaine on protein (%), grain yield (g/m^2) and protein yield (g/m^2) in two wheat cultivars under saline conditions at Ras Sudr

effect of saline stress on wheat yield, there are many researches confirm this negative effect [3, 31, 61]. On the other hand, there are many researches showed that glycinebetaine treatment had a positive effect on yield [14, 34, 87]. In another study, Ibrahim *et al.* [88] showed that foliar application of glycinebetaine improved the growth of sunflower plants at intermediate level of salt stress, whereas higher level of glycinebetaine did not improve the growth. Concerning the positive effect of proline on yield, Bakry *et al*. [89] showed that treatment of proline with humic acid gave the highest values of seed yield, straw yield and oil yield of Flax cultivars. In another study, Khan *et al*. [71] indicated that increase of proline and chlorophyll contents as well as K/Na ratio in wheat genotypes were associated with an increase of grain yield. In the same direction, Rao *et al*. [52] showed that salt tolerant varieties gave high amounts of yield at different salinity levels, where these varieties alleviate the deleterious effect of salt stress by increased production of proline and betaine as well as antioxidant activity.

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