# Distribution and bioaccumulation of radiocarbon ($^{14}C$) into biochemical components of wheat in relation to antioxidant system under saline stress conditions

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**Abstract:** In the current study two wheat cultivars (Masr1 and Gimmeza9) were cultivated in saline soil (6215 ppm) and irrigated with saline water (4654 ppm). After 30 days from cultivation, plants were sprayed with $^{14}C$ labeled glycine (specific activity 0.025 µCi/1ml/plant of 60 ppm glycine); samples were collected after 3, 6, 24, 48 and 96 hours in addition to 7 and 15 days to study the distribution and bioaccumulation of $^{14}C$ labeled glycine into different biochemical components and its relation to antioxidant system. Results revealed the presence of $^{14}C$ in all plant extracts used in the current study, but the distribution and bioaccumulation were different depending on the type of plant extract and the time of sampling. This proves that glycine had clear role in the biosynthesis of many important biochemical components of the two wheat cultivars under saline conditions. The highest amount of $^{14}C$ was found in E3 extract in roots and shoots of two wheat cultivars compared with the other plant extracts. The total recovery percent of $^{14}C$ glycine in the two cultivars decreased by time reaching the lowest value at 15 days of the application (27.53% in Masr1 and 20.81% in Gimmeza9); this may be attributed to the loss of $^{14}C$ glycine as $^{14}CO_2$ through respiration after complete metabolism of the applied glycine. Antioxidant enzymes (superoxide dismutase, catalase and peroxidase) are important enzymes that increase the defense capability of wheat plants, where protect the biomolecules from free radicals damage (detoxification) under glycine treatment conditions. Also, it was noticed the important role of glycine on hydrolyzing enzymes (α and β-Esterase). With respect of free amino acids, data showed that thirty three free amino acids were detected in two wheat cultivars. The most abundant amino acids noticed were serine, asparagine, proline, alanine, cystine, δ-aminobutyric, lysine and arginine. Current research is recommended to activate the use of radioactive carbon $^{14}C$ in wheat plants under saline condition, to stand on the effective role of many important biomolecules. The research also confirms the positive role of glycine acid in salt stress tolerance, through its contribution to the biosynthesis of many important biochemical components in plant cell and its association with the activity of antioxidant defense system.

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**Key words:** $^{14}C$ labeled glycine, distribution, bioaccumulation, biochemical components, wheat, saline stress

## 1. Introduction

Radioactive isotopes of the most prominent discoveries of modern science and the most important achievements of human to dive into the minor world. To answer questions which are difficult to interpret with normal information about material and the secret of its composition. The most important radioisotopes applications in the field of biochemistry are tracing and knowledge of the motion of atoms inside the plants. In this context, the radioactive isotope is used to gain adequate answers related to biochemical systems within the plant cell, especially biosynthesis processes of many biochemical compounds under adverse environmental conditions such as saline stress. There are still many questions that aspiring professionals in the field of biochemistry to answer them, in order to recognize (documented and emphasizing) about the biosynthesis of these compounds and their role within the plant cell and their relationship to the extent of the plant cell ability to tolerant of environmental stress. Under normal conditions, there is a balance between the oxidant forces (such as reactive oxygen species, ROS) and antioxidant forces (enzymatic or nonenzymatic) inside the plant cell, where the antioxidant defense system protects the cellular system from oxidant forces. While under saline stress conditions, oxidant forces are greater than the antioxidant forces therefore oxidative stress appears and this leads to damage of biomolecules such as lipids, proteins, nucleic acids, etc., causing lipid peroxidation, protein denaturing and DNA mutation in plant cell also damage cells and tissues occurs in the long term. In this regard, **Mittler (2002), Candan and Tarhan (2003), Gara *et al.* (2003) and Vaidyanathan *et al.* (2003)** showed that ROS are the main source of damage cells under biotic and abiotic stresses. This negatively affects on plant growth under saline stress and quantity and quality of the economic crop. Many researches proved that plants are equipped with antioxidant enzymes such as

superoxide dismutase, catalase, peroxidase and polyphenol oxidase against oxidative damages by ROS (**Vaidyanathan *et al.*, 2003; Agarwal and Pandey, 2004; Mittova *et al.*, 2004** and **Bahari *et al.*, 2013)**. Modern trends of oxidative (saline) stress tolerance depend on the use of certain chemicals which are safe to the environment and human health such as amino acids. These substances activate the biochemical mechanisms (stimulating genes) which are responsible for plants tolerance against oxidative stress. These substances have a promotive role in the balance between catabolism and anabolism enzymes of biomolecules which increase the plant's ability to stress tolerance and can improve wheat crop productivity under environmental stress conditions of the Egyptian desert. In this concern, wheat is the first strategic crop in Egypt, and there is a clear gap between production and consumption, where local production is not enough to meet consumption needs. The contribution of amino acids in the biosynthesis of biomolecules in plant cell still needs more study and research. In this regard, **Gioseffi *et al.* (2012)** studied the absorption of glycine and glutamine (double labeled 15N and 13C) by wheat roots and their interactions with nitrate and ammonium during uptake. Also, **Owen and Jones (2001)** studied the competition between rhizosphere microorganisms and wheat plant roots for three amino acids ($^{14}$C labeled) namely, glutamate, glycine and lysine. The current research aims to study the distribution and bioaccumulation of $^{14}$C labeled glycine into biochemical components of two wheat cultivars in relation to antioxidant system under saline stress conditions.

## 2. Materials and Methods

### I. Materials

- **Plant**

Wheat grains (Masr1 and Gimmeza9) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

- **Radioactive compound**

Radiolabeled glycine (U-$^{14}$C) in aqueous solution containing 2% ethanol with original specific activity 3.96 G Bq/mmol was purchased from radiochemical laboratory, Amersham, England. It was diluted with non labeled glycine to give final specific activity of 0.025 µCi/1ml of 60 ppm glycine/plant.

- **Saline irrigation water and soil**

Saline water and soil used in the current study were obtained from Ras Sudr Agricultural Experimental Station of Desert Research Center, South Sinai. Chemical analysis is presented in **Table (1)**.

**Table (1): Chemical analysis of the soil and irrigation water from Ras Sudr**

| ppm | pH | Cations (meq/L) | | | | Anions (meq/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ca++ | Mg++ | Na+ | K+ | CO3= | HCO3- | Cl- | SO4= |
| **Chemical analysis of the experimental soil** | | | | | | | | | |
| 6215 | 7.76 | 4.6 | 3.2 | 88.3 | 0.67 | - | 4.95 | 65.7 | 26.1 |
| **Chemical analysis of irrigation water** | | | | | | | | | |
| 4654 | 7.82 | 10.8 | 7.15 | 53.6 | 0.35 | - | 5.30 | 39.1 | 26.8 |

### II. Cultivation and treatments

- **Growth conditions**

A pot experiment was carried out during winter season (2012/2013) at Radioisotopes Department, Nuclear Research Center, Egypt. Wheat grains were sown in the second week of November (2012/2013)., ten grains were sown in polyethylene pot (16 cm) containing 2 kg soil and irrigated with saline water.

- **$^{14}$C glycine application on plants**

After 30 days of cultivation, pots having similar plant growth were chosen for foliar application of 60ppm glycine containing $^{14}$C labeled glycine with a final specific activity 0.025 µCi/1ml (1 ml/plant).

- **Sampling**

Plant samples were collected after 3, 6, 24, 48, 96 hours and after 7 & 15 days of $^{14}$C glycine application, then kept at -80°C until used and subjected for chemical analysis, distribution and bioaccumulation of $^{14}$C labeled glycine into biochemical components.

### III. Methods

- **Antioxidant enzymes**

Polyacrylamide gel electrophoresis (Native PAGE) was used to study different isozymes variations. Peroxidase (POD.) and α & β-Esterase (α & β-Est.) isozymes were performed according to **Stegemann *et al*. (1983).** Also, superoxide dismutase (SOD.) and catalase (CAT.) were estimated according to **Weydert and Cullen (2010).**

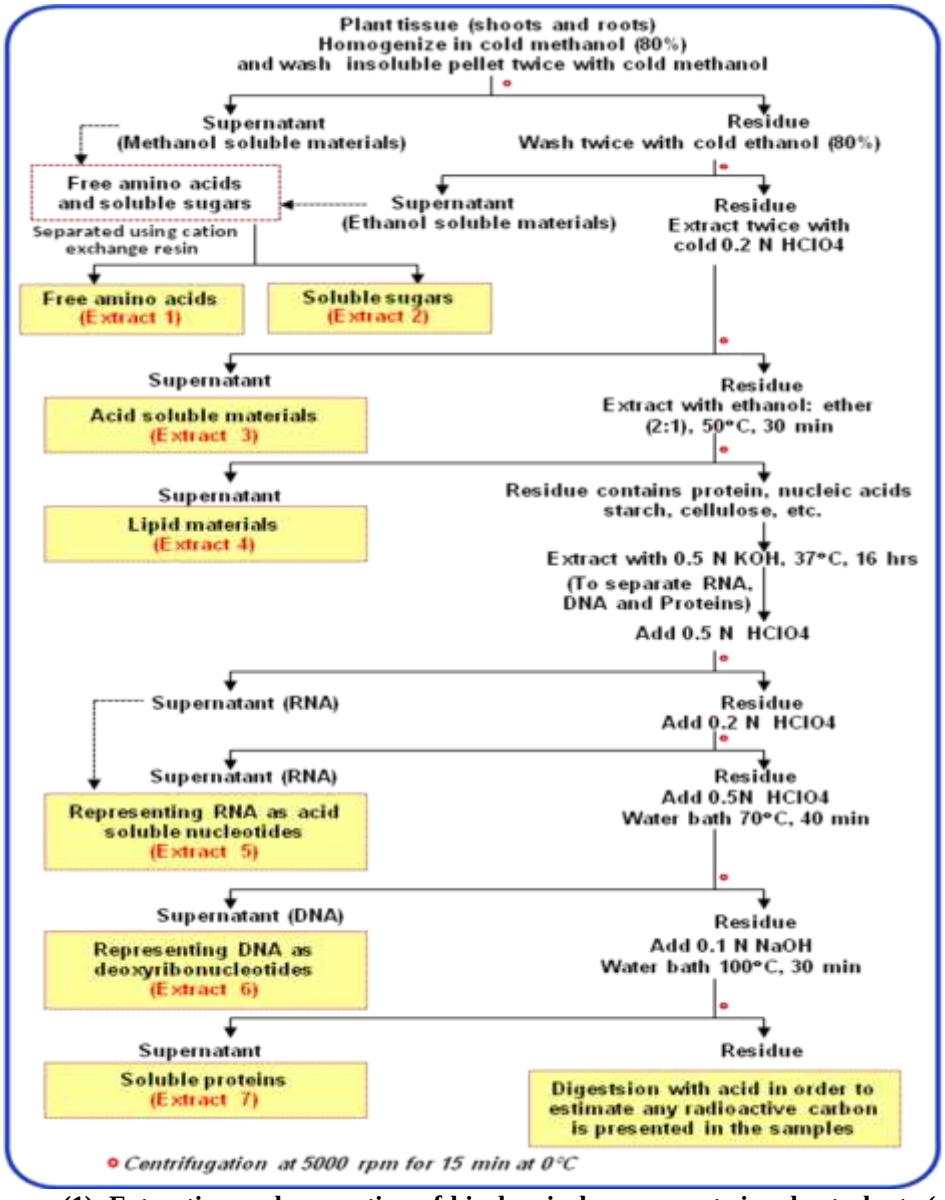
- **Determination of free amino acids**

Free amino acids were determined according to **Awapora (1948), Pellet and Young (1980)** and **Khan and Faiz (2008).** From each fresh samples, 2g were extracted with 70% ethyl alcohol. The ethanolic solution was filtered, concentrated and passed through a column cation exchange resin (Dowex 50). Elution was carried out with 70% ethyl alcohol to take all

carbohydrates, pigments and lipids present, then with ammonia solution for elution of free amino acids. The previous steps were repeated again using HCl instead of ammonia solution to complete elution of free amino acids. Each eluent was concentrated to a small volume by evaporation under vacuum at 45°C and kept deepfreezed till determination of amino acids by Sykam amino acid analyzer.

**• Distribution of $^{14}$C labeled glycine**

The collected plant samples were divided into shoots and roots, then weighted and kept fresh in the deepfreezer for the determination of $^{14}$C washed from shoots as well as $^{14}$C extractable in different extracts and bound in tissues after 3, 6, 24, 48, 96 hours and after 7 & 15 days of $^{14}$C glycine application **(Scheme1)** according to the technique reported by **Charry (1973)**.




**Scheme (1)**: **Extraction and separation of biochemical components in wheat plants (shoots and roots) after foliar application of $^{14}$C labeled glycine under saline conditions**

**• Radiomeasurement**

$^{14}$C activity of different fraction was determined using HIONIC-FLOUR LSC-Cocktail (Perki Elmar) and then counted using Packared Tri-Carb 2300 Liquid Scintillation Counter.

**IV. Statistical analysis**

The $^{14}$C data was subjected to one way ANOVA for mean and standard error using **Duncan's** new multiple range test. The software SPSS, version 10

(SPSS, Richmond, USA) was used as described by **Dytham (1999)**.

## 3. Results and Discussion

### 1. Antioxidant enzymes

- **Superoxide dismutase (SOD.) isozyme**

Superoxide dismutase had a clear role in ridding the plant cell from superoxide radical and thus protects the important biomolecules from oxidative stress damage under saline stress conditions. Data presented in **Table (2)** and **Fig. (1)** showed that, superoxide dismutase banding patterns comprise about five bands with different intensities and this depends on wheat genotype after 7 and 15 days of foliar application. The bands (No.1, 2 and 4) are presented in two wheat genotypes after 15 days from foliar application while they disappeared in the same genotypes after 7 days. On the other hand, band number 3 was detected in two genotypes after 7 days, but being missed after 15 days from foliar application of glycine. Regarding band intensity, band No.5 in samples of Masr1 recorded the maximum value of band intensity after 15 days, while band No.3 in the same genotype recorded the lowest value after 7 days compared with the other bands. In agreement with obtained data, **Hendawey *et al*. (2010)** and **Asmaa (2011)** studied the effect of some amino acids on superoxide dismutase banding patterns in wheat cultivars under New Valley desert conditions. They found that analysis of zymogram gel SOD pattern revealed the presence of about 5 bands in wheat cultivars. It should be pointed out that the appearance or disappearance of SOD bands under the effect of glycine treatment may be due to the difference in the ability of wheat cultivars to salt stress tolerance at different time periods, and associated with other systems that work against oxidative stress.

In addition**, Guan and Scandalios (1998)** and **Mauro *et al*. (2005)** indicated the importance of qualitative nature of SOD system in the scavenging of superoxide radicals, also superoxide dismutase isozyme often respond differentially to various environmental stresses. Also, SODs are metalloproteins and based on their metal cofactor they are classified into three known types: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD) that are localized in different cellular compartment **(Mittler, 2002)**. In addition, the regulation of SODs has been observed in plants subjected to both abiotic **(Boguszewska *et al*., 2010)** and biotic stresses **(Torres, 2010**). Over expression of SODs in transgenic plants was due to high salt or drought tolerance **(Badawi *et al*., 2004)**. Thus, SODS had a critical role in the survival of plants under environmental stresses.

- **Catalase (CAT.) isozyme**

Catalase is one of the important enzymes that increase the antioxidants defense capability in plant cell under oxidative stress conditions, where it has an important role in the elimination of hydrogen peroxide in chloroplast, cytosol, mitochondria and peroxisome of plant cells (**Asada, 2006**). It is evident from the records in **Table (2)** and **Fig. (1)** that catalase patterns of studied wheat genotypes revealed the presence of about five bands after treatment with glycine. In this concern, bands (No.1, 3 and 5) had been observed in two wheat genotypes (Masr1 and Gimmeza9) after 15 days of foliar application, while the same bands in both genotypes showed an opposite trend after 7 days. Also, bands (No.2 and 4) were detected in both genotypes after 7 days of foliar application. On contrary, the same bands in both genotypes took an opposite trend after 15 days of foliar application. In addition, there were detectable changes in band intensity for both cultivars. Band number 1 in Gimmeza9 was exceeded in band intensity after 15 days of foliar application compared with the other bands, while band number 4 in Masr1 was recorded the lowest value of band intensity after 7 days of foliar application. Catalase enzyme is the main scavenger of strong oxidant H2O2 in peroxisomes and it converts hydrogen peroxide to water and molecular oxygen (**Willekens *et al*., 1995**). In this regard, **Bahari *et al.* (2013)** showed that catalase activity increased by amino acids applications**,** also foliar spraying of amino acids can reduce the harmful effects of ROS and improves plant resistant under salt stress conditions. Generally, the increase of CAT activity is a strategy for improving salt tolerance (**Vaidyanathan *et al*., 2003**). Also, many researches proved that there was a correlation between the antioxidant capacity and NaCl tolerance in some plant species **(Gossett *et al*., 1994; Dionisio-Sese and Tobita, 1998** and **Hernandez *et al*., 1999).**

- **Peroxidase (POD.) isozyme**

Plant peroxidases are widely distributed in all higher plants, and involved in various physiological processes. One of the main functions is connected with the role as a part of defense enzyme in cells, ensuring detoxification of activated O2 forms. This function is very important in the formation of metabolic response of plants to different stress factors (**Bakardijieva *et al*., 1996**). From the data presented in **Table (2)** and **Fig. (1)** it's clear that the electrophoretic patterns of POD include a total of five bands. The bands (No. 1, 2, 3 and 4) were detected in two wheat cultivars after 7 and 15 days (except No. 2 and 4 in Masr1 after 15 days). The band No.5 found only in Masr1 after 15 days of treatment with glycine. With respect to band intensity, it has been found that band No.3 in Gimmeza9 recorded the higher value

after 7 days of foliar application. The more intensity of banding pattern indicates high peroxidase activity. In contrast, Masr1 (No.4) gave the lowest value of band intensity after 7 days.

Salinity has a clear effect on the activity of antioxidant enzymes particularly peroxidase, **Esfandiari and Pourmohammad (2013)** showed significant increase in activities of antioxidant enzymes (ascorbate peroxidase and guaiacol peroxidase) in wheat under saline stress, also, **El-Tayeb (2005)** found that peroxidase activity in barley increased in plant organs with increasing of NaCl level. In addition, **Sairam and Srivastava (2002)** and **Sairam *et al.* (2005)** found a higher increase in antioxidant activity of salt tolerant wheat genotype than salt sensitive**.** In this regard, **Harinasut *et al.* (2003)** suggested that under increasing salinity, the primarily prominent peroxidase activity appears to play an active role in scavenging reactive oxygen species in mulberry**.** There are many researches that show the effect of salinity on peroxidase enzyme in wheat such as **Goudarzi and Pakniyat (2009), Moghaieb *et al.* (2010)** and **Hanif and Afshan (2013)** as well as **Esfandiari *et al.* (2011)** and **Ibrahim *et al.* (2014)** on ascorbate peroxidase. In another study, **Edreva (2005)** showed that plants possess a number of antioxidant enzymes such as peroxidase that protects plant cells from these potential cytotoxic effects. Also, **Bakalova *et al.* (2004)** found that stress induced changes in the isozymes profile of peroxidase in wheat, also it was the most specific isozyme profile compared with the other enzymes studied in relation to the stress factors applied.

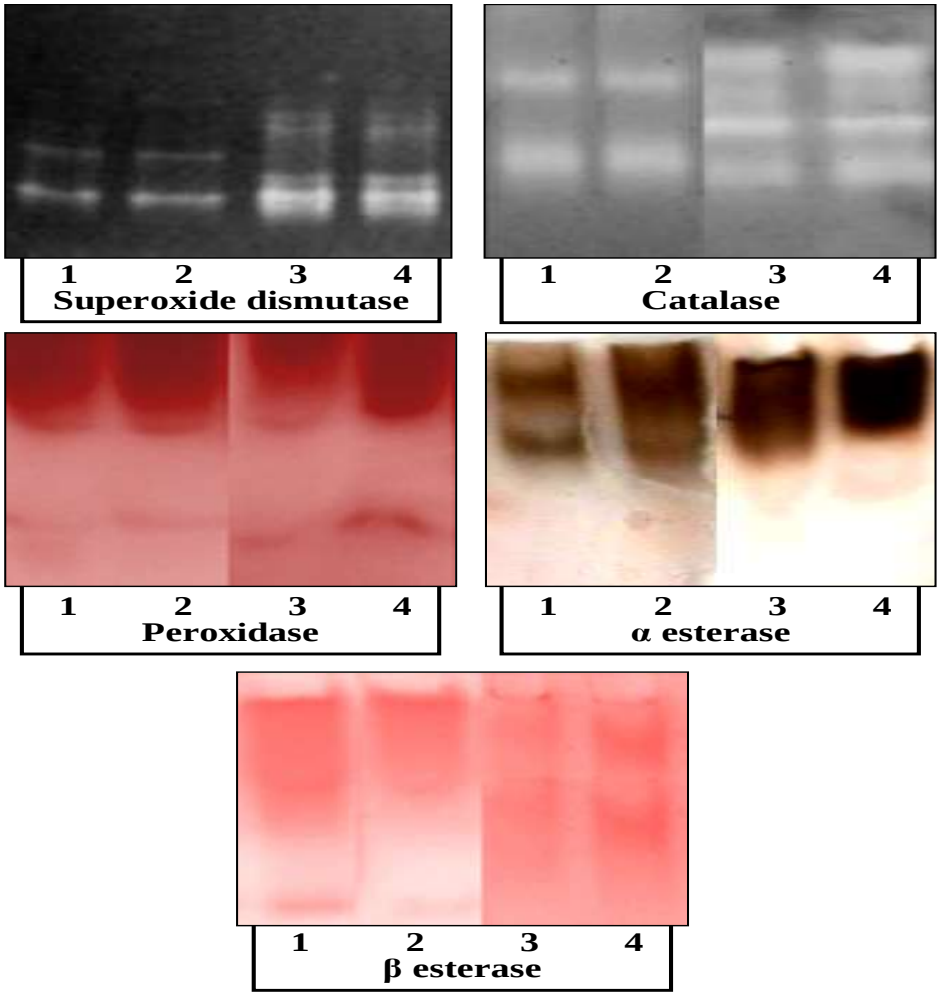



**Fig.(1): Zymogram of some isozymes banding patterns in shoots of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine under saline conditions**

**1** = Masr1 after 7 days **2** = Gimmeza9 after 7 days
**3** = Masr1 after 15 days **4** = Gimmeza9 after 15 days

**Table (2): Profile of some isozymes patterns in shoots of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine under saline conditions**

| Band number | Band intensity | | | |
|---|---|---|---|---|
| | Wheat cultivars | | | |
| | 7 days | | 15 days | |
| | Masr1 | Gimmeza9 | Masr1 | Gimmeza9 |
| **Superoxide dismutase (SOD.)** | | | | |
| 1 | 00 | 00 | 1.50 | 1.15 |
| 2 | 00 | 00 | 1.48 | 1.53 |
| 3 | 1.00 | 1.32 | 00 | 00 |
| 4 | 00 | 00 | 2.43 | 2.61 |
| 5 | 3.15 | 3.88 | 6.05 | 5.12 |
| Total | 2 | 2 | 4 | 4 |
| **Where;** 00= refers to no band, 1.00 = refers to lowest band intensity and 6.05 = refers to highest band intensity | | | | |
| **Catalase (CAT.)** | | | | |
| 1 | 00 | 00 | 2.07 | 2.69 |
| 2 | 1.37 | 1.07 | 00 | 00 |
| 3 | 00 | 00 | 2.29 | 2.24 |
| 4 | 1.00 | 1.60 | 00 | 00 |
| 5 | 00 | 00 | 2.09 | 2.18 |
| Total | 2 | 2 | 3 | 3 |
| **Where;** 00= refers to no band, 1.00 = refers to lowest band intensity and 2.69 = refers to highest band intensity | | | | |
| **Peroxidase (POD.)** | | | | |
| 1 | 1.95 | 2.40 | 2.02 | 1.63 |
| 2 | 2.20 | 2.81 | 00 | 1.61 |
| 3 | 2.52 | 2.91 | 2.04 | 1.92 |
| 4 | 1.00 | 1.33 | 00 | 2.03 |
| 5 | 00 | 00 | 1.75 | 00 |
| Total | 4 | 4 | 3 | 4 |
| **Where;** 00= refers to no band, 1.00 = refers to lowest band intensity and 2.91 = refers to highest band intensity | | | | |
| **α-esterase(α-Est.)** | | | | |
| 1 | 6.06 | 6.28 | 7.01 | 6.94 |
| 2 | 00 | 00 | 6.24 | 4.23 |
| 3 | 4.43 | 3.98 | 00 | 00 |
| 4 | 00 | 00 | 00 | 1.00 |
| Total | 2 | 2 | 2 | 3 |
| **Where;** 00= refers to no band, 1.00 = refers to lowest band intensity and 7.01 = refers to highest band intensity | | | | |
| **β-esterase(β-Est.)** | | | | |
| 1 | 3.52 | 4.16 | 00 | 00 |
| 2 | 00 | 00 | 2.61 | 2.89 |
| 3 | 2.92 | 2.53 | 2.10 | 2.44 |
| 4 | 00 | 00 | 2.09 | 2.77 |
| 5 | 1.38 | 1.00 | 00 | 00 |
| Total | 3 | 3 | 3 | 3 |
| **Where;** 00= refers to no band, 1.00= refers to lowest band intensity and 4.16 = refers to highest band intensity | | | | |

**• α and β-Esterase (α and β-Est.) isozymes**

Esterase is a hydrolase enzyme that splits esters into an acid and an alcohol in a chemical reaction with water called hydrolysis. A wide range of different esterases exists that differ in their substrate specificity, protein structure, and biological function**.** Hydrolysis of the substrate occurs in two stages. First, the acyl group is transferred to the enzyme with the formation

of an acylated enzyme and liberation of alcohol. Second, the acylated enzyme is hydrolyzed yielding the acid and free enzyme **(http://en.wikipedia.org)** and **Chandre *et al*. (2014)**. In the present work, data in **Table (2)** and **Fig. (1)** showed that α-esterase banding patterns comprise four bands with different intensities. The bands (No.2 and 4) were absent in two wheat cultivars after 7 days of foliar application, also band No.3 took the same trend after 15 days. In addition, band number 4 was not presented in Masr1 when treated with glycine (15 days). Concerning band intensity, Masr1 (No.1) was produced the maximum value of band intensity after 15 days of foliar application, recording 7.01 compared with the other bands. Data in the same table and figure showed that β-esterase pattern revealed the presence of about 5 bands after treatment with glycine. The bands (No.1, 3 and 5) were detected in two wheat cultivars after 7 days of foliar application, but bands No.1 and 5 being missed in two wheat cultivars after 15 days. In this regard, the bands number 2 and 4 were absent in wheat cultivar after 7 days of foliar application. Moreover, Gimmeza9 recorded the highest value of band intensity (No.1) after 7 days, while the lowest value (No.5) was achieved in the same cultivar after 7 days.

In general, esterase isozymes patterns were reliable system for discriminating between tolerant and sensitive genotypes under salt stress. In this regard, **Metwali (2012)** indicated that esterase isozymes were reliable molecular markers linked with salt tolerance in barley. In wheat plants, **Metwali *et al.* (2011)** indicated that salt stress increased the accumulation of the esterase enzyme which accelerated in response to salt stress. Also, **Hassanein (1999)** found that the highest number of esterase isozymes was detected under the highest NaCl concentration**.** As reported by **Hanaa *et al.* (2003),** esterase isozyme is good indicator for response to biotic and abiotic stresses. In another study, **El-Sayed *et al*. (2007)** showed that the appearance of addition isozyme bands may be involved for improving salt tolerance in wheat and could be used as biochemical genetic markers for salt tolerance in wheat. There are many researches that show the effect of salinity on esterase enzyme in many plants (non wheat) such as **Radc and Pevalek-kozlina (2010), Abdel Aziz and Mohamed (2011), Al-Shabi *et al.* (2013) and EL-Beltagi *et al.* (2013).**

**2. Free amino acids**

Free amino acids play an important role to push the plants for salt stress tolerance. In this concern, **Rai (2002)** showed that plants subjected to stress show accumulation of amino acids. The role played by accumulated amino acids in plants varies from acting as osmolyte, regulation of ion transport, modulating stomatal opening and detoxification of heavy metals. Amino acids also affect synthesis and activity of some enzymes, gene expression and redox homeostasis. Data presented in **Table (3)** and **Fig. (2)** showed the pattern of free amino acids in wheat plants after treatment with $^{14}$C glycine, it was clear that thirty three amino acids were detected in the tested cultivars. In addition, α-aminoadepic was not detected in all samples under investigation (except Gimmeza9 after 7 days from foliar application). Also, α-aminobutyric and 3-methylhistidine in samples of Masr1 took the same trend after 15 days from foliar application of $^{14}$C glycine. Results indicated that the most abundant amino acids noticed were serine, asparagine, proline, alanine, cystine, δ-aminobutyric, lysine and arginine, where arranged according to the retention time of amino acids which were separated from column of amino acid analyzer apparatus. The highest values of serine, asparagine, proline, cystine, lysine and arginine were observed in Masr1 after 7 days of foliar application. Our results showed that, Gimmeza9 gave the maximum value of alanine after 7 days compared with the other treatments, also δ-aminobutyric in Masr1 was recorded the highest value after 15 days of foliar application. With respect to the accumulation of free amino acids under conditions of salt stress, there are many researches such as **Ranieri *et al*. (1989), Roy-Macauley *et al*. (1992) and Mansour (2000)**, which showed that this accumulation is due to: 1) Inhibition of amino acids degradation. 2) Inhibition of protein synthesis. 3) Protein degradation. In the same direction, **Hayat *et al*. (2012)** summarized the important role of proline in plants under stress conditions in the following: 1) It protects the plants from various stresses and also helps plants to recover from stress more rapidly. 2) Enhanced growth and other physiological characteristics of plants. 3) Scavenges the ROS generated in plants under various biotic and abiotic stresses. 4) Affects plant-water relations by maintaining turgidity of cells under stress and also increases the rate of photosynthesis. 5) Protects plants from harmful stresses such as salinity. Also, **Kavi Kishor *et al*. (2005)** found that accumulation of proline was due to increased synthesis and decreased degradation under a variety of stress conditions. In the current study, phosphoserine, taurine, phosphoethanolamine, citrulline, α-aminobutyric, 1-methylhistidine, carnosine and tryptophan displayed low concentrations in two wheat cultivars compared with the other free amino acids. In this regard, other identified amino acids had concentrations between those extremes and different in their concentrations from cultivar to another and this depends on the interaction between foliar application of $^{14}$C glycine and wheat cultivars under saline conditions.

**Table (3): Patterns of free amino acids (mg/100 g FW) in shoots of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine under saline conditions**

| No. | Free amino acids | Wheat cultivars | | | |
|---|---|---|---|---|---|
| | | 7 days | | 15 days | |
| | | Masr1 | Gimmeza9 | Masr1 | Gimmeza9 |
| 1 | Phosphoserine | 0.05 | 0.15 | 0.002 | 0.20 |
| 2 | Taurine | 0.09 | 0.02 | 0.02 | 0.10 |
| 3 | Phosphoethanolamine | 0.02 | 0.01 | 0.003 | 0.01 |
| 4 | Asparaginic acid | 2.81 | 1.57 | 0.21 | 1.04 |
| 5 | Hydroxyproline | 0.68 | 0.16 | 0.14 | 0.13 |
| 6 | Threonine | 2.83 | 3.29 | 0.28 | 1.16 |
| 7 | Serine | 4.57 | 4.11 | 0.68 | 2.04 |
| 8 | Asparagine | 14.75 | 7.50 | 1.14 | 3.26 |
| 9 | Glutamine | 1.84 | 0.70 | 0.17 | 0.32 |
| 10 | α-Aminoadepic | n.d | 0.03 | n.d | n.d |
| 11 | Proline | 10.63 | 5.26 | 1.51 | 2.56 |
| 12 | Glycine | 3.19 | 3.22 | 2.08 | 1.37 |
| 13 | Alanine | 10.40 | 11.25 | 8.52 | 3.72 |
| 14 | Citrulline | 0.02 | 0.04 | 1.92 | 0.004 |
| 15 | α -Aminobutyric | 0.01 | 0.03 | n.d | 0.01 |
| 16 | Valine | 3.15 | 3.24 | 10.74 | 1.38 |
| 17 | Cystine | 9.18 | 4.33 | 0.07 | 3.44 |
| 18 | Methionine | 0.08 | 0.21 | 0.51 | 0.04 |
| 19 | Isoleucine | 1.89 | 1.77 | 3.98 | 0.79 |
| 20 | Leucine | 3.52 | 3.58 | 3.11 | 1.57 |
| 21 | Tyrosine | 2.50 | 1.73 | 0.11 | 1.30 |
| 22 | Phenylalanine | 1.78 | 2.40 | 1.02 | 1.35 |
| 23 | β –Alanine | 1.38 | 1.15 | 1.54 | 0.15 |
| 24 | β-Aminobutyric | 1.65 | 2.28 | 0.61 | 0.16 |
| 25 | δ-Aminobutyric | 11.13 | 9.10 | 11.90 | 2.12 |
| 26 | Ornithine | 0.98 | 0.95 | 0.35 | 0.35 |
| 27 | Lysine | 16.53 | 14.35 | 14.05 | 8.26 |
| 28 | 3-Methylhistidine | 0.97 | 0.75 | n.d | 0.29 |
| 29 | Histidine | 1.13 | 0.63 | 0.02 | 0.18 |
| 30 | 1-Methylhistidine | 0.02 | 0.02 | 0.11 | 0.004 |
| 31 | Tryptophan | 0.04 | 0.04 | 0.02 | 0.01 |
| 32 | Carnosine | 0.03 | 0.03 | 0.02 | 0.002 |
| 33 | Arginine | 11.92 | 6.30 | 0.01 | 2.52 |
| Total free amino acids | | 119.77 | 90.20 | 64.85 | 39.84 |

**Where;**
Amino acids in the table were arranged according to the retention time (Ascending) of amino acids which separated from column of amino acid analyzer apparatus. **n.d** = not detectable and **FW**= Fresh weight

Effects of salinity on amino acids have been studied by **Abd El-Samad (2013)** in wheat plants**.** Also, **Simon-Sarkadi *et al*. (2002)** showed that the salt stress caused increases in proline, glutamic acid, aspartic acid, arginine, ornithine and α-aminobutyric acid levels in cereals. In the same direction, **Azevedo Netoa *et al.* (2009)** showed that salinity increased the content of the majority of free amino acids in leaves and roots of maize genotypes. In addition, **Kovacs *et al.* (2012)** showed that during the first days of osmotic stress the glutamic, glutamine, aspartic, asparagine, threonine, serine, leucine and histidine concentrations were greater in the tolerant wheat line than in the sensitive one. In light of the importance of amino acids to plant cell, they enter in the synthesis of many biomolecules, **El-Shintinawy and El-Shourbagy (2001)** found that the changes in glutamic and glycine were associated with cystine synthesis under salt stress;

these acids are precursors of glutathione, which plays a vital role in antioxidative system **(Noctor and Foyer, 1998)**. Also, **Miflin and Lea (1977)** found that glutamic is a common substrate for the biosynthesis of several amino acids**.** In addition, **Hammad and Ali (2014)** indicated that the usage of amino acids caused a significant increase in total free amino acids compared with untreated wheat plants.

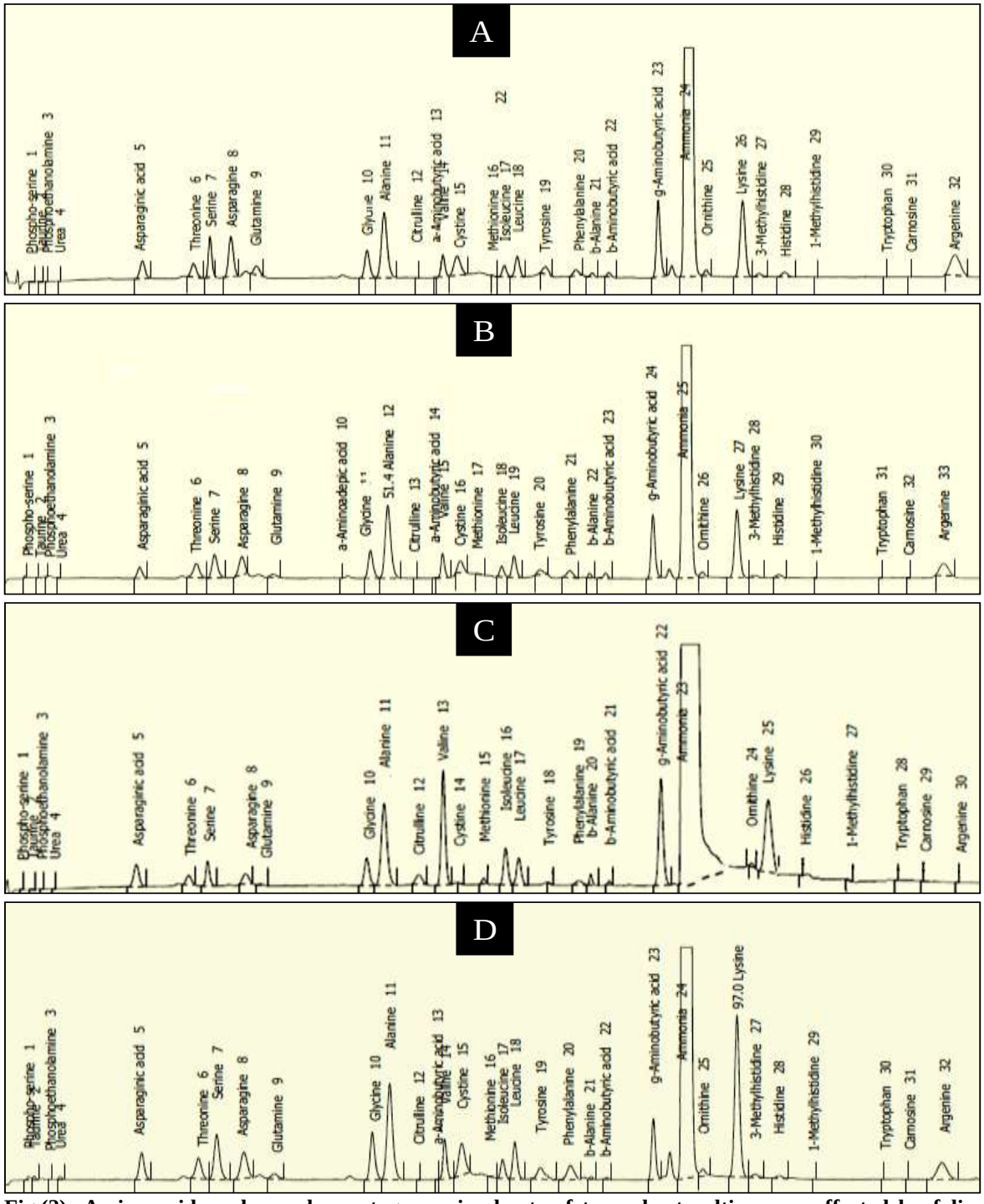



**Fig.(2): Amino acid analyzer chromatograms in shoots of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine under saline conditions**

**A=** Masr1 after 7 days **C=** Masr1 after 15 days

**B=** Gimmeza9 after 7 days **D=** Gimmeza9 after 15 days

**Table (4): $^{14}C$ recovery (dpm/g FW and its % of applied dose) in washed, extractable and residue fractions of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine (0.25µCi/1ml of 60ppm/plant) under saline conditions**

| Plant | | Fractions | 3h | | 6h | | 24h | | 48h | |
|---|---|---|---|---|---|---|---|---|---|---|
| Wheat cultivars | Part | | dpm/g FW | % | dpm/g FW | % | dpm/g FW | % | dpm/g FW | % |
| Masr1 | Shoot | Washed | 4781±267 | 50.18 | 3476±225 | 36.48 | 2396±139 | 25.15 | 2198±145 | 23.07 |
| | | Extractable | 2996±239 | 31.44 | 3290±220 | 34.53 | 2973±149 | 31.20 | 3296±286 | 34.59 |
| | | Residue | 470±15 | 4.93 | 550±19 | 5.77 | 973±22 | 10.21 | 285±7 | 2.99 |
| | Root | Extractable | 2339±107 | 12.67 | 2423±145 | 13.13 | 2526±113 | 13.68 | 3657±237 | 19.82 |
| | | Residue | 658±28 | 3.57 | 703±20 | 3.81 | 2691±188 | 14.59 | 672±20 | 3.64 |
| | Total recovery/plant | | | 102.79 | | 93.72 | | 94.83 | | 84.11 |
| Gimmeza9 | Shoot | Washed | 3902±214 | 31.61 | 3935±94 | 31.88 | 3382±290 | 27.4 | 3307±168 | 26.79 |
| | | Extractable | 5628±247 | 45.59 | 4565±260 | 36.98 | 4084±228 | 33.08 | 3700±225 | 29.97 |
| | | Residue | 555±19 | 4.50 | 703±10 | 5.69 | 634±40 | 5.14 | 1840±71 | 14.91 |
| | Root | Extractable | 4435±292 | 21.71 | 3110±108 | 15.23 | 4320±146 | 21.15 | 3470±194 | 16.99 |
| | | Residue | 562±13 | 2.75 | 416±15 | 2.04 | 897±39 | 4.39 | 859±37 | 4.21 |
| | Total recovery/plant | | | 106.16 | | 91.82 | | 90.16 | | 92.87 |

| Plant | | Fractions | 96h | | 7d | | 15d | |
|---|---|---|---|---|---|---|---|---|
| Wheat cultivars | Part | | dpm/g FW | % | dpm/g FW | % | dpm/g FW | % |
| Masr1 | Shoot | Washed | 1743±59 | 18.29 | 1089±39 | 11.43 | 837±38 | 8.78 |
| | | Extractable | 4454±302 | 46.74 | 1450±75 | 15.22 | 1006±57 | 10.56 |
| | | Residue | 175±5 | 1.84 | 177±4 | 1.86 | 137±4 | 1.44 |
| | Root | Extractable | 2784±153 | 15.09 | 1900±119 | 10.30 | 1046±53 | 5.66 |
| | | Residue | 229±9 | 1.24 | 277±7 | 1.50 | 201±4 | 1.09 |
| | Total recovery/plant | | | 83.20 | | 40.31 | | 27.53 |
| Gimmeza9 | Shoot | Washed | 984±27 | 7.97 | 670±24 | 5.42 | 506±30 | 4.10 |
| | | Extractable | 5191±285 | 42.05 | 2850±202 | 23.09 | 1094±50 | 8.86 |
| | | Residue | 268±7 | 2.17 | 229±8 | 1.86 | 224±7 | 1.81 |
| | Root | Extractable | 2831±12`1 | 13.86 | 1758±112 | 8.61 | 982±33 | 4.81 |
| | | Residue | 182±6 | 0.89 | 419±14 | 2.05 | 265±15 | 1.23 |
| | Total recovery/plant | | | 66.14 | | 41.04 | | 20.81 |

**Where;**
**h** = hours, **d** = days, **FW**= Fresh weight**, % of applied dose** = [(dpm/g FW *Part wt)/55550]/100

### 3. $^{14}C$ recovery % in wheat plants

Data in **Table (4)** showed that, after 3h of $^{14}C$ glycine application about 50% of the applied glycine remained on the shoot of Masr1 while 31.61% remained on the shoots of Gimmeza9. In both experimental cultivars the amount of $^{14}C$ in the washed fraction decreased by time reaching 8.78% and 4.10% of the applied dose in Masr1 and Gimmeza9 respectively after 15 days. The data revealed that the absorption of glycine by Gimmeza9 is higher than that by Masr1. Regarding $^{14}C$ in the extractable fraction in both cultivars, data in the same table revealed that: 1) At all time intervals $^{14}C$ % of the applied glycine in shoots was higher than that in the roots. 2) Extractable $^{14}C$ % of the applied glycine increased by time in shoots up to 96h (46.74% in Masr1 and 42.05% in Gimmeza9) then decreased and reached the lowest percent at 15 days (10.56% in Masr1 and 8.86 % in Gimmeza9). 3) In roots, the increase in $^{14}C$ extractable fraction reached the highest percent at 48h (19.82% in Masr1 and 16.99% in Gimmeza9), then decreased at 7 and 15 days. The highest percent of the $^{14}C$ residue fraction was recorded in shoots and roots of the cultivar Masr1 at 24h, while at 48h in Gimmeza9 then decreased in both cultivars reaching the lowest values at 15 days. The total recovery percent of $^{14}C$ glycine in the two plants decreased by time reaching the lowest value at 15 days of the application (27.53% in Masr1 and 20.81% in Gimmeza9); this may be attributed to the loss of $^{14}C$ glycine as $^{14}CO_2$ through respiration

after complete metabolism of the applied glycine. In this connection, **Kafi (2009)** noticed that wheat cultivars differ in respiration rates in the presence of salinity, also respiration was quite different at different hours of the day. Also, **Moud and Maghsoudi (2008)** found that respiration in wheat plants decrease with increasing salinity level.

**4. Distribution of $^{14}C$ into biochemical components of wheat plants**

The distribution of $^{14}C$ into different biochemical components of two wheat cultivars (E1, free amino acids extract; E2, soluble sugars extract; E3, the acid and other soluble materials extract; E4, lipids extract; E5, representing RNA as acid soluble nucleotides extract; E6, represented DNA as deoxyribonucleotides extract; E7, total soluble protein extract) after treatment with $^{14}C$ labeled glycine are presented in **Table (5)**. From the results obtained, the presence of $^{14}C$ was detected in each plant extracts used in this study, but the distribution was different depending on the type of wheat extracts and the time of sampling. This proves that glycine had a clear role in the biosynthesis of many important biochemical components of two wheat cultivars under saline conditions.

**Table (5): $^{14}C$ distribution % in different extracts of two wheat cultivars as affected by foliar application of $^{14}C$ labeled glycine (0.25µCi/1ml of 60ppm/plant) under saline conditions**

| Wheat cultivars | Extracts | 3h | 6h | 24h | 48h | 96h | 7d | 15d |
|---|---|---|---|---|---|---|---|---|
| Masr1 | | | | Shoot | | | | |
| | E1 | 10.7 | 6.6 | 9.0 | 17.6 | 25.4 | 11.3 | 23.6 |
| | E2 | 8.2 | 13.3 | 14.1 | 10.2 | 14.5 | 10.1 | 8.8 |
| | E3 | 46.3 | 39.5 | 34.2 | 40.4 | 40.3 | 24.9 | 34.8 |
| | E4 | 7.1 | 5.8 | 10.2 | 10.1 | 5.4 | 12.5 | 6.6 |
| | E5 | 11.2 | 15.1 | 14.5 | 11.8 | 7.4 | 18.0 | 7.5 |
| | E6 | 10.0 | 11.8 | 12.4 | 6.2 | 4.9 | 11.3 | 6.1 |
| | E7 | 6.5 | 7.9 | 5.5 | 3.8 | 2.1 | 11.9 | 12.5 |
| | | | | Root | | | | |
| | E1 | 7.1 | 7.1 | 5.5 | 13.0 | 11.1 | 5.7 | 8.9 |
| | E2 | 18.8 | 12.0 | 7.4 | 24.8 | 25.0 | 21.3 | 8.7 |
| | E3 | 29.1 | 21.8 | 28.1 | 30.0 | 27.4 | 25.1 | 20.2 |
| | E4 | 6.7 | 5.1 | 8.6 | 10.2 | 11.0 | 9.1 | 17.1 |
| | E5 | 23.4 | 37.3 | 32.0 | 11.9 | 13.1 | 18.4 | 16.1 |
| | E6 | 9.9 | 10.1 | 11.9 | 5.3 | 7.1 | 10.0 | 11.6 |
| | E7 | 5.0 | 6.6 | 6.5 | 4.6 | 5.3 | 10.4 | 17.4 |
| | | | | | | | | |
| Gimmeza9 | | | | Shoot | | | | |
| | E1 | 12.4 | 13.8 | 5.9 | 14.9 | 21.6 | 19.1 | 12.7 |
| | E2 | 8.2 | 18.9 | 15.6 | 17.7 | 8.5 | 9.8 | 8.4 |
| | E3 | 52.2 | 41.5 | 25.4 | 40.3 | 47.8 | 41.2 | 17.6 |
| | E4 | 9.5 | 7.6 | 14.3 | 9.9 | 7.7 | 11.6 | 17.0 |
| | E5 | 9.1 | 8.9 | 19.7 | 10.0 | 7.4 | 7.7 | 18.6 |
| | E6 | 5.3 | 6.1 | 12.2 | 4.1 | 4.8 | 6.7 | 9.4 |
| | E7 | 3.2 | 3.3 | 6.8 | 3.0 | 2.4 | 4.0 | 16.2 |
| | | | | Root | | | | |
| | E1 | 9.5 | 18.1 | 4.9 | 13.7 | 7.9 | 6.1 | 7.9 |
| | E2 | 11.7 | 15.2 | 9.0 | 13.6 | 18.7 | 13.0 | 16.1 |
| | E3 | 46.1 | 27.7 | 23.3 | 32.7 | 28.2 | 25.3 | 15.5 |
| | E4 | 7.0 | 13.8 | 7.4 | 12.0 | 10.5 | 12.7 | 19.3 |
| | E5 | 14.6 | 11.4 | 32.4 | 16.2 | 16.0 | 23.7 | 14.5 |
| | E6 | 5.7 | 7.6 | 12.6 | 6.4 | 8.7 | 11.9 | 7.4 |
| | E7 | 5.5 | 6.2 | 10.4 | 5.4 | 10.0 | 7.3 | 19.3 |

**Where;**

**E1**= free amino acids extract, **E2**= soluble sugars extract, **E3**= the acid and other soluble materials extract, **E4=** lipids extract, **E5=** representing RNA as acid soluble nucleotides extract, **E6=** represented DNA as deoxyribonucleotides extract, **E7=** total soluble protein extract, **h=** hour **and d=** day**.** Chemicals used in these extracts are described in materials and methods

The highest amount of $^{14}C$ was found in E1 extract in roots and shoots of two wheat cultivars compared with the other plant extracts. It is worth noting that the proportion of $^{14}C$ in this extract

decreased with the time of sampling and this was evident at 15 days compared with the proportion of $^{14}C$ in the same extract at the first times. In this regard, E5 extract recorded the second order to contain $^{14}C$ and this was clear in shoots and roots of Masr1 cultivar as well as the roots of Gimmeza9 compared with the other plant extracts. While, E1 extract recorded the second order in shoots of Gimmeza9 only. The results showed that the lowest amounts of $^{14}C$ were obtained from E7 extract (compared with the other plant extracts) in different plant parts of two wheat cultivars under saline conditions. On the other hand, it was noted a clear increase in $^{14}C$ content in the previous extract at 15 days compared with the other time of sampling for two wheat cultivars. In light of glycine role in the biosynthesis of many biochemical components in current study, **Hendawey (2015)** showed that foliar application of glycine alleviate the adverse effects of saline stress on wheat plants, as well as, it had a positive effect on most of the biochemical components and wheat grain yield. In the same direction, **Gioseffi *et al.* (2012)** studied the absorption of double labeled glycine by wheat roots. Also, **Owen and Jones (2001)** studied the competition between rhizosphere microorganisms and wheat plant roots for $^{14}C$ labeled glycine.

## Conclusion

It is concluded that glycine acid had an effective role in pushing wheat plants for salt stress tolerance, through effective contribution in the biosynthesis of many important biochemical components and its association with the activity of antioxidant defense system under salt stress conditions.

## References


1. Abd El-Samad, H. M. (2013). The physiological response of wheat plants to exogenous application of gibberellic acid (GA3) or indole-3-acetic acid (IAA) with endogenous ethylene under salt stress conditions. Int. J. Plant Physiol. Biochem., 5(4): 58-64.
2. Abdel Aziz, A. and H. I. Mohamed (2011). Brassinolide alleviates salt stress and increases antioxidant activity of cowpea plant (*Vigna sinensis*). Am. Eurasian J. Agric. Environ. Sci., 10(2): 238-247.
3. Agarwal, S. and V. Pandey (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Plant Biol., 48: 555-560.
4. Al-Shabi, J. H., M. A. A. Sallam and N. E. Ahmed (2013). Gene expression analysis of Egyptian and Yemeni sorghum cultivars under salinity stress using isozyme and SDS-PAGE. Global J. Biotechnol. Biochem., 8 (4): 78-83.
5. Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol., 141: 391–396.
6. Asmaa A. Mahdi (2011). Biochemical changes in heat stressed wheat grown at El Wadi El Gedeed. M.Sc. Thesis, Fac. Sci., Ain Shams Univ., Egypt.
7. Awapora, J. (1948). Extraction of free amino acids. Arch. Biochem., 2:172.
8. Azevedo Netoa, A. D., J. T. Priscob and E. Gomes-Filhob (2009). Changes in soluble amino-N, soluble proteins and free amino acids in leaves and roots of salt-stressed maize genotypes. J. Plant Interact., 4(2): 137-144.
9. Badawi, G. H., E. Yamauchi, R. Shimada, N. Sasaki, K. Kawano, K. Tanaka and K. Tanaka (2004). Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tabacco (*Nicotina tabacum*) chloroplast. Plant Sci., 166: 919-928.
10. Bahari, A., H. Pirdashti and M.Yaghubi (2013) The effects of amino acid fertilizers spraying on photosynthetic pigments and antioxidant enzymes of wheat (*Triticum aestivum* L.) under salinity stress. Int. J. Agron. Plant Prod., 4 (4): 787-793.
11. Bakalova, S., A. Nikolova and D. Nedeva (2004). Isoenzyme profiles of peroxidase, catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds. Bulg. J. Plant Physiol., 30 (1-2): 64-77.
12. Boguszewska, D., M. Grudkowska and B. Zagdańska (2010). Drought responsive antioxidant enzymes in potato (*Solanum tuberosum* L.). Potato Res., 53: 373-382.
13. Candan, N. and L. Tarhan (2003). The correlation between antioxidant enzyme activities and lipid peroxidation levels in Mentha pulegium organs grown in Ca, Mg, Cu, Zn and Mn stress conditions. Plant Sci., 163: 769-779.
14. Chandre, M., T. Shilpa, A. Kharde and N. Chavan (2014). Studies on the effect of pH and temperature on the activity of esterase enzyme isolated from *Pisum sativum.* Asian J. Biochem. Pharm. Res., 3 (4):335-341.
15. Cherry, J.H. (1973). Molecular biology of plants. A Test-Manual. Columbia Univ. Press. New York and London. 68-71.
16. Dionisio-Sese, M. L. and S. Tobita (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci., 135: 1-9.
17. Dytham, C. (1999). Choosing and Using Statistics: A Biologist's guide. Blackwell Science Ltd., London,UK.
18. Edreva, A. (2005). Generation and scavenging of reactive oxygen species in chloroplasts: A submolecular approach. Agric. Ecosyst. Environ., 106:119-133.

19. EL-Beltagi, H. S., H. I. Mohamed, A. M. A. Mohammed, L. M. Zaki and A. M. Mogazy (2013). Physiological and biochemical effects of γ-irradiation on cowpea plants (*Vigna sinensis*) under salt stress. Not. Bot. Horti. Agrobo., 41(1):104-114.
20. El-Sayed,O. E., A. A. Rizkalla and S. R. S. Sabri (2007). *In vitro* mutagenesis for genetic improvement of salinity tolerance in wheat. Res. J. Agric. Biol. Sci., 4(5): 377-383.
21. El-Shintinawy, F. and M. N. El-Shourbagy (2001). Alleviation of changes in protein metabolism in NaCl-stressed wheat seedlings by tiamine. Biol. Plant., 44(4):541-545.
22. El-Tayeb, M.A. (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. Plant growth Regul., 45:215-224.
23. Esfandiari, E. and A. Pourmohammad (2013). Evaluation of the salinity effects on some physiological and biochemical characteristics of two wheat cultivars. YYU J. Agr. Sci., 23(2): 141–148.
24. Esfandiari, E., V. Enayati and A. Abbasi (2011). Biochemical and Physiological changes in response to salinity in two durum wheat (*Triticum turgidum* L.) genotypes. Not. Bot. Hort. Agrobot. Cluj., 39(1):165-170.
25. Gara, L. D., M. C. Pinto and F. Tommasi (2003). The antioxidant systems vis-vis reactive oxygen species during plant-pathogen interaction. Plant Physiol. Biochem., 41: 863-870.
26. Gioseffi, E., A. de Neergaard and J. K. Schjoerring (2012). Interactions between uptake of amino acids and inorganic nitrogen in wheat plants. Biogeosci., 9: 1509–1518.
27. Gossett, D. R., E. P. Millhollon and M. C. Lucas (1994). Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. Crop Sci., 34: 706-714.
28. Goudarzi, M. and H. Pakniyat (2009). Salinity causes increase in proline and protein contents and Peroxidase activity in wheat cultivars. J. Appl. Sci., 9(2): 348-353.
29. Guan, L. and J.G. Scandalios (1998). Two structurally similar maize cytosolic superoxide dismutase genes, *Sod4* and *Sod4A*, respond differentially to abscisic acid and high osmoticum. Plant Physiol., 117: 217–224.
30. Hammad, S. A. R. and O. A. M. Ali (2014). Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. Ann. Agric. Sci., 59(1):133–145.
31. Hanaa, H., M. Amal and M. Hussein (2003). Influence of salinity on lipid per oxidation, antioxidant enzymes and electrophoresis patterns of protein and isozymes in leaves of some onion cultivars. Asian J. Plant Sci., 2(17): 1220-1227.
32. Hanif, F. and S. Afshan (2013). Evaluating the response of wheat genotypes to salinity stress. Asian J. Agric. Sci., 5(6): 126-129.
33. Harinasut, P., D. Poonsopa, K. Roengmonghol and R. Charoensatapom (2003). Salinity effects on antioxidant enzymes in mulberry cultivar. Sci. Asia, 29: 109-113.
34. Hassanein, A. (1999). Alternation in protein and esterase wheat in response to salinity stress. Biol. Plant., 42(2): 241-248.
35. Hayat, S., Q. Hayat, M. N. Alyemeni, A. S. Wani, J. Pichtel and A. Ahmad (2012). Role of proline under changing environments. Plant Signaling Behav., 7(11): 1456–1466.
36. Hendawey, M. H. (2015). Biochemical changes associated with induction of salt tolerance in wheat. Global J. Biotechnol. Biochem., 10 (2): 84-99.
37. Hendawey, M.H., Nahla S. Hassan, I. H. Borai and Asmaa A. Mahdi (2010). Biochemical counteraction of heat stress injury in wheat under New Valley desert conditions. Minufiya J. Agric. Res., 35 (4):1223-1253.
38. Hernandez, J. A., A. Campillo, A. Jimenez, J.J. Alarcon and F. Sevilla (1999). Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol., 141: 241-251.
39. Ibrahim, W. M., R. M. Ali, K. A. Hemida and M. A. Sayed (2014) Role of *Ulva lactuca* extract in alleviation of salinity stress on wheat seedlings. The Sci. World J., 1-11.
40. Kafi, M. (2009). The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt tolerant and salt sensitive wheat (*Triticum aestivum* L.) cultivars. J. Agric. Sci. Tech., 11:535-547.
41. Kavi Kishor, P. B., S. Sangam, R. N. Amrutha, P. Sri Laxmi, K. R. Naidu, K. R. S. S. Rao, S. Rao, K. J. Reddy, P. Theriappan and N. Sreenivasulu (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr.Sci., 88 (3): 424-438.
42. Khan, A.S. and F.Faiz (2008). Amino acid analysis using ion exchange resins. Coden Jnsmac, 48(1&2): 1-17.
43. Kovacs, Z., L. Simon-Sarkadi, I. Vashegyi and G. Kocsy (2012). Different accumulation of free amino acids during short and long term osmotic stress in wheat. The Sci. World J., 1-10.
44. Mansour, M. M. F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant., 43(4):491-500.

45. Mauro, S., F. Van Eycken, N. Challou, P. Lucas and M. L'Oiseau (2005). Characterization of new maize chloroplastic copper/zinc superoxide dismutase isoforms by high resolution native two-dimensional polyacrylamide gel electrophoresis. Identification of chilling responsive chloroplastic superoxide dismutase isoforms. Physiol. Plant., 124: 323-335.
46. Metwali, E. M. R. (2012). Molecular studies on some barley genotypes in relation to salt stress tolerance. Life Sci. J., 9(1) :111-123.
47. Metwali, E. M. R., M. H. Eid and T. Y. Bayoumi (2011). Agronomical traits and biochemical genetics markers associated with salt tolerance in wheat cultivars (*Triticum aestivum* L). Aust. J. Basic Appl. Sci., 5(5): 174-183.
48. Miflin, B. J. and P. J. Lea (1977). Amino acid metabolism. Annu. Rev. Plant Physiol., 28:299-329.
49. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7(9): 405-410.
50. Mittova, V., M. Guy, M. Tal and M. Volokita (2004). Salinity up regulated the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J. Exp. Bot., 55: 1105-1113.
51. Moghaieb, R. E. A., N. B. Talaat, A. A. Abdel-Hadi, S. S. Youssef and A. M. El-Sharkawy (2010). Genetic variation for salt tolerance in some bread and pasta wheat genotypes. Arab J. Biotech., 13(1): 125-142.
52. Moud, A. M. and K. Maghsoudi (2008). Salt stress effects on respiration and growth of germinated seeds of different wheat (*Triticum aestivum* L.) cultivars. World J. Agric. Sci., 4(3):351-358.
53. Noctor, G. and C. H. Foyer (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49:249-279.
54. Owen, A. G. and D. L. Jones (2001). Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. Biochem., 33: 651-657.
55. Pellet, P. L. and V. R. Young (1980). Nutritional evaluation of protein foods. Published by the United Nation University.
56. Radc, S. and B. Pevalek-kozlina (2010). Differential esterase activity in leaves and roots of *Centaurea ragusina* L. as a consequence of salinity. Periodicum Biologorum, 112(3): 253–258.
57. Rai, V.K. (2002). Role of amino acids in plant responses to stresses. Biol. Plant., 45(4): 481-487.
58. Ranieri, A., R. Bernardi, P. Lanese and G. F. Soldatini (1989). Changes in free amino acid content and protein pattern of maize seedlings under water stress. Environ. Exp.. Bot., 29(3):351-357.
59. Roy-Macauley, H., Y. Zuily-Fodil, M. Kidric, A. T. P. Thi and J. V. Silva (1992). Effect of drought stress on proteolytic activities in Phaseolus and Vigna leaves from sensitive and resistant plants. Physiol. Plant., 85(1):90-96.
60. Sairam, R. K. and G. C. Srivastava (2002).Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci., 162(6) :897-904.
61. Sairam, R. K., G. C. Srivastava, S. Agarwal and R. C. Meena (2005). Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biol. Plant., 49(1): 85-91.
62. Simon-Sarkadi, L., G. Kocsy and Z. Sebestyén (2002). Effect of salt stress on free amino acid and polyamine content in cereals. Acta Biol. Szeged., 46(3-4):73-75.
63. Stegemann, H., W. Burgermeister, H. Francksen and E. Krogerrecklenfort (1983): Manual of gel electrophoresis and isoelectric focusing with the apparatus PANTA-PHOR. Inst. Biochem., Messeweg 11, D-3300 Braunschweig, West-Germany.
64. Torres, M.A. (2010). ROS in biotic interactions. Physiol. Plant. 138:414-429.
65. Vaidyanathan, H., P. Sivakumar, R. Chakrabarsty and G. Thomas (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. Plant Sci., 165: 1411-141.
66. Weydert, C. J. and J. J. Cullen (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured and tissue. Nat. Protoc., 5(1): 51–66.
67. Willekens, H., D. Inze, M. Van Montagu and W. Van Camp (1995). Catalase in plants. Mol. Breeding, 1: 207– 228.