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Relationship between efficiency of antioxidants in wheat and tolerance to NaCl

Mamdouh M Nemat Alla*, Gaber M Abogadallah, Enas G Badran

Botany Department, Faculty of Science, Damietta University, Damietta, Egypt

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* Corresponding author: (Tel: +20.1069296239; Fax: +20.57.2403868, email: mamnematalla@du.edu.eg)

Abstract

Application of NaCl at 75, 150 and 225 mM to 9-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) for 15 days significantly decreased fresh and dry weights of only Sakha93. Growth parameters were most likely similar in both cultivars grown under normal conditions. Lipid peroxides and H_2O_2 were greatly accumulated in both cultivars particularly in Sakha93; significant increases were only detected in Misr1 treated with 225 mM. On the contrary, 150 and 225 mM NaCl led to great diminution in reducing power of Sakha93; however, there was no significant change in Misr1. In contrast to reducing power the phenolic contents were highly elevated in Sakha93 by NaCl at all concentrations but the effect of NaCl in Misr1 seemed to be nonsignificant. Also, all concentrations of NaCl decreased both protein content GSH in Sakha93 while the decrease in Misr1 was restricted to 225 mM. On the other hand, the activities of catalase (CAT), peroxidase (POD), glutathione-S-transferase (GST) and glutathione reductase (GR) were increased in Misr1 by 75 and 150 mM NaCl but inhibited in Sakha93 by all concentrations. The results of growth parameters suggest that Misr1 is a more tolerant cultivar to NaCl than Sakha93. The concomitant accumulation of MDA and H_2O_2 in Sakha93 and in the meantime the decrease in GSH reveal that ROS scavenging is less efficient in the more susceptible than the tolerant cultivar. Consequently, the cultivar Misr1 seemed to tolerate NaCl may be due to the more efficient induction of potential antioxidants to cope with salinity conditions.

Keywords: enzymatic antioxidants; non-enzymatic antioxidants; salinity; tolerance; wheat

Introduction

Salt stress is one of the major abiotic stresses that affect biochemical and physiological processes in plants, causing growth inhibition and yield loss [1]. NaCl is the most important constituent of a saline environment. An increase in salinity stress induces both a reduction in the percentage of seeds germinating and a delay in the initiation of the germination process, but also can cause complete inhibition of the germination process at salinities beyond the tolerance limits of the species. The negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants. The outcome of these effects may cause membrane damage, nutrient

imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction which ultimately lead to plant death [2]. Plants respond and adapt to stress by complex molecular responses. Salinity generates reactive oxygen species (ROS) in plants. ROS typically result from the excitation of O_2 to form $^1O_2$ or from the transfer of one, two or three electrons to O_2 to form, respectively, a superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO^-) [3-5]. ROS are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids [6-8]. Production of ROS is increased under saline conditions [9] and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in different crop plants [8,10,11]. It was expected that up-regulation of antioxidant system protect plants against NaCl-induced oxidative damage. Salinity treatments caused significant increase in H_2O_2 and lipid peroxidation in wheat seedlings, which were higher in salt-sensitive cultivar than salt tolerant cultivar [12]. Increased lipid peroxidation and levels of H_2O_2 was observed with increased salinity in *B. napus* and *T. aestivum* [13]. These detrimental effects of the overproduction of ROS could limit the plant tolerance to stresses [14]. However, plants have developed a battery of complex defense system to detoxificate and eliminate the generated ROS [3-5,15]. Such system includes nonenzymatic antioxidants as glutathione and enzymatic antioxidants as glutathione reductase (GR), glutathione-S-transferase (GST), catalase (CAT) and peroxidase (POD) [3,16-18]. Therefore, this work aims at studying the potentialities of antioxidants in two cultivars of wheat varied in tolerance to salinity in order to elucidate the relationship of antioxidant efficiency with plant tolerance to salinity.

Materials and methods

Plant materials and growth conditions

The grains of two wheat cultivars (*Triticum aestivum* Misr1 and Sakha93) differentially tolerate NaCl were surface sterilized by immersing in 3% sodium hypochlorite solution for 10 min and thoroughly washed. The grains were soaked for 8 h, germinated in perlite in 7 cm-diameter pots and watered with tap water. On the 7th day, seedlings were thinned to only one per pot. The pots were placed in 40 × 60 cm trays containing 100% long Ashton nutrient solution for 2 days and then divided into 4 sets for NaCl treatments (0, 75, 150 and 225 mM). Each set was represented by 10 replications. The seedlings were distributed randomly into trays and kept for the following 15 days under controlled conditions (25/10 °C day/night regime, 60% RH, 10 h photoperiod and 300 µmol m^{-2} s^{-1} photosynthetic photon flux density). The nutrient solution was consistently made up to the appropriate volume daily and renewed every 4 days. At harvest, the plants were carefully removed from the perlite, washed thoroughly with the relevant nutrient solution, dipped into deionized water and plotted dry. Shoot system was separated and used for determination of fresh and dry weights. Other samples were frozen in liquid N_2 for the subsequent analyses.

Determination of lipid peroxides and H_2O_2 contents

Lipid peroxides were extracted in 150 mM KCl. After centrifugation at 7000 ×g for 15 min, 1 ml of the supernatant was incubated at 37 °C for 2 h with 1 ml of 0.6 M trichloroacetic acid (TCA). One ml of supernatant was taken with 1 ml of thiobarbituric acid and placed in a boiling water bath for 10 min, cooled and diluted with 1 ml distilled water. The absorbance was read at 535 nm [19]. H_2O_2 was extracted in 200 mM perchloric acid and centrifuged at 5000 ×g for 10 min. The assay mixture contained 0.4 ml 12.5 mM 3-dimethylaminobenzoic acid in 375 mM phosphate buffer pH 6.5, 0.08 ml 1.3 mM 3-methyl-2-benzothiazolinone hydrazone and 0.02 ml (0.25 units) horseradish peroxidase. The increase in absorbance at 590 nm was monitored for 3 min [20].

Determination of protein and glutathione (GSH) contents

About 200 mg of frozen leaves from 5 seedlings were homogenized in 80 mM Tris-HCl, pH 7.4. After centrifugation at 14000 xg for 5 min, the extracted protein was precipitated over night at 4ºC by adding 10% chilled trichloroacetic acid in acetone (w/v). Protein pellets were separated by centrifugation at 12000 xg for 15 min and reconstituted in the buffer. Protein was determined using Commassie Brilliant Blue G-250 at 595 nm [21]. GSH was extracted in TCA (5%, w/v) containing 5 mM EDTA and

centrifuged at 12,000 ×g for 15 min [22]. GSH was assayed in 100 mM phosphate buffer, pH 6.8 containing 5 mM EDTA and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was started by adding 1.0 U equine Glutathione-S-transferase (GST) and incubated at 35 °C for 30 minutes. The absorbance was recorded at 340 nm.

Determination of reducing power and phenolic contents

The Fe^{3+} reducing power of the extract was determined according to the method of Oyaizu [23]. The extract (2 ml) was mixed with 0.2 M phosphate buffer, pH 6.6 (2 ml) and 1% potassium ferricyanide (2 ml) and incubated at 50°C for 20 min. The the reaction was stopped by 10% trichloroacetic acid (2 ml) and then centrifuged at 10000 xg for 10 min. The upper layer of supernatant (2 ml) was mixed with distilled water (2 ml) and 0.1% $FeCl_3$ solution (0.5 ml) and the absorbance was measured at 700 nm. Phenolic extraction was performed in methanol at 37°C. The extract was collected and concentrated by evaporation. Total phenolic contents were assessed by using the Folin-Ciocalteu phenol reagent. To 1 ml of the extract, 0.5 ml of 10% Folin-Ciocalteu reagent and 0.5% sodium carbonate were added, thoroughly mixed and allowed to stand for 30 min and absorption at 750 nm was measured. The total phenolic contents were expressed as gallic acid equivalents.

Measurement of antioxidant enzyme activities

The enzyme were extracted in 50 mM sodium phosphate buffer (pH 6.9) containing 2 mM EDTA and 5 mM β mercaptoethanol and centrifuged at 12,000 ×g for 10 min at 4 °C. Glutathione reductase (GR) was assayed in reaction mixture contained 100 mM phosphate, pH 7.5, 0.5 mM EDTA, 0.75 mM 5,5-dithiobis-(2-nitrobenzoic acid), 0.1 mM NADPH and 1 mM oxidized glutathione (GSSG) [24]. The reaction mixture was incubated at 35 °C meanwhile absorbance at 412 nm is measured up to 5 min. Glutathione-S-transferase GST was extracted in 100 Mm Tris-HCL (pH 7.5) containing 2 mM EDTA, 14 mM β-mercaptoethanol and 7.5% (w/v) polyvinylpolyprollidone then centrifuged at 15,000 ×g for 15 min and ammonium sulfate was added to 80% saturation [25]. GST was assayed in 100 mM phosphate buffer (pH 6.5) containing 5 mM GSH and 1 mM CDNB. After incubation for 1 h at 35 °C, 3 ml of 0.33 N HCl were added to stop the reaction and absorbance was measured at 340 nm. GST activity was assayed using the extinction coefficient $E = 9.6\ mM^{-1}\ cm^{-1}$. Catalase (CAT) activity was measured by following the consumption of H_2O_2 at 240 nm in 50 mM sodium phosphate buffer (pH 7.0) containing 5 mM H_2O_2 [26]. The absorbance was read at zero time and after 1 min. Peroxidase (POD) activity was measured in 50 mM sodium phosphate buffer (pH 6.9) containing 3.2 mM guaiacol and 0.4 mM H_2O_2 [27]. The absorbance was measured at 470 nm.

Statistical analysis

All values are means (± SD) of at least six determinations from two independent experiments. The full data were first subjected to analysis of variance (ANOVA) followed thereafter by least significant differences (LSD) at 5% level.

Results

In Fig. 1, treatment with NaCl resulted in significant decrease in fresh weight of Misr1 cultivar only by 225 mM (about 32%). In Sakha93, all concentrations induced significant decreases; NaCl at 75, 150 and 225 mM caused a reduction in fresh weight by about 42, 71 and 85%, respectively. Also, dry weight was greatly affected by salinity; however, 75 mM NaCl seemed not significant in Misr1 while 150 and 225 mM led to significant decrease by about 21 and 28%, respectively. On the other hand, all concentrations of NaCl (75, 150 and 225 mM) induced significant decreases in Sakha93 by about 53, 76 and 79%, respectively.

Treatment with NaCl at all concentrations resulted in great accumulations of lipid peroxides in both cultivars (Fig. 2). The accumulation of lipid peroxides reached in Misr1 about 55, 91 and 180% of control values following treatment with 75, 150 and 225 mM NaCl, respectively and in Sakha93 about 109, 227 and 235%, respectively. Meanwhile, H_2O_2 content was highly increased in both cultivars, following NaCl treatment, although only 75 mM resulted in non significant effect in Misr1 but 150 and 225 mM induced increases of about 33 and 59%. Whilst all concentrations were of significant effect in

Sakha93; 75, 150 and 225 mM led to an accumulation of about 97, 103 and 106%, respectively.

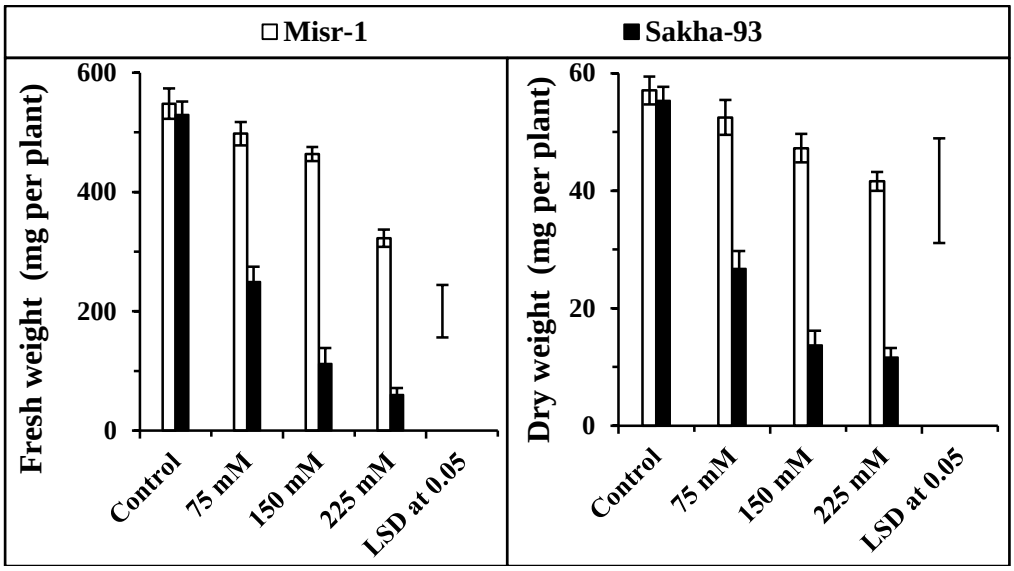

Fig 1. Changes in shoot fresh and dry weight of nine-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) after 15 days from treatment with 75, 150 and 225 mM NaCl. Data are means (±SD) of at least six replications from two independent experiments. Vertical bars represent LSD at 5% level.

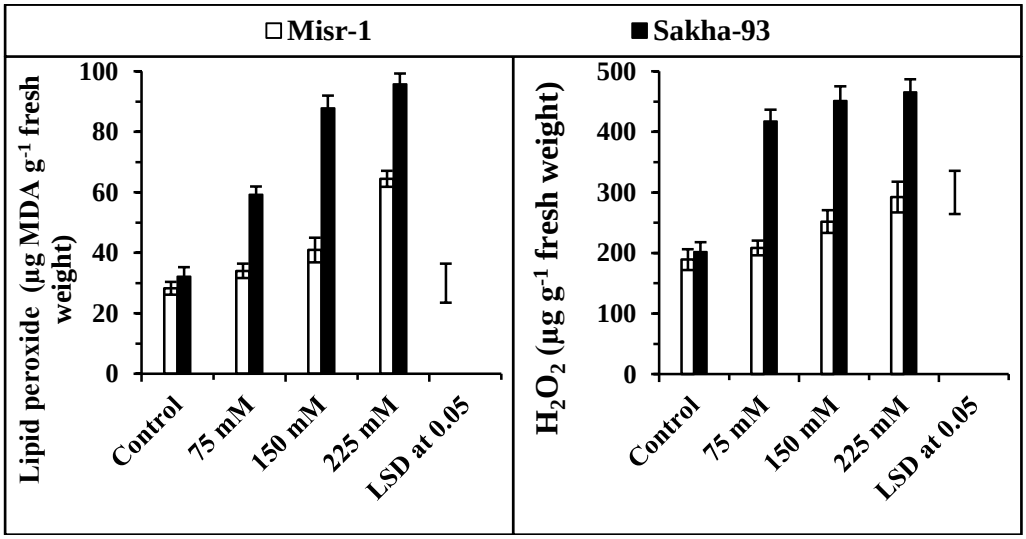

Fig 2. Changes in lipid peroxides as MDA and H_2O_2 of nine-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) after 15 days from treatment with 75, 150 and 225 mM NaCl. Data are means (±SD) of at least six replications from two independent experiments. Vertical bars represent LSD at 5% level.

The results depicted in Fig. 3 represent that reducing power was higher in Sakha93 than in Misr1 by about 11%. However, treatment with NaCl resulted in significant decrease in reducing power in seedlings of both cultivars; the decrease was more pronounced in Sakha93 than in Misr1. Nonetheless, only 225 mM NaCl was of significant effect in Misr1 inducing an inhibition of about 33%. On the contrary, all NaCl concentration resulted in significant inhibition in Sakha93, 75, 150 and 225 mM caused a decrease in reducing power by about 31, 50 and 78%, respectively of the control values. In addition, phenolics content exhibited higher values in Misr1 than in Sakha93 by about 14% (Fig. 3). In this account, phenolics content was further increased by NaCl treatment, the magnitude of increase was greater in Sakha93 than in Misr1.

Only 225 mM NaCl seemed to significantly increase phenolics content in Misr1 by about 47%. On the contrary, all NaCl concentrations induced significant increases in Sakha93, the content of phenolics increased following treatment with NaCl at 75, 150 and 225 mM by about 69, 74 and 102%, respectively of the control values.

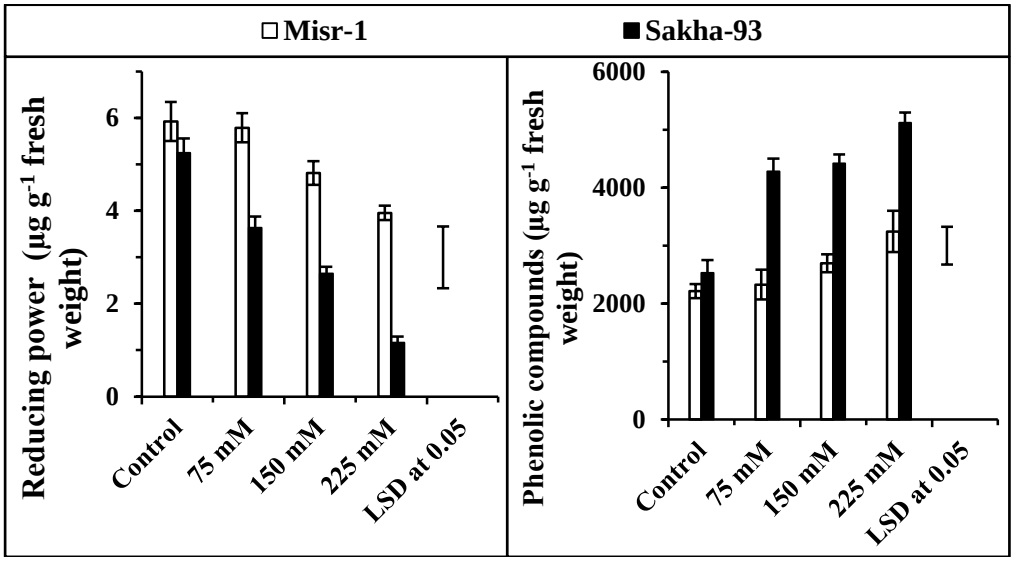

Fig 3. Changes in reducing power and phenolic content of nine-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) after 15 days from treatment with 75, 150 and 225 mM NaCl. Data are means (±SD) of at least six replications from two independent experiments. Vertical bars represent LSD at 5% level.

In Fig. 4, Protein content was only 5% higher value in Misr1 cultivar than in Sakha93 under control conditions. Treatment with 75 mM NaCl seemed with no significant effect upon Protein content of Misr1 seedlings while the other concentrations induced significant reductions as compared to the control values. On the contrary, all concentrations of NaCl resulted in significant reductions Protein content of Sakha93 as compared to the control values. The magnitude of reduction due NaCl treatment was higher in Sakha93 than in Misr1 seedlings and augmented with increasing concentrations. The decrease in Protein content in Misr1 reached about 5, 16 and 28% of control values following treatment with 75, 150 and 225 mM NaCl, respectively whereas these decreases were greater in Sakha93 and reached about 49, 69 and 72%, respectively. The cultivar Misr1 contained higher GSH content than Sakha93 by about 6% under control conditions (Fig. 4). Nonetheless, decreases were detected in these contents following NaCl treatment particularly in Sakha93, the magnitude of decrease augmented with increasing NaCl concentrations. However, GSH content seemed non significantly changed in Misr1. The magnitude of decrease of GSH content decreased in Sakha93 by about 44, 63 and 77%, respectively of the control values following treatment with NaCl at 75, 150 and 225 mM, respectively.

In Fig. 5, there was a marked difference in GR activity among both cultivars normally-grown under control conditions; the activity was higher by 9% in Misr1 than in Sakha93. The treatment with NaCl at all concentrations resulted in non significant effect in GR activity in Misr1 seedlings; however, all concentrations significantly inhibited the enzyme activity in Sakha93. NaCl at 75, 150 and 225 mM caused a significant inhibition in GR activity of Sakha93 seedlings by about 57, 64 and 67%, of the control value respectively. The activity of GST was higher in Misr1 than in Sakha93 by only about 7% (Fig. 5).

Nonetheless, NaCl at all concentrations seemed with no significant effect upon GST activity in only Misr1 cultivar; however, the activity was significantly inhibited in Sakha93. The activity of GST decreased following treatment with NaCl at 75, 150 and 225 mM by about 44, 63 and 77%, respectively of the control values in Sakha93. The activity of CAT was most likely similar in magnitude in both cultivars. Treatment with NaCl seemed with no significant effect upon GST activity in only Misr1 cultivar, however, the enzyme activity was significantly inhibited in Sakha93 by all concentrations. The

inhibition in CAT activity reached about 64, 66 and 67% of control values in Sakha93 following treatment with 75, 150 and 225 mM NaCl, respectively. There was a higher activity of POD in Misr1 than in Sakha93 by only about 8% (Fig. 5). Nonetheless, the POD activity in Misr1 cultivar seemed to be non-significantly affected by NaCl at all concentrations but exhibited significant inhibition in Sakha93. The activity of POD in Sakha93 was inhibited following treatment with NaCl at 75, 150 and 225 mM by about 58, 62 and 64%, respectively of the control values.

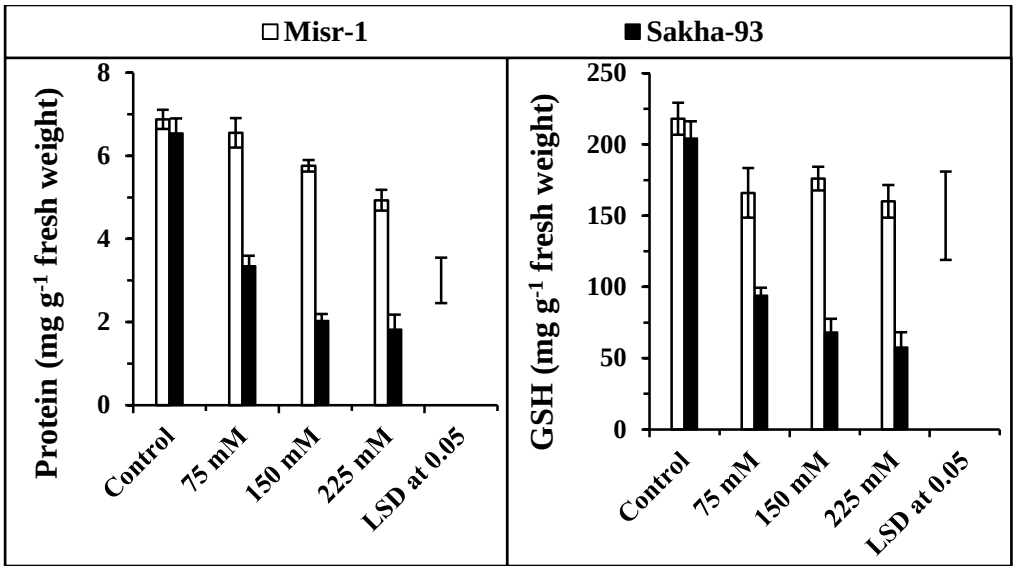

Fig 4. Changes in protein and GSH content of nine-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) after 15 days from treatment with 75, 150 and 225 mM NaCl. Data are means (±SD) of at least six replications from two independent experiments. Vertical bars represent LSD at 5% level.

Discussion

The general response of plants to salinity is reduction in growth [28]. In spite of having most likely similar values of growth parameters under normal conditions in the present study, the growth of the wheat cultivar Sakha93 was more inhibited than Misr1. These findings could conclude that Sakha93 might be considered as a more sensitive cultivar to NaCl than Misr1. This varied sensitivity to NaCl could be related to deteriorations in various processes. Parida and Das [29] indicated that salt stress induces various biochemical and physiological responses in plants and affects almost all plant metabolic processes. Salinity causes alterations in the integrity of cell membranes [30] and inhibition of different enzymatic activities [31]. Moreover, one of the most effects of salinity stress is the accumulation of ROS that would react with lipids, proteins and pigments causing lipid peroxidation and membrane damage [3,5,32]. ROS initiate a variety of autooxidative chain reactions on membrane unsaturated fatty acids, producing lipid hydroperoxides and thereby cascade of reactions ultimately leading to destruction of organelles and macromolecules [16].

In the present results, the accumulation of lipid peroxides (as MDA) in both wheat cultivars could point to an occurrence of an oxidative stress status induced due to salinity treatment. However, MDA was more accumulated by NaCl in Sakha93 than in Misr1 concluding that the oxidative stress is related to the sensitivity to salinity. Therefore, a relationship could be established between the deleterious effects of NaCl and the sensitivity of plants to salinity. Yasar *et al*. [33] reported that NaCl treatments led to a gradual increase in the levels of MDA in green bean and its accumulation was higher in the sensitive cultivar than tolerant one. Also NaCl caused greater accumulation of H_2O_2 in the more sensitive cultivar than in the less sensitive one. In confirmation, the reducing power was greatly affected by NaCl more in Sakha93 than in Misr1. Moreover, salinity highly induced induction in phenolic compounds Sakha93 concluding that this cultivar is more sensitive to NaCl relative to Misr1. Similar effects were also

detected regarding protein content revealing that salinity has a negative effect on the structural or functional protein of Sakha93.

To cope with oxidative stress, plants develop an antioxidant system which plays an important role in the defense mechanism against stress. The correlation between the resistance to environmental stresses and the efficiency of the antioxidant system has been established [33]. Plants are endowed with an array of non-enzymatic antioxidants such as GSH and enzymatic antioxidants such as GR, GST, CAT and POD for removal of ROS [3,5,16]. The present results indicate greater effects of NaCl on GSH of Sakha93 than Misr1 pointing out that antioxidants are less efficient in the less tolerant cultivar than in the more tolerant one.

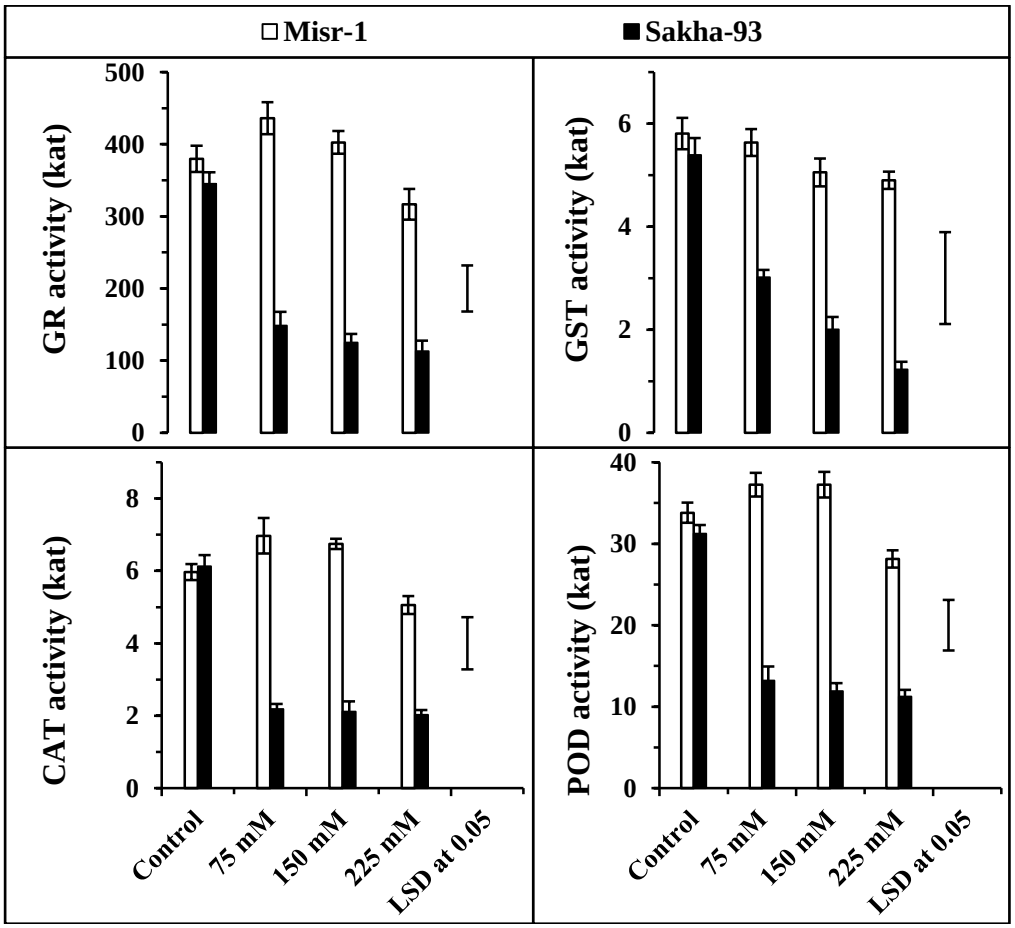

Fig 5. Changes in activities of glutathione reductase (GR), glutathione-S-transferase (GST), catalase (CAT) and peroxidase (POD) of nine-d-old seedlings of two wheat cultivars (Misr1 and Sakha93) after 15 days from treatment with 75, 150 and 225 mM NaCl. Data are means (±SD) of at least six replications from two independent experiments. Vertical bars represent LSD at 5% level.

GSH is the most abundant low molecular weight non-enzymatic antioxidants in plant cells participating in ROS scavenging through the AsA-GSH cycle [4]. Nonetheless, during ROS scavenging, GSH is oxidized to GSSG while GR maintains glutathione in its reduced form. GST activates the protection of plants from some xenobiotics and ROS [3]. Therefore, the decrease in GSH content and in activities of GST and GR in Sakha93 reveals that the scavenging of ROS is less efficient in the less tolerant cultivar suggesting that there is a severe state of stress imposed by NaCl occurs particularly in Sakha93, the more sensitive cultivar.

In conclusion, both cultivars, Misr1 and Sakha93, responded differentially to NaCl in spite of having similar values of growth parameters. The results concluded that Sakha93 is more sensitive to NaCl treatment than Misr1. Greater was the induction of lipid peroxides and H_2O_2 by NaCl in Sakha93 than in Misr1. Similar increases were also detected regarding phenolic compounds. On the contrary, proteins as well as antioxidants either enzymatically or non-

enzymatically were inhibited by salinity more in Sakha93 than in Misr1. So, Sakha93 could be concluded to suffer from NaCl treatment more than Misr1. These findings support that Sakha93 is more sensitive to NaCl than the other cultivar, Misr1 which can tolerate and combat salinity tolerance. The ability of the plant to combat environmental stress is determined by its efficiency to sense the stress and activate its defense machinery. These results confirm that there is a relationship between the potential antioxidants and NaCl stress tolerance in wheat.

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الملخص العربى

العلاقة بين كفاءة المواد المضادة للأكسدة في القمح وتحمله لكلوريد الصوديوم

ممدوح نعمة الله، جابر مختار أبو جاد الله، ايناس بدران
قسم النبات - كلية العلوم - جامعة دمياط - مصر

تم معاملة بادرات صنفين من القمح (مصر1 وسخا93) بتركيزات 75 و 150 و 225 مللى مولار من كلوريد الصوديوم لمدة 15 يوما. وجد أن كل التركيزات أدت إلى انخفاض كبير في الأوزان الرطبة والجافة فقط فى صنف سخا93، بينما بدا صنف مصر1 لا يتأثر كثيرا. وكانت دلالات النمو في البادرات النامية فى ظل الظروف الطبيعية متماثلة لكلا الصنفين. وتراكمت البيروكسيدات الدهنية وفوق أكسيد الهيدروجين في كلا الصنفين وكان التراكم أكثر وضوحا في صنف سخا93. أدت المعاملة بالتركيزين 150 و225 مللى مولار إلى الحد من القدرة الاختزالية فى صنف سخا93، وكانت المحتويات الفينولية مرتفعة للغاية بينما كان أن التغير طفيفا في صنف مصر1. وبدا أن كلا الصنفين تحت الظروف الطبيعية لا يختلفان فى القدرة الاختزالية أو المحتويات الفينولية. وانخفض البروتين والجلوتاثيون في صنف سخا93 في حين اقتصر الانخفاض في صنف مصر1 على المعاملة بتركيز 225 مللى مولار. ولم يكن هناك فرق كبير سواء في محتوى البروتين أو الجلوتاثيون بين بادرات كلا الصنفين النامية في ظل الظروف الطبيعية. وزادت أنشطة مضادات الأكسدة الإنزيمية في صنف مصر1 بفعل كلوريد الصوديوم عند التركيزات 75 و150 مللي مولار ولكنها ثبطت في صنف سخا93. وتشير النتائج إلى أن صنف مصر1 هو أكثر تحملا لكلوريد الصوديوم من صنف سخا93. وتؤكد عن كفاءة أقل في التخلص من مسببات الإجهاد التأكسدى فى الصنف الأقل تحملا للملوحة بينما تحمل صنف مصر1 لكلوريد الصوديوم قد يكون بسبب كفاءة أكثر لمضادات الأكسدة للتغلب على الإجهاد التأكسدى الناتج عن الملوحة.