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Original Article

Response of ROS-Scavenging Systems to Salinity Stress in Two Different Wheat (*Triticum aestivum* L.) Cultivars

Ezatollah ESFANDIARI[1]*, Gholamreza GOHARI[2]

[1]*University of Maragheh, Faculty of Agriculture, Department of Agronomy and Plant Breeding, Madar Square, Maragheh, Iran; esfand1977@yahoo.com (*corresponding author)*

[2]*University of Maragheh, Faculty of Agriculture, Department of Horticulture, Madar Square, Maragheh, Iran; gholamreza.gohari@gmail.com*

Abstract

Salinity leads to oxidative stress in plant cells due to increased production of reactive oxygen species. The response of two wheat (*Triticum aestivum* L.) cultivars, salt sensitive ('Darab2') and salt-tolerant ('Arta') were studied to salinity-induced oxidative stress (0, 75 and 150 mM NaCl). Increasing of lipid peroxidation caused oxidative stress in both sensitive and tolerant cultivars. The result showed that reactive oxygen species (ROS) viz., superoxide and hydrogen peroxide increased in leaves of 'Darab2' under salinity stress. Under salinity stress, the salt-tolerant cv. 'Arta' showed higher activity of the ROS scavenging enzymes like ascorbate peroxidase and peroxidases than 'Darab2'. Furthermore, in sensitive cv. 'Darab2' the activities of these enzymes in leaves were unable to prevent the scavenging of H_2O_2. Unlike 'Arta', there were no significant differences in superoxide dismutases and glutathione reductase activities in sensitive cv. 'Darab2' under salinity stress. The amount of reduced glutathione, reduced/oxidized glutathione ratio in leaves of 'Darab2' was lower than 'Arta' under saline conditions. It seems that in salt tolerant cultivars like 'Arta', both enzymatic and non-enzymatic ROS scavenging machineries is critical point to overcome salinity-induced oxidative stress.

Keywords: antioxidant enzymes, hydrogen peroxide, lipid peroxidation, reduced and oxidized glutathione, superoxide radical, wheat genotypes

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in Iran, which plays a special role in people's nutrition. Abiotic stresses, such as salinity, decrease wheat growth and yield by reducing water uptake and cause nutrient disorders and ion toxicity. Salinity same as water stress, leads to oxidative stress in plant cells due to increased production of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) (Yadava *et al*., 2013; AbdElgawad *et al*., 2016; Asadi Karam and Keramat, 2017). ROS's are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration. In the other word, chloroplasts, mitochondria and peroxisomes are the major sources of ROS production in plant cells (Sharma *et al*., 2012). ROS are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid etc., causing lipid peroxidation, protein denaturing and DNA mutation, respectively (Kaur and Zhawar, 2016). Recent evidence suggests the cell membranes are the primary effect sites in salinity stress (Bita and Gerats, 2013) because reaction of ROS and unsaturated fatty acids cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles (Bita and Gerats, 2013). Peroxidation of plasmalemma leads to the leakage of cellular contents, rapid desiccation and cell death. Intracellular membrane damage can affect respiratory activity in mitochondria, causing pigment to break down and leading to the loss of the carbon fixing ability in chloroplasts (Scandalios, 1993).

ROS generated by abiotic stresses such as salinity must be scavenged for security of essential macromolecules from destructive effects of ROS and maintenance of normal growth (Caverzan *et al*., 2016; Kaur and Zhawar, 2016). Superoxide dismutase (SOD: EC 1.15.1.1) is the primary scavenger, which localized in chloroplasts, mitochondria, peroxisomes and cytosol. SOD catalyses the disproportion of two $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 (Perry *et al*. 2010). H_2O_2 is detoxified to two molecules of water in glutathione-ascorbate cycle by ascorbate peroxidase (APX: EC 1.11.1.11) in cooperation with dehydroascorbate reductase (DHAR: EC 1.8.5.1) and glutathione reductase (GR: EC1.6.4.2), and regenerate the ascorbic acid (Shereefa and Kumaraswamy, 2016). Moreover, catalase is the other enzyme which converts two molecules of H_2O_2 to water and oxygen (CAT: EC 1.11.1.6). It has less impact than glutathione-ascorbate cycle in H_2O_2 detoxifying

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(Bhutta, 2011). Changes in activities of different antioxidant enzymes under salinity were reported in wheat (Sairam *et al*., 2005; Bhutta, 2011), potato (Rahnama and Ebrahimzadeh, 2005), tobacco (Çelik and Atak, 2012), pea (Hernandez *et al*., 2000), corn (Carrasco-Ríos and Pinto, 2014) and rice (Pushpalatha *et al*., 2013). These reports show that the induction of ROS-scavenging enzymes, such as SOD, POD and CAT, is the most general defense mechanism of salinity tolerance for detoxifying ROS generated.

As mentioned before, salinity has the most important role in decreasing wheat yield in west and north-west of Iran. Moreover, wheat has a significant role in food security in our country. These reasons indicate the importance of research about salinity tolerance mechanisms, especially ROS scavenging systems in wheat. In this research two wheat cultivars were selected ('Arta' and 'Darab2'), which were tolerant and sensitive to salinity, respectively, to evaluate the effect of salt stress on various antioxidant enzymes activity and their relation in terms of salinity stress tolerance in differentially tolerant and sensitive wheat genotypes under hydroponic culture conditions.

Materials and Methods

Plant material and salt treatments

Two salt sensitive and salt tolerant wheat cultivars ('Darab2' and 'Arta') seeds were selected among 52 cultivars of our previous study (Shokrpour and Esfandiari, 2014). Seedlings were grown hydroponically in specifically designed plastic trays. Two seeds were placed in each hole, and then put in other rectangular plastic trays containing 2.0 L of Hoagland's solution (Hoagland and Arnon, 1950). These solutions were continuously aerated by electrical pumps (Resun, AC 9904, China) and renewed each 7 days. The experiment was carried out in an one-layer polyethylene covered greenhouse under natural sunlight.

For evaluate of different salinity levels on two cultivar of wheat, 75 mM and 150 mM NaCl added to the solution was employed for the nourishment of related treatments. pH and EC of Hoagland's nutrient solutions were adjusted at 6-6.5 and 2 dSm^{-1} by HCl or NaOH and water respectively.

Samples were obtained from 1th mature and well-expanded leaf (2nd leaf from top) and soaked in liquid nitrogen immediately. The samples were preserved in -20 °C until the measuring of physiological parameters.

Enzyme extraction and assay

For SOD, CAT and GPX extraction, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4 °C for 15 min at 15000×g. The supernatant was used for enzyme activity assay (Esfandiari *et al*., 2015). SOD activity was determined according to Sen Gupta *et al*. (1993) by measuring the inhibition of NBT (nitroblue tetrazolium) reduction at 560 nm. One enzyme unit was defined as the amount of enzyme which could cause 50% inhibition of the photochemical reaction. CAT and POX activities were assayed as described by Aebi (1984) and Panda *et al*. (2003), respectively. Method of Yoshimura *et al*. (2000) was employed to assay APX. GR activity was assayed according to the Sairam *et al*. (2005) by recording the formation of 2-nitro-5-thiobenzoic acid formation in the presence of oxidized glutathione (GSSG) and 5,5-dithio-bis-2-nitrobenzoic acid (DTNB).GSH and GSSG content were determined according to Griffith (1980). Superoxide radical content and hydrogen peroxide levels were determined according to Elstner *et al*. (1975) and Sergiev *et al*. (1997), respectively. Moreover, MDA was determined by Stewart and Bewley (1980).

Statistical analysis

All physiological and biochemical parameters were recorded with five replications. The data were analyzed by MSTATC software in the complete randomized design (CRD). Mean comparison were carried out by LSD method. Standard error of mean was also calculated for displaying in figures.

Results and Discussion

The occurrence of oxidative stress can be assessed by the amount of superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) generation under salinity conditions. Our results indicated that, production of $O_2^{\cdot-}$ and H_2O_2 in leaves of control seedlings of 'Darab2' (sensitive cultivar) (211% and 140% respectively) was more than 'Arta' (tolerant cultivar) (Table 1). When salinity level increased to 75 mM in leaves of 'Darab2', the production of $O_2^{\cdot-}$ increased considerably, but H_2O_2 content remained unchanged. Enhancing salinity up to 150 mM, increased production of ROS ($O_2^{\cdot-}$ and H_2O_2) and reached to highest level in 'Darab2' (Table 1). Unlike 'Darab2', salinity did not affected on the amount of $O_2^{\cdot-}$ and H_2O_2 in 'Arta' (Table 1). The amount of $O_2^{\cdot-}$ and H_2O_2 in leaves of 'Arta' at 150 mM NaCl were less than 'Darab2' at control level. In other words, the content of $O_2^{\cdot-}$ and H_2O_2 in 'Darab2', was about 4 and 2 times more than 'Arta' in 150 mM NaCl, respectively (Table 1). According these results increasing of $O_2^{\cdot-}$ under salinity stress has been reported by Jiang and Zhang (2001), Li *et al*. (2004) and Kukreja *et al*. (2006) in corn, wheat and pea, respectively. Moreover, Bhutta (2011) and Esfandiari and Javadi (2014) have been reported that salinity stress increased of H_2O_2 level in wheat. It seems that the significant increase of ROS ($O_2^{\cdot-}$ and H_2O_2) under saline conditions in 'Darab2' may play an important role in inducing oxidative and membrane permeability by attacking membrane lipids in this variety.

The present results indicate that salinity caused more severe oxidative stress in salt-sensitive cultivar in compared to salt-tolerant ones, as showed by generation of ROS and MDA content (Table 1). Moreover, MDA content increased in both 'Arta' and 'Darab2' cultivars but in 'Arta' there was no significant difference in MDA content between all treatments (Table 1). Sairam *et al*. (2005), Esfandiari and Javadi (2014), Rahmani and Padervand (2016) and Kaur and Zhawar (2016) reported that MDA content increased in salt tolerant and sensitive wheat cultivars. Although, these researchers observed that the severity of damage in cell membranes or MDA content was more in salt sensitive cultivars as compared to salt tolerant ones.

SOD activity remained unchanged with increase in salinity in 'Darab2', but its increased in 'Arta' and showed the highest activity in 150 mM NaCl (Fig. 1A). In control, the activity of SOD was 504.9 and 198.2 units g^{-1} FW in 'Arta' and 'Darab2' respectively, which indicated that the basal level of SOD activity in 'Arta' was also significantly higher than 'Darab2' (Fig. 1A). Our results are in agreement with those of Kumar *et*

Table 1. Effect of salinity on superoxide radical and peroxide hydrogen content in leaves of tolerant ('Arta') and sensitive ('Darab2') cultivars of wheat

Variant	Superoxide radical content (nmoles g^{-1} FW)		Hydrogen peroxide content (mmoles g^{-1} FW)		MDA content (nmoles g^{-1} FW)	
	'Arta'	'Darab2'	'Arta'	'Darab2'	'Arta'	'Darab2'
Control	88.7±3.85e	118±16.86d	294.3±18.41c	409.3±2.99b	17.93±2.99d	37.90±1.05c
75 mM NaCl	105d±3.60e	260±10.44b	302.4±30.07c	418.2±7.42b	19.50±2.56d	62.29±1.24b
150 mM NaCl	164.2±13.0c	310.4±19.0a	310.9±21.75c	597.2±7.23a	21.23±2.01d	83.68±1.59a

al. (2006) in *Brassica juncea* and Carrasco-Ríos and Pinto (2014) in corn who also observed that basal activity of SOD was higher in salt-tolerant cultivars compared to that sensitive ones. Decreasing in SOD activity in salt-sensitive cultivar and increment in salt-tolerant cultivar of wheat reported by Mandhania *et al*. (2006) which are according with our results. While, Bhutta (2011) have been reported that SOD activity increased in both salt sensitive and tolerant cultivars of wheat under salinity stress. It seems that the increase of SOD activity in leave of 'Arta' lead to reduced cell damage and low oxidative stress. Unlike to 'Arta', in the 'Darab2', unchanged in SOD activity may reduced the metabolic capacity of the plant, which will lead to further cell damages and cause to oxidative stress. It seems that in salt sensitive cultivar, the increasing of ROS accumulation by salinity cause to decrease of SOD activity, it could be restrict the capacity of scavenging $O_2^{\cdot-}$ which resulting in accumulation of ROS and ultimately led to cell membrane damage in seedling.

According to Fig. 1B the activity of CAT in the leaves of 'Arta' (8.4 units g^{-1} FW) was higher than 'Darab2' (4.55 units g^{-1} FW) under non-salt stressed condition. There was no significant difference in CAT activity in both sensitive and tolerant cultivars at both levels of salinity in comparison to non-stressed condition. In non-stressed condition in 'Arta' the basal level activity of POX was higher than 'Darab2'. Furthermore POX activity was enhanced in both sensitive and tolerant cultivars in 75 mM NaCl (Fig. 1C). Increasing the salinity levels to 150 mM, POX activity remained unchanged in 'Darab2' but increased in 'Arta'. There were some reports that POX activity has been shown to increase in both sensitive and tolerant cultivars of wheat (Manndhania *et al*., 2006; Bhutta, 2011) and rice (Kumar *et al*., 2006). Although Esfandiari *et al*. (2015) have been reported that POX activity increased only in tolerant cultivars and decreased in sensitive cultivars of wheat under salinity stress.

Similar to other antioxidative enzymes, APX activity in the leaves of 'Arta' was higher than 'Darab2' under non-stressed condition and its activity enhanced by increasing salinity to 75 mM NaCl in both cultivars (Fig. 1D). With increase of salinity levels to 150 mM, APX activity remained unchanged in 'Arta' and increased in 'Darab2'. Benavides *et al*. (2000) reported that the level of APX activity in salt tolerant potato clones more than sensitive clone, however in present research the activity of this enzyme in 'Arta' was more than 'Darab2' about 2.5 times. Furthermore the activity of POX and APX enhanced in both cultivars under salinity stress but it was more evidence in 'Darab2', on the other hand in 'Arta' the basal levels activity of theses enzymes were multifold higher than 'Darab2', which may have result in faster depletion of H_2O_2 in 'Arta'. Rahnema and Ebrahimzadeh (2005) reported that APX activity increased in salt-sensitive variety of potato under salinity stress. Esfandiari *et al*. (20115) reported that the increasing of APX in salt tolerant cultivars of wheat was more than sensitive cultivars. Amor *et al*. (2007) and Esfandiari and Javadi (2014) suggested that, APX activity by detoxifying H_2O_2 which generated in upon exposure of plants to saline conditions, lead to increase of plant resistance to abiotic stress.

GR Activity declined in 'Darab2' (approximately 49% and 42% in 75 mM and 150 mM NaCl, respectively) by salinity stress. But the activity of this enzyme was increased in salinity and the highest activity was observed in 150 mM in 'Arta' (Fig. 1E). There were similar results in different plant such as pea (Hernandez *et al*., 2000) and green bean (Yaser *et al*., 2008) in which salt tolerance was corrected with elevated GR activity. But Çelik and Atak (2012) showed that GR activity increased in both salt-sensitive and salt-tolerant cultivars of tobacco.

With imposition of salinity treatment, activities of SOD and GR increased in leaves of 'Arta', whereas in 'Darab2', these enzymes activities remained unchanged and decreased, respectively. Although, POX and APX activities enhanced in both cultivars though CAT activity remained unchanged under salinity stress in 'Arta' and 'Darab2'. Furthermore, in 'Arta' the basal level of APX, POX and CAT were higher than 'Darab2' (Fig.1 A-E).

Glutathione is a low molecular weight antioxidant, which is a potent regulator of major cell functions. Glutathione can react directly with ROS such as $O_2^{\cdot-}$ and H_2O_2 and prevent inactivation of enzymes due to oxidation of essential thiol groups (Griffith, 1980). In 'Arta', the analysis of GSH content in leaves, showed a linear increase in GSH with increasing salinity level approximately 2 and 4 folds in 75 mM and 150 mM NaCl, respectively (Table 2). However GSH content remained unchanged in 'Darab2' at both levels of salt stress (Table 2). In 'Arta', GSSG content in leaves declined linearly but its increased 'Darab2' with the increase in salinity level (Table 2). Unlike 'Arta', the ratio of GSH/GSSG decreased in 'Darab2' under salinity stress. It seems that sensitivity to salt stress could be due to reduced antioxidative capacity in detoxification of ROS. Our results are in agreement with those of Mittova *et al*. (2003), Vaidyanathan *et al*. (2003) and Meneguzzo *et al*. (1999) who also observed that GSH content and GSH/GSSG ratio significantly elevated in tolerant cultivars as compared to sensitive cultivars of tomato, rice and wheat respectively under salinity stress.

Table 2. Effect of salinity on reduced glutathione and oxidized glutathione content in leaves of tolerant ('Arta') and sensitive ('Darab2') cultivars of wheat.

Variant	Reduced glutathione (GSH) (nmoles g^{-1} FW)		Oxidized glutathione (GSSG) (nmoles g^{-1} FW)	
	'Arta'	'Darab2'	'Arta'	'Darab2'
Control	2.62±0.17c	1.06±0.15c	2.18±0.21b	1.50±0.12d
75 mM NaCl	5.67±0.44b	2.77±0.13c	1.92b±0.13c	2.63±0.17a
150 mM NaCl	10.54±0.56a	2.66±0.16c	1.75±0.11cd	2.79±0.13a

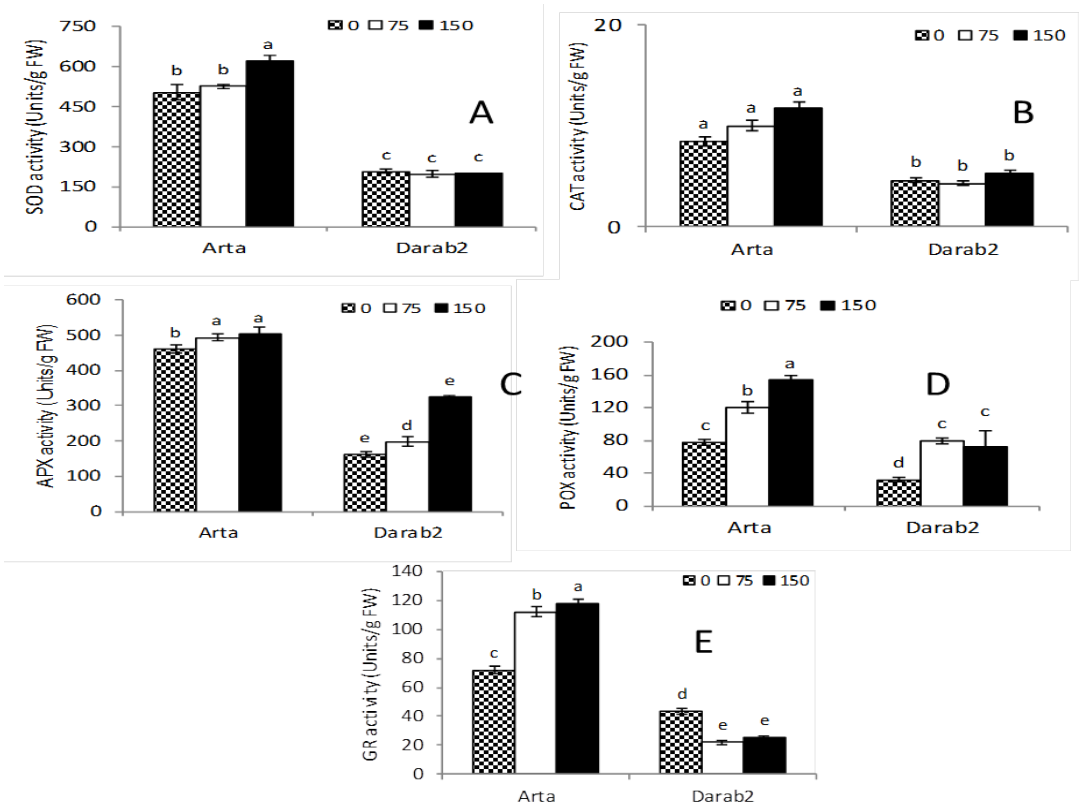

Fig. 1. Effect of salinity on antioxidant enzymes activity in leaves of tolerant ('Arta') and sensitive ('Darab2') cultivars of wheat. (A) Superoxide diamutase, (B) Catalase, (C) Ascorbate peroxidase, (D) Peroxidases, (E) Glutathione reductase

Conclusions

To avoid the negative effects of ROS and prevent to occurrence oxidative stress and damage to biomolecules, the existence of balance between the production of ROS and ROS-scavenging mechanisms is necessary. Finally, the activities of antioxidant enzymes such as SOD, APX, POX and GR by high GSH/GSSG ratio increased in 'Arta' under salinity stress which led to scavenging of ROS and finally, this cultivar could be decrease the negative effects of ROS and it would be a tolerant cultivar under salinity stress.

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