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EVALUATION OF SALINITY STRESS EFFECTS ON CHANGES IN PHOTOSYNTHETIC PIGMENTS, HYDROGEN PEROXIDE AND OSMOLYTES IN SENSITIVE AND TOLERANT CULTIVARS OF WHEAT CROP

Rasoul Khodavirdivand Keshtiban[1], Hassan Soltanloo[2], Seyedeh Sanaz Ramazanpour[3] and Vahid Shariati[4]
[1]Department of Plant Breeding, Gorgan University of Agricultural Sciences and Natural Resources, Iran
[2, 3]Associate Professor, Department of Plant Breeding and Biotechnology,
Gorgan University of Agricultural Sciences and Natural Resources, Iran
[4]Assistant Professor, Department of Plant Molecular Biotechnology,
National Research Institute of Genetic Engineering and Biotechnology, Iran

ABSTRACT

Investigation of wheat response to salinity stress can help to better understand the effective defense mechanisms of salinity stress tolerance. For this purpose, biochemical and physiological traits related to salinity tolerance in wheat cultivars were evaluated at Gorgan University of Agricultural Sciences and Natural Resources in 2019. Experimental factors, included two wheat crop cultivars (Sarc and Chinese spring as tolerant and susceptible wheat cultivars, respectively) and sampling time series (zero or control, 24, 48, 72, and 96 h) were examined in a factorial experiment based on a completely randomized design with three replications. Salinity stress was applied with sodium chloride at a concentration of 250 mM to uniform 10-day-old seedlings at the two-leaf stage, followed by sampling of shoot tissue. The studied traits were hydrogen peroxide (H_2O_2), chlorophyll a, chlorophyll b, total chlorophyll, chlorophyllase, carotenoids, proline, and total carbohydrates. Results of analysis of variance (ANOVA) indicated significant effects of genotype, time, and interaction of genotype × time (except H_2O_2 and total carbohydrates) on all the studied traits. Results of interaction of genotype × time showed although the trend of changes in the studied traits, depending on the type of cultivar and the sampling time were different, but generally, the susceptible Chinese spring cultivar contained higher levels of chlorophyllase and carotenoids than the control time at the end of sampling time and also higher H_2O_2 levels than the Sarc tolerant cultivar, while the Sarc tolerant cultivar, on the other hand, contained higher levels of chlorophyll a, chlorophyll b, total chlorophyll, and proline than the control time at the end of sampling time and also greater total carbohydrates than the susceptible Chinese spring cultivar. The results confirm the higher capacity of the antioxidant defense system of Sarc tolerant cultivar than the susceptible Chinese spring cultivar. Therefore, the osmolytes of proline and total carbohydrates are reliable for crop screening, particularly wheat crop, in salinity stress studies.

Keywords: Sodium chloride, Proline, Chlorophyllase, Chlorophyll, Carotenoids

Introduction

Wheat has nowadays become the most important strategic crop worldwide (Shiran Tafti *et al*., 2019). In Iran, it is also one of the most important cereals (Alipour *et al*., 2019) and provides 47% of daily calories for people (Hosseini *et al*., 2007). In Iran, the area under wheat cultivation is 6.70 million hectares (FAO, 2019) while the area of saline soils is about 24 million hectares (Jafari, 1994), the total area of irrigated land is 7.3 million hectares, and the total area of agricultural lands with different salinities of soil, water or both is estimated to be 3.5 million hectares (Banaei *et al*., 2004). According to forecasts, more than 50% of the world arable land will be saline by 2050 (Mohamed *et al*., 2006). A high level of sodium chloride is the main cause of soil salinity in most of these areas (Tejera *et al*., 2006).

On the other hand, it is estimated that the average crop yield reduction may reach more than 50% in saline areas (Qureshi *et al*., 2007). In these conditions, there are generally two methods to deal with salinity. The first method is to improve saline soils, which is based on the use of drainage and irrigation systems that are high-cost and require fresh water. The second is the biological method in which tolerance to salinity in plants can be increased by cultivating resistant plants or using physiological information, selection criteria, breeding methods, and biotechnological techniques (Almansouri *et al*., 2001; Munns, 2002; Blum, 1988).

In general, salinity stress induces various biochemical and physiological responses in plants and affects almost all plant functions from photosynthesis to growth and crop production (Daneshmand & Oloumi, 2014). One of the important biochemical changes that occurs upon exposure of the plant to saline environment is an increase in the production of reactive oxygen species (ROS) (Wang *et al*., 2003). The types of oxygen free radicals include superoxide (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) (Sairam & Tyagi, 2004). Elevations of these radicals lead to the oxidation of lipids, changes in the structure of proteins, inactivation of enzymes, discoloration of chlorophyll, and degradation of nucleic acids (Nayyar & Gupta, 2006).

Plants have a high-performance defense system, including enzymatic and non-enzymatic mechanisms, to deal with induced oxidative stress (Loggini *et al*., 1999). Carotenoids are considered as non-enzymatic systems (Ozkur *et al*., 2009) and include key pigments of the antioxidant system in plants being very sensitive to oxidative damage (Kafi *et al*., 2011). These pigments are involved in neutralization singlet oxygen (Ashraf & Mc Neilly, 2004). In addition to carotenoids, chlorophylls are also affected by salinity stress, so that the degradation of chlorophyll molecule is another damage of oxidative stress (Yasar *et al*., 2006). Degradation of chloroplast structure and reduction of chlorophyll content are influenced by increased chlorophyllase activity due to altered nitrogen metabolism associated with the production of such compounds as proline that play a role in osmotic regulation (Borzouei *et al*., 2011). Reduction of leaf total chlorophyll under salinity stress generally results in decreased leaf photosynthetic efficiency and consequently plant growth (Emadi *et al*., 2009).

In addition to the antioxidant enzymatic defense mechanism, compatible compounds (osmolytes), such as proline and carbohydrates, improve plant tolerance to salinity (Heydari *et al*., 2010). Accumulation of compatible compounds helps to detoxify ROS, and chaperone-like activities of these compounds maintain and stabilize the structure and function of proteins and cellular structures (Apse & Blumwald, 2002). An increase in proline concentration is the most frequent and common response observed upon the development of stress (Suriyan & Chalermpol, 2009). As a soluble substance, proline increases cellular osmotic potential, preserves cell turgor, and stabilizes the shape of proteins, thereby protecting the stability of cell membranes (Verslues *et al*., 2006). Stress-resistant plants have a greater ability to synthesize proline and, consequently, have more membrane stability, which results in less water loss through cell membranes (Valentovic *et al*., 2006).

Accumulation of soluble sugars as compatible osmolytes also increases the resistance of plants to salinity stress (Setayesh Mehr & Esmaeilzadeh Bahabadi, 2013). Degradation and hydrolysis of larger molecules, such as starch, and their conversion into sugar compounds, such as sucrose, and then smaller molecules, such as glucose and fructose, due to salinity stress cause more negativity of water potential in cells and osmotic regulation (Bartels & Sunkar, 2005).

Wheat cultivars respond very differently to salinity stress and the study of defense mechanisms is obviously of paramount importance. The amounts and variations of photosynthetic pigments, H_2O_2, and compatible compounds were compared and evaluated in the present study to better understand the effect of salinity stress on susceptible and tolerant cultivars of wheat crop.

Materials and Methods

Planting and sampling methods

This research was conducted in the laboratory of the Faculty of Plant Production in Gorgan University of Agricultural Sciences and Natural Resources during 2019. Experimental factors, namely two crop wheat cultivars (Sarc 6 and Chinese spring as tolerant and susceptible wheat cultivars, respectively) as the first factor, and five sampling time series (zero or control, 24, 48, 72, and 96 h) as the second factor were examined in a factorial experiment as a completely randomized design with three replications. To plant and apply salinity stress at the seedling stage, seeds were first disinfected using a solution of sodium hypochlorite and 70% ethanol. The uniformly germinated seeds were then transferred to hydroponic growth conditions using Hoagland's solution (Hoagland & Arnon, 1950). Planting containers were placed in a controlled environment with 16 h light at 25 °C and 8 h darkness at 20 °C. The nutrient solution was changed every three days and its pH was adjusted between 5.5 and 6.5 using sodium hydroxide (NaOH). Salinity stress was applied with NaCl at a concentration of 250 mM to uniform 10-day-old seedlings at the two-leaf stage. Calcium chloride ($CaCl_2$) was added to NaCl solution to maintain a Na/Ca ratio of 10: 1. To measure the traits, leaf samples of each cultivar were harvested in three replications before salinity stress at time zero and then at 24, 48, 72, and 96 h after salinity stress.

Extraction and measurement of H_2O_2

The amount of H_2O_2 was measured using a spectrophotometer based on absorbance at 390 nm (Sergiev *et al*., 1997) and expressed in micromoles per gram of fresh weight.

Extraction and measurement of chlorophyll and carotenoids

Chlorophyll and carotenoids were measured based on the adsorption values of the solutions through spectrophotometry at 480 and 510 nm for carotenoids and 645 and 663 nm for chlorophyll a and b (Arnon, 1949), and calculated using the following formulas:

Chl. a (mg/g FW) = [12.7 (A663) – 2.69 (A645)] × V/W

Chl. b (mg/g FW) = [22.9 (A645) –4.68 (A663)] × V/W

Total Chl. (mg/g FW) = [20.2 (A645) + 8.02 (A663)] × V/W

Car. (mg/g FW) = [7.6 (A480) – 1.49 (A510)] × V/W

In the above equations, A663, A645, A480, and A510 are the absorbance read at 663, 645, 480, and 510 nm, respectively, V is the final volume (ml) of consumed acetone, and W is the weight of fresh plant tissue. The contents of chlorophyll and carotenoids were expressed in mg/g of fresh weight.

Extraction and measurement of chlorophyllase

Chlorophyllase was extracted using the modified method of Fernandez-Lopez *et al*. (1992), followed by measuring chlorophyllase through calculation of chlorophyllidea spectrophotometrically at a wavelength of 665 nm using a extinction coefficient of 54.1 mmol/cm (Tanaka *et al*. (1982). The amount of chlorophyllase was then expressed in nanomoles per gram of fresh weight.

Measurement of proline content

Proline content was measured using the method described by Bates (1973). According to this method, the upper phase is harvested from two phases formed in the reaction solution, and finally proline content in the samples was determined quantitatively using a standard curve spectrophotometrically at a wavelength of 520 nm. Proline content was then expressed in micromoles per gram of fresh weight.

Extraction and measurement of total carbohydrates

Total carbohydrate was extracted using the phenol-sulfuric method (Dubois *et al*., 1956). Accordingly, total carbohydrate was measured spectrophotometrically using glucose as a standard solution based on the absorbance at 490 nm. Total carbohydrate content was then expressed in micromoles per gram of fresh weight.

Statistical analysis

Data were analyzed statistically, including analysis of variance (ANOVA) and comparison of mean values of the studied traits, using SAS software. Mean values were compared based on the LSD method and significant differences were considered at levels of 5% and 1%.

Results and Discussion

Hydrogen peroxide

According to ANOVA results (Table 1), salinity stress had a significant effect on H_2O_2 contents in the studied cultivars as well as on sampling time series. However, the interaction of genotype and time was not significant. Accordingly, the diagrams of simple effects of genotype factors and sampling times (Fig. 1) revealed that H_2O_2 contents decreased from zero to final times by the application of salinity stress, with the lowest level in 96 h after salinity stress, which was higher in susceptible Chinese spring cultivar than Sarc tolerant cultivar. In similar studies on wheat, it was reported that the accumulation of oxygen free radicals reflects the cultivar susceptibility to salinity stress, which was attributed to lower H_2O_2 contents in tolerant cultivars than sensitive cultivars (Sarvajeet & Narendra, 2010). In rice plant, changes in H_2O_2 content was examined under salinity stress (at 0, 12, 24, 48, and 72 h after stress), and decreased H_2O_2 content was reported in most of tolerant and sensitive cultivars at the end of sampling times (Kurd Rostami *et al*. 2016). The reduction of H_2O_2 from zero to final times can be attributed to the superiority of the antioxidant defense system in both studied wheat cultivars in overcoming oxidative stress. In different plant genotypes have been reported to utilize different antioxidant capacities to deal with oxidative stress-induced damage (Moharramnejad & Valizadeh, 2015), with tolerant cultivars possessing a better defense mechanism against oxidative stress than sensitive cultivars (Yildiz & Terzi, 2013).

Table 1 : Analysis of variance on the studied traits under salinity stress

S.O.V	df	Mean of Square (MS)							
		H_2O_2	Chlorophyll			Chlorophyllase	Carotenoid	Proline	Carbohydrate
			a	b	Total				
Genotype	1	0.406**	0.00032**	0.0009**	0.0015**	288.9**	0.000086**	461.4**	165.06**
Time	4	8.93**	0.0054**	0.0018**	0.0112**	139.6**	0.00064**	142.1**	12.96**
Genotype × Time	4	0.038^{ns}	0.0009**	0.0019**	0.0050**	3.84*	0.00032**	17.61**	0.089^{ns}
Error	20	0.019	0.0000065	0.0000066	0.00003	1.17	0.000002	0.923	0.139
C.V (%)		4.25	4.88	7.08	6.41	4.39	6.49	5.49	2. 71

ns: not significant; * and **: significant at probability levels of 5% and 1%, respectively

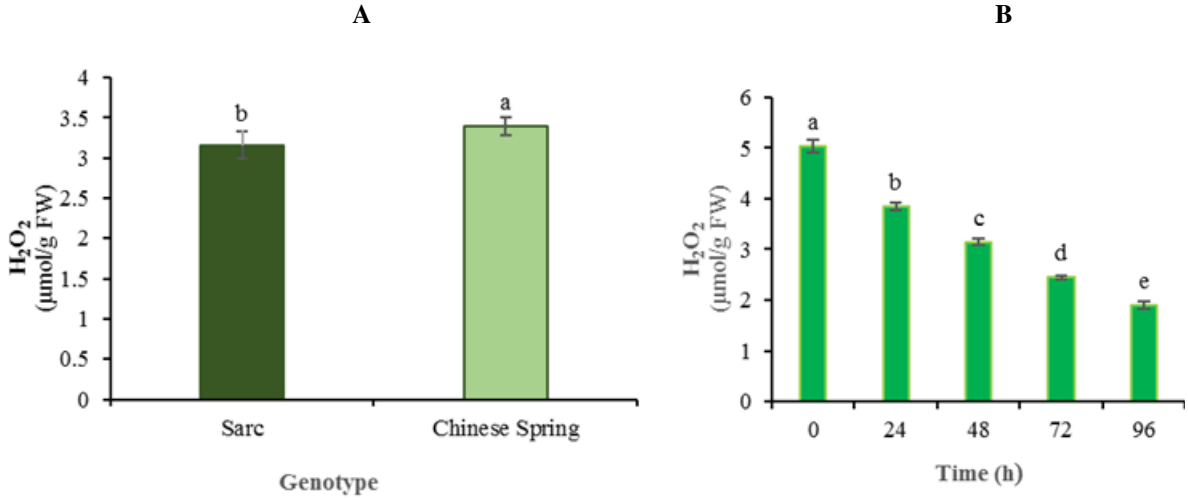

Fig. 1 : Comparison of mean simple effects of genotype (A) and sampling time (B) on H_2O_2 trait in wheat cultivars

Chlorophyll and chlorophyllase contents

Based on the results of ANOVA, the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and chlorophyllase in the studied genotypes, sampling times and interactions of genotype and time were affected significantly by the salinity stress. Comparison of mean interactions between chlorophylls a and b contents (Figs. 2 and 3) revealed that the Sarc tolerant cultivar contained the highest chlorophyll a content at 96 h, which was not significantly different from the susceptible Chinese spring cultivar at the same time. The lowest amount belonged to Chinese spring cultivar at 72 h, which was not significantly different from both cultivars at 48 h and also the control time of Chinese spring cultivar. The highest chlorophyll b content belonged to Sarc cultivar at control time (zero) and the lowest content was recorded in Chinese spring cultivar at 72 h after salinity stress, which was not different significantly from Sarc cultivar at 24 h. For total chlorophyll trait, the interaction results showed that the uppermost and lowermost total chlorophyll levels belonged to Sarc and Chinese spring cultivars at 96 h and 72 h after salinity stress, respectively.

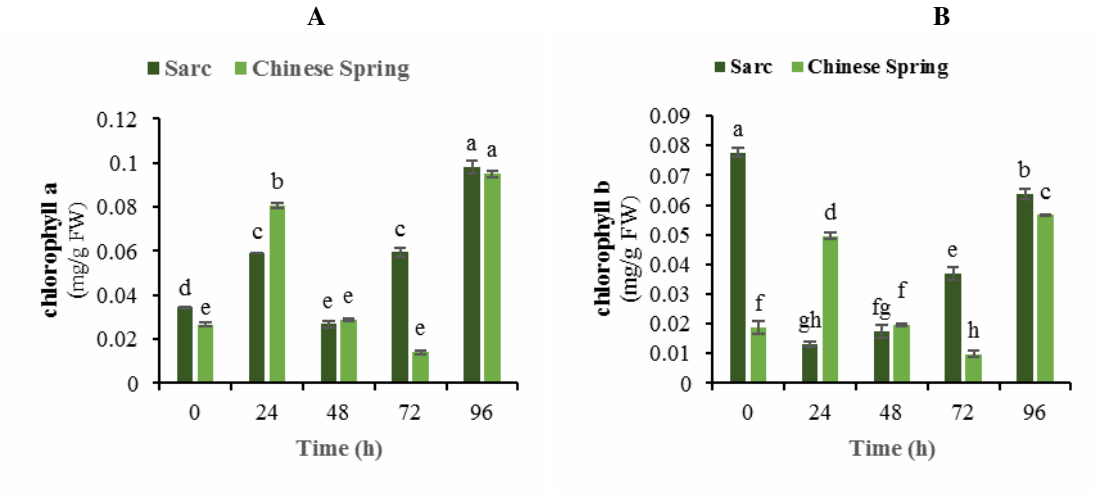

Fig. 2 : Comparison of mean interactions chlorophyll a (A) and chlorophyll b (B) in wheat cultivars under salinity stress

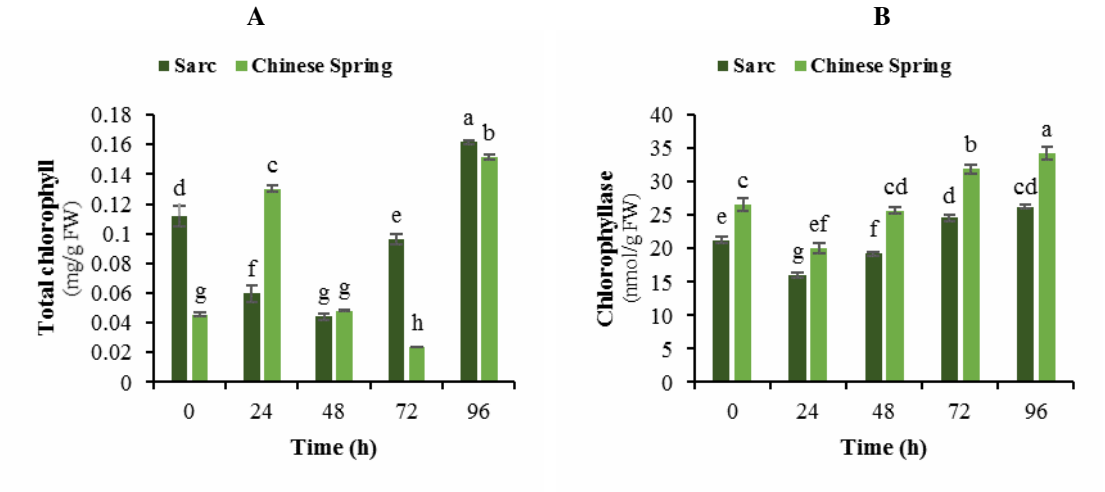

Fig. 3: Comparison of mean interactions total chlorophyll (A) and chlorophyllase (B) in wheat cultivars under salinity stress

In general, the results of genotype and sampling time interaction on chlorophyll a, chlorophyll b, and total chlorophyll showed that the application of salinity stress led which was attributed to no regular decrease or increase relative to the control time. The general trend, however, indicated the elevated contents of chlorophyll a, chlorophyll b and total chlorophyll at 96 h after the stress compared with other sampling times, particularly at zero time (except chlorophyll b levels in Sarc cultivar, its value was less than zero, despite an increase in 96 h). Elevation of traits in the Sarc tolerant cultivar was more than that of the sensitive Chinese spring cultivar.

Comparison of mean chlorophyllase interactions revealed that Chinese spring cultivar contained the highest chlorophyllase level at 96 h after stress and the lowest level belonged to Sarc cultivar at 24 h after stress. In general, the results of genotype and sampling time interaction on this trait showed that the general trend of changes in this trait was regular after salinity stress. After a significant reduction of this enzyme at 24 h after stress (compared to time zero), the changes had an increasing trend until the end of sampling and finally the highest amount of enzyme was obtained in both cultivars at 96 h. However, the amount of this enzyme was significantly higher in the susceptible Chinese spring cultivar than the tolerant Sarc cultivar at all the sampling times.

Both chlorophyll a and b are believed to be sensitive to stress (Farooq *et al*., 2009). Salinity stress leads to changes in the amounts of these molecules in plant cells (Arvin, 2015). Salinity stress causes chloroplast degradation, chlorophyll decomposition, and photosynthetic pigment reduction through decreasing the activity of enzymes involved in chlorophyll synthesis (Vieira Santos, 2004), stimulating chlorophyllase production by increasing growth regulators such as abscisic acid and ethylene (Drazkiewicz, 1994), and increasing nitrogen utilization by proline synthesis (Bybordi, 2012). However, the stability of photosynthetic pigments under salinity stress conditions is considered as a Criteria for plant resistanceto salinity stress (Sevengor *et al*., 2011). In addition, chlorophyll concentrations increase in mild salinity stresses and decrease in severe stresses (Nemati *et al*., 2013). Researchers believe that plants respond differently to osmotic potential and its effect on the minimum or maximum reduction of photosynthetic pigments during salinity stress (Vojodi Mehrabani *et al*., 2017). There are currently various reports of decreasing or increasing chlorophyll content in plants under salinity stress. These include decreasing

chlorophyll content in wheat (Ehsanzadeh *et al*., 2009), safflower (Siddiqi *et al*., 2009), sugar beet (Emadi *et al*., 2009), and rice (Kanawapee *et al*., 2012), and increasing chlorophyll content in wheat (Jam Barandozi *et al*., 2012), tobacco (Locy *et al*., 1996), sugar beet (Dadkhah, 2011), and safflower (Karimi *et al*., 2015) under salinity stress. Movahhedy Dehnavy *et al*. (2004) attributed The reason an increase in chlorophyll is the decreased leaf surface area and accumulation of chlorophyll at a lower leaf surface area, while Borzouei *et al*. (2011) explained decreased leaf surface area and an increase in chlorophyll content as a stress prevention mechanism. Papp *et al*. (1983) also reported that leaf thickness increased at all salinity levels and this change in leaf thickness increased chlorophyll levels. In similar results to this study, Sadat Musavizadeh *et al*. (2018) reported significant effects of genotype, time, and their interactions on chlorophyll a and chlorophyll b contents of rice under salinity stress. They also observed no regular trend of changes in these traits at sampling times of 0, 6, 24, 48, 120, 72, and 168 h after salinity stress, but most of these traits occurred at the final time (168 h) after stress.

On the other hand, the results of this study showed that despite the increased chlorophyllase levels in both tolerant and sensitive cultivars, the chlorophyll content increased at the final time of stress. This indicates the effectiveness of defense and antioxidant mechanisms of wheat and chlorophyll stability in dealing with salinity stress given the decreasing trend of H_2O_2. However, chlorophyll stability is considered as an indicator of plant resistance to salinity stress so that tolerant and sensitive cultivars have higher and lower stability indices, respectively (Mohan *et al*., 2000).

It should also be emphasized that salinity stress tolerance is not a function of a plant organ or trait, but a result of most plant traits (Akbari Ghogdi *et al*., 2011). Undoubtedly, several enzymatic and non-enzymatic mechanisms contribute to the resistance or sensitivity of plants to salinity stress (Kordrostami *et al*., 2016).

Carotenoid content

The results of ANOVA revealed that salinity stress had significant effects on the carotenoid content in the studied genotypes, sampling time, and the interaction of genotype and sampling time. Accordingly, comparison of mean interactions of carotenoid content (Figure 4 A) indicated that the Chinese spring cultivar contained the highest and lowest carotenoid content at 96 and 72 h after stress, respectively. The results of genotype and sampling time interaction on this trait showed that the general trend of changes in this trait was different and opposite in the studied cultivars after applying salinity stress. After a significant reduction of this enzyme in Sarc cultivar at 24 and 48 h after stress (compared to time zero), the changes had an uptrend until the final time of sampling. In Chinese spring cultivar, on the other hand, a significant increase in this enzyme at 24 h after stress (compared to time zero) was followed by declining changes until the penultimate time of sampling. Similarly, Sadat Mousavizadeh *et al*. (2018) reported an increase in rice carotenoid content at final times (120 and 168 h after stress) after a decreasing trend at initial times of sampling. Karimi *et al*. (2015) reported an increase in carotenoid concentrations in safflower cultivars at different levels of salinity stress, and concluded that elevated carotenoid concentrations was part of the plant defense mechanisms against salinity stress. Jam Barandozi *et al*. (2012), In the study of salinity resistance of wheat cultivars, reported that carotenoid content increased in some wheat cultivars and decreased in others by applying salinity stress in comparison to control conditions. On the other hand, as fat-soluble antioxidants in chloroplast membranes, carotenoids play an important role in plant processes, including tolerance to oxidative stress (Lovdel *et al*., 2010). In this study, the susceptible Chinese spring cultivar seems to be more inclined to use this defense mechanism to overcome oxidative stress while being exposed to higher H_2O_2 and chlorophyllase levels than Sarc tolerant cultivar. It is believed that genotypes select different antioxidant activities in response to salinity stress and this difference in defense mechanisms varies not only in different species, but sometimes in the genotypes and cultivars of a single plant species (Dastneshan & Sabokdast, 2020).

Content of osmolytes

According to the results of ANOVA, proline and carbohydrate contents in the studied genotypes and sampling time were affected significantly by salinity stress. However, the interaction of genotype and time was significant on proline but not on total carbohydrates. Comparison of mean interactions for proline content (Fig. 4 B) showed the highest proline content belonged to the Sarc tolerant cultivar at 96 h, which was not significantly different from that of 72 h, and the lowest level was recorded in the Chinese spring cultivar at time zero. In general, the results of genotype and sampling time interaction on proline revealed that salinity stress led to an increasing trend in proline changes of the Sarc tolerant cultivar, which was more regular than that of the sensitive Chinese spring cultivar. However, proline content increased in the Chinese spring cultivar compared to the control time (similar to Sarc cultivar) at the time series, but it decreased after 72 h of stress application. Due to the non-significant interaction between genotype and time on carbohydrate content, simple effects diagrams of genotype and sampling time factors (Fig. 5) showed that carbohydrate content increased from time zero to the final times by applying salinity stress. It reached the uppermost level at 96 h after salinity stress and was less abundant in the susceptible Chinese spring cultivar than in the Sarc tolerant cultivar.

Researchers reported similar results on increased proline (Martin *et al*., 1993; Heydari *et al*., 2010) and soluble carbohydrates (Hamada & Khalea, 2010; Farhoudi, 2014) in wheat under salinity stress. In a similar research on rice plant, proline content increased significantly with increasing after stress time and the tolerant cultivar contained higher levels than the sensitive cultivar (Sadat Musavizadeh *et al*., 2018). Kerepesi and Galiba (2000) stated increased carbohydrate concentrations in wheat seedlings to be a criterion for the selection of salinity-tolerant wheat cultivars. During salinity stress, resistant plants are able to maintain cellularturgescence by producing osmotic compatible compounds such as proline and sugars (Ashraf & Harris, 2004).

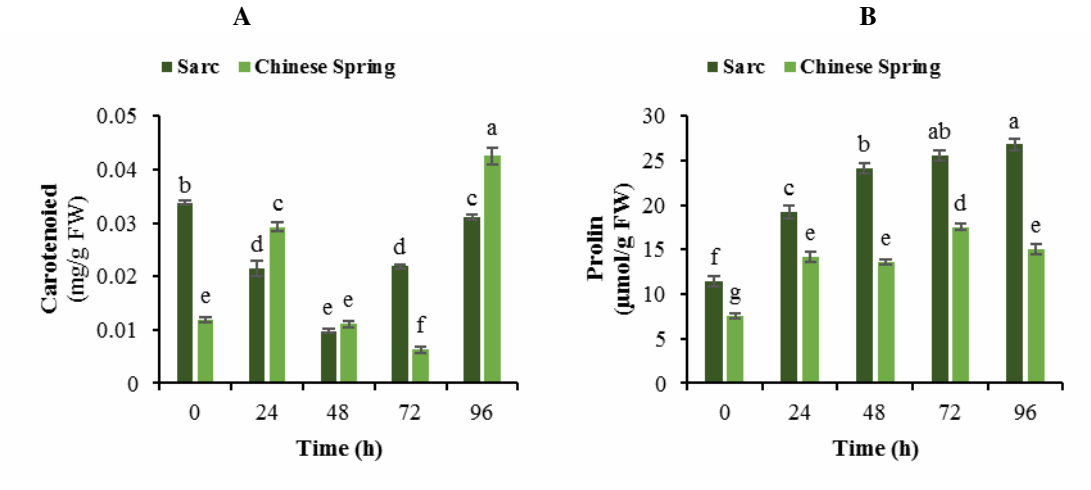

Fig. 4 : Comparison of mean interactions of carotenoid (A) and proline (B) contents in wheat cultivars under salinity stress

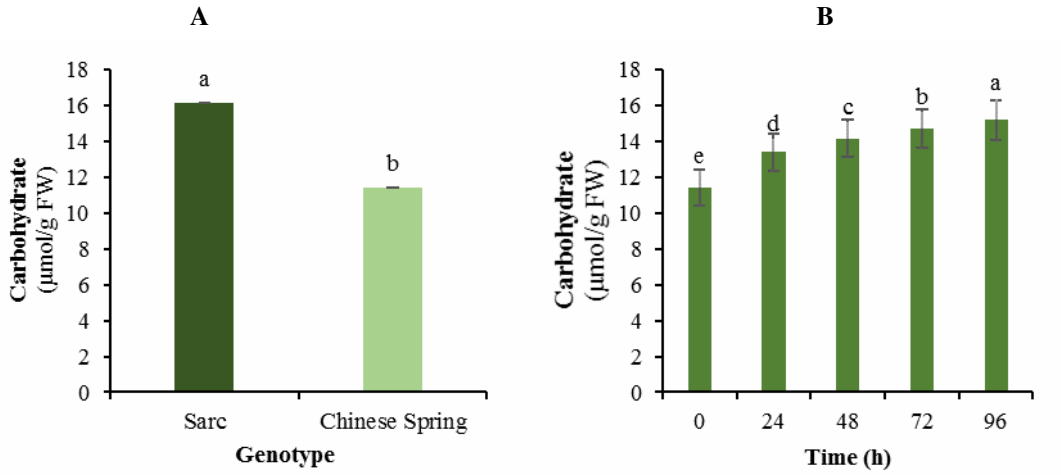

Fig 5 : Comparison of mean simple effects of genotype (A) and sampling time (B) on carbohydrate content in wheat cultivars

These osmolytes support plants by detoxification of ROS and stabilization of the quaternary structure of proteins (Chinnusamy *et al*., 2006). As such, proline is considered as a source of energy, carbon, and nitrogen for damaged tissues during stress (Najafi *et al*., 2010) while insoluble sugars are broken down and decrease the risk of cellular dehydration through the production of soluble sugars (Parvaiz and Satyawati, 2008).

Conclusion

In this study, salinity stress caused physiological and biochemical changes in wheat cultivars so that it had significant effects on all traits in the studied cultivars and on the sampling time series. According to the results, although tolerant and susceptible genotypes of wheat utilize various defense mechanisms to overcome the effects of exposure to salinity stress, the Sarc tolerant cultivar had a higher capacity, efficiency, and ability to utilize defense mechanisms, in particular the non-enzymatic antioxidant system, than the sensitive Chinese spring cultivar. Therefore, the biomarkers of proline and soluble carbohydrate are reliable for crop screening, particularly wheat crop, in salinity stress studies.

References

Akbari Ghogdi, E., A. Izadi-Darbandi, A. Borzouei and A. Majdabadi (2011). Evaluation of morphological changes in some wheat genotypes under salt stress. *Journal of Water and Soil Science*, 1(4): 71-83.

Alipour, H., H. Abdi, Y. Rahimi and M. R. Bihamta (2019). Investigating grain yield and yield stability of wheat cultivars introduced in Iran over the last half century. *Cereal Research*, 9(2): 157-167.

Almansouri, M., J. M. Kinet and S. Lutts (2001). Effect of Salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf). *Plant Soil*, 231: 243-254.

Apse, M.A. and E. Blumwald (2002). Engineering salt tolerance in plants. *Current Opinion in Biotechnology*, 13: 146-150.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24: 1-15.

Arvin, P. (2015). Effect of gibberellin on some morphological traits, photosynthetic pigments content and proline in savory (*Satureja hortensis* L.) under salinity stress conditions. *Journal of Crop Production Research,* 7(2): 89-105.

Ashraf, M. and P.J.C. Harris (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166: 3-16.

Ashraf, M. and T. Mc Neilly (2004). Salinity tolerance in Brassica oil seeds. *Critical Reviews in Plant Sciences*, 23: 157–174.

Banaei, M. H., A. Moameni, M. Baybordi and M. J. Malakouti (2004). Iran Soils: New transformations in

the identification, management and operation. Soil and Water Research Institute, Tehran.

Bartels, D. and R. Sunkar (2005). Drought and salt tolerance in plants. *Plant Science*, 24: 23-58.

Blum, A. (1988). Plant breeding for stress environment. Florida: CRC Press, 212 p

Borzouei, A., M. Kafi, H. Khazaei, A. Khorasani and A. Majdabad (2011). The Study of Physiological Characteristics and Enzyme Superoxide Dismutas Activity in Two Wheat (*Triticum aestivum* L.) Cultivars at Different Growth Stages under Irrigation Water Salinity. *Iranian Journal of Field Crops Research*, 9(2): 190-201.

Bybordi, A. (2012) Study effect of salinity on some physiologic and morphologic properties of two grape cultivars. *Life Science Journal*, 9(4): 1092-1101.

Chinnusamy, V., J. Zhu and J.K. Zhu (2006). Salt stress signaling and mechanisms of plant salt tolerance. *Genetic Engineering*, 27: 141-177.

Dadkhah, A. (2011). Effect of salinity on growth and leaf photosynthesis of two sugar beet (*Beta vulgaris* l.) cultivars. *Journal of Agricultural Science and Technology*, 13: 1001-1012.

Daneshmand, F. and H. Oloumi (2014). The effect of salt stress and interaction with 5-aminolevulinic acid (ALA) on the activity of enzymatic antioxidants in (*Lycopersicun esculentum* Mill.) tomato plants. *Journal of Plant Process and Function*, 3(8): 123-132.

Dastneshan, S. and M. Sabokdast **(**2020**).** Evaluation of Tolerance Rate of Some Genotypes of Beans (*Phaseolus Vulgaris* L.) To Salinity Stress. *Journal of Crop Breeding,*11(32):184-194.

Drazkiewicz, M. (1994). Chlorophyllase: Occurrence functions, mechanisms of action, effects of external and internal factors. *Photosynthesis*, 30: 321-331.

Ehsanzadeh, P., M.S. Nekoonam, J.N. Azhar, H. Pourhadian and S. Shaydaee (2009). Growth, chlorophyll, and cation concentration of tetraploid wheat on a solution high in sodium chloride salt: hulled versus free-threshing genotypes. *Journal of Plant Nutrition*, 32: 58-70.

Emadi, A.R., H. Nourani Azad and A. Borzoo (2009). Response of some physiological traits to salinity in sugar beet (*Beta vulgaris* L.). *Plant and Ecosystem Fall*, 1(19): 17-26.

FAO (2019). Statistical data. Food and Agriculture Organization. From www.faostat.org (October 1, 2019).

Farhoudi, R (2014). Investigation the salinity tension effect on growth and physiological characteristics of nine wheat cultivars at vegetative growth stage. *Crop Physiology Journal*, 5(20): 71-86.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra (2009). Plant droughtstress: effects, mechanisms and management. *Agronomy for Sustainable Development*. 29: 185-212.

Fernandez-Lopez, J.A., L. Almela, M.S. Almanza and J.M. Lopez-Roca (1992). Partial purification and properties of chlorophyllase from chlorotic *Citrus limon* leaves. *Phytochemisrty*, 31: 447–449.

Hamada, A.M. and Khalea, E.M. (2010). Effect of salinity and heat-shock on wheat seedling and content of carbohydrates, proteins and amino acids. *Biologia Plantarum*. 37(3): 399-404.

Heydari, M., F. Mesri and Z. Keykha (2010). Effects of Salinity Stress on Nucleic Acid Metabolism, Antioxidants Enzyme Activity, Chlorophyll Fluorescence and Osmotic Adjustment in Five Canola Genotypes. *Iranian Journal of Field Crops Research*, 41(3): 491-502.

Hoagland, D. R., and D. I. Arnon (1950). The water culture method for growing plants without soil. Circular. California Agricultural Experiment Station. 347 (2), 32.

Hosseini, S.M.T., A. Sioseh Mardeh, P. Fathi and M. Sioseh Mardeh (2007). Application of artificial neural networks and multiple regression for estimating assessing performance of dry farming wheat yield in ghorveh region, Kurdistan province. *Journal of Agricultural Research*, 7(1): 41-54.

Jafari, M. (1994). Salinity image and halophytes. Forest and Rangeland Research Institute, 62p.

Jam Barandozi, A., I. Ghaffari and R. Sohrabi Meshkabadi (2012). Evaluation of salinity resistance of wheat using photosynthetic pigments and lipid peroxidation in seedling stage. Third National Conference on Combating Desertification and Sustainable Development of Desert Wetlands in Iran, Arak, Iran, 337-340.

Kafi M, A. Bagheri, J. Nabati, M. Zare Mehrjerdi and A. Masomi (2011). Effect of salinity on some physiological variables of 11 chickpea genotypes under hydroponic conditions. *Journal of Science and Technology of Greenhouse Culture*, 1 (4): 55-70.

Kanawapee, N., J. Sanitchon, W. Lontom, and P. Threerakulpisut, P. (2012). Evaluation of salt tolerance at the seedling stage in rice genotypesby growth performance, ion accumulation, proline and chlorophyll content. *Plant and Soil*, 358: 235-249.

Karimi S., A. Arzani and G. Saeidi (2015). Effect of salinity stress on antioxidant enzymes and chlorophyll content of salt-tolerant and salt-sensitive safflower (*Carthamus tinctorius* L.) genotypes. *Journal of Plant Process and Function*, 4 (13): 25-35.

Kerepesi, I. and G. Galiba (2000). Osmotic and salt Stress-Induced Alteration in soluble carbohydrate content in wheat seeding. *Crop Science*, 40:482-487.

Kordrostami, M., B. Rabiei and S.H. Hassani Kumleh (2016). Biochemical characteristics and expression analysis of some oxidative pathway genes in rice (*Oryza sativa* L.) under salt stress conditions.*Cereal Research*, 6(1): 15-30.

Locy, R. D., C. Chang, B.L. Nielsenand and N.K. Singh (1996). Photosynthesis in salt adapted hetrophic tobaco cells and regenerated plants. *Plant physiology*, 110: 321-328.

Loggini, B.A. Scartazza and E. Brugnoli (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology*, 119: 1091-1099.

Lovdel, T., K.M. Olsen, R. Slimestad, M. Verheul andC. Lillo (2010). Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. *Phytochemistry*,71: 605-613.

Martin, M., F. Miceli, J.A. Morgan, M. Scalet and G. Zerbi (1993). Synthesis of osmotically active substrates in winter wheat leaves as related to drought resistance of

different genotypes. *Journal of Agronomy and Crop Science*, 171: 176-184.

Mohan, M.M., S.L. Narayanan and S.M. Ibrahim (2000). Chlorophyll stability index (CSI): its impact on salt tolerance in rice. *International Rice Research Notes*, 25(2):38-39.

Moharramnejad, S. and M. Valizadeh (2015). Variation of Pigment Content and Antioxidant Enzyme Activates in Pinto Bean (*Phaseolus vulgaris* L.) Seedlings under Salt Stress. *Journal of Crop Ecophysiology*, 9(1): 153-166.

Mokhamed, A. M., G.N. Raldugina, V.P. Kholodova and V.V. Koznetov (2006) Osmolyte accumulation in different rape genotypes under sodium chloride salinity. *Russian Journal of Plant Physiology*, 53: 649-655.

Movahhdy Dehnavy, M., S.A.M. Modares Sanavi, A. Soroushzadeh and M. Jalali (2004). Changes in proline, total soluble sugars, chlorophyll (SPAD) and chlorophyll fluorescence in winter safflower cultivars under drought stress and foliar application of zinc and manganese. *Desert*, 9(1): 93-109.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment, 25: 239-250.

Najafi, F., R.A. Khanvari-Nejad and M. Siah Ali (2010). The effect of salt stress on certain physiological parameters in summer savory (*Satureja hortensis*L.). *PlantJournal of Stress Physiology and Biochemistry*, 6: 13-21.

Nayyar, H., and D. Gupta. (2006). Differential sensitivity of C3 and C4plants to water deficitstress: Association with oxidative stress and antioxidants. *journal of Experimental Botany*. 58: 106-113.

Nemati, I., S. Gholizadeh and F. Moradi (2013). Study of different salts effect and their interaction on concentration of organic and inorganic solutes and antioxidant enzymes activity in alfalfa seedlings. *Electronic Jornal of Crop Production,*5 (4): 39-61.

Ozkur, O., F. Ozdemir, M. Bor and I. Turkan (2009). Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environmental and Experimental Botany*, 66: 487-492.

Papp, J.C., M.C. Ball and N. Terry (1983). A comparative study of the effects of NaCl salinity on respiration, photosynthesis, and leaf extension growth in *Beta vulgaris* L. (Sugar beet). *Plant, Cell and Environment*, 6: 675-677.

Parvaiz, A. and S. Satyawati (2008). Salt stress and phyto-biochemical responses of plants. a review. *Plant, Soil and Environment*, 54: 88-99.

Qureshi, A.S., M. Qadir, N. Heydari, H. Turral, and A. Javadi (2007). A review of management strategies for salt-prone land and water resources in Iran. International Water Management Institute. Colombo, Sri Lanka. 30p (IWMI Working Paper 125)

Sadat Musavizadeh, Z., H. Najafi Zarini, S.H. Hashemi Petroudi and S.K. Kazemitabar. (2018). Assessment of Proline, Chlorophyll and Malondialdehyde in Sensitive and Tolerant Rice (*Oryza sativa* L.) Cultivars under Salt Stress. *Journal of Crop Breeding*, 10(25): 28-35

Sairam, R.K. and A. Tyagi (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, 86(3): 407-42.

Sarvajeet, S.G. and T. Narendra (2010). Reactive oxygen species and antioxidant machinery in a biotic stress tolerance in crop plants. Annual Review. *Plant Physiology and Biochemistry*, 3: 1-22.

Sergiev, I., V. Alexieva and E. Karanov (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *Proceeding of the Bulgarian Academy of Sciences*, 51: 121-124.

Setayesh Mehr, Z. and S. Esmaeilzadeh Bahabadi (2013). Effect of salt stress on some physiological and biochemical characteristics in *Coriandrumsativum* L. *Journal of Plant Production*, 20(3): 111-128.

Sevengor, S., S. Kusvuran, and S. Elliaitioglu (2011). The effect of salt stress on growth, chlorophyll content, lipid peroxidation and anti-oxidative enzymes of pumpkin seedling. *African Journal of Agricultural Research*, 6: 4920-4924.

Shiran Tafti, M., H. Pirasteh-Anosheh and A. Amini Sefid (2019). Determining threshold salinity tolerance of wheat promising lines under greenhouse and field conditions. *Cereal Research*, 9(3): 235-248.

Siddiqi, E. H., M. Ashraf, M. Hussain and A. Jamil (2009). Assessment of inter-cultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange characteristics as selection criteria. *Pakistan Journal of Botany*, 41: 2251-2259.

Suriyan, C.H. and K. Chalermpol (2009). Proline accumulation, photosynthetic abilities and growth characters of sugarcane (*Saccharum officinarum* L.) plantlets in response to iso-osmotic salt and water deficit stress. *Agricultural Sciences in China*, 8: 51-58.

Tanaka, K., T. Kakuno, J. Yamashita and J. Horio (1982). Purification and properties of Chlorophyllase from greened rye seedlings. *Journal of Biochemistry*, 92(6): 1763–1773.

Tejera, N.A., M. Soussi and C. Luch (2006). Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic conditions. *Environmental and Experimental Botany*, 58(1-3): 17-24.

Valentovic P., M. Luxova, L. Kolarovic and O. Gasparicova (2006). Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant, Soil and Environment*, 52: 186-191.

Verslues, P.E., M. Agarwal, S. Katiyar-Agarwal, J.H. Zhu and J.K. Zhu (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant Journal*, 45: 523–539.

Vieira Santos, C. (2004) Regulation ofchlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scientia Horticulturae*,103(1): 93-99.

Vojodi Mehrabani, L., M.B. Hassanpour Aghdam and R. Valizadeh Kamran (2017). Growth and Some Physiological Characteristics of Savory (*Satureja hortensis* L.) as Affected by Salinity Stress. *Journal of Crop Ecophysiology*, 11(1): 99-110.

Wangxia, W., B. Vinocur and A. Altman (2003). A plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, 218: 1–14.

Yasar, F., S. Kusvuran and S. Ellialtıoğlu (2006). Determination of anti-oxidant activities in some melon (*Cucumismelo* L.) varieties and cultivars under salt stress. *Journal of Horticultural Science and Biotechnology*, 81(4): 627-630.

Yildiz, M. and H. Terzi (2013). Effect of NaCl stress on chlorophyll biosynthesis, proline, lipid peroxidation and antioxidative enzymes in leaves of salt-tolerant and salt-sensitive barley cultivars. *Journal of Agricultural Sciences*, *19*: 79-88.