Plant Physiol. Rep. (July–September 2019) 24(3):370–375
https://doi.org/10.1007/s40502-019-00461-z

ORIGINAL ARTICLE

The effect of NaCl on some physiological and biochemical parameters in *Triticum aestivum* L. genotypes

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Received: 18 July 2018 / Accepted: 26 September 2019 / Published online: 4 October 2019

Abstract Effects of salinity caused by different concentrations of NaCl (100 and 200 mM) have been studied in two genotypes of *Triticum aestivum* L. (salt tolerant Saratovskaya-29 and salt sensitive Gyrmyzygul-1) with contrasting salt tolerance. Stress caused by salinity influenced differently on the content of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) in 14 to 16-day-old seedlings. In plants exposed to 100 mM NaCl an increase in quantity of photosynthetic pigments was observed in leaves, while 200 mM concentration of NaCl caused a reduction of the pigment content in leaves. A slightly higher amount of photosynthetic pigments were observed in the salt tolerant genotype Saratovskaya-29 at 200 mM NaCl. Lipid peroxidation level was higher in the sensitive Gyrmyzygul-1 genotype compared with tolerant Saratovskaya-29. It was found that, salinity stress caused an accumulation of soluble sugars and secondary metabolites—phenolic compounds. The amounts of soluble sugars and phenolic compounds were high in the salt sensitive genotype exposed to salt stress.

Keywords *Triticum aestivum* L. · Salt stress · Photosynthetic pigments · Soluble sugars · Malondialdehyde · Phenolic compounds

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Abbreviations

ROS	Reactive oxygen species
MDA	Malondialdehyde
TBA	Thiobarbituric acid
TCA	Thrichloracetic acid

Introduction

Soil salinity is one of the widespread abiotic factors that limits the productivity and geographical areas of plants. High concentrations of soluble salts in the soil have a strong impact on growth and development of plants by affecting photosynthesis, respiration, protein and lipid metabolism processes (Evelin et al. 2009). As a result of intense salinity, approximately 30–50% of world's arable lands are expected to lose the planting utility by the middle of the twenty-first century (Wang et al. 2003).

High salinity leads to plant death by creating hyperosmotic and ion stress. The reduction of water potential due to accumulation of salts in the soil impedes the absorption of water from roots of plants leading to the creation of osmotic stress. Plant response to the osmotic stress caused by salt is identical to water stress induced by drought (Abbasdokht 2011). On the other hand, accumulation of ions such as Na^+ and Cl^- adversely affects plants. The excessive amount of these ions entered the plant eventually rises to toxic level in older transpiring leaves. This leads to a decrease in the amount of the produced assimilates and reduction in the assimilates transported to the growing tissues. Thus, salt stress causes premature senescence (Munns and Tester 2008).

The plants respond to salinity with complex changes in their molecular and physiological status (Tomar et al. 2012; Mehta et al. 2010a, b). Synthesis of osmolities such as soluble carbohydrates, proteins and free amino acids is considered as the most effective salt-tolerance mechanism. The adaptability of plant species to high salinity by lowering tissue osmotic potential was accompanied by accumulation of these osmotic solutes. Osmolities also act as an osmoprotectors. Salt stress causes oxidative stress in plant tissues by increasing reactive oxygen species (ROS). Osmolities prevent oxidative stress by scavenging ROS (Gadallah 1999).

Secondary metabolites (carotenoids, tocopherols, phenolic compounds, tocotrienols) also play an important role in response of plants to environmental changes. One of the largest groups of these metabolities, phenolic compounds, being a structural component of cell walls, regulate growth and developmental processes and are involved in the plant response to known abiotic stresses (Cheynier et al. 2013; Gould and Lister 2006). Phenolic compounds are mainly synthesized from phenylacrylic acid which is converted into cinnamic acid (Parr and Bolwell 2000).

Soil salinity is a major threat for agriculture (Xu et al. 2000). Thus, studying the effects of salinity on plants and the development of salt-tolerant genotypes are important. The purpose of this study was to identify differences in the physiological and biochemical responses to the stress caused by NaCl by a comparative analysis of two wheat genotypes with contrasting salt tolerance.

Materials and methods

Plant material

Two winter wheat (*Triticum aestivum* L.) genotypes known as Saratovskaya-29 (salt tolerant) and Gyrmyzygul-1(salt sensitive) from the genebank of the Azerbaijan Research Institute of Crop Husbandry have been taken as a research material. Seeds uniform in size (38.5 ± 2.87 mg) were selected for the experiment. Seeds were germinated in darkness for 24 h at 25 °C on wet filter paper in Petri dishes. Germinated seeds then were covered with a filter paper to minimize evaporation and growth continued for 24 h at 25 °C. After 72 h seedlings were transferred to pots containing Knop nutrient solution and cultivation continued in the growth chamber at a photosynthetically active photon flux density of 80 µmol m^{-2} s^{-1} with 14 h/10 h (light/dark) photoperiod. After the emergence of the second leaf, plants were exposed to the sudden salt stress (except control) by supplementing the nutrient solution with 100 and 200 mM NaCl. Growth of seedlings continued at 25 °C, for 14 h (day) and at 22 °C for 10 h (night) for the next 7 days.

Determination of photosynthetic pigments

The photosynthetic pigments, chlorophyll *a* and *b* (chl *a* and chl *b*), carotenoids (car) are determined using spectrophotometric method. Chl *a*, chl *b* and car were extracted from leaves by 80% acetone. Amounts of chlorophylls *a* and *b* were estimated by measuring optical density of the extract at 663 and 645 nm, respectively (MacKinney 1941). Carotenoids were determined by measuring optical density at 440.5 nm (Wettstein 1957).

Determination of malondialdehyde

Malondialdehyde (MDA) was determined through the thiobarbituric acid (TBA) reaction described earlier (Kumar and Knowles 1993). The 500 mg of leaves were homogenized in 2 ml of 5% thrichloracetic acid (TCA). Homogenate was centrifuged during 10 min at 1000×*g*. The supernatant was added to 4 ml of medium containing 0.5% TBA and 20% TCA and the mixture was kept in the water bath at 95 °C for 30 min. Then the sample was transferred into the ice bath and after cooling precipitated at 1000 g for 15 min. MDA was determined by measuring optical density of the last supernatant at wavelengths of 532 and 600 nm.

Determination of soluble sugars

Soluble sugars were determined by the anthrone-sulfuric acid method (Fales 1951). The 100 mg of dry ground sample was dissolved in 10 ml of 80% ethanol. The samples were shaken for 24 h and filtered. The homogenate was centrifuged at 5000×*g* for 10 min, then 2.5 ml of anthrone reagent (2 mg/ml anthrone, dissolved in sulphuric acid) was added to 0.5 ml of supernatant and the mixture was kept in the water bath, warmed to 40 °C, for 30 min. After cooling, the absorbance of the sample was measured at 625 nm. The calibration curve was depicted using pure sucrose solution.

Determination of phenolic compounds

The phenolic compounds were determined using the method reported earlier (Folin and Ciocalteu 1927). 50 mg of leaves were dried and ground, dissolved in 80% ethanol, and incubated in the water bath at 40 °C for 30 min. The homogenate was centrifuged at 12,000×*g* for 10 min. The reaction mixture including 0.5 ml of supernatant, 2.5 ml of Folin–Ciocalteu reagent and 2 ml of Na_2CO_3 (75 g/l) was kept at room temperature for 2 h, and then the optical

density was measured spectrophotometrically at 765 nm. The calibration curve was depicted using Gall acid.

The experiments were performed in 3 replicates, mean values and standard deviations were estimated. Statistical analysis was performed using Microsoft Excel.

Results and discussion

The cell membrane is the first organelle to be affected by salt stress (Xu et al. 2010). Polyunsaturated fatty acids are among the main membrane lipid components susceptible to peroxidation during stress (Elkahoui et al. 2005). Membrane integrity is disrupted as the result of lipid peroxidation due to increasing membrane permeability (Mosahebeh et al. 2016). Lipid peroxidation in salt sensitive plants is more obvious compared with salt-tolerant ones. The formation of MDA is believed to be the indicator of lipid peroxidation (Radi et al. 2013; Mehta et al. 2010c).

In our study we used two genotypes of *Triticum aestivum* L. with different salt tolerance: salt tolerant Saratovskaya-29 and salt sensitive Gyrmyzygul-1. The amount of malondialdehyde increased in both sensitive and tolerant genotypes in response to NaCl stress. As shown in Fig. 1 a clear difference between two genotypes affected by 100 mM NaCl was not observed: compared with control, the amount of MDA increased ~ 2.0 and ~ 1.7 times in 100 mM NaCl-treated genotypes Saratovskaya-29 and Gyrmyzygul-1, respectively. However, at the high concentration of salt (200 mM NaCl) the differences were obvious between two genotypes. In the salt sensitive genotype Gyrmyzygul-1 at 200 mM NaCl, MDA increased ~ 3.5 times compared with control plants, whereas genotype Saratovskaya-29 showed 2.2-fold increase in MDA at 200 mM NaCl.

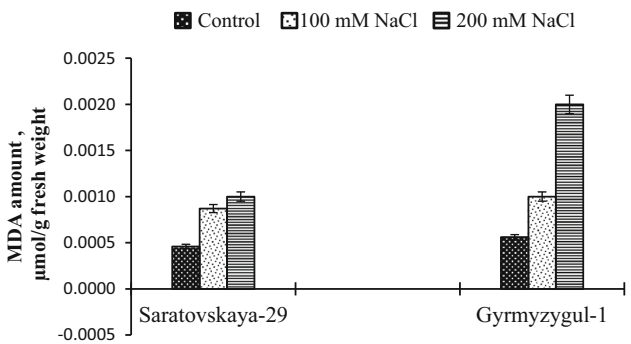

Fig. 1 Effect of different concentrations of NaCl on the malondialdehyde quantity determined in wheat genotypes with contrasting salt tolerance: salt tolerant Saratovskaya-29 and salt sensitive Gyrmyzygul-1. Quantitation of MDA is described in the section "Materials and methods"

Increased concentrations of Na^+ and Cl^- affect the content of photosynthetic pigments in plant leaves (Sabra et al. 2012). Long-term effect of salt causes the disruption of the formation of chlorophyll-protein-lipid complex (Akbari et al. 2012). Chlorophylls *a* and *b* carry out the absorption of visible light and excitation energy transfers to the photosynthetic reaction centers in the light phase of photosynthesis. The amount of chlorophylls in leaves has a positive correlation with the photosynthetic activity of plants (Salama et al. 1994). In our study, the quantity of photosynthetic pigments was determined in the 14 to 16-day-old seedlings, and the results presented in Fig. 2 shows that the total amount of chlorophyll and carotenoids slightly increased in both genotypes after treatment with 100 mM NaCl. In Saratovskaya-29, the content of Chl *a* increased approximately 2%, chl *b* 20% and carotenoids 30%. In Gyrmyzygul-1, the increase was 18% in the content of Chl *a*, 21% in the content of Chl *b* and 17% in the content of carotenoids. Different changes in pigment content were observed in 200 mM NaCl-treated plants. In the salt-tolerant genotype Saratovskaya-29 the amount of pigments was slightly higher (chl *a* and *b* ~ 3.5%, car ~ 15%) compared with control plants. However, in the salt sensitive genotype Gyrmyzygul-1at 200 mM NaCl the amount of pigments was significantly lower (the decrease was 18% in the content of chl a, 25% in chl b and 17% in the content of carotenoids) compared with control plants.

Different opinions concerning the effect of NaCl on the chlorophyll content in plants were reported earlier. Sabater and Rodriguez (1978) show that the depressive effect of NaCl on the chlorophyll biosynthesis can be due to the stimulation of chlorophyllase activity which causes chlorophyll degradation. According to Jaleel et al. (2008) the reduction in the amount of chlorophyll under stress could be ascribed instability of the pigment-protein complexes. In the presence of Na^+ and Cl^- ions the tonoplast permeability increases, and the acids of cell sap pass through chloroplast membrane and destroy chlorophyll. On the other hand, the reduction in the chlorophyll content is possible due to a decrease of ALA (5-aminolionic acid) synthesis (Santos 2004). This acid is a precursor of protochlorophyllide, which converts to chlorophyll under illumination. It was also shown that, the reduction in the content of chl *a* and *b* and carotenoids was correlated with the increase of Na^+ content in plant shoots, and the major ion causing the pigment reduction was Na^+ (Sabra et al. 2012).

The accumulation of soluble carbohydrates in plants has been widely reported as a response to drought or salinity (Popp and Smirnoff 1995; Murakeözy et al. 2003; Radi et al. 2013). In our study salt stress stimulated the accumulation of soluble sugars (Fig. 3A). The amount of soluble sugars noticeably increased in both studied genotypes

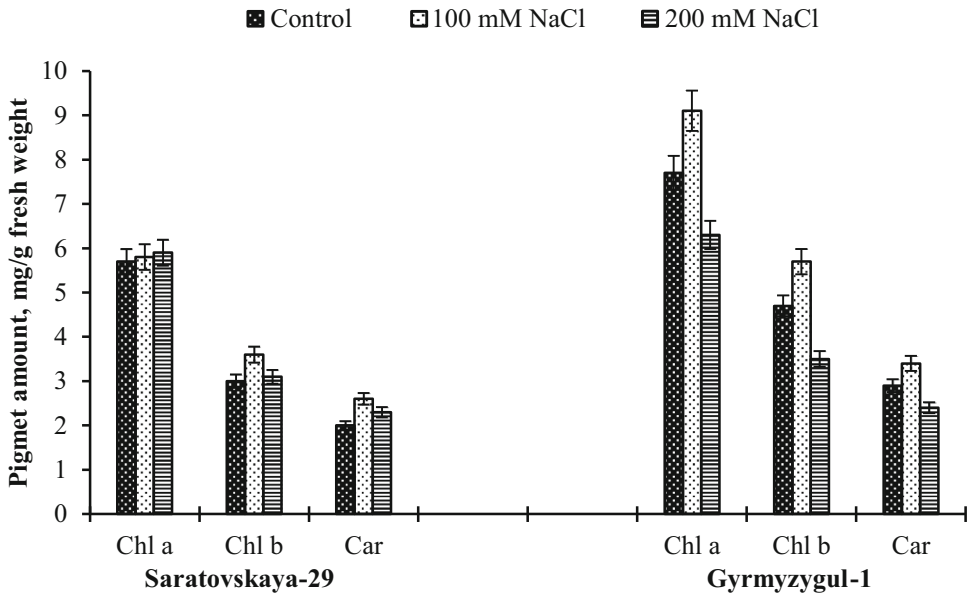

Fig. 2 Effect of different concentrations of NaCl on the amount of photosynthetic pigments Chl a and b, and carotenoids in wheat genotypes with contrasting salt tolerance: salt tolerant Saratovskaya-29 and salt sensitive Gyrmyzygul-1. Determination of pigments is described in the section "Materials and methods"

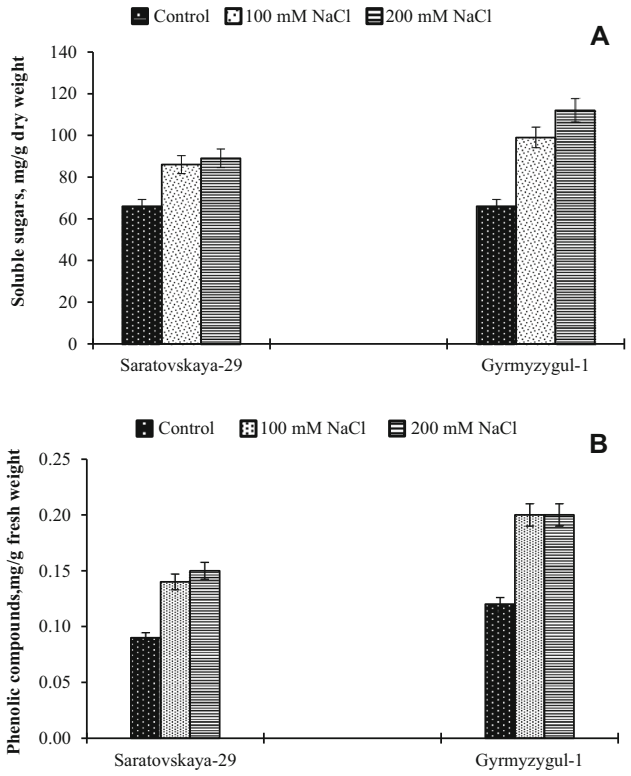

Fig. 3 Effect of different concentrations of NaCl on the amount of soluble sugars (**A**) and phenolic compounds (**B**) in wheat genotypes with contrasting salt tolerance: salt tolerant Saratovskaya-29 and salt sensitive Gyrmyzygul-1. Determination of sugars and phenols is described in the section "Materials and methods"

exposed to 100 and 200 mM NaCl. Compared with the control plants the increase in the amount of soluble sugars was 30–35% in salt tolerant Saratovskaya-29 and 50–70% in the salt sensitive Gyrmyzygul-1 genotype.

Biochemical responses have a tendency to correlate with physiological adaptations. An earlier study suggested that soluble sugars are involved in osmotic regulation of plants grown under salinity (Cheeseman 1988). According to Jouve et al. (2004) carbohydrates may also have an antioxidant function and protect the membrane stability. It has been shown that the increase in the content of monosaccharides might be due to the deceleration in sugar transfer from the mesophyll to phloem. Accumulation of soluble sugars under stress regulates osmotic regulation in the cell (Hellebusi 1976; Gadallah 1999), and contributes to the stability of cell structure and functions by interacting with macromolecules (Rhodes 1987; Smirnoff and Cumbes 1989).

Phenolic compounds such as phenolic acids, flavonoids and proanthocyanidins are involved in scavenging free radicals formed in the cells (Ksouri et al. 2007; Arora et al. 2000; Mehta et al. 2010c). The increase in ROS is mainly directly related to changes in the C chain, which has a strong effect on carbon-based secondary compounds, especially on biosynthesis of leaf polyphenols (Radi et al. 2013). Recent studies show that the synthesis of polyphenols depends on abiotic factors (Ksouri et al. 2008; Naffeti et al. 2011). Results presented in Fig. 3B show that NaCl at concentrations of 100 and 200 mM stimulates the synthesis of phenolic compounds in both salt-tolerant and salt-sensitive genotypes. The increase was higher in the salt sensitive Gyrmyzygul-1 genotype compared with the salt tolerant Saratovskaya-29. The results obtained in our study agreed with the results of previous studies, showing that the phenolic compounds increased more in sensitive plants (Radi et al. 2013; Keutgen and Pawelzik 2008). Our results agree with the reports showing an increase of the content of

phenolic compounds under salinity. The increase in phenolic compounds during germination and under salinity can be attributed to the response of seedlings to adverse environmental conditions (Cevallos-Casals and Cisneros-Zevallos 2010), or to oxidative stress caused by salinity (Turkan and Demiral 2009; Lim et al. 2012).

Summary

The results presented in this study show that salinity causes formation of secondary oxidative stress due to increasing amount of MDA in leaves. As seen from the results, lipid peroxidation was higher in the salt-sensitive genotype compared with the tolerant genotype. Phenolic compounds participate in plant responses to all types of abiotic stresses along with the growth and development processes. They accumulate in the cell against reactive oxygen species under salt stress. By increasing cell antioxidant activity, phenolic compounds participate in the inactivation of free radicals. The increasing amount of phenolic compounds in leaves also could be an indicator for plant response to oxidative stress. The accumulation of different amounts of soluble sugars in the studied salt-sensitive and salt-tolerant genotypes demonstrates their contribution to osmotic regulation under salt stress. Apparently, tolerant plants have more effective defense mechanisms for their normal growth and development under stress.

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