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SALINITY IMPACT ON PHOTOSYNTHETIC PIGMENTS, CHLOROPLAST ULTRASTRUCTURE AND CARBOHYDRATE POOL IN LEAVES OF TOMATO PLANTS

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ABSTRACT

Salinity is thought to be a limitation for growth of several economic crops worldwide. In this work we examine the effects of different concentrations of NaCl (0, 50 and 100 mM) on photosynthetic pigments, chloroplast ultrastructure and carbohydrate pool of two tomato cultivars differing in salt tolerance, a salt sensitive variety (GS12) and a salt tolerant cultivar (Adora). Salt stress caused a decrease in Chl a content and a significant increase in Chl b content, this effect being most pronounced in Adora leaves. GS12 chloroplasts were affected adversely due to salinity treatment as they appeared elongated with less garana and much developed starch grains. Meanwhile Adora chloroplasts seemed to tolerate salinity stress being oval in shape with well-stacked grana. Carbohydrate pool in both cultivars was decreased due to salinity treatment but 50 mM NaCl treated leaves in both cultivars showed increased total carbohydrate than that of control leaves. It is apparent that Adora plants tolerated salinity stress well as the photosynthetic machinery and chloroplast ultrastructure appeared to be able to accommodate with such stress in a very impressive manner.

Keywords: salt stress, total soluble sugars, chloroplast, starch, chlorophyll a

1. INTRODUCTION

Salt stress causes alterations in plant cell structure and function even if it occurred over a short period of time. This leads to reduced yield in arid and semi-arid regions across the globe (Mäkelä et al., 2000). During the past few years, our understanding of plant response to salinity has been significantly improved by studying model plants. Salt tolerance is believed to be affected by

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many different genes involved in different pathways, such ion selectivity, compatible solute synthesis and reactive oxygen species (ROS) scavenging (Zhu, 2003; Munns & Tester, 2008).

Cultivated tomato, one of the most important vegetable crops in the world, is moderately sensitive to salt stress. The existence of halotolerant accessions of several wild tomato species has made tomato a model crop for comparative studies on the mechanisms of salt tolerance. Previous studies have focused on the physiological and genetic characterization of wild tomato species in comparison with tomato cultivars (Sun et al., 2010).

Photosynthesis and carbohydrates of many plants are inhibited under salinity stress, and the sensitivity and tolerance against the NaCl concentration depend upon plant species, intracellular ionic status, and environment. Under salinity, net photosynthetic CO2 uptake decreases mainly because NaCl treatment decreases stomatal conductance, and consequently less CO2 is available for carboxylation reaction in the photosynthetic apparatus. Also, the rate of photochemical reactions is inhibited. NaCl stress also changes thylakoid membrane structure and decreases the contents of chlorophylls and carotenoids. The production and transport of saccharides in the plants are both affected by NaCl treatment (Khavari-Nejad & Mostofi, 1998).

In this study we investigate the effects of different concentrations of NaCl (0, 50 and 100 mM) on two tomato cultivars differing in salt tolerance, a salt-sensitive variety GS12 and a moderately salt-tolerant cultivar Adora, on photosynthetic pigments, chloroplast ultrastructure and carbohydrate pool.

2. MATERIALS AND METHODS

2.1 Plant material and growth conditions

This work contains the results of two large scale experiments which were conducted during the two consecutive late summer seasons of 2013 and 2014 in the botanical garden of the faculty of Science at Mansoura University, Egypt to examine the response of two tomato cultivars which are different in salt tolerance, a salt sensitive variety "GS12" and a moderately salt-tolerant cultivar "Adora", to different concentrations of NaCl (0, 50 and 100 mM). Adora seeds were purchased from Gaara seeds supplier in Egypt (Adora seeds are a hybrid known to be salt tolerant to 20 ppm soil salinity) while GS12 seeds were purchased from Syngenta seeds supplier in Egypt.

Sowing of seeds was done on the 4th of August, in 84 cells, foam trays filled with growing media of peat and vermiculite (1:1, v/v) and transplanted into pots containing 8 kg of clay-sandy soil (1:1, w/w) on 8th September on both growing seasons.

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Seedlings were watered with half strength Hoagland solution (Hershey, 1995) regularly in two days intervals until transplantation. After one week from transplantation, the three sets of plants were watered with Hoagland nutrient solution containing 50 or 100 mM NaCl while control plants were watered as before. Each treatment at each experiment was arranged in a completely randomized design with four replicates; each replicate included five plants (20 plants per treatment). Salinization took place every two weeks until the end of the experiment. Plants were watered with distilled water if needed in between salt treatments. Tomato plants were harvested and sampled after 49 d of the start of salt treatment. Leaves were harvested, oven-dried at 80˚C, ground to a fine powder and kept in dry place until use. Other leaves samples were treated with a fixative and were transferred for transmission electron microscopy investigation.

2.2 Chlorophyll and carotenoid determination

Chlorophylls (a, b) and carotenoids were determined spectrophotometrically in 80% acetone according to Arnon (1949) and Davis (1976).

2.3 Ultrastructural studies using transmission electron microscope (TEM)

To verify the different changes resulting in NaCl treatment, the fifth leaf of all GS12 and Adora plants were taken for transmission electron microscopy. Small parts (about 1 mm2) of freshly harvested leaves were cut with a sharp razor blade under 2.5% (v/v) glutaraldehyde. Leaf tissues were transferred to vials of 2.5% (v/v) glutaraldehyde in 1M phosphate buffer at pH 7.5 at 4°C for 24 h. Following fixation, the specimens were were embedded in gelatine capsules and left in an oven at 60°C for 60 h. The gelatine capsules were dissolved in boiling water for 1-2 h. Ultra-thin sections were cut on a Reichert ultra-microtome using glass knife. Silver or pale gold interference sections were picked up on the dull surface of formvar-coated 100 or 200 mesh copper grids (Juniper et al., 1970). The grids with sections were left on a clean filter paper to dry. Ultra-thin sections were stained by 2% aqueous uranyl acetate (Juniper et al., 1970). A drop of stain was put in a clean plastic Petri dish and the grids were gently floated, with the sections facing down, on a drop of the stain. The grids were washed by a stream of distilled water and then transferred to drops of lead citrate (Reynolds, 1963) which were placed on a wax plate in a Petri dish. Pellets of sodium hydroxide were placed in the Petri dish to remove carbon dioxide. The grids were left in lead citrate for 10-20 min and then rinsed by distilled water, dried under a bench lamp and stored in a grid box. The stained sections were examined and photographed with a JEOL 1010 transmission electron microscope at 80 kV in the Transmission Electron Microscopy Unit at Mansoura University.

2.4 Determination of Carbohydrates

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Carbohydrates were extracted from dry leaves of tomato plants in warm water. Concentration of total soluble sugars and starch were determined based on methods of Dubois et al. (1956) and Jeffries et al. (1998) respectively.

2.5 Statistical analysis

Data were subjected to one-way ANOVA analysis for each parameter. When the effect was significant ($P \leq 0.05$), differences between means were evaluated for significance by using the LSD test ($P \leq 0.05$).

3. RESULTS

Table 1 shows the amounts of the various pigment fractions namely: Chl a, Chl b, Chl a+b, Chl a/b, Car and total pigments in the leaves of the differently treated tomato plants after 49 d of the start of salt treatment. It is apparent that leaves of control tomato plants in both cultivars GS12 and Adora showed significant increases in all pigment fractions as compared with those of salt-treated plants. These changes were associated with a significant increase in Chl a/b ratio. On the other hand, in both cultivars it is apparent that salinity caused a decrease in Chl a but induced a significant increase in Chl b amount, this effect being most pronounced in Adora leaves. As for the amount of Car, in GS12 leaves there was a decrease at 50 mM NaCl and an increase at 100 mM NaCl treatment meanwhile in Adora leaves at both concentrations, there were significant increases above that of control (Table 1).

Table 1: The effect of different concentrations of NaCl on photosynthetic pigments (µg / g fresh weight) of salt sensitive GS12 and salt tolerant Adora tomato plants. * Mean values are significantly different from control at $p \leq 0.05$.

Parameters / Treatments		Chl a	Chl b	Chl a+b	Chl a/b	Car	Total pigments
GS12	Control	1.82	30.28	32.10	0.06	1.10	33.20
	50 mM NaCl	1.15*	15.08*	19.23*	0.28*	1.02*	20.25*
	100 mM NaCl	0.60*	17.28*	17.88*	0.03	2.05*	19.93*
Adora	Control	7.07	26.38	33.45	0.27	2.24	35.69
	50 mM NaCl	0.66*	24.91*	25.57*	0.03*	2.60*	28.17*
	100 mM NaCl	1.80*	37.45*	39.25*	0.05*	4.03*	43.28*

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Figure 1 shows the transmission electron micrograph of leaves of both cultivars GS12 and Adora control and treated plants. In GS 12 control leaves, the chloroplasts from mesophyll cells of leaves appeared in normal shape (oval or elliptical). These chloroplasts contained well-defined granal stacks with parallel thylakoids and few starch grains (Figure 1a). Figure 1b shows the chloroplast of 50 mM NaCl treated GS 12 leaves, in which the chloroplast appeared elongated with large starch grains and less granal stacks. Figure 1c shows how 100 mM NaCl adversely affected the chloroplast structure of the salt sensitive variety GS 12. The chloroplast appeared so elongated with few grana and a large starch grain occupying most of the stroma space.

Figure 1d shows the chloroplast of control Adora leaves with normal shape and well-defined grana with few starch grains. In 50 mM NaCl treated Adora plants; the chloroplast appeared quite oval with well-defined granal stacks and a moderate starch grain occupying the stroma (figure 1e). In Adora plants which are moderately tolerant to NaCl, 100 mM NaCl appeared to be well-tolerated by the chloroplasts of such variety, as the chloroplasts appeared spherical to oval in shape with increased number of starch grains and with quite moderate stacking of grana (figure 1f).

The pattern of changes in the amounts of the various carbohydrate fractions of the control as well as of the variously treated tomato plants are recorded in table 2. Total soluble sugars and starch of both cultivars were significantly decreased as compared with control plants, the following sequence of treatments was displayed Control > 50 mM NaCl > 100 mM NaCl for total soluble sugars and starch of both GS12 and Adora plants. As for total carbohydrates, the following sequence of treatments was displayed 50 mM NaCl > Control > 100 mM NaCl for both GS12 and Adora cultivars.

4. DISCUSSION

Salt stress is considered one of the most harmful effects that hinder plant growth in the field in arid and semi-arid regions of the world. Salinity causes deleterious effects to cell structure, function and may even in severe cases and severe sensitive species lead to cell death. Tomato plants being sensitive to salt stress have been widely studied to develop ways to overcome such stress and the losses that occur in the field if a wave of salinity or drought has struck a field of tomatoes (Barhoumi et al., 2007).

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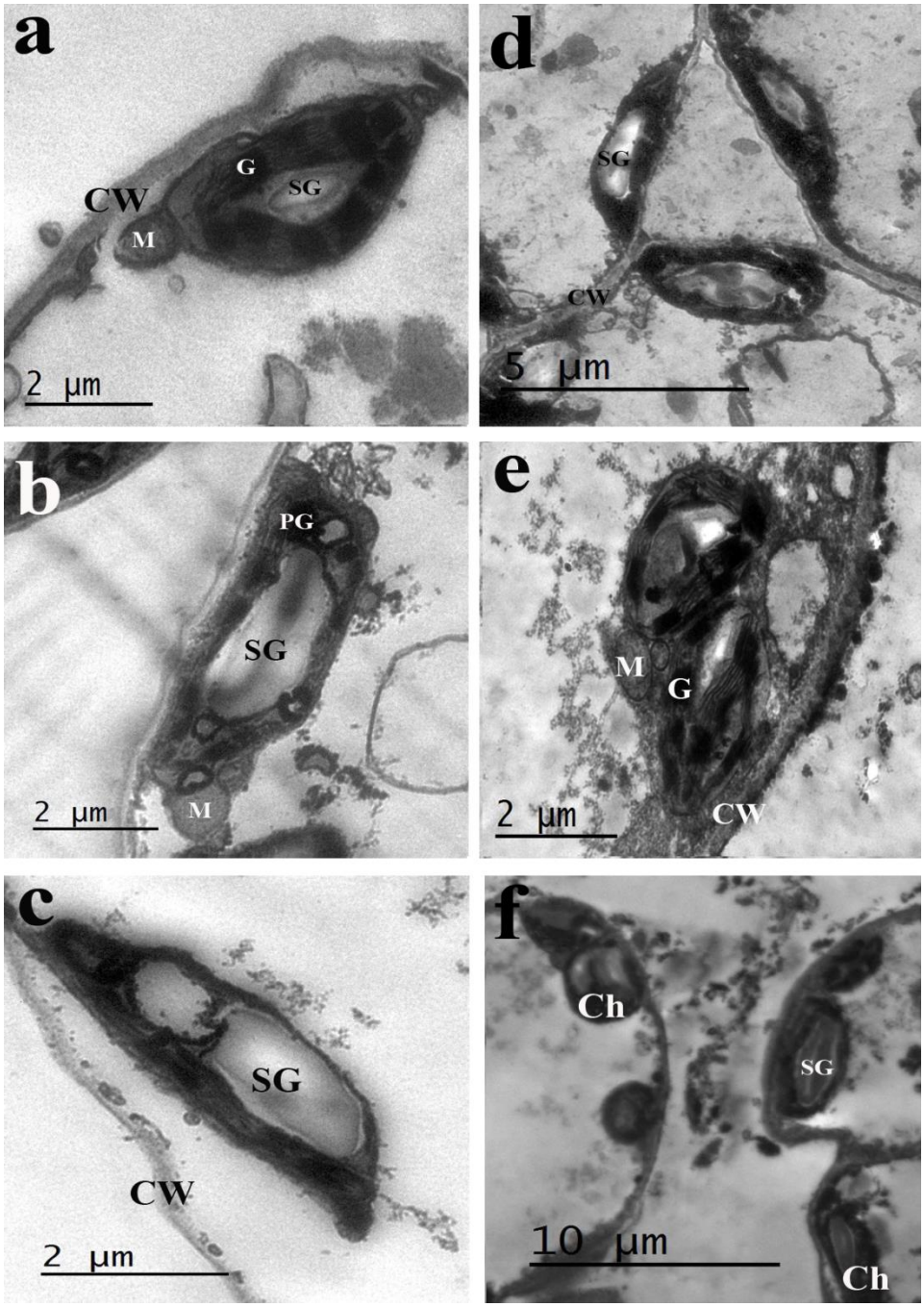

Figure 1: Transmission electron micrograph of chloroplasts of GS12 leaves (a) control, (b) 50 mM NaCl and (c) 100 mM NaCl, and Adora leaves (d) control, (e) 50 mM NaCl and (f) 100 mM NaCl. Ch: Chloroplast; CW: cell wall; G: grana; M: mitochondria; PG: plastoglobule; SG: starch grain.

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Table 2: The effect of different concentrations of NaCl on carbohydrate content (mg glucose equivalent/ 100 g dry weight) of salt sensitive GS12 and salt tolerant Adora tomato plants. * Mean values are significantly different from control at $p \leq 0.05$.

Parameters / Treatments		Total soluble sugars	Starch	Total carbohydrate
GS12	Control	25.38	10.01	35.39
	50 mM NaCl	24.82*	12.23*	37.05*
	100 mM NaCl	23.13*	7.27*	30.40*
Adora	Control	25.32	5.98	31.30
	50 mM NaCl	24.23*	11.74*	35.97*
	100 mM NaCl	23.07*	7.03*	30.10*

The ability to develop tolerant species of tomato is the main struggle of genetic engineering techniques nowadays. Tolerant species are now developed with moderate to high ability to sustain severe concentrations of salt stress. This approach opens up a new era in the future of salt stress studies and applications (Sun et al., 2010).

In our study, there appeared to be significant decreases in the photosynthetic pigments with special reference to Chl a and an increase in Chl b which could contribute to the behavior of such pigments under salinity. In such a way Chl a is not able to withstand salt stress with special reference to 100 mM NaCl which caused much decrease in Chl a. In a defensive way to accommodate with such negative effect, Chl b was increased in a manner to balance the Chl a/b ratio and to sustain such salinity to overcome the deleterious effects of NaCl. The net photosynthesis was decreased but the products of such activity were efficiently observed in chloroplasts of treated leaves. In accordance with our above mentioned results and arguments, Mäkelä et al. (2000) found that Chl a decreased as a consequence of salt stress while Chl b was more stable in their research on four tomato cultivars.

Chloroplasts were adversely affected with salinity in GS12 and were more stable in Adora plants. Chloroplasts of higher plants contain thylakoid membranes differentiated into cylindrical

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granum stacks of appressed (stacked) membranes which are surrounded by non-appressed (unstacked) helically organized stroma thylakoids (Mustárdy & Garab, 2003). The PSII and PSI complexes deferentially embedded in granum and stroma membranes (Danielsson et al., 2004), are organized into large supercomplexes with specific peripheral antenna complexes, chlorophyll a/b light-harvesting complexes. Changes in the degree of thylakoid membrane stacking observed in response to environmental factors, e.g. under variable salt stress conditions and are closely related to the rearrangement of chlorophyll–protein (CP) super complexes (Kirchhoff et al., 2004).

Salinity stress causes various structural alterations of chloroplasts. The most common one is the loss of grana, the decrease of the total thylakoid volume, and even the disintegration of thylakoids (after exposure to severe salinity) (Kutík et al., 2004). Increase in the size and number of starch inclusions in chloroplasts has been also depicted as a symptom of salinity stress in tomato (Miyake et al., 2006). The shape and size of photosynthetic organelles often change as well: chloroplasts become more rounded and sometimes swell up (Kutík et al., 2004). All the above mentioned alterations well support those alterations herein presented for chloroplasts of GS12 and Adora plants in response to salinity treatment (Figure 1a-f).

In support to the present results of carbohydrate pool, Kafiet et al. (2003) and Younis et al. (2008) observed a significant decrease in soluble sugars content of wheat and lettuce plants treated with NaCl. Furthermore, Timpa et al. (1986) found that the salt-stressed cotton plants showed two to three times greater amounts of carbohydrates (glucose and sucrose) over the values determined for the control samples. These carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration.

Thus, from our present results, we can conclude that Adora plants appeared to tolerate salinity more than GS12 plants this being evident from the chloroplast ultrastructure stability of Adora leaves. Also, the maintenance of the carbohydrate pool and the integrity of the photosynthetic machinery under salt stress were maintained to a much well developed manner in Adora leaves. From our study we can conclude that Adora tomato plants can be used as a tolerant variety in slightly salty soils with impressive yield abilities to be expected. Further studies in slightly salty soils are needed to observe the ability of Adora plants to grow and give good produce in such lands.

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