Genetics and Plant Physiology – 2014, Volume 4 (3–4), pp. 163–173
Special Issue (Part 2) – Conference "Plant Physiology and Genetics – Achievements and Challenges"
24 - 26 September 2014 – Sofia, Bulgaria

Available online at http://www.ifrg-bg.com

EFFECT OF SOIL SALINITY ON GROWTH, GAS EXCHANGE AND ANTIOXIDANT DEFENSE OF TWO *PAULOWNIA* LINES

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Received: *24 October 2014* Accepted: *13 January 2015*

Summary: This study was conducted to assess the tolerance of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4) to salt stress based on some physiological and biochemical parameters at the vegetative stage. The effect of salinity on gas exchange and activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and guaiacol peroxidase (GPOX, EC 1.11.1.7) in two year-aged *Paulownia* plants (*P. tomentosa x fortunei* - TF01 and *P. elongata x elongata* - T4) grown on different types of soil for a period of four months was investigated. The soil samples were collected from two areas of the village Belozem near Plovdiv city, Bulgaria. The sodium adsorption ratio (SAR) for the first and second type of soil was 0.187 and 0.856, respectively. It was found that the increase of soil salinity led to a more pronounced reduction of total dry biomass, leaf area, the total leaf area/leaf number ratio as well as the leaf K/Na ratio in TF01 than in T4. Salinity significantly enhanced the level of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), as well as the activities of SOD, CAT and GPOX in TF01. The increased enzyme activities in TF01 suggested that they might play a possible role in the tolerance of this line to salinity. With increasing salinity level, the net photosynthetic rate (P_N) and stomatal conductance (G_s) were reduced, while transpiration rate (E) increased insignificantly in both lines. Respiration rate (R_d) was enhanced in T4, but not in TF01. Water use efficiency (WUE) was not improved at high salinity level and the decreasing trend was more pronounced in TF01 than in T4. It was evident that soil salinity affected gas exchange, water relations and antioxidant defense in both lines in a different way.

Citation: Ivanova K., V. Dimitrova, T. Georgieva, Y. Markovska, 2014. Effect of soil salinity on growth, gas exchange and antioxidant defense of two *Paulownia* lines. *Genetics and Plant Physiology*, Conference "Plant Physiology and Genetics – Achievements and Challenges", 24-26 September 2014, Sofia, Bulgaria, Special Issue (Part 2), 4(3–4): 163–173.

Keywords: growth, antioxidants, photosynthesis, *Paulownia*, salinity.

INTRODUCTION

Natural or "primary salinity" is more widespread in arid and semi-arid regions of the world. It results from the accumulation of soluble salts in soils or groundwater over long geological periods. The occurrence of so-called secondary salt-affected soils is due to application of different

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agricultural practices and continues to grow. According to FAO Land and Plant Nutrition Management Service the total surface of the saline regions in the world is almost $397x10^6$ ha and 2% of them are salt-affected (Dajič, 2006).

Salinity in soil or water is one of the major negative factors of the environment, and especially in arid and semiarid regions, can severely limit crop production. That is why, the problem for feeding the population in the regions with natural salinity may be decided though improvement of salinity tolerance of important agricultural crops. In recent decades, investigations have been focused on studying the biochemical and physiological traits (Dajic, 2006; Munns and Richards, 2007; Koyro et al., 2014), identification of key genes and/ or discovering of distinctive molecular markers in a great number of crops (Pardo, 2010; Kosova et al., 2011).

Deeper soil pollution and salinization require using as an alternative fast-growing woody species with a deep root system and ability to grow on nutrient-poor soil. Some of them (poplar, willow, black locust, ash, alder, paulownia) are successfully used for remediation of substrates contaminated with inorganic (or organic) pollutants. Munns (2011) has proposed that increased salt tolerance of perennial species (such as woody species) used for fodder or fuel production is a key component in reducing the spread of secondary salinity, while increased salt tolerance of crops will directly improve production in soils with primary salinity. By using of salt tolerant species, areas of degradated soils can be reduced and change their purpose for cultivation of high-yielding plants producing woody biomass, bio-fuel or economic important bioactive products.

Woody species from the genus *Paulownia* (Paulowniaceae) are native from China. *Paulownia tomentosa* has been introduced into Asia, USA, Australia and Europe as a high-yielding plant (Woods, 2008). These trees can be used for the production of energy, paper pulp and wooden building materials. Over the last two decades, *Paulownia* species has been extensively studied due to its ability to uptake nitrates and land contaminants, namely heavy metals (Doumett et al., 2008; 2011; Miladinova- Georgieva, 2014).

Paulownia tomentosa (Thunb.) Stend, as well as other species, belonging to the genus *Paulownia* (*P.elongata, P.fortunei*) and hybrid (*P. fortunei* x *tomentosa)* are used for afforestation of Eastern Black Sea region of Turkey due to its high adaptive abilities combined with continuous growth and formation of abundant leaf biomass (Yavuzsefik et al., 2001).

Elaboration of a system of suitable physiological and biochemical markers can help understanding the mechanisms of salt resistance of *Paulownia*. Our preliminary investigations with five selected *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01, *P. elongata x fortunei* – EF02, *P. elongata x fortunei x elongata* - T2, *P. elongata x elongata* - T4, *P. elongata x kawakamii* - EK), grown *ex vitro* as hydroponic cultures at three levels of salinity – 50, 100 and 200 mmol l^{-1} sodium chloride (NaCl) solution showed different salt resistance. Net photosynthetic rate (P_N) was enhanced in TF01 and T4, whereas it was reduced in T2 and EF02. Stomatal conductance (G_s) and transpiration rate (E) were strongly reduced in the five lines with increasing

salinity levels. These results indicated that under salinity conditions TF01 line was characterized with the highest rate of photosynthesis as well as the lowest water use efficiency, while for T4 the tendency was opposite (Ivanova et al., 2014). Studies of salinity effects on *in vitro* and *ex vitro* growth of *Paulownia* species are scarce (Ayala – Astorga et al., 2010).

In the present study, a pot experiment was carried out to investigate the differences in growth and leaf K/Na ratio between *P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4 plants grown on two types of salinized soil. The effects of salinity on plant morphology, photosynthetic gas exchange, antioxidant production and protective enzyme activities were studied.

MATERIALS AND METHODS

Sampling site, plant materials and pot experiments

The sampling area was situated in the vicinity of the village Belozem near Plovdiv city, Bulgaria. A sampling strategy was carried out consisting of the collection of soil aliquots (about 10 kg) from the surface and at depths of 30 to 60 cm in two different locations of the area. The soil samples were combined (full weight about 60 kg), air dried, sieved through nylon mesh and then mixed with sand in a 3:1 ratio. The agrochemical characteristics of the two soil types are shown in Table 1.

Two-year-old plantlets of *P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4 derived from *in vitro* micropropagated seedlings as proposed by Ivanova et al. (2013) were initially cultivated in plastic pots (d=10 cm) filled with a peat-perlite mixture (2:1, v/v), placed in a greenhouse (temperature 25°C day/17°C night, relative humidity max 70%) and irrigated daily prior to being transplanted into pots.

Soil aliquots with 2.5 kg dry weight were used to fill 28 pots. One plantlet from each line was planted in a pot. All pots were adjusted daily by weight to 65% water holding capacity with tap water to maintain vigorous plant growth. There were seven replications of each treatment. The experiment was conducted in a glasshouse supplied with natural sunlight from 20th April to 7th July, 2013. The glasshouse temperatures ranged from 15°C to 35°C, and relative humidity varied between 40% and 65%. Plants were harvested at the end of the experiment.

Growth parameters

Seven plants from each treatment were harvested to determine dry mass of plants (leaf, petiole, stem) gravimetrically after heating at 60°C until a constant weight was obtained. Leaf area (LA) was calculated using the software program SigmaScan Pro 5. The following indices were calculated: total leaf area/leaf number (MLA).

Gas exchange measurements

Net photosynthetic rate (P_N, μmol m^{-2} s^{-1}), transpiration rate (Tr, mmol m^{-2} s^{-1}), respiration rate (R_d– μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (G_s, mol m^{-2} s^{-1}) were measured simultaneously using a portable gas analyzer Li-6400 (Li-Cor Inc., Lincoln, NE, USA), with a red-blue LED light source. Measurements were done in the late morning hours between 10:00 a.m. and 12:00 a.m. under standardized conditions (air temperature

25.0°C, humidity 50% inside the gas exchange cuvette, 1000 μmol m^{-2} s^{-1} PAR and 350 μmol ambient concentration of CO_2). Three to five fully developed, attached leaves of plants from each treatment were measured and averaged. The following indices were calculated: water use efficiency (WUE, μmol $mmol^{-1}$ = P_N/Tr).

Ion content

To determine the content of K^+ and Na^+ (mg g DW^{-1}), 0.25 g dry leaf samples were extracted after acidic digestion with Suprapur grade Fluka reagents and analyzed using an atomic absorption spectrophotometer (Perkin-Elmer 5000, UK). The K^+/Na^+ ratio was calculated based on the content of K^+ and Na^+.

Metabolite assays

For determination of MDA and H_2O_2 content, 0.15 g FW of leaves were homogenized in a mortar at 4°C with 0.1 % (w/v) trichloroacetic acid and centrifuged for 20 min at 15 000 x g (4°C).

For malondialdehyde (MDA) estimation, 0.5 ml of the supernatant were mixed with 0.5 ml phosphate buffer (pH 7.4) and after the addition of 1 ml 0.5 % (w/v) thiobarbituric acid dissolved in 20 % trichloroacetic acid, the samples were boiled for 30 min (Dhindsa et al. 1981). After rapid cooling of the samples in an ice-bath, the absorption was measured at 532 and 600 nm using the extinction coeficient 155 $mM^{-1}.cm^{-1}$ (Heath and Packer, 1968).

For hydrogen peroxide (H_2O_2) assay, 0.5 ml of the supernatant were mixed with 0.5 ml phosphate buffer (pH 7.4) and after the addition of 1 ml of 1 M KI, samples were incubated in the dark for 60 min and the absorption was measured at 390 nm. The content was calculated using a standard curve of H_2O_2 in the range 1–100 nmol/ml of H_2O_2 (Jessup et al., 1994).

Antioxidant enzyme assays

In order to prepare crude extracts for determination of the enzymes SOD, GPOX and CAT leaves were ground at 4°C with 0.05 g PVP and 4 ml of extraction buffer (100 mM potassium phosphate buffer, pH 7.8, 2 mM EDTA, 8 mM DTT) added to 0.3 g of tissue powder. The suspensions were centrifuged for 25 min at 15 000 x *g* (4ºC) (Yuan et al., 2002). All enzymes were assayed spectrophotometrically by tracing the changes in absorbance at 27°C using a UV-VIS spectrophotometer (Boeco S22, Germany) in a 3 ml reaction mixture as described below. In each case the reaction was initiated by the compound listed last. The assay conditions for all enzymes tested were modified from the referred sources to give optimum activities in leaf extracts from *Paulownia* plants.

SOD (EC 1.15.1.1): 50 mM Tris-succinate buffer (pH 8.2), 8 mM pyrogallol, 0.1 ml extract. The decomposition of pyrogallol was determined by following the increase in absorbance at 412 nm for 3 min (Marklund and Marklund, 1974).

GPOX (EC 1.11.1.7): 100 mM potassium phosphate buffer (pH 7.0), 20 mM quaiacol, 200 μl extract, 1 mM H_2O_2. The oxidation of quaiacol was measured by following the increase in absorbance at 470 nm for 2 min (Polle et al., 1994).

CAT (EC 1.11.1.6): 100 mM potassium phosphate buffer (pH 7.0), 50 μ1 extract, 15 mM H_2O_2. The decomposition of H_2O_2 was determined by following the decline in absorbance at 240 nm for 3 min (Aebi, 1984).

The protein content was determined after Lowry et al. (1951).

Statistical data analysis

Data are expressed as means ±SE. Comparison of means was performed by Fisher's LSD test ($P \leq 0.05$) after performing ANOVA analysis (Statgraphics Plus, v. 2.1).

RESULTS

On the basis of agrochemical characteristics the second type of soil might be determined as middle alkaline (Referative basic data, 2009). The content of exchangeable Na was 5.3 times higher and SAR was about 4.6 times higher for this soil type in comparison with the first non-saline soil (Table 1).

Growth parameters

The effects of salinity on some growth parameters (total dry mass, leaf area, total leaf area/leaf number) are shown in Table 2. Higher salinity gradually decreased total dry mass, leaf area and MLA. The reduction was more pronounced in TF01 than in T4.

Gas exchange measurements

The results showed that salinity increased R_d in TF01 by 26%, while in T4 this parameter was decreased by 33%.

Table 1. Basic agrochemical soil characteristics in the experimental soils.

Soil characteristics	Soil	
	NS-G(1)	S-A(2)
Soil type	non-saline	saline
Soil use	grassland	arable
CEC [meq 100 g^{-1} soil]	14.680	14.980
pH [H_2O]	8.000	8.920
Conductivity [mS cm^{-1}]	6.300	14.000
C_{org} [%]	0.980	1.920
Exchangeable K [meq 100 g^{-1} soil]	0.800	0.960
Exchangeable Na [meq 100 g^{-1} soil]	0.490	2.600
Sodium adsorption ratio (SAR) [meq 100 g^{-1}soil]	0.187	0.856

Table 2. Mean values ± SE (n=7) of total dry mass [g], leaf area [cm^2] (LA) and total leaf area/leaf number ratio [cm^2 $leaf^{-1}$] (MLA) of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4) grown on non-saline [NS-G(1)] and saline [S-A(2)] soils.

Lines	Total dry mass [g]	LA [cm^2]	MLA [cm^2 $leaf^{-1}$]
TF1	14.197±2.217^a	1281.12±271.95^a	128.11±24.72^a
T41	11.103±4.012^a	817.32±226.15^a	90.81±2.26^a
TF2	5.714±2.124^b	800.67±241.87^b	90.98±2.02^b
T42	8.270±4.486^a	575.33±275.67^b	65.38±3.32^b

Values with the same letter are not significantly different when means are separated by Fisher`s LSD test (P<0.1).

Net photosynthetic rate declined in TF01 by 24%, however, in T4 the change was negligible (by 2%). Transpiration rate rose insignificantly – by 5% and 9% in TF01 and in T4, respectively. Stomatal conductance was reduced by 83% and 56% in TF01 and T4, respectively. WUE declined in both lines, but in TF01 the decrease was more pronounced (by 28%) than in T4 (by 10%) (Table 3).

Ion concentrations and K^+/Na^+ ratio

The effects of salt stress on K^+ and Na^+ contents and the K^+/Na^+ ratio are shown in Table 4. The content of K^+ increased with increasing soil salinity in the leaves of both lines. Higher values were measured in T4 than that in TF01. The content of Na^+ in the leaves of both lines grown on different types of soil remained the same. The K^+/Na^+ ratio was reduced with increasing soil salinity in TF01, however, it was enhanced in T4**.**

MDA and H_2O_2 content

The lipid peroxidation level in fully developed leaves of both lines, measured as the content of MDA, is shown in Fig. 1A. The MDA content rose progressively due to salinity, but this increase was not significant in the leaves of TF01. The MDA content was higher in TF01 and T4, while H_2O_2 content increased more in TF01 and slightly in T4 (Fig. 1B).

Table 3. Changes in respiration rate (R_d– μmol CO_2 m^{-2} s^{-1}), net-photosynthetic rate (P_N - μmol CO_2 m^{-2} s^{-1}), transpiration (Tr- mmol H_2O m^{-2} s^{-1}), stomatal conductance (G_s - mmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE- μmol CO_2 $mmol^{-1}$ H_2O) in fully developed leaves of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4) grown on non-saline [NS-G(1)] and saline [S-A(2)] soils.

Lines	R_d	P_N	Tr	G_s	WUE
TF1	1.32±0.23[a]	10.50±2.09[a]	2.12±0.23[a]	20.80±3.45[a]	4.95
T41	1.91±0.35[a]	10.20±1.67[a]	2.09±0.35[a]	39.00±3.67[a]	4.88
TF2	1.67±0.37[a]	7.98±0.55[b]	2.23±0.38[a]	3.48±0.26[b]	3.58
T42	1.28±0.21[b]	10.00±2.69[a]	2.28±0.26[a]	17.20±2.32[b]	4.38

Data are expressed as means ±SE, where n=20-40. Comparison of means was performed by Fisher's LSD test ($P \leq 0.05$) after performing ANOVA analysis (Statgraphics Plus, v. 2.1).

Table 4. Effects of salt stress on K^+ and Na^+ contents (mg g^{-1} DW) and K^+/Na^+ ratio in fully developed leaves of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4) grown on non-saline [NS-G(1)] and saline [S-A(2)] soils.

Lines	K^+ content	Na^+ content	K^+/Na^+ ratio
TF1	10.4±1.2[a]	0.14±0.02[a]	74.29
T41	7.5±0.6[b]	0.14±0.01[a]	53.57
TF2	11.8±1.4[a]	0.19±0.02[b]	62.11
T42	10.7±0.9[a]	0.19±0.03[b]	56.32

Data are expressed as means ±SE, where n=5. Comparison of means was performed by Fisher's LSD test ($P \leq 0.05$).

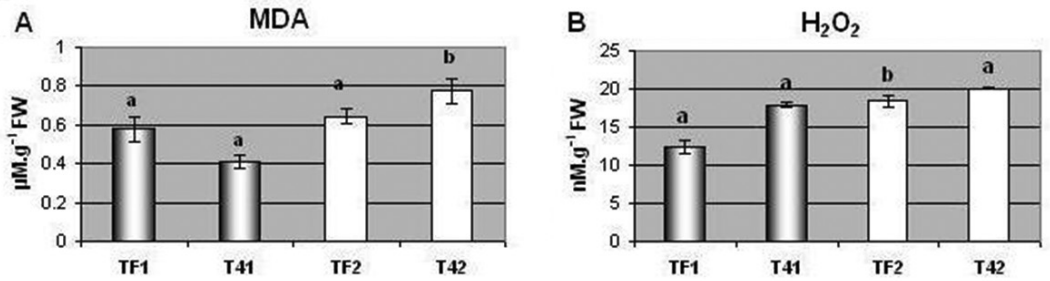

Figure 1. Changes in MDA (A) and H_2O_2 (B) in fully developed leaves of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4), grown on non-saline [NS-G(1)] and saline [S-A(2)] soils.

Activities of antioxidant enzymes

Antioxidant enzyme activities in fully developed leaves of both *Paulownia* lines showed differences in their response to soil salinity (Fig. 2A, 2B, 2C). SOD activity increased significantly in TF01, while in T4 it declined slightly (Fig. 2A). The same tendency was observed for CAT activity, but the changes found in T4 were significant (Fig. 2B). GPOX activity rose in both lines with increasing soil salinity. A lower increase in GPOX activity was observed in TF01 (Fig. 2C).

DISCUSSION

Increased soil salinity caused a marked reduction in vegetative growth of the *Paulownia* hybrid lines (Table 2). Our study showed that two-year old plants possessed different potential for growth. Plants from TF01 grown on non-saline soil had higher dry biomass, total leaf area and MLA ratio than those from T4. With increasing soil salinity the reduction of growth parameters of T4 was slight in comparison with TF01. Investigations have shown that plant growth is limited under salt stress (Maeda and Nakazawa, 2008). Usually lower concentrations of NaCl (50 mM l^{-1}) in

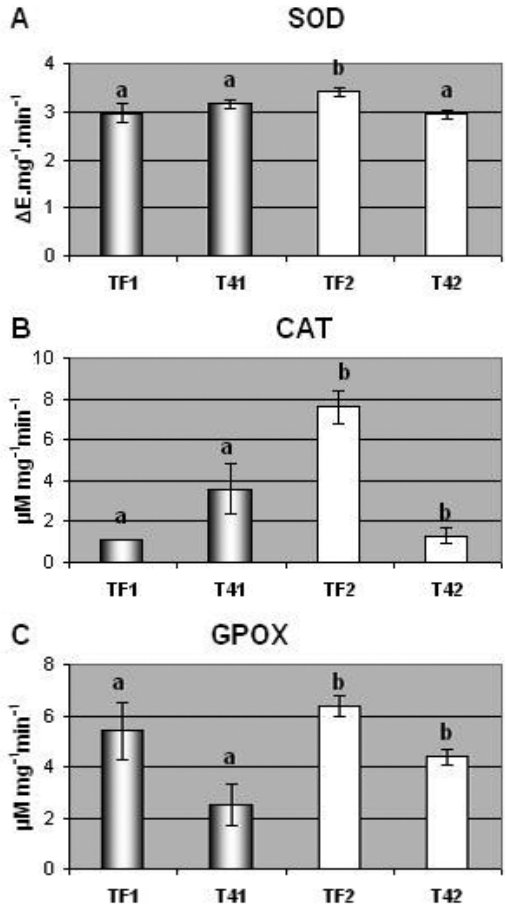

Figure 2. Changes in superoxide dismutase (A), catalase (B) and quaiacol peroxidase (C) activities in fully developed leaves of two *Paulownia* hybrid lines (*P. tomentosa x fortunei* – TF01 and *P. elongata x elongata* - T4), grown on non-saline [NS-G(1)] and saline [S-A(2)] soils.

hydroponic experiments stimulated, while higher concentrations (200 mM l^{-1}) reduced growth parameters (Sun et al., 2011). Our preliminary investigations with five selected *Paulownia* hybrid lines (TF01, EF02, T2, T4 and EK), grown as hydroponics at three levels of salinity – 50, 100 and 200 mmol l^{-1} NaCl showed that TF01 possessed the best growth parameters (total leaf area/ total dry mass ratio), while in T4 the growth parameters were worse. With increasing salt level (from 50 to 200 mmol l^{-1} NaCl) the MLA ratio was enhanced in T4, but declined in TF01 (Ivanova et al., 2014). In the rhizosphere, increasing salinity influenced plant growth and development by limiting the intake of water and nutrients from the soil. The salinity response of plants comprises two phases: 1/ rapid, osmotic phase, in which the growth of young leaves is inhibited; 2/ slower, ionic phase, in which the senescence of mature leaves is accelerated. The first phase starts with increasing the concentration of NaCl in the rhizosphere zone to a threshold of 40 mM for most plants. It is characterized with a decrease in the rate of shoot growth. In the second phase increasing Na^+ and Cl^- levels compete with nutrients such as K^+, Ca^{2+}, and NO_3^- and inhibit nutrient uptake or induce imbalances (Hu and Schmidhalter, 2005). In many studies it has been reported that salinity affects plant nutrients (Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-), growth, membrane integrity, osmotic adjustment, photosynthetic activity, and antioxidant activity (Yang et al., 2008; Dogan et al., 2010; Erdal and Çakirlar, 2014; Koyro et al., 2014).

Along with plant growth, photosynthesis is another essential physiological process affected by salt stress. Salinity reduced P_N and G_s in most plant species (El-Shihaby et al., 2002; Munns and Tester, 2008), but these parameters were enhanced under low salinity level - 50 mmol l^{-1} NaCl in *Periploca sepium* Bunge (Sun et al., 2011). Our results showed that P_N, G_s and WUE were reduced to a higher extent in TF01 than in T4 plants grown on saline soil (Table 3). The R_d values increased only in TF01. Contrary to these results, Ivanova et al. (2014) reported that TF01 line was characterized with enhanced net photosynthesis, a non-significant decrease of G_s, E and increased WUE in comparison with the other four lines under increased salinity levels in a hydroponic experiment. The results showed that the balance between water loss and CO_2 uptake helped to find a weak spot in the mechanism of adjustment of photosynthesis to high salinity. Biomass production of plants depended mainly on the ability to keep high net photosynthesis, minimum transpiration, high stomatal resistance, and minimum internal CO_2 concentration at their threshold salinity tolerance. In the case of *S. portulacastrum* net photosynthesis and WUE increased, but stomatal resistance decreased (Koyro, 2002).

Besides osmotic effects, salt stress affects plant growth and development by causing accumulation of ions in detrimental concentrations in tissues (Na^+ and Cl^-) and alterations in the nutritional content of essential ions (Ca^{2+} and K^+) (Rejili et al., 2007). Our results showed that K^+ and Na^+ content increased in mature leaves of the treated lines. The changes in K^+ and Na^+ concentrations affected the K^+/Na^+ ratio in both lines (Table 4). This ratio is an important selection criterion for salt tolerance (Morant-Manceau et al.,

2004; Ashraf and Orooj, 2006). The K^+/Na^+ ratio was decreased due to increasing salt concentrations in safflower cultivars grown hydroponically, but the highest values determined more tolerant to salt stress plant species (Erdal and Çakirlar, 2014). The values for the K^+/Na^+ ratio were higher in TF01 than in T4 (Table 4).

Peroxidation of membrane lipids, known as MDA, caused by salt stress has been reported in various species such as *Arabidopsis thaliana* (M`rah et al., 2006), *Vigna radiata* (Hayat et al., 2010) and *Periploca sepium* Bunge (Sun et al., 2011). T4 showed a higher increase in MDA content, but the increase in H_2O_2 content was lower in response to soil salinity (Fig 1A and Fig. 1B). Net photosynthesis remained unchanged in this line grown on saline soil.

Generally, salt tolerance is related to higher activity of antioxidant enzymes (Koyro et al., 2014). In our study, salt stress caused a significant increase in the activities of the examined antioxidant enzymes (SOD, CAT, GPOX) in TF01 and only in GPOX activity in T4 (Fig. 2A, 2B, and 2C).

In conclusion, assessment of the *Paulownia* hybrid lines responses to salt stress and development of more tolerant lines are important. The results of this study showed that salt stress affected more negatively growth, K^+/Na^+ ratio, photosynthesis and water use efficiency in TF01 than in T4 at the vegetative stage. However, TF01 line tried to withstand higher salinity conditions by upregulating protective mechanisms. Although TF01 and T4 showed similar results for accumulation of MDA and H_2O_2, TF01 exhibited a much better response in terms of increased antioxidant enzyme activities.

ACKNOWLEDGMENTS

This work was supported by grant 049/14 of Science Fund of University of Sofia, Bulgaria.

REFERENCES

Aebi H, 1984. Catalase *in vitro*. In: (Eds. Colowick SD, Caplan NO), Methods in Enzymol, 105, pp 120–126, Academic Press, New York.

Ashraf M, Orooj A, 2006. Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Truchyspermum ammi* [L.] Spraque). J Arid Environ, 64: 209–220.

Ayala-Astorga GI, Alcaraz-Meiendez L, 2010. Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seeman & Hemsley) grown *in vitro*. J Biotechn, 13(5): 1–15.

Dajic Z, 2006. Salt stress. In: (Eds. Rao SM, Raghavendra R, Reddy SJ), Physiology and molecular biology of stress tolerance in plants, pp 41–101, Springer, Dordrecht.

Dhindsa R, Plumb-Dhindsa T, Thorpe T, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot, 32: 93–101.

Dogan M, Tipirdamaz R, Demir Y, 2010. Salt resistance of tomato species grown in sand culture. Plant Soil Environ, 56: 499–507.

Doumett S, Lamperi L, Checchini L,

Azzarello E, Mugnai S, Mancuso S, 2008. Heavy metal distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere,72: 1481–1490.

Doumett S, Fibbi D, Azzarello E, Mancuso S, Mugnai S, Petruzzelli G, Del Bubba M, 2011. Influence of the application renewal of glutamate and tartarate on Cd, Cu, Pb and Zn distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study. Int J Phytorem: 13: 1–17.

El-Shihaby QA, Nemat Alla MM, Younis ME, El-Bastawisy ZM, 2002. Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or seawater – treated *Vigna sinensis* and *Zea mays* plants. Plant Biosyst, 136: 277–290.

Erdal SÇ, Çakirlar H, 2014. Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars. Turk J Biol, 38: 549–650.

Hayat S, Hasan SA, Yusuf M, Hayat Q, Ahmad A, 2010. Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. Environ Exp Bot, 69: 105–112.

Heath RL, Packer L, 1968. Photoperoxidation in isolated choroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys, 125:189–190.

Hu Y, Schmidhalter U, 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci, 168: 541–549.

Ivanova K, Tzvetkova N, Georgieva T, Markovska Y, 2014. Photosynthesis and growth responses of five *Paulownia* lines to salt stress. Compt. Rend. Acad. Bulg. Sci, 67: 1101–1106.

Jessup W, Dean RT, Gebicki JM, 1994. Iodometric determination of hydroperoxides in lipids and proteins. Method Enzymol, 233: 289–303.

Kosova K, Vitamivas P, Prasil IT, Renaut J, 2011. Plant proteome changes under abiotic stress – contribution of proteomics studies to understanding plant stress response. J. Proteom, 74: 1301–1322.

Koyro H-W, 2002. Ultrastructural effects of salinity in higher plants. In: (Eds. Läuchli A, and Lüttge M), Salinity: Environments – Plants - Molecules, pp 139–157, Kluwer Academic Publishers, Dordrecht, the Netherlands.

Koyro H-W, Huchzermeyer B, Zorb Ch, 2014. Effects of hyperosmotic salinity on protein patterns and enzyme activities, In: (Ed. Pessarakli M), Handbook of plant and crop physiology, pp 487–507, CRC Press.

Lowry OH, Rosenbough NJ, Farr AL., Randall RJ, 1951. Protein measurement with the Folin phenol reagent, J Biol Chem, 193: 265–275.

Maeda Y, Nakazawa R, 2008. Effects of the timing of calcium application on the alleviation of salt stress in the maize, tall fescue, and reed canarygrass seedlings. Biol. Plant, 52: 153–156.

Marklund S, Marklund G, 1974.

Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur J Biochem, 47: 469–474.

Miladinova-Georgieva, K., Growth and accumulation potential of two *Paulownia* lines, cultivated on heavy metal polluted soil, PhD Thesis, Faculty of Biology, University of Sofia, 2014.

Morant-Manceau A, Pradier E, Tremblin G, 2004. Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parential species under salt stress. J Plant Physiol, 161: 25–33.

M`rah S, Querghi Z, Berthomieu C, Havaux M, Jungas C, Haji M, Grignon C, Lachaál M, 2006. Effects of NaCl on the growth, ion accumulation and photosynthetic parameters of *Thellungiella halophila*. J Plant Physiol, 163: 1022–1031.

Munns R, 2011. Plant adaptations to salt and water stress: differences and commonalities, Adv Bot Res, 57: 1–32.

Munns R, Richards RA, 2007. Recent advances in breeding wheat for drought and salt stresses. In: (Eds. Jenks MA, Hasegawa PM, Jain SM), Advances in molecular breeding towards salinity and drought tolerance, pp 565–585, Springer, New York.

Munns R, Tester M, 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol, 59: 651–681.

Pardo JM, 2010. Biotechnology of water and salinity stress tolerance, Curr Opinion Biotechn, 21: 185–196.

Polle A, Otter T, Seifert F, 1994. Apoplastic peroxidases and lignification in needles of norway spruce (*Picea abies* L.). Plant Physiol, 106: 53–66.

Rejili M, Vadel AM, Guetet A, Neffatti M, 2007. Effect of NaCl on the growth and the ionic balance K^+/Na^+ of two populations of *Lotus creticus* (L.) (Papilinaceae). S Afr J Bot, 73: 623–631.

Referative basic data for the soils in Bulgaria, Agricultural Academy, Sofia, 2009 (in Bulgarian)

Sun JK, Li T, Xia JB, Tian JY, Lu ZH, Wang RT, 2011. Influence of salt stress on ecophysiological parameters of *Periploca sepium* Bunge. Plant Soil Environ, 57: 139–144.

Woods VB, 2008. *Paulownia* as a novel biomass crop for Northern Ireland? Publ. No 7, AFBI Hilsborough.

Yang CW, Shi DC, Wang DI, 2008. Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge). Plant Growth Regul, 56: 179–190.

Yavuzsefik Y, Cicek E, Cetin B, 2001. Discussion of foreign species in Turkey and early results of *Paulownia* seedlings in Duzce, Turk J Forestry Engineer, 4: 5–10.

Yuan YJ, Li C, Hu ZD, Wu JC, Zeng AP, 2002. Fungal elicitor-induced cell apoptosis in suspension cultures of *Taxus chinensis* var. *mairei* for taxol production. Process Biochem, 38: 193–198.