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Research article

Effects of salinity on the photosynthetic apparatus of two *Paulownia* lines

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ABSTRACT

The effects of soil salinity on the functional activity of photosynthetic apparatus and pigment composition of two *Paulownia* lines (*Paulownia tomentosa x fortunei* and *Paulownia elongata x elongata*) were investigated. PAM chlorophyll fluorescence measurements revealed that salinity leads to: (i) an increase of the photochemical quenching coefficient (qP) and the linear electron transport rate (ETR) in both lines of *Paulownia*, while the maximum quantum yield of the primary photochemistry of PSII in the dark adapted state (F_v/F_m) was unaffected; (ii) improved the efficiency of the photochemical energy conversion (Φ_{PSII}); (iii) an impact on the chlorophyll fluorescence decrease ratio (R_{Fd}), which correlates to the net CO_2 assimilation rate; (iv) an impact on Q_A^- reoxidation. The analysis of the kinetics of $P700^+$ reduction upon turning off far-red irradiation revealed that salinization lead to a delay of the cyclic electron transport around PSI in both studied lines as the effect on this process is more pronounced in *P. tomentosa x fortunei* than in (in comparison with) *P. elongata x elongata*. The present experimental results suggested high salt tolerance of the studied lines *Paulownia*, but *P. tomentosa x fortunei* is more tolerant to salinity than *P. elongata x elongata*. Molecular mechanisms involved in the *Paulownia* response to the soil salinity are discussed.

1. Introduction

Soil salinity is one of the most important environmental problems in the world, which limits plant growth and development and thus decreases the crop yields (Krasensky and Jonak, 2012). Today about 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinization (Zhu, 2001). Salinization has a major impact on photosynthesis, which is substantial for plant growth and development (Ioannidis et al., 2009; Ashraf and Harris, 2013). The decrease of the efficiency of the photosynthesis is a result from structural changes in the photosynthetic apparatus. Electron microscopic studies have shown that increasing the amount of the salts leads to destruction of the chloroplast envelope, damage to stromal thylakolds and a disintegration of the granal thylakoid systems (Shu et al., 2012). All these changes are associated with a decrease in the amount of the chlorophylls and carotenoids (Misra et al., 1997). The decrease in the amount of the pigments depends on the degree of salinity, its duration and plant species (Ashraf and Harris, 2013).

Many plants have mechanisms either to exclude salts from their cells or to endure their presence in the cells (Parida and Das, 2005). The salt concentrations, which are harmful for one plant species, could not be stressful for another (Krasensky and Jonak, 2012; Khan et al., 2015). In addition, differences in the polypeptide composition of the most sensitive place in the photosynthetic apparatus, photosystem II (PSII) complex, were detected (Wang et al., 2009). The study of rice varieties with different sensitivity to high salinity revealed that amounts of 43 kDa (polypeptide internal antenna PSII) and 33 kDa (polypeptide of the oxygen evolving system) are greater in the salt-resistant in comparison to the salt-sensitive plants. The different sensitivity of the plant species and different

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Abbreviations: EE_0, *Paulownia elongata x elongata* grown in soil type 0; EE_1, *Paulownia elongata x elongata* grown in soil type 1; EE_2, *Paulownia elongata* x elongata grown in soil type 2; TF_0, *Paulownia tomentosa x fortunei* grown in soil type 0; TF_1, *Paulownia tomentosa x fortunei* grown in soil type 1; TF_2, *Paulownia tomentosa x fortunei* grown in soil type 2; ETR, linear electron transport rate; Fv/Fm, maximum quantum yield of PSII photochemistry; Fv'/Fm', effective quantum yield of PSII photochemistry; Φ_{PSII}, the effective quantum yield of photochemical energy conversion of PSII; qN, non-photochemical quenching coefficient; qP, photochemical quenching coefficient; R_{Fd}, chlorophyll fluorescence decrease ratio.

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experimental conditions are the reasons for the contradictory results concerning the effects of the high salt concentration on the PSII. Some studies have shown that salt stress inhibits the PSII activity (Loreto et al., 2003; Akram and Ashraf, 2011) whereas others have demonstrated that activity of PSII is unchanged at a high concentration of salts (Lu et al., 2002, 2003).

A widespread approach to decrease of the soil salinity is phytoremediation, which is low-cost and low-impact technology, which receives increasing attention in the last several years. Plants, used for phytoremediation of polluted soils, should be accumulated high amounts of these pollution, should be tolerant to them and should also produce great quantity of biomass in contaminated conditions (Clements et al., 2002; Hsiao et al., 2007). In recent years, species belonging to the genus *Paulownia* are used for phytoremediation. *Paulownia* trees are native to China, but they are characterized with high tolerance to various environmental conditions, which make them attractive to other countries (Wang and Shogren, 1992). It has been found that the sensitivity to salinization varies in different *Paulownia* species (Ayala-Astorga and Alcaraz-Melendez, 2010; Ivanova et al., 2014a,b). The investigations with *Paulownia imperialis* and *Paulownia fortunei* show that chlorophylls, β-carotene and violaxanthin are significantly decreased after exposure to higher sodium concentrations (Ayala-Astorga and Alcaraz-Melendez, 2010). In addition, an influence on the protein content, lipid peroxidation and proline was registered. Recently it has been also shown that the salinity leads to increase of the activity of the antioxidant enzymes as well as influences on the total dry biomass, leaf area, stomatal conductance and transpiration rate in different hybrid lines of *Paulownia* (Ivanova et al., 2014 a,b).

The aim of the present study is to assess the effect of soil salinity on the activity of the photosynthetic apparatus and pigment composition of two new hybrid lines *Paulownia* (*Paulownia tomentosa x fortunei* and *Paulownia elongata x elongata*), which it will enable to assess their salt tolerance and to assess the possibility of their application for remediation of saline soils. The obtained results provide new information for molecular mechanisms involved in the response of *Paulownia* to high salinity.

2. Materials and methods

2.1. Plant material and soil sampling

Two hybrid lines of *Paulownia: P. tomentosa x fortunei* (*TF_0, TF_1, TF_2*) and *P. elongata x elongata* (*EE_0, EE_1, EE_2*) were chosen as plant material for this study. The plants were grown on non-saline (type 0) and two saline soils (type 1 and 2). The indices denote the type of the soil on which plants are grown. The plants were grown in a greenhouse under controlled conditions (temperature 30 °C day/15 °C night, relative humidity 40%–65%). Details for the cultivation of the plants are given in Ivanova et al. (2014a). For the experiments mature leaves from two-year-old plants were used. *Paulownia* seedlings were obtained from the Bulgarian company “Bio Tree”.

The soils used in this study were taken from an area located in the vicinity of the village Belozem, Plovdiv region, Bulgaria (42° 20′ N, 25° 3′ E). A sampling strategy was carried out from the surface and at depths of 30–60 cm in different locations of the areas. As a control soil (type 0) peat moss-perlite (2:1, v:v) was used. The characteristics of the soils are given in Table 1. The analysis of the soils was made by the Institute of Soil Science “Pushkarov”, Sofia, Bulgaria.

2.2. Pigment analysis

The pigments were extracted from leaves with 80% acetone. Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*) and carotenoids (Car) were determined spectrophotometrically according to the equations developed by Lichtenthaler (1987). Measurements of the pigment composition were made on Specord 210 Plus, Edition 2010 (Analytik Jena AG, Germany).

2.3. Room temperature chlorophyll a fluorescence

Chlorophyll *a* fluorescence was measured on leaf discs by PAM fluorimeter (H. Walz, Effeltrich, Germany, model PAM 101-103). Leaves were dark adapted for 30 min. The F_0 level was measured at instrument frequency of 1.6 kHz and measuring beam set at 0.02 μmol m^{-2} s^{-1} PFD. For evaluation of maximal fluorescence level, saturating flashes of 2500 μmol m^{-2} s^{-1} PFD were provided by Schott lamp KL 1500 (Schott Glaswerke, Mainz, Germany). The actinic light illumination (250 μmol m^{-2} s^{-1} PFD) was provided by second Schott lamp KL 1500 for induction of the photosynthesis. The time interval between two consecutive flashes was 60 s. The steady state level (Fs) was estimated after 9–10 min after turn on of the actinic light. The following parameters were calculated: the maximum quantum yield of PSII photochemistry, $F_v/F_m = (F_m - F_0)/F_m$ (Kitajima and Butler, 1975); the effective quantum yield of PSII photochemistry, $Fv'/Fm' = (Fm' - F_0')/Fm'$ (Rohacek, 2002); the effective quantum yield of photochemical energy conversion of PSII, $\Phi_{PSII} = \Delta F/F_m' = (F_m' - F_s)/F_m'$; the photochemical quenching coefficient, $qP = (F_m' - F_s)/(F_m' - F_0')$, (Schreiber et al., 1986); the non-photochemical quenching coefficient, $qN = (F_v - F_v')/F_v$ (Bilger and Bjorkman, 1990), the linear electron transport rate $ETR = \Phi_{PSII} \times PFD \times 0.5$ (Genty et al., 1989). The chlorophyll fluorescence decrease ratio R_{Fd}, $R_{Fd} = F_d/(F_m - F_d)$ where F_d is fluorescence decrease from F_m to steady state chlorophyll fluorescence, was measured using continuous saturated light (Lichtenthaler et al., 2005). The half-times of decay components of the variable chlorophyll fluorescence relaxation after excitation by a saturating light pulse in dark adapted leaves also were determined.

2.4. P700 redox state measurements

The redox state of P700 was investigated on leaf discs with a dual wavelength (820 nm) unit (Walz ED 700DW-E) attached to a PAM101E main control unit in the reflectance mode. The measurements were made as in (Dankov et al., 2009).

2.5. Statistical analysis

The results were mean values from seven independent experiments. The statistical differences between the means were determined using ANOVA. Values of $P < 0.05$ were considered as significant differences.

3. Results

3.1. Pigment composition

The pigment composition of studied lines of *Paulownia* in non-saline and saline soils is shown in Table 2. Our results revealed that the amount of the chlorophylls in *TF* line is increased in the plants grown in saline soils in comparison to those grown in non-saline soils, while in the other studied line (*EE*) changes in the chlorophyll amount are not detected. The increase of the chlorophylls in *TF* was similar in both saline soils (type 1 and type 2). Data also showed that salinization does not influence on the carotenoid content in both studied lines of *Paulownia*. The variation in the chlorophyll content in *TF* line was accompanied by an increase of the Chl *a*/*b* ratio and decrease of the Car/Chl ratio.