INFLUENCE OF SALT STRESS ON *EX VITRO* GROWTH AND ANTIOXIDATIVE RESPONSE OF TWO *PAULOWNIA* CLONES

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The morphological response of Paulownia tomentosa x fortunei clone TF 01 and Paulownia elongata x fortunei clone EF 02, grown in hydroponic at three levels of salinity, 50 mmol/l , 100 mmol/l, 200 mmol/l NaCl was compared. The content of malondialdehyde (MDA) was enhanced with increasing of salt stress. Activity of phenylalanine ammonia - lyase (PAL), which participate in the control of phenolic metabolism, was increased more in the leaves of Paulownia elongata x fortunei. Under salt stress phenolic and flavonoid contents were increased in the leaves of both clones selected. The results were discussed from the view of changes in synthesis of secondary metabolites and participation of separate classes of them in antioxidative response, characterizing different salt tolerance of Paulownia tomentosa x fortunei and Paulownia elongata x fortunei.

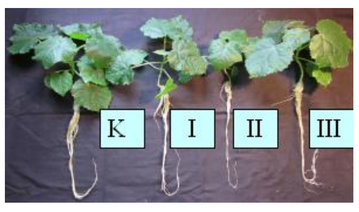
Paulownia – promising investment

Materials and methods

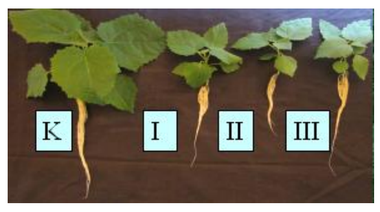
***Plant material*. For induction of shoots, explants are cultured on Murashige and Skoog (MS) nutrient medium included 2.5% (w/v) sucrose, 0.8% (w/v) agar and vitamins. For a multiplication of shoots MS medium was used supplemented with 4.439 µM 6-benzylaminopurine (BAP) and 0.537 µM indolilacetic acid (IAA). The medium included 3.0% sucrose (w/v), 0.8% agar and vitamins. After multiplication the shoots were transferred to rooting medium based on half strength basal salts MS medium, 2 % sucrose, 6 % agar and vitamins supplemented with 4.92 µM indole-3-butyric acid (IBA) and 1.075 µM IAA. The pH of all media was adjusted to 5.7 using 0.1 N HCl and 0.1 N NaOH before autoclaving. All cultures were incubated under controlled conditions – 16 h photoperiod, light intensity of 35 µmol m-2 s-1 and 24/18 1ºC day/night temperature.**

***Hydroponic experiment*. The experiments were set as four treatments including control, each treatment with 3 replications. The uniform explants were selected and transplanted to polyethylene vessels containing 1.2 l of 1/4 Hellriegel solution with an addition of A-Z microelements after Hoagland (pH 5.9) in growth chamber(with a 16-h photoperiod (PAR 100 µmol m-2 s-1 on the upper leaf surface, 25/23 1 ºC day/night temperature, relative humidity 60/70%). Each vessel contained two explants which represented one replication. After 21 days of cultivation the plants were transferred to 1/2 Hellriegel solution with the addition of A-Z microelements (pH 5.9). The salt treatment was applied on the 48th day after transplanting of explants when the plants had adapted to the conditions of 1/2 Hellriegel nutrient solution and 0 (control), 50, 100, and 200 mM/l NaCl was added. The solutions were aerated every day and were changed every 3 d to prevent depletion of nutrients and NaCl. Plants were harvested after 10 d of treatment. At the end of the experiment the plant samples were collected, washed with tap water and rinsed with distilled water before being separated into leaf, petiole, stem and root and fresh mass of each plant sample were measured gravimetrically. Dry mass of shoots and roots were determined after oven-drying (60oC) for 2 days until constant weight was obtained. Leaf area was calculated using software program.**

***Enzyme and metabolite assays*. For measurements of phenylalanine ammonia-lyase (PAL) the method of Yuan et al. (2002) were used. Protein content was measured with Folin reagent (Lowry et al., 1951). For determination of malondialdehyde (MDA) the method of Dhindsa et al. (1981) were used. For determination of the phenols and flavonoids, dry samples from third basal leaf of plants at each salt treatment were ground and exhaustively extracted with 96% (v/v) methanol. Total phenolics were determined by the Folin & Ciocalteu's colorimetric method (Pfeffer et al.,1998). The absorbtion was measured at λ=730 nm and the total phenolics were calculated by means of a calibration curve of chlorogenic acid (in the range of 30µg/ml to 100 µg/ml) and expressed as mg of chlorogenic acid equivalent per 1 g DW of the sample. Total flavonoids content of the whole shoot samples of the plant was measured using a colorimetric assay (Zhishen, 1999). The total flavonoids of the samples were expressed in mg of (+) catechin equivalent per 1 g DW of the sample. All measurements were performed in triplicate with three repetitions.**



Paulownia elongata x fortunei clone EF 02



Paulownia tomentosa x fortunei clone TF 01

High salinity is the most widespread abiotic stress and constitutes the most stringent factor in limiting plant distribution and productivity Salinity affects plant growth, metabolism and photosynthetic efficiency of crop plants. The study of plant stress tolerance is suggested for understanding and transfer of tolerance traits from tolerant species to sensitive crop plants in future.

***Paulownia* is native from China. *Paulownia tomentosa* has been introduced into USA and Europe as an ornamental plant and is still widely used for this purpose. Trees introduced in Bulgaria reach 12 m average height and 13.4 cm average diameter during 7 years. Over the last two decades *Paulownia* species has been extensively studied due to its ability to uptake nitrates and land contaminants, namely heavy metals. This high-yielding tree can be used for the production of energy, paper pulp and wooden building materials. The genetically tissue–cultured *Paulownia* seedlings produced by The World Paulownia Institute (WPI) allow production of biofuels after introducing of cultivars without detrimental impacts on food supply or the environment. Research on *in vitro* propagation of *P. elongata* and *P. fortunei* has been reported. Application of this technology for micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody biomass production and it is effective way to maintain the genetic gain.**

Plants used in the current paper are propagated and rooted according technology registered of Biotree Ltd., Bulgaria. This laboratory is largest producer and supplier of genetically superior *Paulownia* tissue-cultures – *in vitro* seedlings. The farmers preferred *Paulownia tomentosa* x *fortunei* clone TF 01 due to fast development a uniform regular growth. *Paulownia elongata x fortunei* clone EF 02 is less branchy for the purpose of wood material formation. There is no information about its tolerance to salt stress and possibilities to improvement of saline soil utilization with these clones. The results derived from tissue cultures can be used to predict the responses of plants to environmental contaminants and to improve the design and thus reduce the cost of subsequent conventional whole plant experiments.

In this research, the effect of NaCl on growth, activity of phenylalanine ammonia - lyase, phenolic and flavonoid contents in leaves of *Paulownia tomentosa x fortunei* clone TF 01 and *Paulownia elongata x fortunei* clone EF 02, grown in hydroponic after transfering the explants were compared so as to provide fundamental base for vegetation restoration in contaminated soils.

Results and discussion

***Effect of salt stress on plants growth*. In our study, with increasing salinity levels, the root and stem length, leaf number and total leaf area in both plants were reduced (Table 1). The root and stem length of Paulownia tomentosa x fortunei clone TF 01 was reduced more than that of Paulownia elongata x fortunei clone EF 02. The leaf number of Paulownia tomentosa x fortunei clone EF 02 rose, but total leaf area declined sharply with increasing salinity levels. With increasing salinity levels total leaf area was reduced by 40%; 28% and 36%, respectively, compared to control. Total leaf area showed the capability of a plant in forming of photosynthetic surface.**

***Effect of salt stress on plants malondialdehyde*. MDA concentration was enhanced with increasing salinity levels, the maximum values was observed at 200 mM/l NaCl (Table 2). The results indicated that salt stress produced more reactive oxygen species, resulting in more increased lipid peroxidative products and oxidative stress in Paulownia tomentosa x fortunei clone than in Paulownia elongata x fortunei clone.**

***Effect of salt stress on plants secondary metabolism*. PAL is involved in the control of phenolic metabolism and provides precursors for lignin biosynthesis. It is known that the level of PAL are affected by age, light, phytochrome, wonding, infection. Our results showed that PAL activity in the leaves of Paulownia elongata x fortunei was three time higher compared to that in the leaves of Paulownia tomentosa x fortunei (Fig. 1). PAL activity was enhanced in both plants with increasing salinity levels. Flavonoids and phenolic substances isolated from wide range of vascular plants, act in plants as antioxidants and antimicrobials. Our results showed that PAL activity, phenolic and flavonoid content rose with increasing salinity levels. The maximum values were observed at 100 and 200 mM/l NaCl. Phenolics including various flavonoids play pivotal roles in absorbing free radicals, quenching singlet oxygen, and decomposing peroxides. Paulownia elongata x fortunei clone TF 01 is more tolerant to salt stress than Paulownia tomentosa x fortunei clone EF 02. It is characterized with smaller reduction of root and stem length, total leaf area and higher PAL activity, total phenolic and flavonoid content in the leaves.**

Table 1. Mean values SD (n = 5-6) of root and stem length, leaf number and total leaf area of Paulownia tomentosa x fortunei clone TF 01 and Paulownia elongata x fortunei clone EF 02, grown in hydroponic in response to salt stress

Treatments	Root length [cm]	Stem length [cm]	Leaf number	Leaf area [cm²]
Paulownia tomentosax fortunei				
Control	28.25±2.12b	8.57±0.91b	12±1.7b	429±16b
50mM/l NaCl	19.06±2.87a	6.13±0.71a	8±0.6a	126± 5a
100 mM/l NaCl	21.71±3.29a	6.53±0.64a	9±1.5a	109±22a
200 mM/l NaCl	18.31±3.56a	5.63±0.66a	11±1.5b	87± 4a
Paulownia elongate x fortunei				
Control	37.51±2.12b	10.51±0.51b	10±0.8a	502±39b
50mM/l NaCl	26.81±1.85a	8.41±0.33a	9±1.1a	300±40a
100 mM/l NaCl	26.71±1.61a	9.11±1.91a	8±1.2a	360±23a
200 mM/l NaCl	27.71±1.67a	10.11±1.51b	8±0.7a	321±67a

Table 2. Mean values SD (n = 5-6) of MDA content (in nM/gFW), determined in leaves of *Paulownia tomentosa x fortunei* clone TF 01 and *Paulownia elongata x fortunei* clone EF 02, grown in hydroponic in response to salt stress

Treatments	*Paulownia tomentosa x fortunei*	*Paulownia elongata x fortunei*
Control	0.017±0.000b	0.017±0.000b
50 mM/l NaCl	0.298±0.005a	0.146±0.021a
100 mM/l NaCl	0.281±0.034a	0.128±0.024a
200 mM/l NaCl	0.396±0.031c	0.191±0.013c

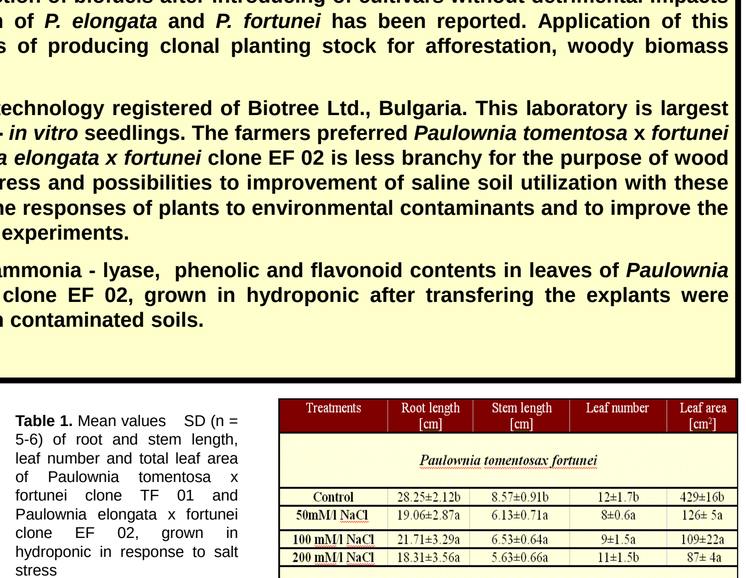


Figure 1. Changes in the PAL activity, phenol and flavonoid content, determined in leaves of *Paulownia tomentosa x fortunei* and *Paulownia elongata x fortunei* clones, grown in hydroponic in response to the addition of NaCl

THE TOLERANCE OF PAULOWNIA ELONGATA X FORTUNEI CLONE TF 01 FOR HIGH-SALINITY ENVIRONMENTS MAKES IT A POSSIBLE CANDIDATE FOR STUDYING THE MOLECULAR MECHANISMS BY WHICH PLANTS RESPOND TO SALINITY STRESS. CHANGES IN THE LEVELS OF SOME NONENZYMATIC ANTIOXIDANTS, SUCH AS PHENOLICS AND FLAVONOIDS MAY ESTIMATE FOR THEIR USE AS MARKERS OF SALT TOLERANCE IN GENETICALLY DIVERSE PAULOWNIA CLONES.