Environmental and Experimental Botany 129 (2016) 48–56

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

The conserved salt-responsive genes in the roots of *Populus* × *canescens* and *Arabidopsis thaliana*

Jie Luo[a,b], Wenguang Shi[a], Hong Li[c], Dennis Janz[d], Zhi-Bin Luo[a,d,*]

[a] State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Silviculture of the State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China
[b] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China
[c] Postgraduate School, Chinese Academy of Forestry, Beijing 100091, China
[d] Büsgen-Institut, Department of Forest Botany and Tree Physiology, Georg-August Universität, Büsgenweg 2, 37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form 14 December 2015
Accepted 17 December 2015
Available online 21 December 2015

Keywords:
Transcriptome
Salinity
Populus
Co-expression
Hub genes
cis-Elements

ABSTRACT

To dissect the salt responsive genes in the roots of *Populus* × *canescens* after salt exposure and to characterize the conserved transcripts with differential expression in the roots of *P.* × *canescens* and *Arabidopsis thaliana* under salinity, *P.* × *canescens* grown in sandy soil was exposed to 150 mM NaCl for 18 days and the transcriptional profiling was analyzed in the roots by using a whole genome poplar array chip. The raw data of the Affymetrix *Arabidopsis* genome arrays were obtained from the online array data bank and statistically analysed as that of the poplar. In the roots of *P.* × *canescens* exposed to salinity, about 860 genes displayed significantly differential expression, including 647 up-regulated and 213 down-expressed genes. In the roots of *A. thaliana*, 1292 genes were up- and 718 down-expressed. Among the differentially expressed genes between *P.* × *canescens* and *A. thaliana*, a set of common genes (128 genes) showed the same change pattern in response to salinity, including 114 induced and 14 repressed genes. A salt-responsive co-expression network was constructed with 98 common genes. Among the co-expressed genes, 22 genes were defined as hub genes which were involved in fundamental biological processes such as abiotic stimulus and signal transduction. Moreover, the *cis*-regulatory elements were found in the conserved motifs of hub genes. These results suggest that *P.* × *canescens* and *A. thaliana* possess conserved salt-responsive genes and that *cis*-elements in the conserved motifs of hub genes play a crucial role in coordinating the co-expression of the common genes underlying the physiological acclimation to salinity in the roots of *P.* × *canescens* and *A. thaliana*.

1. Introduction

Soil salinity is one of the most serious abiotic stresses which constrain plant growth and ecosystem productivity worldwide. High salinity in the soil induces osmotic and ion-specific stresses which affect physiological, biochemical and molecular activities of plants leading to growth limitation and decrease in productivity (Munns and Tester, 2008; Polle and Chen, 2014). Responses of plants to salinity include stress sensing and signalling, ion homeostasis, osmo-regulation, detoxification and growth arrest (Munns and Tester, 2008; Polle and Chen, 2014). At the physiological level, plants generally display alterations in ion flux of roots and transpiration and carbon assimilation of leaves in response to salinity (Chen et al., 2003; Sun et al., 2009). At the metabolic level, plants may display changes in phytohormones such as abscisic acid (ABA), auxin, salicylic acid (SA) and jasmonic acid (JA), accumulation of osmolytes such as soluble sugars, sugar alcohols and amino compounds and increases in membrane lipid oxidation under salinity (Bolu and Polle, 2004; Ding et al., 2010; Dluzniewska et al., 2007; Luo et al., 2009a, 2011; Sanchez et al., 2008).

Transcriptional profiling has enabled us to better understand the molecular mechanisms underlying physiological and biochemical activities of plants in response to salt stress. For example, *Arabidopsis thaliana*, a glycophyte displays substantial changes in gene differential expression under salinity in comparison with salt cress (*Thellungiella halophila*), a closely related halophyte (Gong et al., 2005; Taji et al., 2004). Upon salt exposure, *Arabidopsis* exhibited a global activation of defenses requiring massive induction of genes involved in signalling, transport, hormone metabolism and stress acclimation, while salt cress only a few

* Corresponding author at: State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Silviculture of the State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China.
E-mail address: luozbbill@163.com (Z.-B. Luo).

http://dx.doi.org/10.1016/j.envexpbot.2015.12.008
0098-8472/

genes responded with differential expression (Gong et al., 2005; Taji et al., 2004). In *Populus* species, comparative analysis of transcriptional profiles of the salt sensitive *popularis* and the salt tolerant *Populus euphratica* showed rapid upregulation of genes related to antioxidant enzymes in leaves of *P. euphratica*, whereas over-expressed genes mediating K^+/Na^+ homeostasis and decreased transcript levels of genes involved in scavenging of superoxide radicals and H_2O_2 are found in leaves of *P. popularis* exposed to long-term salt stress (Ding et al., 2010). In another study the transcriptomes of mature unstressed leaves of the salt sensitive *P.* × *canescens* and tolerant *P. euphratica* were compared and showed that genes involved in transport processes, energy metabolism and secondary metabolism are enriched, whereas genes related to nucleus, RNA or DNA binding, kinase activity and transcription factor activity are depleted in *P. euphratica* (Janz et al., 2010). In contrast to *P.* × *canescens*, a general activation of stress relevant genes has not been found in *P. euphratica* (Janz et al., 2010). Furthermore, salt stress induces more tension wood formation to adapt to hydraulic stress caused by salinity in *P.* × *canescens* than in *P. euphratica* (Janz et al., 2012). The results from these studies indicate that there is a stress-anticipatory preparedness at the transcript level in halophytes, but this readiness is lacking in glycophytes.

Previous transcriptional profiling studies in salt-exposed herbaceous plants displayed a common set of salt responsive genes in many plant species in addition to specific responses of different species (Gong et al., 2005; Ma and Bohnert, 2007; Ma et al., 2006; Peng et al., 2014; Taji et al., 2004). Conserved salt responsive genes in plants with different salt tolerance are important for breeding because these genes may serve as candidates to improve salt tolerance. There is now a wealth of information on the salt transcriptomes of herbaceous plants and woody plants (Bazakos et al., 2012; Brinker et al., 2011; Brosche et al., 2005; Ding et al., 2010; Janz et al., 2010, 2012; Yoon et al., 2014), but whether there are overlapping, conserved sets of salt-responsive genes between herbaceous and woody species has not yet been investigated.

In a previous study, we used *P.* × *canescens* (*Populus tremula* × *Populus alba*) to characterize anatomical, physiological and molecular acclimation of the roots to salinity conditions (Luo et al., 2009a). Additionally, the leaf physiology under salt stress were assessed in *P.* × *canescens* (Luo et al., 2011). The physiological and biochemical data obtained in the roots and leaves of *P.* × *canescens* under either non-salinity or salinity conditions in both studies (Luo et al., 2009a; Luo et al., 2011) provided a good basis for further transcriptomic studies. Here, we used this hybrid poplar with high salt sensitivity (Bolu and Polle, 2004; Ehlting et al., 2007) to characterize the changes in the transcriptomic profile of roots to salinity. Using online available array data of *A. thaliana* exposed to 0 or 150 mM NaCl (https://www.arabidopsis.org/servlets/TairObject?type=expression_set_full&id=1007966888), the salt responsive genes involved in important functional categories in the roots were compared between the woody plant *P.* × *canescens* and the herbaceous plant *A. thaliana*. The aims of this study are (i) to identify the salt responsive genes in the roots of *P.* × *canescens* after salt exposure and (ii) to characterize the conserved transcripts with differential expression in the roots under salinity between the woody and herbaceous plants. We hypothesize that the common salt-responsive genes in the roots of the woody and herbaceous plants form a tightly co-expressed network.

2. Materials and methods

2.1. Cultivation of plants and salt exposure

Plantlets of *P.* × *canescens* (syn. *P. tremula* × *P. alba*) were multiplied by micropropagation (Leple et al., 1992). To acclimate the plants to ambient conditions, rooted plantlets were cultivated in hydroponic nutritional solutions for 3 weeks in a growth room (21 °C, 50–60% relative air humidity, 16 h of light per day, 150 μmol photons m^{-2} s^{-1} of photosynthetic active radiation at plant height) before transfer to a climatized cabinet with the same environmental conditions as decribed earlier by Luo et al. (2009a).

The growth condition and soil substrate of poplars were described in detail previously (Luo et al., 2009a, 2011). Briefly, the growth medium consisting of 5 parts peat, 5 parts fine sand (grain size: 0.1–0.3 mm) and 10 parts coarse sand (grain size: 1.2–2.0 mm) was mixed as described elsewhere (Luo et al., 2009b). For plant cultivation growth tubes (5 cm diameter, 41 cm height) with a nylon mesh at the bottom were filled with the rooting medium. Subsequently, poplar plantlets were planted in growth tubes. The plants were randomized twice a week. Each plant was irrigated daily with 20 ml sterile nutritional solution in the morning and 20 ml sterile water in the evening. Poplar plants were grown in a climatized room for 13 weeks. Subsequently, 12 plants were irrigated with low-nitrogen-nutrient solution once a day and the other 12 plants with low-nitrogen-nutrient solution containing additionally 150 mM NaCl.

2.2. Harvest

After 18 days of salt exposure, the roots of all plants were harvested and immediately frozen in liquid nitrogen and subsequently stored at −80 °C. Frozen samples were milled to fine powder with a ball mill (Retsch, Haan, Germany) pre-cooled in liquid nitrogen.

2.3. RNA isolation and DNA chip hybridization

Within each treatment, equal weights of the root powder from 4 harvested plants of *P.* × *canescens* were pooled to form a biological replicate. Three independent biological replicates (i.e., comprising 12 plants) per treatment were analyzed. Total RNA was isolated from about 1 g root powder according to the method with minor modification (Chang et al., 1993). No spermidine was applied in the extraction buffer, and 2% β-mercaptoethanol was used. An additional extraction step was performed after precipitation with 2.5 M LiCl. Total RNA was purified according to the RNeasy mini protocol (Qiagen GmbH, Hilden, Germany). The purity and integrity of RNA were assessed according to the protocol of technical manual (Affymetrix, 2008) of Genechip expression analysis of the supplier (Affymetrix Inc., Central Expressway, Santa Clara, USA). Further processing of the RNA and cRNA hybridization using the Affymetrix poplar genome array was accomplished at the Microarray facilities (Eberhard Karls University, Tübingen, Germany, http://www.microarray-facility.com/). For each treatment, 3 arrays were hybridized, thus, yielding 6 arrays. The raw data are available under accession number: E-MEXP-1874, ArrayExpress depository: http://www.ebi.ac.uk/microarray-as/aer/entry;jsessionid=50F7A8619BE733EB58BD0C5-F3E3CF8F7.

2.4. Statistical analysis

For gene expression analysis, the Affymetrix CEL files generated at the Microarray Service Facilities (Tübingen, Germany) containing the raw probe intensity values from 6 arrays were imported into R (http://www.r-project.org) and further analysis was computed in R. The data were normalized by the quantile normalization method and adjusted for background correction by using the robust multiarray average (RMA) method (Irizarry et al., 2003). Statistical analysis of significantly differentially regulated genes was accomplished by using SAM (significance analysis of microarrays) according to the