Environmental and Experimental Botany 141 (2017) 60–73

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Transcriptomic and hormonal control of boron uptake, accumulation and toxicity tolerance in poplar

Kubilay Yıldırım

Gaziosmanpasa University, Department of Bioengineering, Tokat, Turkey

ARTICLE INFO

Keywords:
Salicylic acid
Boron toxicity
Boron uptake
Boron detoxification
Poplar

ABSTRACT

Despite its low abundance in soils, Boron (B) could be highly toxic for plants in especially arid and semi-arid environments. In the current study, physiological, transcriptomic and hormonal regulations behind B toxicity response were investigated comparatively among poplar species. Previously identified clones of *Populus nigra* (P.n) and *Populus alba* (P.a) having contrasting B accumulation and leaf B toxicity symptoms were treated with elevated soil B supply in a pot trial. Physiological results of the treatment indicated better biomass growth, higher leaf chlorophyll content and more than three folds lower B accumulation in the leaves of P.a compared to P.n for all soil B supply. Microarray-based transcriptomic analysis revealed 1902 and 1006 differentially regulated transcripts for the leaves and roots of P.a under B toxicity, respectively. Several transcripts responsible for salicylic acid (SA) production (salicylic acid binding protein 2) and SA-dependent gene regulation (PR proteins, WRKYs, chitinases, proteases, lipases and protease inhibitors) were strongly upregulated specifically in P.a tissues with B toxicity. Furthermore, endogenous increase in SA content with a soil B concentration-dependent manner was measured in the roots and leaves of P.a while there was no significant alteration in concentration of the same hormone for P.n. Therefore, increase in endogenous SA concentration was strongly attributed to lower B uptake and B toxicity tolerance in P.a. In addition to SA-mediated gene regulation, genes responsible for external excretion process (mannitol dehydrogenase, UGTs, sugar transporters) were also supposed to be functional in P.a for the reduction of tissue B content under toxic conditions. On the other hand, transcriptome profiling of P.n under B toxicity revealed 1624 and 1419 altered transcripts for the leaves and roots, respectively. Several ATP binding cassette B type transporters functional in auxin transport were specifically upregulated in only P.n under B toxicity. However, endogenous auxin content was not altered in both poplar tissues in response to B toxicity. Therefore, induction of these transporter proteins in P.n without any increase in auxin content was attributed to directional transport of excess B to edge of the leaves to regulate cellular ion homeostasis in photosynthetic part of the leaves. Other P.n-specific induced transcripts such as glutathione S transferases and metallochaperones were supported this suggestion and revealed internal excretion of excess B in P.n that could be related with much higher B uptake from the roots, directional transport to the leaves and detoxification under toxic B conditions.

1. Introduction

Boron (B) is an essential micronutrient required for proper vegetative and reproductive growth in plants. B concentration in soil and plant tissues is highly critical for the yield and productivity (Nable, 1997). If B concentrations are greater than 2.0 ppm in the soil or exceed 200 ppm in plant tissues, toxicity symptoms such as marginal and tip necrosis in leaves as well as reduction in growth are likely to appear depending on plant sensitivity to excess B (Schnurbusch et al., 2010a; Pallotta et al., 2014). Nearly all plants, even those somewhat tolerant to higher soil B concentrations, exhibit B toxicity symptoms or lethality when soil B content is greater than 5 ppm (Miwa and Fujiwara., 2010). Increased B mining practices in recent years and extensive usage of B in agricultural chemicals (fertilizers, herbicides and insecticides) and industrial products (detergents, cleaning products, ceramics, glass etc.) released a great amount of B to the environment (Hasenmueller and Criss, 2013). Therefore, B toxicity in the soil and water became a widespread environmental problem throughout the world and cause significant and often substantial reductions in quality and yield of agricultural plants (Princi et al., 2016). Restrictions on agricultural production due to B pollution of the soils have been reported throughout the world including Turkey, South Australia, USA and Chile (Türker et al., 2016).

Uptake and accumulation of excessive B in plant tissues lead to

E-mail address: kubilay.yildirim@gop.edu.tr.

http://dx.doi.org/10.1016/j.envexpbot.2017.07.004
Received 7 April 2017; Received in revised form 30 June 2017; Accepted 7 July 2017
Available online 10 July 2017

several physiological disturbances such as reduction in root growth affecting water and nutrient uptake and enhanced necrotic zones on leaves impairing photosynthesis (Reid and Fitzpatrick, 2009). Therefore, B toxicity tolerance in plants have been attributed to (1) low tissue B accumulation with the rejection of excess B from roots by the activity of B efflux transporters and (2) the existence of internal tolerance mechanisms including binding of B to non-toxic compounds for detoxification and compartmentation into cell wall or vacuole once it reaches to excess concentrations in plant tissues (Princi et al., 2016). Until now, just a few reports indicated detoxification of toxic B in plant tissues (Zhao et al., 2015, Yıldırım and Uylaş, 2016). Generally, the former mechanism has been studied and accepted for B toxicity tolerance in plant species (Reid, 2014; Princi et al., 2016). In this tolerance mechanism, B exporter proteins detected on the outer (soil-facing) membranes of root epidermal cells in Arabidopsis (Miwa et al., 2007) was found to be highly important for B directional export to the soil, limiting B accumulation in growing cells and xylem (Miwa and Fujiwara, 2010). Many other B transporters and aquaporin proteins have been recently suggested to involve in B toxicity tolerance in Arabidobsis (AtTIP5), rice (OsBOR1-4, OsPIP2;4 and OsPIP2;7), barley (HvBot1, HvNIP2;1), maize (ZmBot1), and wheat (TaBOR1) (Pang et al., 2010; Reid 2014; Kumar et al., 2014; Wakuta et al., 2016). The consensus functions of these transporter proteins were reduction in the expression of multifunctional channel proteins, limiting B entry into roots and transport to the leaves (Reid, 2014). However, transporter protein-dependent tolerance was confusing until now due to increased expression of the same transporter genes under toxic conditions in some B toxicity tolerant genotypes (Princi et al., 2016). Furthermore, some overexpression studies on these genes did not positively affected the crop yield in B-contaminated field trials (Emebiri et al., 2009). These aspects deeply limited the importance of B efflux transporters in terms of B toxicity tolerance.

Coordinated regulations of hormones on genetic control have been previously reported to play crucial roles in adaptation of plants to toxic mineral concentrations (reviewed in Zhou et al., 2016). Recently, the roles of phytohormones in response to limited or excessive nutrients (including nitrogen, phosphorus, potassium, sulfur, and many others) and toxic heavy metals have been well-characterized in the literature (reviewed in De Smet et al., 2015). Furthermore, spraying exogenous phytohormones or knocking out the phytohormone biosynthesis/signaling-related genes were reported to alter the expression of some genes implicated in mineral uptake and transport, which may, to a certain extent, lead to the restoration of phenotypes caused by mineral deficiency or toxicity (He et al., 2013; Luo et al., 2016; Wang et al., 2016, 2017). Therefore, exogenous application of plant hormones is recently gaining importance for specifically enhancement of mineral toxicity or deficiency tolerance in plant species (De Smet et al., 2015; Shi et al., 2015). Interestingly, there is growing evidence suggesting the possible association between B deficiency and phytohormones such as ethylene and auxin (Camacho-Cristóbal et al., 2011; Zhou et al., 2016). Some recent studies revealed inhibition of root cell elongation via an ethylene/auxin/ROS-dependent pathway in Arabidopsis (Camacho-Cristóbal et al., 2015) and ethylene mediated root responses to B deficiency (González-Fontes et al., 2015). However, contributions of phytohormones on B toxicity tolerance and gene regulation network have not been investigated in detail, yet. Just a few studies revealed contribution of exogenously applied salicylic acid to enhanced growth and B toxicity tolerance in carrot (Eraslan et al., 2007), spinach (Eraslan et al., 2008), barley (El-Feky et al., 2012) and maize (Gunes et al., 2007) by modulating the B uptake and metabolism in plant tissues. One of the most suitable species for these types of investigations could be the poplar trees which were known to be accumulated large and varied amount of B in their tissues without any toxicity symptoms (Robinson et al., 2007; Rees et al., 2011; Yıldırım and Uylaş, 2016). Especially after sequencing the whole genome of *Poplar* species, they were accepted as model organisms offering opportunities to use genomic tools such as high-density arrays or whole-genome microarrays for investigation of any type of environmental stress (Yıldırım and Kaya, 2017). As a matter of fact, internal B toxicity tolerance including detoxification processes was firstly described in black poplar, recently (Yıldırım and Uylaş, 2016). Therefore, we aimed to identify phytohormones and phytohormone regulated genes in poplar to understand transcriptomic and hormonal control of B uptake, accumulation and toxicity tolerance

2. Materials and methods

2.1. Plant materials and B toxicity treatments in a greenhouse trial

In our previous study, several clones of poplar species that were collected at around B mines and polluted areas of Turkey were subjected to soil B toxicity to select the most tolerant hyper-B accumulator poplar individuals that can be used for afforestation purposes in B contaminated soils and mine areas of the country (not published yet). In this study, one clone of *Populus nigra* (P.n) (PN.92.237) and *Populus alba* (P.n) (PA.29.126) were recorded as the highest and the lowest B accumulator plants, respectively. Therefore, we decided to use these two poplar clones in a microarraybased transcriptome study to identify genes, transcriptional networks and phytohormone-related molecular pathways that could be responsible for differential tissue B accumulation and toxicity tolerance. For B toxicity treatment, one hundred hardwood cuttings of *P.a* and P.n (15 cm in length, 1–2 cm in diameter) were obtained from Poplar and Fast Growing Forest Trees Research Institute (İzmit/Turkey). Cuttings were firstly planted into the plastic bags (1 L) as a single cutting per bag on 15 April 2015. When the rooted cuttings had 15–20 mature leaves, the best growing 50 rooted cuttings/clone were selected and transferred individually to 20 L plastic pots (bottom closed) having five different soil boric acid concentrations (1.5, 5,10,15 and 20 ppm) with 10 replication. Each pot contained 5 kg perlite and soil mixture (4:1) (pH 7.1) that have 0.0062 dS/m EC, 1.9% organic carbon and 1.48 (control) initial soil B concentration. In total, the experiment included 100 saplings for B toxicity treatment (2 different poplar clones x 5 soil B supply x 10 replications = 100 pots). In two day intervals, the pots were weighted and transpired water replenished with tap water (B content; 0.068 ppm) to keep the water content at a constant level. Experiment was carried out for five weeks in a fully controlled greenhouse at the Gaziosmanpasa University (Tokat/Turkey) with a 25/22 °C day/night temperatures. The height growth of the saplings was measured periodically during the B toxicity treatments and was accomplished when the shoot growth of the clones, grown at 20 ppm B containing pots, stopped completely. Here, the soil B supply and duration of the experiment were chosen based B accumulation rates in poplar tissues in our previous study (Yıldırım and Uylaş, 2016). At the end of the experiment, leaf chlorophyll contents of saplings were measured from 10 leaves found on the top, middle and bottom of the stems with CCM-200 plus chlorophyll content meter (Opti-science) and represented as average Chlorophyll Content Index (CCI). Total numbers of the leaves found on the saplings were counted to find out the rate of abscised leaves of poplars in response to B toxicity. Some fresh root and leaf materials were immediately frozen in liquid nitrogen for microarray experiment and other biochemical measurements. The remaining plant materials were oven-dried to estimate dry weight (biomass growth) and B concentrations in mature leaves (fully expanded at the beginning of the experiment), whole stems and roots tissues. Distribution pattern of B accumulation on poplar mature leaves were also determined by dividing them into equal squares from the leaf base to the margins as represented in Fig. 1. The necrotic spots and zones of the leaves appeared at 10 ppm and higher soil B treatments were isolated from the leaves to compare the amount of accumulated B in these necrotic parts with the green parts of the leaves.