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Expression profiling of genes encoding ABA route components in response to dehydration or various light conditions in poplar buds and leaves

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ABSTRACT

In this report, the members of PP2C, SnRK2a and Rboh oxidase families from Arabidopsis and poplar were studied *in silico*, and the expression profiles of the some of them were specified in *Populus tremula* buds and adult leaves. In poplars, the counterparts of ABI1- and ABI2-like protein phosphatases are lacking, but poplar genomes encode three HAB-like proteins denoted in this work as HAB1, HAB3a and HAB3b, and the counterparts of the two latter ones are absent in Arabidopsis. Nonetheless, they may be present in other species. In poplars, SnRK2 subclass III includes two SnRK2.6-like protein kinases denoted by us as SnRK2.6a and SnRK2.6b, and only one SnRK2.2 corresponding to SnRK2.2 and SnRK2.3 ones from Arabidopsis. In contrast to Arabidopsis, the poplar Rboh family involves two RbohD- and RbohF-like proteins denoted here as RbohD1 and RbohD2, and RbohF1 and RbohF2, respectively.

The expressions of genes encoding the above components of the ABA route were studied in *Populus tremula* dehydrated buds and adult leaves not subjected to stress but exposed to natural daylight or to darkness, and to inhibition of ethylene biosynthesis or signaling route by cobalt or silver ions, respectively. In leaves, the light conditions seemed to be the most pronounced factor, from among the studied stimuli, controlling the expression *Ptre-HAB3a*, *Ptre-HAB1*, *Ptre-SnRK2.6a* and *Ptre-RbohF2* genes, their expression was upregulated in darkness. This observation implies that these genes may be important for dark-induced stomatal closure regulation. Ethylene negatively affected the expression of three studied Rboh genes and *Ptre-HAB1*one but only at daylight, whereas its positive effect on the of *Ptre-HAB3a* was shown in the dark exposed leaves.

In buds, three studied Rboh genes took part in the early response to dehydration, however their participation involved the visibly highest level of the *Ptre-RbohD1* transcripts, followed by *Ptre-RbohF2* and the lowest one of *Ptre-RbohF1*. Nonetheless, the further stress-induced superoxide anion generation seemed to depend on the enhanced expression of the *Ptre-RbohD1* and *Ptre-RbohF2* genes only, still with a significantly higher level of the *Ptre-RbohD1* one. *Ptre-RbohD2* transcripts were found neither in leaves nor in buds. The expression of the other genes discussed in the present work was either slightly upregulated at moderate stress or did not significantly change in response to dehydration. The protein kinase activity of overexpressed *Ptre-SnRK2.6a* and *Ptre-SnRK2.6b* was confirmed in *in vitro* protein kinase assay and compared to that of SnRK2.6/OST1 one from Arabidopsis.

1. Introduction

ABA signaling starts from ABA-bound receptors that suppress Protein Phosphatase 2C-mediated (PP2C) dephosphorylation of Sucrose non-fermenting 1-Related protein Kinases 2 (SnRK2s) and enable their activation which targets ABA-responsive genes in the nucleus and ion

Abbreviations: ABA, abscisic acid; ABI1 or 2, ABA-insensitive 1 or 2; ABI5, ABA-insensitive 5; ACO, 1-aminocyclopropane-1-carboxylate oxidase; ARR2, arabidopsis response regulator 2; AtI-2, arabidopsisInhibitor-2; CTR1, constitutive triple response 1; EIN2, ethylene insensitive 2; EIN3, ethylene insensitive 3; EIN4, ethylene insensitive 4; ERS1, ethylene response sensor 1; ETR1, ethylene receptor 1; HAB1, homology to ABI1; HAI1, 2 and 3 highly ABA induced 1, 2 and 3; OST1, open stomata 1 protein kinase; PP2C, protein phosphatase type 2C; Rboh, respiratory burst oxidase homologue of gp91phox NADPH oxidase; ROS, reactive oxygen species; SnRK2, sucrose non-fermenting 1-related protein kinases 2; TPP1, type one protein phosphatase 1

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channels in cytosol (reviewed in Fujita et al., 2011; Joshi-Saha et al., 2011; Klingler et al., 2010; Melcher et al., 2010; Santiago et al., 2012; Umezawa et al., 2010; Yoshida et al., 2015). Direct dephosphorylation of SnRK2s and their inactivation by PP2Cs (HAB1, Homology to ABI1; ABI1, ABA-insensitive 1 and ABI2, ABA-insensitive 2) has been evidenced (Soon et al., 2012; Vlad et al., 2009). In ABA-insensitive PP2C hypermorphic mutants (abi1-1; abi1-2 and hab1-1) from Arabidopsis (reviewed in Santiago et al., 2012), ABA signaling is disturbed (Koornneef et al., 1984) because the deregulated PP2Cs are no longer under ABA-dependent control of ABA receptors, whereas PP2Cs interactions with downstream targets such as SnRK2s are not blocked (Vlad et al., 2009). In contrast, ABA-hypersensitive phenotype was shown in the loss of function pp2c triple mutant (inactive ABI1, ABI2 and HAB1) (Rubio et al., 2009). The plant SnRKs are divided into subfamilies: SnRK1, SnRK2 (2a and 2b), and SnRK3 (Coello et al., 2011). In Arabidopsis, SnRK2a members such as SnRK2.2, SnRK2.3 and SnRK2.6 (synonyms: SRK2D, SRK2I and SRK2E/OST1, respectively), are strongly activated by ABA, and the loss of function snrk2.2/2.3/2.6 triple mutant exhibits an extreme ABA-insensitive phenotype (Fujii and Zhu, 2009). From among SnRK2s, SnRK2.6 acts as a key positive regulator of ABA signalosome (Ding et al., 2015; Fujii et al., 2011; Ma et al., 2009; Mustilli et al., 2002; Vlad et al., 2009; Yoshida et al., 2006, 2015). A phosphoproteomic study has revealed 32 (Umezawa et al., 2013) or even 84 phosphopeptides (Wang et al., 2013) being SnRK2.2/2.3/2.6 potential targets. Recently, the other components such as Type One Protein Phosphatase 1 (TOPP1) and its regulatory protein, Arabidopsis Inhibitor-2 (AtI-2) inhibiting SnRK2.2/2.3/2.6-mediated ABA signaling have been reported by Hou et al. (2016). Moreover, the putative role of SnRK2.2/2.3/2.6 as a link between ABA route and the regulatory function of microRNA has been shown, (Yan et al., 2017). In the nucleus, SnRK2.6 (and/or related ABA-responsive SnRK2a) controls the expression of a broad spectrum of ABA-responsive genes (Sirichandra et al., 2010; Wang et al., 2013). However, various stimuli may control the expression of SnRK2 genes and for example, such a regulation has been recently reported for *SnRK2s* from *Populus trichocarpa* (Yu et al., 2017). Despite this, ABA is a root-derived molecule, and its water loss induced biosynthesis in leaf vascular tissues and its subsequent transport to guard cells have been documented (reviewed in Seo and Koshiba, 2011). Moreover, in some species, ABA level increases in leaves exposed to darkness, especially at the beginning of the dark period (Novakova et al., 2005; Reynolds-Henne et al., 2010; Tallman, 2004). SnRK2.6 function has been shown to be required for rapid stomatal closure in response to reduced air humidity, darkness, CO_2 enrichment and ozone, (Merilo et al., 2013), as well as for the regulation of metabolic homeostasis in plants, (Zheng et al., 2010). ABA promotes stomatal closure and inhibits stomatal opening *via* SnRK2.6 action (reviewed in Hedrich and Geiger, 2017; Kollist et al., 2014); inhibition of light-induced stomatal opening is reduced in Arabidopsis *snrk2.6* mutants and they are insensitive to ABA-induced stomatal closure, while SnRK2.6 overexpressing plants are hypersensitive to these responses (Acharya et al., 2013). ABA-induced closure is suppressed in ethylene-overproducing mutants, in contrast to the ABA promoted stomatal closure, ethylene can promote either stomatal opening *via* inhibition of ABA-mediated closure or stomatal closure, (reviewed in Acharya and Assmann, 2009; Wilkinson and Davies, 2009, 2010).

In plants, an increase in the ABA endogenous level coordinates the action of stress-related pathways, among them the ROS signaling route, (Hu et al., 2006; Kwak et al., 2003; Sirichandra et al., 2009). Plasma membrane localized NADPH-dependent respiratory burst oxidases (Rbohs) catalyze extracellular production of superoxide anion which dismutates to H_2O_2; the Rboh proteins represents their key enzymatic subunit (Kwak et al., 2003; Sagi and Fluhr, 2006; Torres and Dangl, 2005; Torres et al., 2002). In Arabidopsis, two (RbohD and F) of ten functioning Rbohs (RbohA-J) have been shown to be involved in the ABA route (Bright et al., 2006; Toum et al., 2016; Zhang et al., 2009). In Arabidopsis, the SnRK2.6/OST1 interaction with RbohF and its phosphorylation, (Acharya et al., 2013; Sirichandra et al., 2009), the putative involvement of SnRK2.6/OST1 in flagellin induced RbohD phosphorylation (Benschop et al., 2007; Melotto et al., 2006; Nuhse et al., 2007), and the physical interaction between them, have been reported (Acharya et al., 2013). In Arabidopsis guard cells, stomatal closure requires the binding of RbohF-derived H_2O_2 by ethylene receptors, however the signaling through ETR1 invoked by H_2O_2 is different from that invoked by the presence of ethylene, (Desikan et al., 2006, 2005). The ethylene signaling through ETR1, ERS1 and EIN4 inactivates CTR1 protein kinase and then releases Gα protein-dependent H_2O_2 production, while H_2O_2 signaling *via* ETR1 and ERS1, and downstream located EIN2, EIN3, and ARR2-dependent routes promotes stomatal closure, (Desikan et al., 2006; Ge et al., 2015; Shi et al., 2015).

In this report, the comparative *in silico* study of Arabidopsis SnRK2a-, PP2C- and Rboh oxidase family members, and their counterparts from poplar (*P. trichocarpa*, *P. euphratica* and *P. tremula*) led to the conclusion about differences in gene organization between Arabidopsis and poplars. The expression profiling of some of discussed genes was carried out on breaking buds and adult leaves of *P. tremula*; on the former ones in response to dehydration, whereas on these latter ones, not subjected to stress, in response to their exposition to daylight or darkness, and in response to various ethylene conditions. In our study on adult leaves the pre-treatments with cobalt or silver ions were performed. Both types of pre-treatment limit the regulatory role of ethylene, however the final effect of each of them on gene expression does not have to be the same. The cobalt ions block ethylene production *via* inhibition of the enzyme catalyzing the last step in ethylene biosynthesis, ACC oxidase (ACO), (Garcia et al., 2010; Kim et al., 2008; Merritt et al., 2001). In poplars, ACO seems to play a critical role in ethylene production, (Andersson-Gunneras et al., 2003; Love et al., 2009). In Arabidopsis, silver ions disrupt ethylene signaling *via* ethylene receptors subfamily I (ETR1 and ERS1) but not *via* subfamily II ones (ETR2, ERS2 and EIN4) (McDaniel and Binder, 2012). In poplars, subfamily I includes three receptors (ETR1: POPTR_0001s21120g and POPTR_0003s02680g; ERS1: POPTR_0002s20260g), whereas subfamily II includes four ones (ERS2: POPTR_0010s08510g and POPTR_0008s16430g; EIN4: POPTR_0013s04140g and POPTR_0019s02220g). It has been shown that the expression of *P. tremula* genes representing the counterparts of these Arabidopsis ones involved in the ABA signalosome may be controlled in a different manner by light conditions and/or ethylene, or not affected. However, light conditions seemed to be the most potent stimulus. The protein kinase activity of overexpressed Ptre-SnRK2.6a and Ptre-SnRK2.6b was confirmed in *in vitro* protein kinase assay and compared to that of SnRK2.6/OST1 one from Arabidopsis.

2. Materials and methods

2.1. Bioinformatical analysis

BLASTP program searches of NCBI databases using protein sequences of known members of PP2C, SnRK2 and Rboh family from Arabidopsis were performed to find their counterparts from poplars and some other species. The phylogenetic trees were constructed from the complete protein sequence alignment of 58 PP2C- like polypeptides (Supplementary materials 4), 50 SnRK2-like polypeptides (Supplementary materials 5), and 30 Rboh oxidase −like polypeptides (Supplementary materials 6) by the Neighbor-Joining method (Saitou and Nei, 1987) with bootstrapping analysis (500 replicates) (Felsenstein, 1985). The numbers beside the branches indicate the bootstrap values that support the adjacent node. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the JTT matrix-based method (Jones et al., 1992). Evolutionary analyses were conducted in MEGA 6 (Tamura et al., 2013).