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BZIP transcription factors as negative regulators of secondary cell wall formation in plant fibers

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Motivation and aim: Plant cell walls play a crucial role in growth and development by protecting plants from environmental stress and facilitating directed growth. They are generally categorized into two types: primary walls, which are dynamic structures that support the expansion of growing plant cells and are essential for plant morphogenesis, and secondary walls, which are formed after cell expansion and provide structural support and rigidity. Secondary walls make up the majority of a plant's biomass and are primarily composed of cellulose, hemicelluloses, and lignin. Plant fiber form quite remarkable cell walls, especially as many fiber crops, including flax, depositing an additional layer – a tertiary cell wall, also called the G-layer. The distinguishing features of such cell walls are high cellulose content, axial orientation of cellulose microfibrils, the virtual absence of xylan and lignin, and presence of rhamnogalacturonan I having a special structure and properties. The transcriptional network consisting of master transcriptional switches and their downstream transcription factors (TFs) that regulate secondary cell walls biosynthesis in plant fibers is well established, while TFs regulate tertiary cell wall deposition are not known. We performed transcriptome profiling of phloem flax fibers at different stage of development and revealed the upregulation of expression of a number of genes encoding TFs of different families, including the BZIP44 TF. To establish the possible role of BZIP44, we used the Arabidopsis *nst1nst3* mutants, which lack secondary cell wall in fascicular fibers. These mutant plants are convenient models for investigation of phenotypes induced by expressing TFs of unknown function driven by the NST3 promoter, which is specific for xylary fibers.
Materials and methods: *Arabidopsis thaliana* ecotype Columbia-0 and the *nst1nst3* double mutants were transformed with constructs containing ATBZIP44 under the control of NST3 promoter and fused to strong transcriptional activation (VP16) or inhibition domains (SRDX). The phenotypes of the obtaining transgenic plants were analyzed using light microscopy. The expression of a set of genes encoding key TFs involved in secondary cell wall formation was analyzed using qPCR. Transcriptome analysis of the inflorescence stems of transgenic lines was also performed.
Results: Transformation of the *nst1nst3* mutants by NST3::ATBZIP44_SRDX induced recovery of cell wall deposition in interfascicular fibers. In the reconstructed thickened cell wall in interfascicular fibers of NST3::ATBZIP44_SRDX (*nst1nst3* background), the distribution of cellulose and xylan was similar to that of the wild type, while in Arabidopsis plants Col-0 transformed by NST3::ATBZIP44_VP16, a reduction of secondary cell walls in interfascicular fibers was observed, and the phenotype of these transgenic plants resembles the *nst1nst3* phenotype. The observed phenotype changes were confirmed by gene expression analysis using qPCR. Downregulation of genes involved in the biosynthesis of cellulose, lignin and xylan was revealed in wt_NST3::ATBZIP44_VP16. The expression pattern of the analyzed genes was very similar to that of the *nst1nst3* mutants. Conversely, in *nst1nst3* mutant plants transformed by NST3::ATBZIP44 with the repressor SRDX, upregulation of genes involved in secondary cell wall development occurred. TFs associated with secondary cell wall formation in interfascicular fibers were suppressed in wt_NST3::ATBZIP44 (NST1,2,3 and SDN2, 3), while the TFs involved in vessel elements formation (VND6 and VND7) did not change their expression. In *nst1nst3*_NST3::ATBZIP44_SRDX plants, the upregulation of the NST2 was revealed. The gene for WRKY12 known as a negative regulator of NST2 was not affected in the ATBZIP44 transgenic plants. Since VP16 chimeric constructs activate expression of target genes and SRDX constructs act as suppressor of target gene expression, the obtained data suggest that ATBZIP44 activates an unknown inhibitor (except WRKY12) that usually is not expressed in Arabidopsis inflorescence stem tissues; this inhibitor suppresses secondary cell wall formation in wt_NST3::ATBZIP44 transgenic plants. Suppression of the proposed inhibitor in *nst1nst3* mutant plants transformed with NST3::ATBZIP44_SRDX stimulates secondary cell wall deposition, which is regulated rather by NST2 TF, but not NST1 and NST3. In the report data of transciptomic analysis for the analyzed transgenic plants will be presented as well to provide a deeper understanding of the observed phenomenon and to reveal new participants in cell wall regulation.
Conclusions: In the course of the current research work, a novel transcription factor, BZIP44, involved in thickened cell wall formation was identified. This factor acts as a negative regulator of secondary cell wall formation, but could be associated with tertiary cell wall formation since the downregulation of the secondary cell wall is required to start the deposition of the tertiary cell wall layer.