SND1 Transcription Factor–Directed Quantitative Functional Hierarchical Genetic Regulatory Network in Wood Formation in Populus trichocarpa

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Wood is an essential renewable raw material for industrial products and energy. However, knowledge of the genetic regulation of wood formation is limited. We developed a genome-wide high-throughput system for the discovery and validation of specific transcription factor (TF)–directed hierarchical gene regulatory networks (hGRNs) in wood formation. This system depends on a new robust procedure for isolation and transfection of Populus trichocarpa stem differentiating xylem protoplasts. We overexpressed Secondary Wall-Associated NAC Domain 1s (Ptr-SND1-B1), a TF gene affecting wood formation, in these protoplasts and identified differentially expressed genes by RNA sequencing. Direct Ptr-SND1-B1–DNA interactions were then inferred by integration of timecourse RNA sequencing data and top-down Graphical Gaussian Modeling–based algorithms. These Ptr-SND1-B1-DNA interactions were verified to function in differentiating xylem by anti-PtrSND1-B1 antibody-based chromatin immunoprecipitation (97% accuracy) and in stable transgenic P. trichocarpa (90% accuracy). In this way, we established a Ptr-SND1-B1–directed quantitative hGRN involving 76 direct targets, including eight TF and 61 enzyme-coding genes previously unidentified as targets. The network can be extended to the third layer from the second-layer TFs by computation or by overexpression of a second-layer TF to identify a new group of direct targets (third layer). This approach would allow the sequential establishment, one two-layered hGRN at a time, of all layers involved in a more comprehensive hGRN. Our approach may be particularly useful to study hGRNs in complex processes in plant species resistant to stable genetic transformation and where mutants are unavailable.

This work was supported by the Office of Science (Biological and Environmental Research), Department of Energy Grant DE-SC000691 (to V.L.C.).