

# Fishing out regulators of the stilbene pathway in Scots pine



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## Stilbenes contribute to decay resistance of Scots pine heartwood

Decay resistance of Scots pine (*Pinus sylvestris* L.) heartwood is a high heritability trait of significant breeding interest. Decay resistance positively correlates with the amount of heartwood extractives of which the contribution of the stilbene pinosylvin stands out. Pinosylvin biosynthesis peaks during heartwood formation at the sapwood – heartwood boundary (transition zone) in mature trees (Fig.1) and under various abiotic stresses in young trees. The pinosylvin biosynthesis pathway (Fig.2) is a branch of the phenylpropanoid pathway originating from phenylalanine.

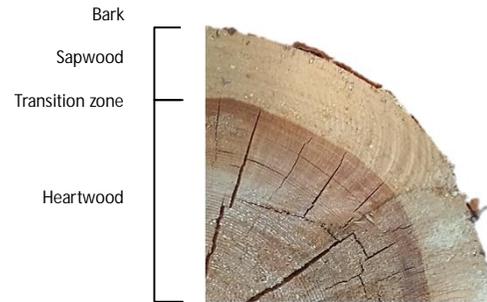


Figure 1. Scots pine heartwood forms the decay resistant, central part of the trunk. During the development of heartwood, the stilbene pinosylvin is abundantly synthesized at the sapwood opposing transition zone.

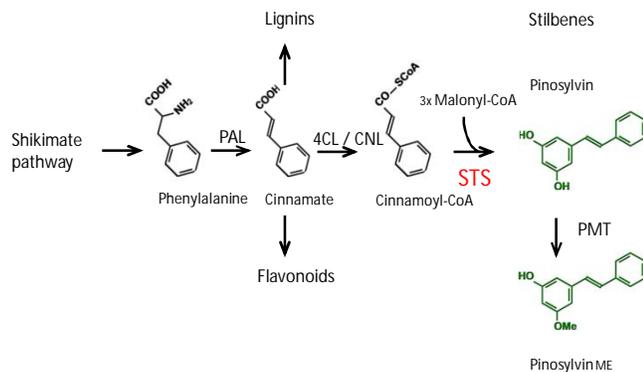


Figure 2. The biosynthesis pathway of Scots pine stilbene pinosylvin starts from phenylalanine, forming a branch of the phenylpropanoid pathway. PAL, phenylalanine ammonia lyase; 4CL, 4-coumaroyl-CoA ligase; CNL, cinnamate-CoA ligase; STS, stilbene synthase; PMT, pinosylvin methyl transferase.

## MYB transcription factors potentially regulate stilbene biosynthesis

We aim to identify and characterize the transcriptional regulators of the pinosylvin synthase promoter *PST-1* in order to speed up conifer wood quality breeding. The transcriptome data from mature wood, from wounded wood of young trees, and from needles of UV-C treated seedlings implicate seven MYB domain transcription factors as potential regulators of *PST-1* (Fig. 3).

Analysis of *PST-1* promoter activation will be performed by luciferase reporter assays in transiently transformed *Nicotiana benthamiana* L. leaves utilizing agroinfiltration (Fig. 4A). The activator and repressor domain fusions to MYBs (Fig. 4B, 4C) will overcome the need of additional transcription factors in MYB mediated regulation.

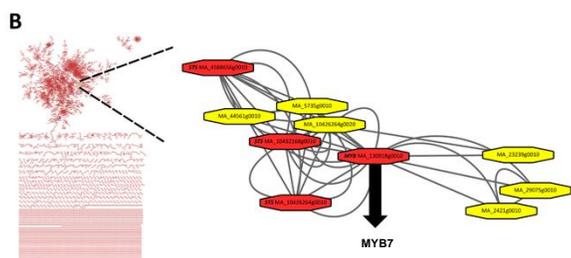
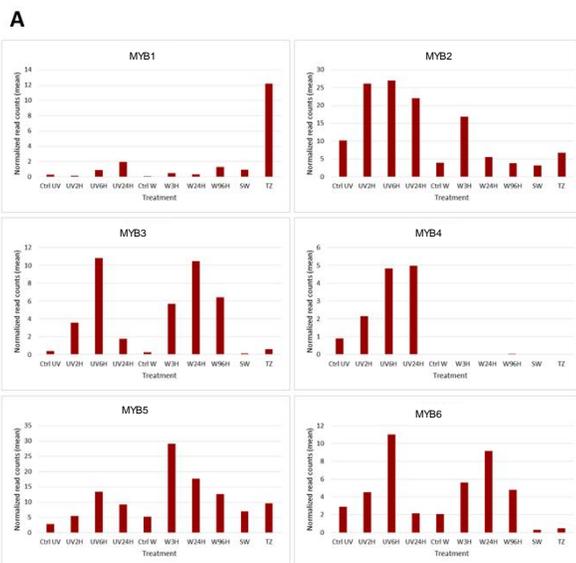


Figure 3. Transcription factors (TFs) for co-infiltration analyses were chosen based on their expression patterns in Scots pine (Lim et al. 2016) or Norway spruce (Jokipii-Lukkari et al. 2018) RNAseq data. A) The expressions of TF genes in Scots pine under the conditions that are known to induce stilbene pathway. UV, ultraviolet; W, wounding; SW, sap wood; TZ, transition zone. B) The co-expression neighborhood of Norway spruce MYB MA\_130918g0010, the homolog of pine MYB7, included several gene models that putatively encode stilbene synthase genes (*STS*).

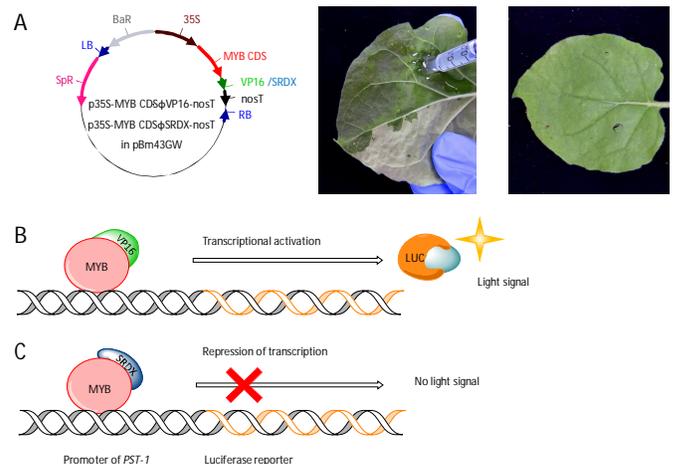


Figure 4. The MYB domain transcription factors are cloned into overexpression vectors and co-infiltrated into tobacco leaves with a reporter construct containing *PST-1* promoter fused to luciferase (A). If the MYB binds to the *PST-1* promoter, it will affect luciferase expression. The VP16 domain fusion will turn the MYB into an activator causing luciferase production and detectable light signal (B). The SRDX repressor domain fusion turns the MYB into a repressor and reduces the light signal compared to the agrobacterium triggered background level of luciferase expression (C).

## References

Lim K-J, Paasela T, Harju A, Venäläinen M, Paulin L, Auvinen P, Kärkkäinen K, Teeri TH. 2016. Developmental changes in Scots pine transcriptome during heartwood formation. *Plant Physiology* 172: 1403-1417.

Jokipii-Lukkari S, Delhomme N, Schiffthaler B, Mannapperuma C, Prestele J, Nilsson O, Street NR, Tuominen H. 2018. Transcriptional roadmap to seasonal variation in wood formation of Norway spruce. *Plant Physiology* 176: 2851-2870.