Muilu-Mäkelä R, Vuosku J, Saarinen M, Hamberg L, Ruotsalainen S, Häggman H, Sarjala T (2016)
**Coping with spring frost-effects on polyamine metabolism of Scots pine seedlings**
iForest - Biogeosciences and Forestry – doi: 10.3832/ifor2003-009

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# Supplementary Material

**Fig. S1** - Phenylpropanoid pathway. R2R3-MYB transcription factors affect expression of phenylalanine ammonia lyase (*PAL*) gene. 4-coumarate:CoA ligase (4CL) ligates coenzyme A to simple phenolic acids, who can conjugate with PAs and form HCCAs. Stilbenes are formed by pinosylvin synthase (psSTS). Pinosylvin methyl transferase (PMT) forms pinosylvin monomethyl ether. The names of the molecules and genes indicated in black were investigated.

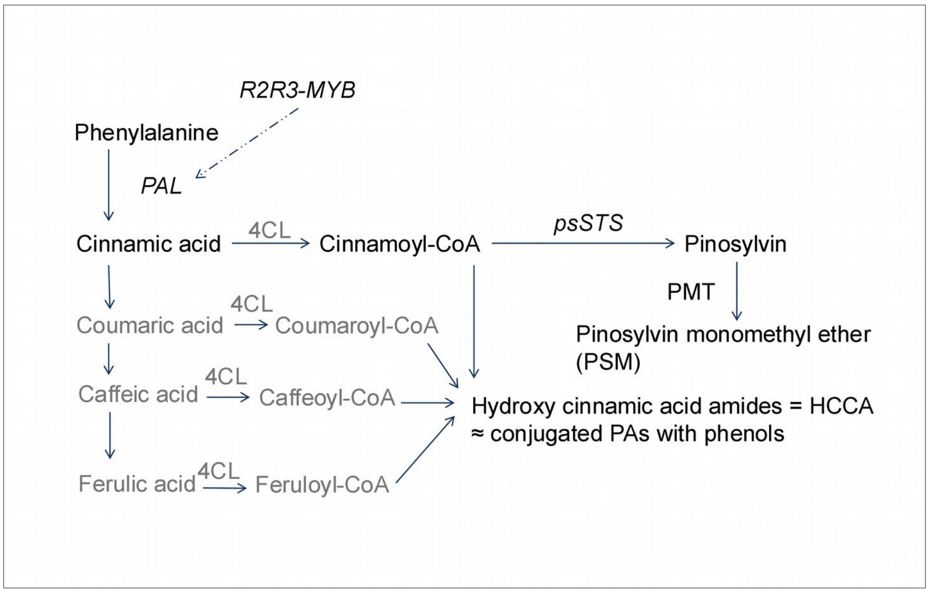

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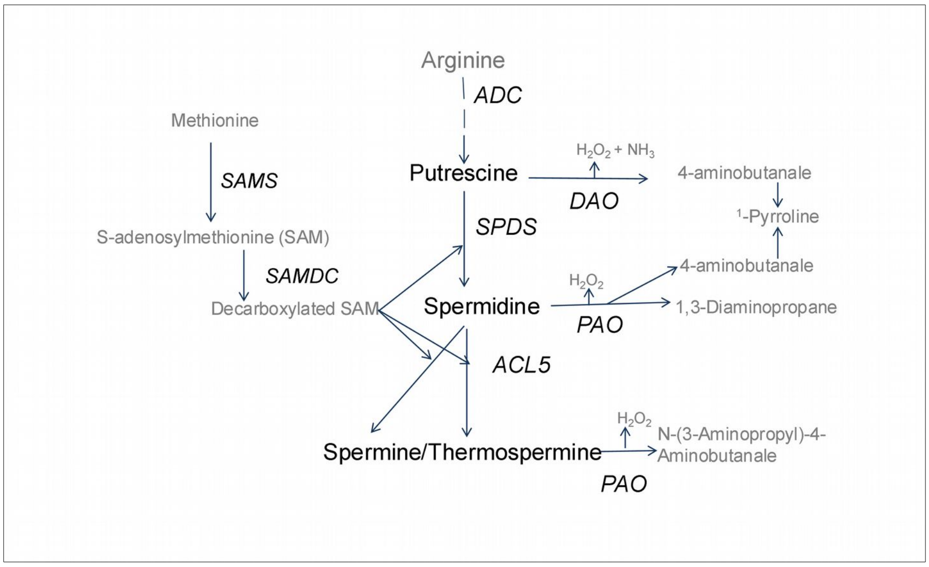
**Fig. S2** - PA metabolism pathway in Scots pine. [ADC=arginine decarboxylase, SPDS= spermidine synthase, ACL5= thermospermine synthase, SAMS=S-adenosyl methionine, SAMDC=s-adnosyl methionine decarboxylase, DAO= diamino oxidase, PAO=polyamine oxidase, $H_2O_2$= hydrogen peroxide, $NH_3$=ammonia]. The names of the molecules and genes indicated in black were investigated.



**Tab. S1** - PCR primers for the real-time PCR amplification of the Scots pine polyamine metabolism (*ADC, SPDS, ACL5, DAO, PAO* and *SAMDC*), stress (*SAMS, LEA, CAT, ICE1*), phenylpropanoid synthesis (*R2R3-MYB, PAL* and *psSTS*) and reference genes (*UBQ* and *TUBA*) in Scots pine and the Gene Bank accession number of the sequences where the primers are based.

| Gene | Forward primer | Reverse primer | GenBank Accession number |
|---|---|---|---|
| *ADC* | 5´-AGTCCGTGTGGCCTGTAATC-3´ | 5´-TGCACAGACACAACGTCAAA-3´ | HM236823 |
| *SPDS* | 5´-CCAACGTCCCATTAACCCTA-3´ | 5´-TGGCAAACAAAATGATGCTG-3´ | HM236827 |
| *ACL5* | 5´-ACTGCTCACATTCCGTCCT-3´ | 5´-TTCGCCTTTGATTCTCTGCT-3´ | HM236828 |
| *DAO* | 5´-AATGGGGAAGTTGGGAGTTC-3´ | 5´-CCCTCCTCAGTTTTCCAGTG-3´ | HM236829 |
| *PAO* | 5´-CGAAATTGCAGAACCTCCAC-3´ | 5´-CGGCCACGAACTACTCATCT-3´ | HM236830 |
| *SAMDC* | 5´-GCTTCGGCGAGGAAATATCTTA-3´ | 5´-TGTTTGCGGTCCAGTTG-3´ | HM236826 |
| *SAMS* | 5´-ACTGCAAAGTGCTGGTT-3´ | 5´-ATGGGTCAGTGGCATAAG-3´ | JQ970126 |
| *LEA* | 5´-ACCCTCGCAGAGGTTACAGACA-3´ | 5´-TTGGCCTTCACTGACCCAGGA-3´ | FJ201571 |
| *CAT* | 5´-GGGAGGCAAACCTATGTGAA-3´ | 5´-TTGGTTGCATGACTGTGGTT-3´ | EU513163 |
| *ICE1* | 5´-TTA GCT TGC TCT GCC CGA AA-3´ | 5´-TCA CTT CCC AGT CCC AAT GC-3´ | DR384895.1 |
| *R2R3-MYB8* | 5´-CTT CCT GGA AAG ATT TAA TAG TGT-3´ | 5´-GGA GCC TGC AAT ACC CAT A-3´ | DQ399057 |
| PAL | 5´-GAGGGAATTTCCAGGGCACA-3´ | 5´-GATCTCGGCCCCTTTCAGTC-3´ | AF353986.1 |
| *psSTS* | 5´-ATGTTTCCGTACTCGCTCATAAC-3´ | 5´-ACAAGTTCAAGCGAATATGTGAA-3´ | S50350.1 |
| *UBQ* | 5´-GAAGGAGCAGTGGAGTCCTG-3´ | 5´-CAATTTCAGGGACGAGAGGA-3´ | AF461687 |
| *TUBA* | 5´-TGGCCGCATCTTCCTTGCCG-3´ | 5´-GATGGCCAGATGCCCAGCGA-3´ | FN546172 |

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**Fig. S3** - Alignment of the predicted amino acid sequence of the *Pinus sylvestris* putative CBF (C-repeat binding factor) 1 inducer protein (ICE1) with *Citrus sinensis* (XP_006485105.1), *Arabidopsis lyrata* (NP_189309.2), *Amborella trichopoda* (XP_006844542.1), *Theobroma cacao* (XP_007038116.1) and *Phalaenopsis aphrodite* (AIE56161.1). Identical and similar amino acids are highlighted in black and grey, respectively.

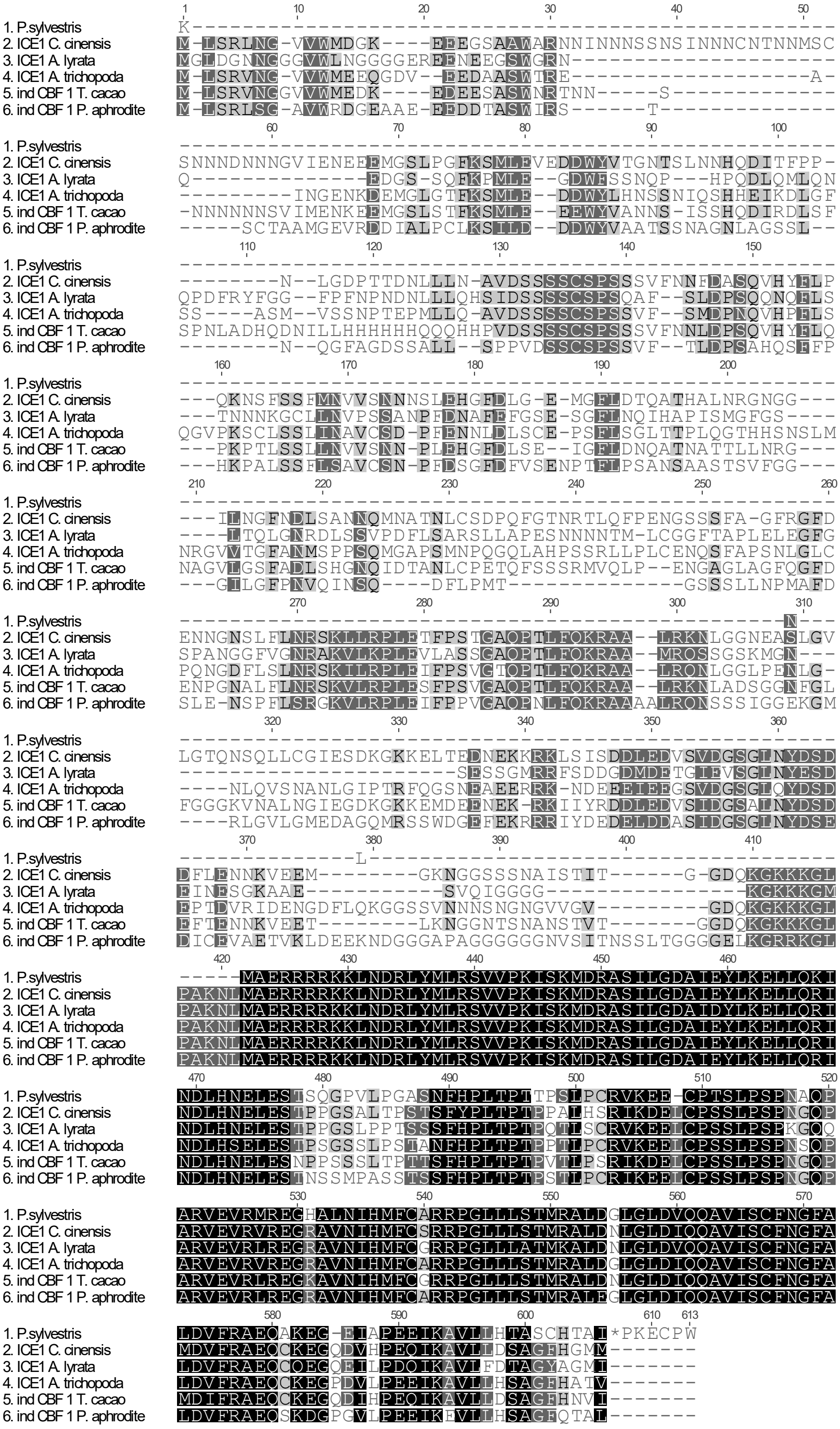

**Fig. S4** - Alignment of the predicted amino acid sequence of the *Pinus sylvestris* putative phenylpropanoid synthesis related transcription factor (R2R3-MYB8) with *Pinus taeda* (ABD60280.1), *Pinus pinaster* (CBM40481.1) and *Picea glauca* (ABQ51224.1). Identical and similar amino acids are highlighted in black and grey, respectively.