

Figure S1. A schematic presentation of the pRT99/35S-Pt4CL1-a plasmid vector used in the biolistic transformation of silver birch clones A, E5382 and E5396.

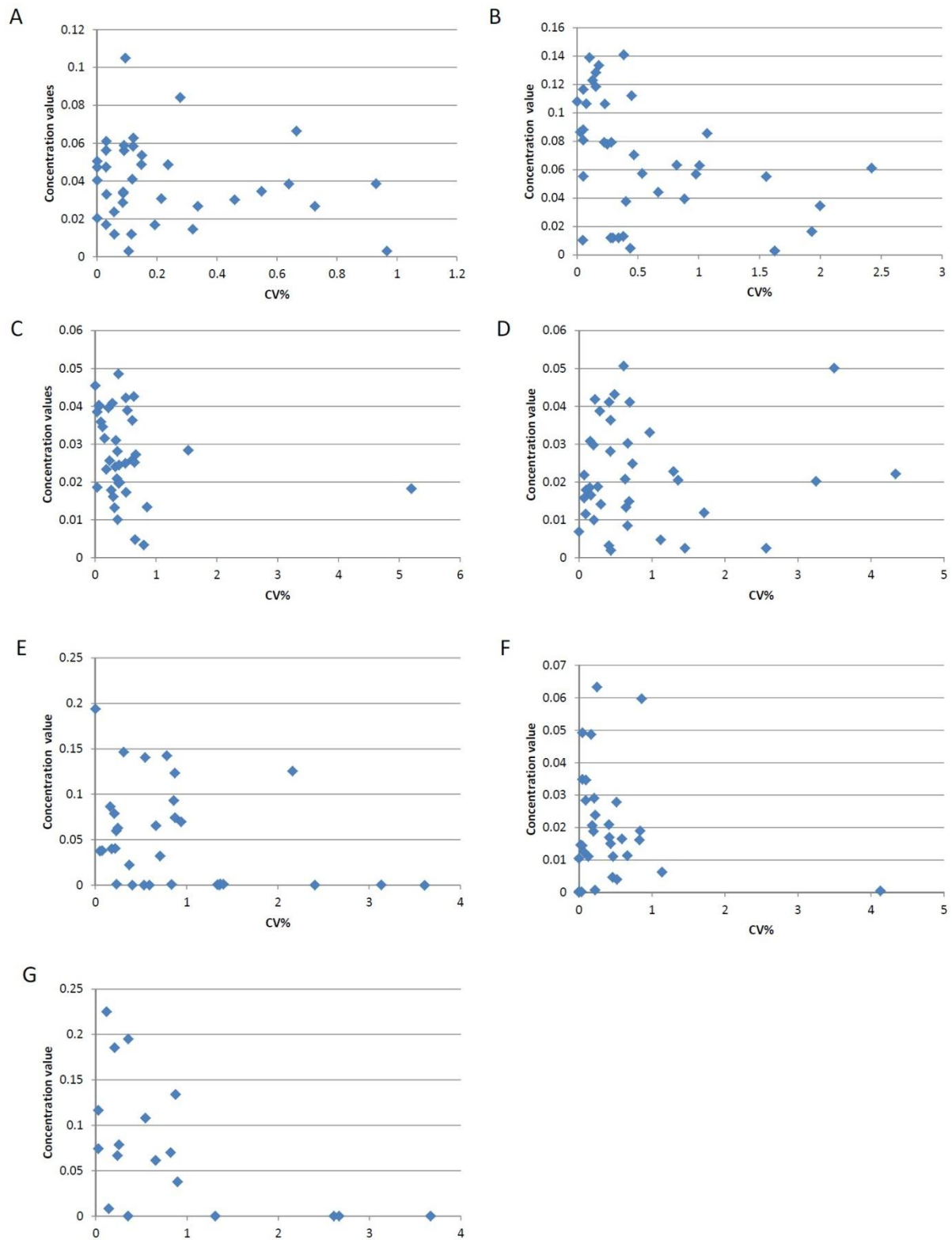


Figure S2. The scatterplots presenting CV% and concentration values of stem samples. The CV% ($SD_{Cq}/mean_{Cq}$) values were calculated from two technical replicates of each utilized primer pair and the concentration values were generated with Abs Quant/2nd Derivative Max for All Samples Analysis of Lightcycler 480 Software release 1.5.0 SP3. A, *Atub*; B, *PP2A*; C, *Bp4CL1*; D, *Bp4CL2*; E, *Bp4CL3*; F, *Bp4CL4*; G, *Pt4CL1*.

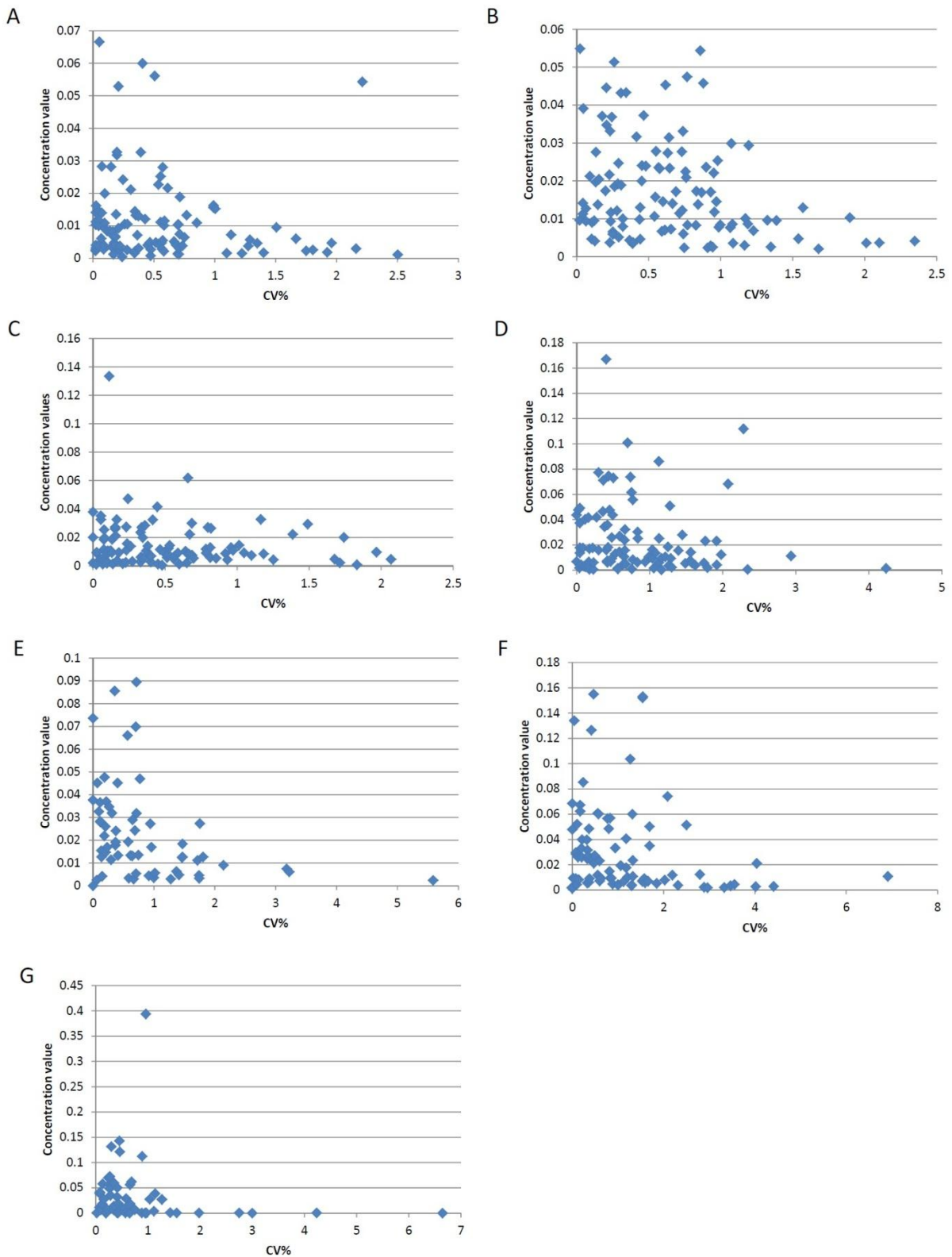


Figure S3. The scatterplots presenting CV% and concentration values of leaf samples. The CV% ($SD_{Cq}/mean_{Cq}$) values were calculated from two technical replicates of each utilized primer pair and the concentration values were generated with Abs Quant/2nd Derivative Max for All Samples Analysis of Lightcycler 480 Software release 1.5.0 SP3. A, *Atub*; B, *PP2A*; C, *Bp4CL1*; D, *Bp4CL2*; E, *Bp4CL3*; F, *Bp4CL4*; G, *Pt4CL1*.

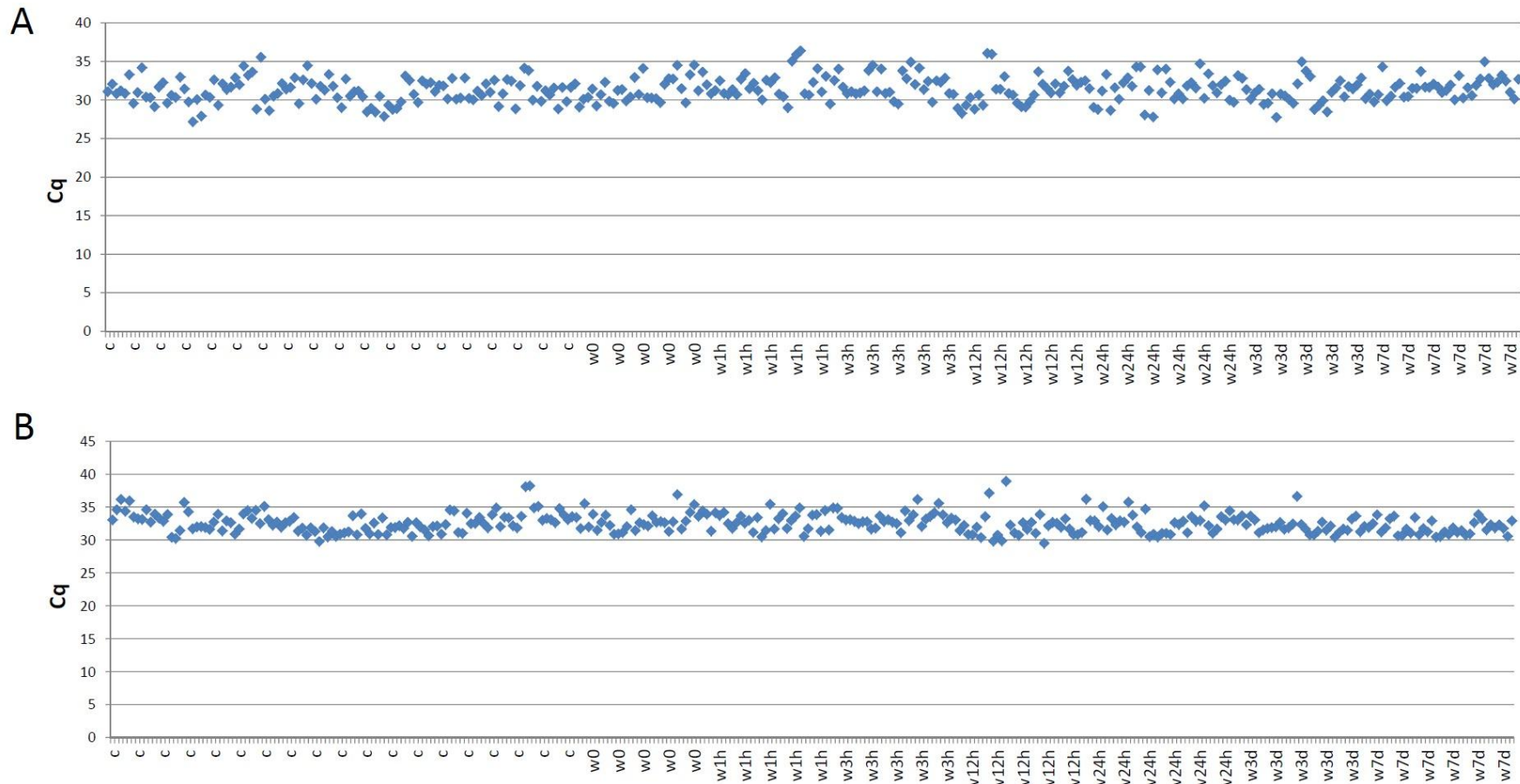


Figure S4. The Cq values of *Atub* and *PP2A* in undamaged and wounded leaves. The Cq values represent crossing point values generated with Abs Quant/2nd Derivative Max for All Samples Analysis of Lightcycler 480 Software release 1.5.0 SP3 of *Atub* (A) and *PP2A* (B) in non-treated leaves (c) and leaves collected immediately after mechanical wounding (w0) and 1 (w1h), 3 (w3h), 12 (w12h), 24 (w24h), 72 (w3d) and 168 (w7d) hours after the wounding treatment.

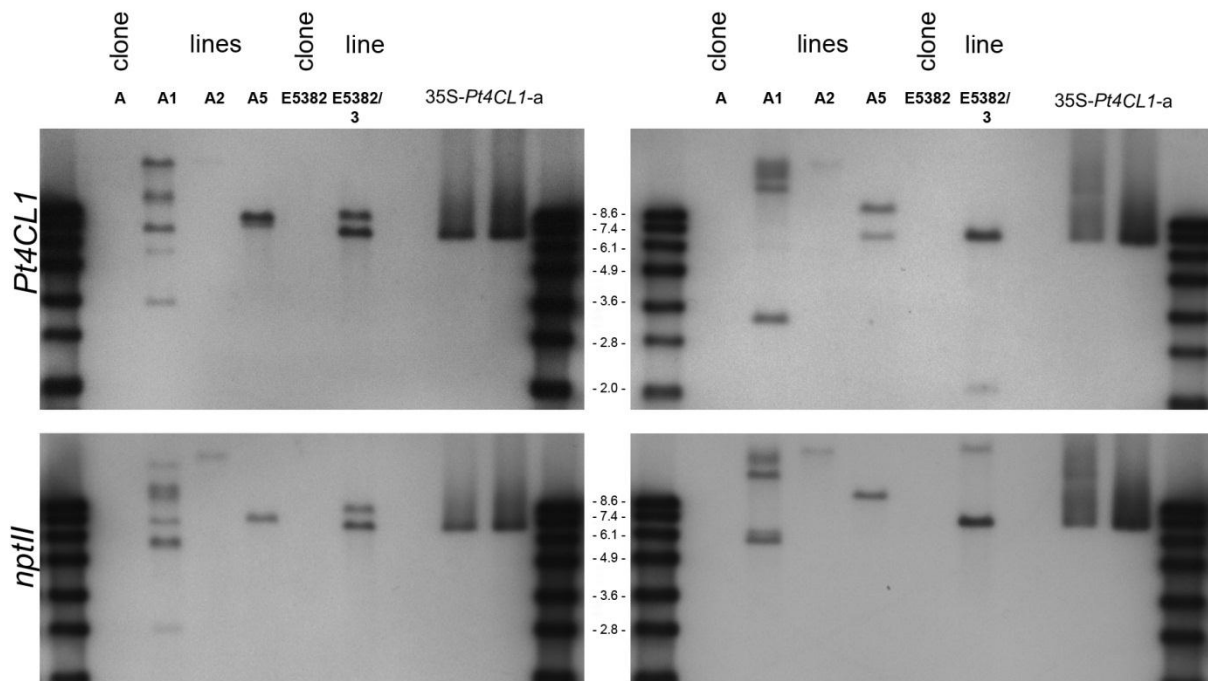


Figure S5. Southern blot analysis of regenerated silver birch lines. Lines A1, A2, A5 and E5382/3 were transformed with pRT99/35S-*Pt4CL1*-a plasmid vector and probed for the presence of the 35S-*Pt4CL1* and *nptII*. A genomic DNA sample of 15 μ g digested with *Bam*HI (figures on the left) or *Xba*I (figures on the right) was loaded to each lane.

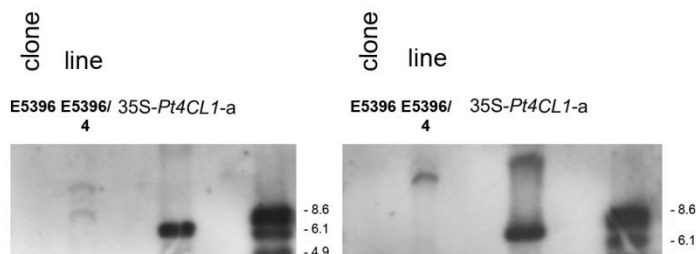


Figure S6. Southern blot analysis of regenerated silver birch line E5396/4. Line was transformed with pRT99/35S-*Pt4CL1*-a plasmid vector and probed for the presence of the *nptII*. A genomic DNA sample of 15 μ g digested with *Bam*HI (figures on the left) or *Xba*I (figures on the right) was loaded to each lane.

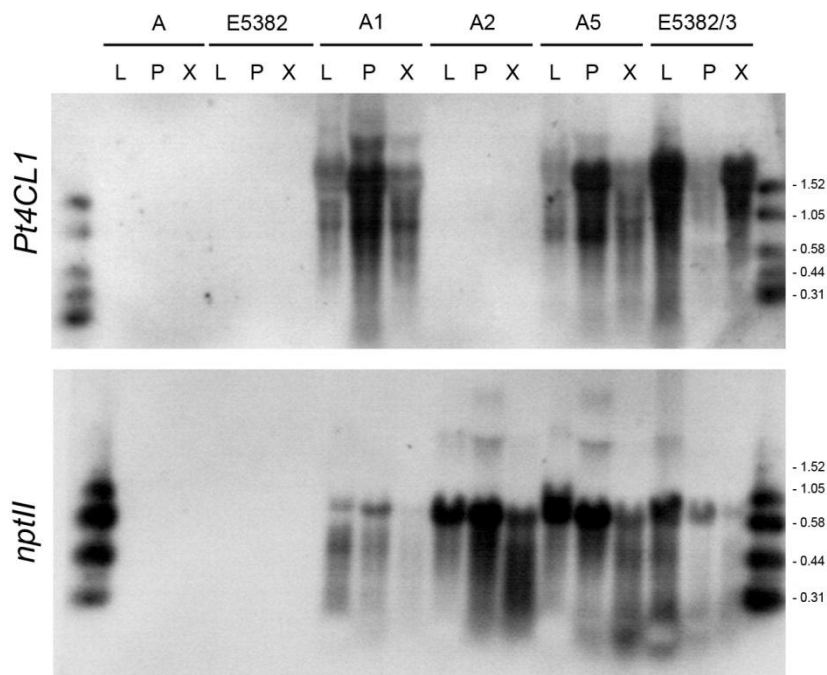


Figure S7. Northern blot analysis of transgenic silver birch lines. RNA samples of 15 μ g were isolated from the leaf (L), phloem (P) and xylem (X) of clones A and E5382 and lines A1, A2, A5 and E5382/3. The probes were 1.1 and 0.8 kb for *Pt4CL1* and *nptII*, respectively.



Figure S8. The leaf morphology of *Pt4CL1a* line E5382/3. During the first growing season in the greenhouse, the leaf margins of *Pt4CL1a* line E5382/3 (on the left) differed in from clone E5382 (on the right).



Figure S9. The autumn leaf senescence of silver birch clones and lines. The clone A, line A2, line A5, line E5382/3, and clone E5382 (from left to right) at the end of first growing season at the greenhouse of Finnish Forest Research Institute Punkaharju Unit (photographed on the 6th of October).

Bp1 4CL1	1	--	MAIQAKEQEFIFRSKLPDIYIPKHLPIHSYCFENISKVGSRPCLINGLTGKVVYTYADV
Bp4CL1	1	--	MAIQAKEQEFIFRSKLPDIYIPKHLPIHSYCFENISKVGSRPCLINGLTGKVVYTYADV
B1 4CL	1	--	MAIQAKEQEFIFRSKLPDIYIPKHLPIHSYCFENISKVGSRPCLINGLTGKVVYTYADV
Pt 4CL1	1	--	MNPQEFIFRSKLPDIYIPKHLPLHSYVLENLSKHSSKPCLINGANGDVYTYADV
Poptr 4CL3	1	-	MDAIMNSQEFIFRSKLPDIYIPKHLPLHSYVLENLSKYSSKPCLINGANGDVCTYADV
Poptr 4CL5	1	-	MDITIKQKEEFIFRSKLPDIYIPKGLPLHSYVFNFSKYPSKPCLINGANGDVYTYADV
Me4CL2	1		METQLINQQGEFIFRSKLPDIYIPKHLPLHSYIFENISSHSSRPCLINGATGDVYTYAEV
Me4CL3	1		METQVLKQGEFIFRSKLPDIYIPKHLPLHSYIFENISSHSSKPCLINGVTGNVYTYSEV
Gm4CL2	1	--	MADDGSRRELFIFRSKLPDIYIPKHMPLHSYCFENLRECGSRPCLINAPTDGVVYSYHEV
Gm4CL6	1	--	MAVVERRRELFIFRSKLPDIYIPKHLPLHTYCFENLPEYGARPCLINAPTDGVVYSYEEV
Bp1 4CL1	59		ELTARKVASGLSKLGIQKGDVVMLLPNSPPEFAFVFLGASYLGAMTTAANPFCTAGEVSK
Bp4CL1	59		ELTARKVASGLSKLGIQKGDVVMLLPNSPPEFAFVFLGASYLGAMTTAANPFCTAGEVSK
B1 4CL	59		ELTARKVASGLSKLGIQKGDVVMLLPNSPPEFAFVFLGASYLGAMTTAANPFCTAGEVSK
Pt 4CL1	55		ELTARRVASGLNKLGIQQGDVIMLFLPSSPEFVLAFLGASHRGAMITAAANPFSTPAELAK
Poptr 4CL3	60		ELTARRVASGLNKLGIQQGDVIMLFLPSSPEFVLAFLGASHRGAMITAAANPFSTPAELAK
Poptr 4CL5	60		ELTARRVASGLNKLGIQQGDVIMLFLPSSPEFVLAFLGASHRGAMITAAANPFSTPAELAK
Me4CL2	61		ELTARRVASGLNKLGIQQGDVIMLFLHNSPQFVLSFLGASFRGAMITAAANPFSTPAELAK
Me4CL3	61		ELTARRVASGLNKLGIQQGEVIMLFLHNSPEFVLSFLGASFRGAMITAAANPFSTPAELAK
Gm4CL2	59		DSTARVKVARGLKKEGVEQGOVIMLFLPNCPEFVFSFLGASHRGAMITAAANPFSTPAELAK
Gm4CL6	59		ESTARKVARGLKKEGVEQGOVIMLFLPNCPEFVFSFLGASHRGAMITAAANPFSTPAELAK
Bp1 4CL1	119		QAKSANAKIVVTOACYYDRVKDYTNENGVKIIICIDSPPE-----DCLHFSELTKADEND-
Bp4CL1	119		QAKSANAKIVVTOACYYDRVKDYTNENGVKIIICIDSPPE-----DCLHFSELTKADEND-
B1 4CL	119		QAKSANAKIVVTOACYYDRVKDYTNENGVKIIICIDSPPE-----DCLHFSELTKADEND-
Pt 4CL1	115		HAKASRAKLLITQACYEKKVDFARESDVKVMCVDSAPD-----GASLFRHITKADENE-
Poptr 4CL3	120		HAKASRAKLLITQACYEKKVDFARESDVKVMCVDSAPD-----GCLHFSELTKADENE-
Poptr 4CL5	120		QAKSNAKLLITQACYYDKVDAQQNDVKKVMCVDSAPD-----VCLHFSELTKADEND-
Me4CL2	121		QAKSNTKLLITQAAAYAEKVKDLARDHGIKVLICIDSPD-----GCLHFSSELSEADERD-
Me4CL3	121		QAKSNTKLLITQAAAYAEKVKDLARDYGIKILICIDSPD-----GCLHFSSELSEADERD-
Gm4CL2	119		QAHASNAKLLITQASYYDKVKDLR---DIKLVFVDSCPPHTEEKQHLHFSHLCEDNGDAD
Gm4CL6	119		QAHASNAKLLITQASYYDKVKDLR---HIKLVFVDSCPP-----QHLHFSQLCEDNG---
Bp1 4CL1	173	-	VAEVDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHSEVDILC
Bp4CL1	173	-	VAEVDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHSEVDILC
B1 4CL	173	-	VAEVDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHSEVDILC
Pt 4CL1	169	-	VPOVDISPDDVVALPYSSGTTGLPKGVMITHKGLITVAQQVDGDNPNLYYHSEVDILC
Poptr 4CL3	174	-	VPOVDISPDDVVALPYSSGTTGLPKGVMITHKGLITVAQQVDGDNPNLYYHSEVDILC
Poptr 4CL5	174	-	MPQVDISPDDVVALPYSSGTTGLPKGVMITHKGLITVAQQVDGDNPNLYYHSEVDILC
Me4CL2	175	-	MPDVNISSEDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHSEVDILC
Me4CL3	175	-	MPDVIDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHSDVDILC
Gm4CL2	176	-	VDVDVIDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHCHDTILC
Gm4CL6	168	-	DADVIDISPDDVVALPYSSGTTGLPKGVMITHKGLVTSVAQQVDGDNPNLYYHCHDTILC
Bp1 4CL1	232		VLPLFHIYSLNSVFLCGLRAGASLILPKFIEVSLQLIKHKVTVMPIVPPIVLAI TKF
Bp4CL1	232		VLPLFHIYSLNSVFLCGLRAGASLILPKFIEVSLQLIKHKVTVMPIVPPIVLAI TKF
B1 4CL	232		VLPLFHIYSLNSVFLCGLRAGASLILPKFIEVSLQLIKHKVTVMPIVPPIVLAI TKF
Pt 4CL1	228		VLPMFHIYALNSIML CGLRVGASLIMPKFIEVSLGLIEKYKVSIPVPPVMMVAI AKS
Poptr 4CL3	233		VLPMFHIYALNSIML CGLRVGASLIMPKFIDITLLGLIEKYKVSIPVPPVMMVAI AKS
Poptr 4CL5	233		VLPMFHIYALNSIML CGLRVGAALIMPKFIEVSLGLIEKYKVSIPVPPVMMVAI AKS
Me4CL2	234		VLPMFHIYALNSIML CGLRVGAALIMPKFIDIGALLQLIKYKIVTAVPIVPPIVLAI AKS
Me4CL3	234		VLPMFHIYALNSIML CGLRVGAALIMPKFIEVSLGLIKYKIVTAVPIVPPIVLAI AKS
Gm4CL2	236		VLPLFHIYSLNSVLLCGLRAKATILMPKFIDINSLLALIKHKVTVIAPVPPIVLAI SKS
Gm4CL6	227		VLPLFHIYSLNSVLLCGLRAKATILMPKFIDINSLLALIKHKVTVIAPVPPIVLAI SKS
Bp1 4CL1	292		PDLDKYDLSSVKMLKSGGAPLGKEIEETVKKAKFPNALFGQGYGMEAGPVLAMCLAFAKE
Bp4CL1	292		PDLDKYDLSSVKMLKSGGAPLGKEIEETVKKAKFPNALFGQGYGMEAGPVLAMCLAFAKE
B1 4CL	292		PDLDKYDLSSVKMLKSGGAPLGKEIEETVKKAKFPNALFGQGYGMEAGPVLAMCLAFAKE
Pt 4CL1	288		PDLDKHDLSSLRMLKSGGAPLGKELEDTVRAKFPQARLFGQGYGMEAGPVLAMCLAFAKE
Poptr 4CL3	293		PDLDKHDLSSLRMLKSGGAPLGKELEDTVRAKFPQARLFGQGYGMEAGPVLAMCLAFAKE
Poptr 4CL5	293		PDLDKHDLSSLRMLKSGGAPLGKELEDTVRAKFPQARLFGQGYGMEAGPVLAMCLAFAKE
Me4CL2	294		PDLTKYDLSSLRMLKSGAAPLGKELEDTVRAKFPQATLFGQGYGMEAGPVLAMCLAFAKE
Me4CL3	294		PDLTKYDLSSLRMLKSGAAPLGKELEDTVRAKFPQATLFGQGYGMEAGPVLAMCLAFAKE
Gm4CL2	296		PDLHKYDLSSLRVFKSGGAPLGKELEDLRAKFPNAKLFGQGYGMEAGPVLAMSLAFAKE
Gm4CL6	287		PDLHNYDLSSLRVFKSGGAPLGKELEDLRAKFPNAKLFGQGYGMEAGPVLAMSLAFARE

Figure S10. Alignment of predicted amino acid sequence of silver birch (*Betula pendula*) putative 4-coumarate:CoA ligase (Bp4CL1, KM099195). Bp4CL1 aligned with the predicted amino acid sequences of *Betula luminifera* (Bp14CL1, AY792353), *Betula platyphylla* (B14CL, FJ410448), *Glycine max* (Gm4CL2, Glyma13g44950; Gm4CL6, Glyma15g00390), *Manihot esculenta* (Mes4CL2, cassava4.1_005014m; Mes4CL3, cassava4.1_005006m), *Populus tremuloides* (Pt4CL1, AF041049), and *Populus trichocarpa* (Poptr4CL3, grail3.0100002702 LG I; Poptr4CL5, fgenesh4_pg.C_LG_III001773 LG III).

Pt 4CL2	1	MMSVATVE-----PPKPELSPPQONAPSSHET-----DHI FRSKLP
Popt r 4CL4	1	MMSVATVE-----PPKPELSPPQONAPSSHET-----DHI FRSKLP
Mes 4CL1	1	MSIASLELE-----PSKPEVSPPHNSDA-----HI FRSKLP
Bp4CL2	1	MISVANSST-----PONQEFSPKIPGPTQPSET-----ACVFKSKLP
Ri 4CL3	1	MISASNNNNNSVVVETPTKPEISPNISDVISSTSQIQPEKQQPPTTTTHHVFKSKLP
At 4CL3	1	MITAAALHPQ-----IHKPTDTSVVSDDVLPSPPT-----PRI FRSKLP
Gra 4CL3	1	MSEAAATSFETQHSSTESCVPKTTTSSDVSSSPQT-----HI FRSKLP
Pt 4CL2	38	DIIT-LSNDLPLHAYCFENLSDFSDRPCLISGSTGKTYSFAETHLSRKVAAGLSNLGK
Popt r 4CL4	38	DIIT-LSNHLPLHAYCFENLSDFSDRPCLISGSTGKTYSFAETHLSRKVAAGLSNLGK
Mes 4CL1	33	DIPI-LSNHLPLHAYCFENLSDFAADRPCLISGSTGKTYSFAETHLSOKCAAGLSNLGK
Bp4CL2	42	EIP-LSDHLPLHTYCFEFLAEFSDRPCLISGSTGKTYSFAETHLSOKIAAGLSNLGK
Ri 4CL3	61	DLPL-LSNHLPLHTYCFENLSDFSERPCLISGSTGKSYTFSETRLSOKTGVGLSKLGIHK
At 4CL3	41	DIID-LSNHLPLHTYCFEKLSSVSDKRPCLISGSTGKSYTYGETHLCRRFVASGLYKLGIRK
Gra 4CL3	47	DIPI-LPNLPLHKYCFCLKPEIADRPCLISAGS--RKYTYGETHVTSSRRVAAGMSKLGIOK
Pt 4CL2	97	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSQSEIFKQFSASRAKLIITOSQYVN
Popt r 4CL4	97	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSQSEIFKQFSASRAKLIITOSQYVN
Mes 4CL1	92	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSNEIFKQFSASRAKLIITOSQYVD
Bp4CL2	101	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSAEVFKQLNSSKAKLIITOSQYVD
Ri 4CL3	121	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSASEIFKQLEASNAKLIITOSQYVD
At 4CL3	100	GDVIMTLLQNCPEFVFSFNGASMGAVITANPFYTSQELYKQLKSSGAKLIITOSQYVD
Gra 4CL3	104	GDVIMTLLPNSEPEFVFSFMASSMLGAVITANPFYTAELTKQLAASKAKLVVTLTSAHVH
Pt 4CL2	157	KLGDSDCHENNQKPGEDFIVITIDDP--PENCLHFNVLVEASESEMP--TVSILPDDPVA
Popt r 4CL4	157	KLGDSDCHENNQKPEEDFIVITIDDP--PENCLHFNVLVEANSESEMP--TVSILPDDPVA
Mes 4CL1	152	KLKDS--QENQPKLGGDFNVIITIDDP--PENCLHFTVLSEAKSEIP--DVTIHPDDPVA
Bp4CL2	161	KLRETG--ENFPKLGEDFTVITVDDP--PEKCLHFSVISEANEGEFSSTVSIHPDDPVA
Ri 4CL3	181	KLKQP-----GQHFQVVTIDDP--PENCLHFSVLSDANEINELP--QVSIHPDDPVA
At 4CL3	160	KLKLN-----GENLTLITIDDEPT--PENCLPFSTLITDDEITNPFQETVDIHGDDAAA
Gra 4CL3	164	KLKQDQ-----QGLKVVTVDEPAADENCMSEFR--EGESEVA--EVELISADAVA
Pt 4CL2	213	LPFSSGTTGLPKGVLTHKSLITSVAQQVDGEIPNLYLKQDDVLCVLPFHI FSLNSVL
Popt r 4CL4	213	LPFSSGTTGLPKGVLTHKSLITSVAQQVDGEIPNLYLKQDDVLCVLPFHI FSLNSVL
Mes 4CL1	206	LPFSSGTTGLPKGVLTHKSLITSVAQQVDGENPNLYLKQEDVLCVLPFHI YSLNSVL
Bp4CL2	217	LPFSSGTTGLPKGVVLTTHKSLITSVAQQVDGENPNLYLNPDVLCVLPFHI YSLNSVL
Ri 4CL3	228	LPFSSGTTGLPKGVLTHKSLITSVAQQVDGENPNLYLKQDDVLCVLPFHI FSLNSVL
At 4CL3	210	LPFSSGTTGLPKGVVLTTHKSLITSVAQQVDGDNPNLYLKSNDVLCVLPFHI YSLNSVL
Gra 4CL3	209	LPFSSGTTGLAKGVVLTTHKSLVTVGVAQNMEGENPNVYLKEEDVLCVLPFHI FSIHNSVM
Pt 4CL2	273	LCSLRAGSAVLLMQKFEIGSLELEIKQHNSVAAVPPPLVLAALAKNPLVAVDFLSSIRVV
Popt r 4CL4	273	LCSLRAGSAVLLMQKFEIGSLELEIKQHNSVAAVPPPLVLAALAKNPLVAVDFLSSIRVV
Mes 4CL1	266	LCSLRAGAAVLVMQKFEIGALLELEIKHNSVAAVPPPLVLAALAKNPLVAVDFLSSIRVV
Bp4CL2	277	LCSLRAGAAVLVMQKFEIGALLELEIKHRVNSVAAVPPPLVLAALAKNPLVAVDFLSSIRVV
Ri 4CL3	288	LCSLRAGAAVLVMQKFEIGTLELEIKRYRVFCGGWCLAGDSAGEESVADYDLSSIRVV
At 4CL3	270	LNSLRSGATVLLMHKFEIGALLDLIKRHRVTIAALVPPPLVLAALAKNPLVAVDFLSSIRVV
Gra 4CL3	269	MCALRAGSAIILLIEKFEIRALLELEIKRHRVTIAAVVPPPLVLAALAKNPLVAVDFLSSIRVV
Pt 4CL2	333	LSGAAPLGKLEEDALRSRVPOAIGGGYGMT EAGPVLMSCLAFS KQPFPTKSGSCGT VVR
Popt r 4CL4	333	LSGAAPLGKLEEDALRSRVPOAIGGGYGMT EAGPVLMSCLAFS KQPFPTKSGSCGT VVR
Mes 4CL1	326	LSGAAPLGKLEEDALRSRVPOAIGGGYGMT EAGPVLMSCLGF AKQPFPTKSGSCGT VVR
Bp4CL2	337	LSGAAPLGKLEEDALRN RVPOAIGGGYGMT EAGPVLMSCLGF AKQPFPTKSGSCGT VVR
Ri 4CL3	348	LSGAAPLGKLEEDALRN RVPOAIGGGYGMT EAGPVLMSCLAF AKQPFPTKSGSCGT VVR
At 4CL3	330	LSGAAPLGKLEEDALRRRLPQALGGGYGMT EAGPVLMSLGF AKQPFPTKSGSCGT VVR
Gra 4CL3	329	MSGAAPLGKLEEDALRNRLPNAIGGGYGMT EAGPVLAMCLGF AKQPFPTKSGSCGT VVR
Pt 4CL2	393	NAELKVIDPETGRSLGYNQPGEICIRGSI MKGYLNDAEATANTIDVEGWLHTGDI GYVD
Popt r 4CL4	393	NAELKVIDPETGRSLGYNQPGEICIRGSI MKGYLNDAEATANTIDVEGWLHTGDI GYVD
Mes 4CL1	386	NAELKVIDPETGCSLGYNQPGEICIRGQI MKGYLNDLEATANTIDVEGWLHTGDI GYVD
Bp4CL2	397	NAELKVIDPETGCSLGYNQPGEICIRGSI MKGYLNDDAATATIDVEGWLHTGDI GYVD
Ri 4CL3	408	NAELKVIDPETGRSLGYNQPGEICVRGSI MKGYLNDGEATATIDVEGWLHTGDI GYVD
At 4CL3	390	NAELKVIDPETGRSLGYNQPGEICIRGQI MKGYLNDPEATATIDVEGWLHTGDI GYVD
Gra 4CL3	389	NAELKVIDPETGRSLGYNQPGEICIRGQI MKGYLNDKATATIDVEGWLHTGDI GYVD

Figure S11. Alignment of predicted amino acid sequence of silver birch (*Betula pendula*) putative 4-coumarate:CoA ligase (Bp4CL2, KM099196). Bp4CL2 aligned with the predicted amino acid sequences of *Arabidopsis thaliana* (At4CL3, At1g65060), *Rubus idaeus* (Ri4CL3, AAF91308), *Glycine max* (Gm4CL3, NM_001250341), *Manihot esculenta* (Mes4CL1, cassava4.1_004658m; Mes4CL4, cassava4.1_004136m), *Populus tremuloides* (Pt4CL2, AF041050), and *Populus trichocarpa* (Popt r 4CL4, grail3.0099003002 LG IX).

Poptr ACS1 1 ----- MEKSGYGRDGI YRSLRPTLVLPKDPNLSLVSFLFRNSNSYPHKPALI DADLS-IT
 Poptr ACS2 1 ----- MEKSGYGRDGI YRSLRPLVLKDPNLSLVSFLFRNSNSYPHKPALI DADLS-IT
 Bp4CL3 1 ----- MEKSGYGRDGI YRSLRPLVLKDPNLSLVSFLFRNSNSYPHKPALI DADLS-IT
 At ACS6 1 ----- MEKSGYGRDGI YRSLRPTLVLPKDPNLSLVSFLFRNSNSYPHKPALI DADLS-IT
 Os ACS1 1 MASASVPAAGYGADGVYRSLRPPAPVASDPGLSLTDLRLRRADACPSAVALADAAAGGRA

Poptr ACS1 55 LSFSELSKSI VIKFAHGLLN-LGI SKNDVLI FAPNSYQFPI CFLAITSI GAVATTANPLY
 Poptr ACS2 55 LSFSELSKSI VIKVAHGLLN-LGI SKNDVLI FAPNSYQFPI CFLAITSI GAVATTANPLY
 Bp4CL3 55 LSFSELSKSI VIKVAHGLLN-LGI SKNDVLI FAPNSYQFPI CFLAITSI GAVATTANPLY
 At ACS6 55 LSFSELSKSI VIKVAHGLLN-LGI SKNDVLI FAPNSYQFPI CFLAITSI GAVATTANPLY
 Os ACS1 61 LTFSELSKSI VIKVAHGLLN-LGI SKNDVLI FAPNSYQFPI CFLAITSI GAVATTANPLY

Poptr ACS1 114 TTSSELSKQI KDSNPKLVI TVPELWDKVKGFNLPAVFLGP--KEVSLPLESGSRI RFSFHS
 Poptr ACS2 114 TTSSELSKQI KDSNPKLVI TVPELWDKVKGFNLPAVFLGP--KRVSLPLESGSRI RFSFHS
 Bp4CL3 114 TVHELARQYKDSNPKLVI TVPELWDKVKGFNLPAVFLGSG--NHSS---SSNVI HFDDL
 At ACS6 114 TVNEVSKQI KDSNPKLVI TVPELWDKVKGFNLPAVFLGSG--DTVEI PPGSNSKILSFDNV
 Os ACS1 121 TPREI AKQVSDARAKLVI TISALVPKI AGLRRLPVI LDDANAAAASLPDPATVTLYTNL

Poptr ACS1 172 MELGG-----SNSEFPVSDVKQSDI ATLLYSSGTTGVSKGVI LTHGNFI AASLMVMSMDQ
 Poptr ACS2 172 VGLGG-----SNSQFPSSNVKQSDI STLLYSSGTTGVSKGVI LTHGNFI AASLMVMSMDQ
 Bp4CL3 168 VNLGGTWSGSRSGSDFPDSNVKQNDT AALLYSSGTTGVSKGVI LTHGNFI AASLMVMSMDQ
 At ACS6 173 MELSEP-----VSEYFPFVEI KQSDT AALLYSSGTTGVSKGVI LTHGNFI AASLMVMSMDQ
 Os ACS1 181 VAGVK-----EADYRRPPI KQSDT AALLYSSGTTGVSKGVI LTHGNFI AASLMVMSMDQ

Poptr ACS1 226 VMAGEI HNVFLCFLPMFHVFGGL AVI TYSQLQMGNAVVS MGKFEFEMVLRITIEKYRVTHMW
 Poptr ACS2 226 AMAGEMHNVFLCFLPMFHVFGGL AVI TYSQLQMGNAVVS MGKFEFEMVLRITIEKYRVTHMW
 Bp4CL3 228 ELSGEAHNVFLCFLPMFHVFGGL AVI TYSQLQMGNAVVS MGKFEFEMVLRITIEKYRVTHMW
 At ACS6 227 DLMGEYHGVFLCFLPMFHVFGGL AVI TYSQLQMGNAVVS MGKFEFEMVLRITIEKYRVTHMW
 Os ACS1 234 DERREGPNVFLCFLPMFHVFGGL AVI TYSQLQMGNAVVS MGKFEFEMVLRITIEKYRVTHMW

Poptr ACS1 286 VVPPVILALAKQDMVKKYDLSLRLNIGS GAAPL GKDL MEECAKNLPDITII QGFGMTETC
 Poptr ACS2 286 VVPPVILALAKQDMVKKYDLSLRLNIGS GAAPL GKDL MEECAKNLPDITII QGFGMTETC
 Bp4CL3 288 VVPPVILALAKQDMVKKYDLSLRLNIGS GAAPL GKDL MEECAKNLPDITII QGFGMTETC
 At ACS6 287 VVPPVILALAKQDMVKKYDLSLRLNIGS GAAPL GKDL MEECAKNLPDITII QGFGMTETC
 Os ACS1 294 CVPPVILALAKHGKAGKYDLSLRLNIGS GAAPL GKDL MEECAKNLPDITII QGFGMTETC

Poptr ACS1 346 GIVSLEDPRIG-VRHSGSAGI LNAIGEAQI SVETAKPLPPNQLGEI WVRGPNMMRGYFN
 Poptr ACS2 346 GIVSLEDPRIG-VRHSGSAGI LNAIGEAQI SVETAKPLPPNQLGEI WVRGPNMMRGYFN
 Bp4CL3 348 GIVSLEDPRIG-VRHSGSAGI LNAIGEAQI SVETAKPLPPNQLGEI WVRGPNMMRGYFN
 At ACS6 347 GIVSLEDPRIG-VRHSGSAGI LNAIGEAQI SVETAKPLPPNQLGEI WVRGPNMMRGYFN
 Os ACS1 354 GIVSLEDPRIG-VRHSGSAGI LNAIGEAQI SVETAKPLPPNQLGEI WVRGPNMMRGYFN

Poptr ACS1 405 NPQATKDTIDKKGWHTGDLGYFDGQGFVVDRI KELI KYKGFQVAPAELEGLLVSHPE
 Poptr ACS2 405 NPQATKDTIDKKGWHTGDLGYFDGQGFVVDRI KELI KYKGFQVAPAELEGLLVSHPE
 Bp4CL3 407 NPQATKDTIDKKGWHTGDLGYFDGQGFVVDRI KELI KYKGFQVAPAELEGLLVSHPE
 At ACS6 406 NPQATKDTIDKKGWHTGDLGYFDGQGFVVDRI KELI KYKGFQVAPAELEGLLVSHPE
 Os ACS1 414 NPQATKDTIDKKGWHTGDLGYFDGQGFVVDRI KELI KYKGFQVAPAELEGLLVSHPE

Poptr ACS1 465 ILDAVVI PYPDAEAGEVPIAYVVRSPNSALTEEDVQKFI SDQVAPFKRLRRTVFI NSVPK
 Poptr ACS2 465 ILDAVVI PYPDAEAGEVPIAYVVRSPNSALTEEDVQKFI SDQVAPFKRLRRTVFI NSVPK
 Bp4CL3 467 ILDAVVI PYPDAEAGEVPIAYVVRSPNSALTEEDVQKFI SDQVAPFKRLRRTVFI NSVPK
 At ACS6 466 ILDAVVI PYPDAEAGEVPIAYVVRSPNSALTEEDVQKFI SDQVAPFKRLRRTVFI NSVPK
 Os ACS1 473 ILDAVVI PYPDAEAGEVPIAYVVRSPNSALTEEDVQKFI SDQVAPFKRLRRTVFI NSVPK

Poptr ACS1 525 SASGKILRREL VQVSKSKM-
 Poptr ACS2 525 SASGKILRREL VQVSKSKM-
 Bp4CL3 527 SASGKILRREL VQVSKSKM-
 At ACS6 526 SASGKILRREL VQVSKSKM-
 Os ACS1 533 SASGKILRREL VQVSKSKM-

Figure S12. Alignment of predicted amino acid sequence of silver birch (*Betula pendula*) putative 4CL-like acyl-CoA synthetase (ACS) (Bp4CL3, KM099197). Bp4CL3 aligned with the predicted amino acid sequences of *Arabidopsis thaliana* (AtACS6, At4g05160), *Oryza sativa* (OsACS1, Os03g05780) and *Populus trichocarpa* (PoptrACS1, eugene3.01230068; PoptrACS2, estEXT_fgenesh1_pg_v1.C_LG_IV0024).

Bp4CL4	1	ME-PQK----DLQEFIFRSKLPDIYI PNHLPLHTYCFENLSQFKDRPCLINGADGVTYTY
Ri4CL2	1	MENKHQ---DDHEFIFRSKLPDIYI PNHLPLHTYCFENLSQFKDRPCLINGNTGETFTY
Poptr4CL1	1	MEAEKD---QAQEFIFRSKLPDIHI PNHLPLHTYCFENLSRFKDRPCLINGPTGETFTY
Poptr4CL2	1	MEANKD---QVQEFIFRSKLPDIYI PNHLPLHTYCFEKLSQFKDYPCLINGPTGDIYTY
Gm4CL7	1	MEKQPTQPQHDFIFRSKLPDIYI PTHLPLHTYLFQNLQSFKDRPCLINAATGETFTY
Gm4CL8	1	MEEQQ---AHHDFIFRSKLPDIYI PSHLPLHTYLFQNLQSFKDRPCLINGTTGETFTSY
Gm4CL1	1	MAPSPQ-----ETIFRSPLPDIPIPTHLPLYSYCFQNLQSKFDRPCLIDGDTGETLTY
Gm4CL9	1	MELSPQ-----EFIFRSPLPDIPIPTHLPLYSYCFQKLSQFDRPCLIDGDTSETLTY
Bp4CL4	56	AQVELTARKVASGLDKLGIKQGEVIMLLLQNCPEFAFAFLGASYIGAVSITANPFYTPAE
Ri4CL2	57	AEVELTSRRVAAGLDKLGIQONDVVMLLLQNCPEFAFAFLGASYIGAMSTANPFYTPAE
Poptr4CL1	57	ADVELTSRKVASGLNKLGIKQGDVIMLLLQNSPEFVFAFLGASTIGASITANPFYTPAE
Poptr4CL2	57	ADVELTSRKVASGLYKLGVOGDVIMLLLQNSPEFVFAFLGASTIGASITANPFYTPAE
Gm4CL7	61	AAVELTARKVASGFNKLGIKQGDVIMLLLQNCPEFVFAFLGASYRGATVITANPFYTPAE
Gm4CL8	56	HAIQLTARRVASGLNKLGIKQGDVIMLLLQNCPEFVFAFLGASYRGATVITANPFYTPAE
Gm4CL1	54	ADVDLAARRIASGLHKIGIRQGDVIMLVLRNCPQFALAFLGATHRGAVVITANPFYTPAE
Gm4CL9	54	ADVDLAARRIASGLHKIGICQGDVIMLVLRNCPQFALAFLGATHRGAVVITANPFYTPAE
Bp4CL4	116	VAKQAKASNTKLIITQSSYVDKVKDFAKENG-VKVMCIDSDS----SLDDFLHFSQLTQADE
Ri4CL2	117	VAKQAKASNAKLIITQSAVYVDKVKDFAKLND-VKVMCVDVET----SSEDVLHFSSELVSADE
Poptr4CL1	117	VAKQATASKAKLIITQAVYAEKVQQFVKEVDHVKIIVTVDS----PPENYLHFSSELVTSDE
Poptr4CL2	117	VAKQATASKAKLIITQAVYAEKVQQFAQENDHVKIMTIIDSDS----LTENCLHFSSELVTSDE
Gm4CL7	121	VAKQATASNSKLIITQASVYVDKVKDFAREND-VKVICVDS----APDGYLHFSVLTQADE
Gm4CL8	116	VAKQATASNSKLIITQASVYVDKVKDFAREND-VKVICVDS----APEGYLHFSSELVTEADE
Gm4CL1	114	LAKQATATKTRLVITQSAVYVEKIKSFADSSDVMVMCIDDDFSYENDGVLHFSVLTQNADE
Gm4CL9	114	LAKQAMATKTRLVITQSAVYLEKIKSFADDS-DVMVMCIDDDYSSENDGVLHFSVLTQNADE
Bp4CL4	171	NDIPAVKINPDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFHS GDVIL
Ri4CL2	173	SETPAVKINPDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFHKE DVI L
Poptr4CL1	173	DDIPAVKINPDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFHE KDVI L
Poptr4CL2	173	NEIPAVKINPDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFHERDVI L
Gm4CL7	176	GDIIPAVKISQDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFRS DDVVV
Gm4CL8	171	GDIIPAVKISQDDVVALPYSSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFRS DDVLL
Gm4CL1	174	TEAPAVKINPDELVALPFSSTGSLPKGVMLSHKNLVTTIAQLVDGENPHQYTHSE DVL L
Gm4CL9	173	REAPAVKINPDDLVALPFSSTGSLPKGVMLSHENLVTTISQLVDGENPHQYTHSE DVL L
Bp4CL4	231	CVLPLFHIYSLNSVFLCGLRVGASILIMQKFEIVKLELQVQYKVTIAPFVPPIVLAI AK
Ri4CL2	233	CVLPLFHIYSLNSVFLCGLRVGAAILIMQKFEINLLELVEKEKVTIAPFVPPIVLSI AK
Poptr4CL1	233	CVLPLFHIYSLNSVLLCGLRVGSAIILIMQKFEIVTLMELVQYKVTIAPFVPPIVLVA AK
Poptr4CL2	233	CVLPLFHIYSLNSVLLCGLRVGSAIILIMQKFEIVSLMDLVQYKVTIAPLVPPIVLAI AK
Gm4CL7	236	CVLPLFHIYSLNSVLLCGLRVGAAVLIVPKFEEVALLLELVQKHNVSVAPFVPPIVLAI AK
Gm4CL8	231	CLLPLFHIYALNSVLLCGLRVGASVLIIVPKFEEITLLELIVQKHVSTAPFVPPIVLVA AK
Gm4CL1	234	CVLPMFHIYALNSILLCGIRSGAAVLIIVQKFEITLLELIVQKVTIVASFVPPIVLALVK
Gm4CL9	233	CVLPMFHIYALNSILLCGIRSGAAVLIIVQKFEITLLELIVQKVTIVASFVPPIVLALVK
Bp4CL4	291	SPDVQWYDVSSI RTVMSGAAPMGKELEDAVRAKLPNAKLGQGYGNTAEGPVLAMCLAF AK
Ri4CL2	293	CPDLHRYDLSSI RMVMSGAAPMGKELEDTVRAKLPNAKLGQGYGNTAEGPVLAMCLAF AK
Poptr4CL1	293	CPVVDKYDLSSI RTVMSGAAPMGKELEDTVRAKLPNAKLGQGYGNTAEGPVLAMCLAF AK
Poptr4CL2	293	SPVVDQYDLSSI RTVMSGAAPMGKELEDTVRAKLPNAKLGQGYGNTAEGPVLAMCLAF AK
Gm4CL7	296	SPDVERYDVSSI RMTMSGAAPMGKELEDSVRAKLPNATLGQGYGNTAEGPVLAMCLAF AK
Gm4CL8	291	SPDLERYDLSSI RMTMSGAAPMGKELEDSVRAKLPNAI LGQGYGNTAEGPVLAMCLAF AK
Gm4CL1	294	SGETHRYDLSSI RAVVTGAAPLGGELQEAVKARLPHATFGQGYGNTAEGPVLAMCLAF AK
Gm4CL9	293	SGETHRYDLSSI RAVVTGAAPLGGELQEAVKARLPHATFGQGYGNTAEGPVLAMCLAF AK
Bp4CL4	351	EAFETIKSGACGTVVRNAQVKIVDPDTGASLPRNQAGEICIRGSIKMGYLNDEPEATERTI
Ri4CL2	353	EPYETIKSGACGTVVRNAENKIVDPDTNESLPRNOSGEICIRGSIKMGYLNDEPEATERTI
Poptr4CL1	353	EPFETIKSGACGTVVRNAENKIVDPDTGRSLPRNQAGEICIRGSIKMGYLNDEPEATERTI
Poptr4CL2	353	EPFETIKSGACGTVVRNAENKIVDPDETGDSQPRNKAGEICIRGSIKMGYLNDEPEATERTI
Gm4CL7	356	EPVQVKSACGTVVRNAENKIVDPDTGASLPRNQAGEICIRGNQIKMGYLNDEPEATERTI
Gm4CL8	351	EPVQVKSACGTVVRNAENKIVDPRTGASLPRNQAGEICIRGNQIKMGYLNDEPEATERTI
Gm4CL1	353	VPSKIKPACGTVVRNAENKIVDETETGDSLPRNKHGEICIRGTVKMGYLNDEPEATERTI
Gm4CL9	352	EPSKIKPACGTVVRNAENKIVDETETGDSLPRNKSGEICIRGAKVMGYLNDEPEATERTI

Figure S13. Alignment of predicted amino acid sequence of silver birch (*Betula pendula*) putative 4-coumarate:CoA ligase (Bp4CL4, KM099198). Bp4CL4 with the predicted amino acid sequences of *Rubus idaeus* (Ri4CL2, AAF91309), *Glycine max* (Gm4CL7, Glyma17g07170; Gm4CL8, Glyma17g07180; Gm4CL1, Glyma17g07190; Gm4CL9, Glyma13g01080), and *Populus trichocarpa* (Poptr4CL1, estExt_fgenesh4_pg.C_1210004 scaffold3; Poptr4CL2, gw1.XVIII.2818.1 LG_XVIII).

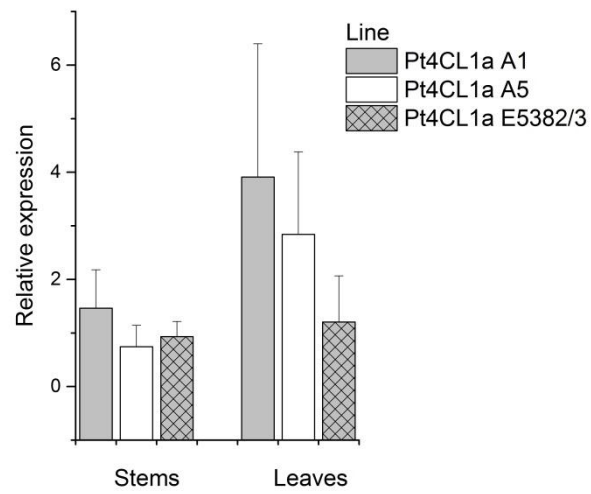


Figure S14. Relative expression of *Pt4CL1* in stems and leaves of *Pt4CL1a* lines A1, A5 and E5282/3. Values represent means and standard deviations calculated using *PP2A* gene as reference gene (n = 3–14).

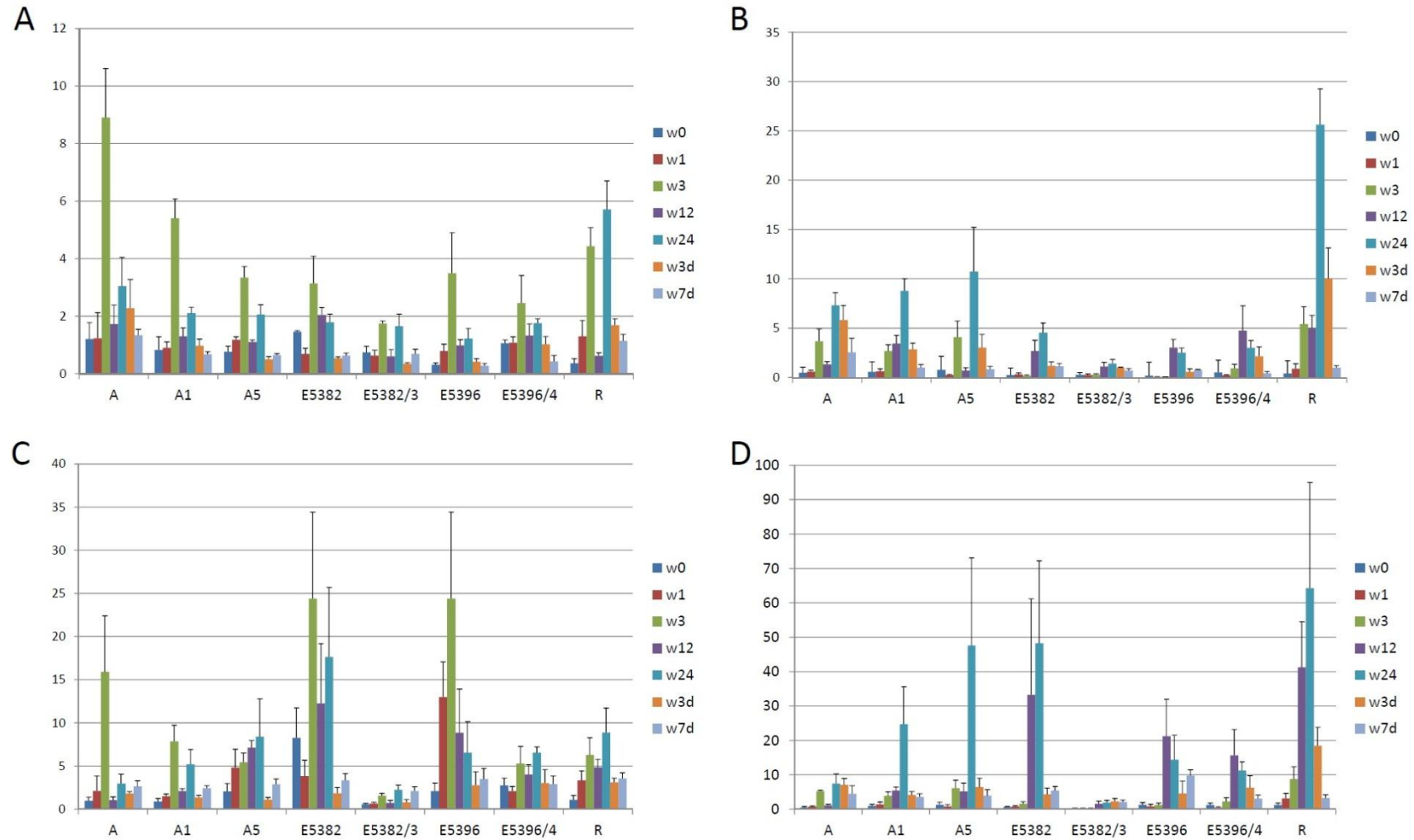


Figure S15. Relative expression of *Bp4CL1-4* genes in mechanically wounded silver birch (*Betula pendula*) leaves. The *Bp4CL1* (A, C) and *Bp4CL2* (B, D) expression in leaves collected immediately (w0) and 1 (w1), 3 (w3), 12 (w12), 24 (w24), 72 (w3d), and 162 (w7d) h after mechanical wounding of clones A, E5382, E5396 and R and transgenic lines A1, A5, E5382/3 and E5396/4. Values represent means and standard errors calculated from the target/reference ratios. A and C, *PP2A* was used as the reference gene; B and D, *Atub* was used as the reference gene.

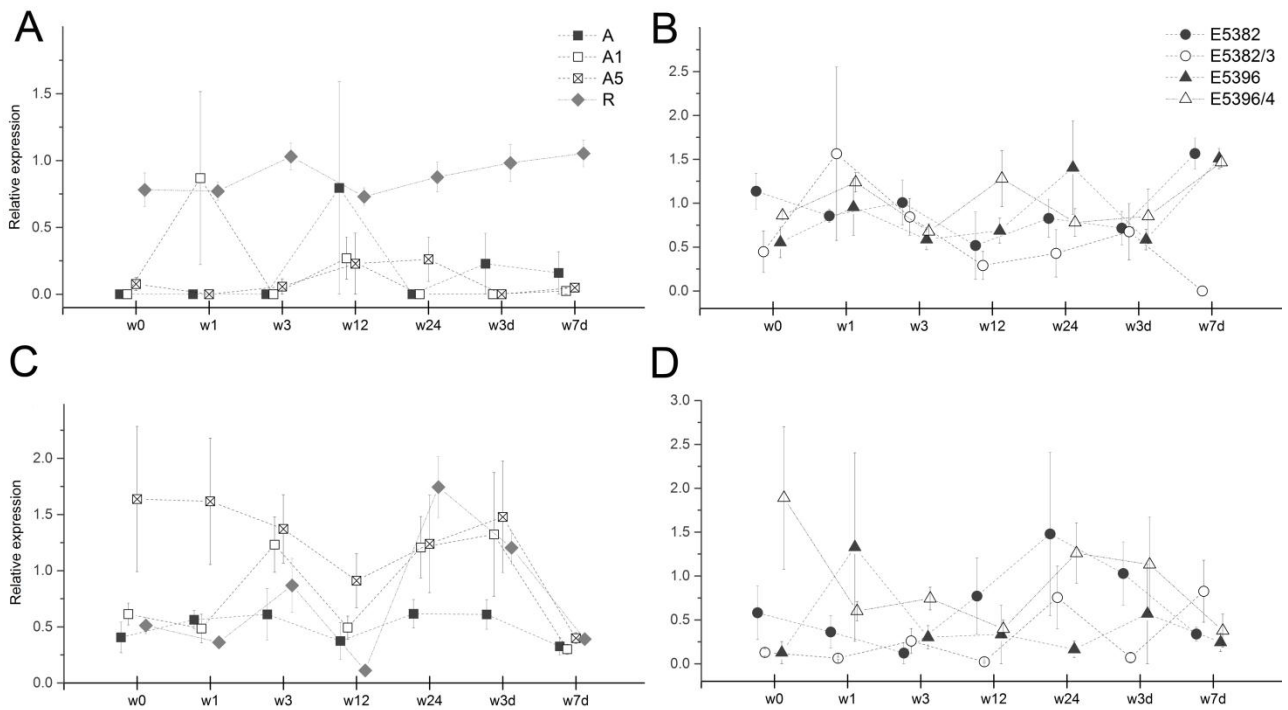


Figure S16. Relative expression of *Bp4CL3* (A, B), and *Bp4CL4* (C, D) in wounded leaves. The leaves were collected immediately (w0) and 1, 3, 12, 24, 48, and 162 h after mechanical wounding of silver birch (*Betula pendula*) clones A and R and transgenic lines A1 and A5 (A, C) and clones E5382 and E5396 and transgenic lines E5382/3 and E5396/4 (B, D). Values represent means and standard errors calculated using the mean of control leaves as normalizer within each line/clone and *PP2A* as reference.