Supplementary data

Arabidopsis membrane-associated acyl-CoA-binding protein ACBP1 is involved in stem cuticle formation

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Supplementary Figure S1.



Figure S1. Expression analysis of wax biosynthetic genes (A), cutin biosynthetic genes (B), and genes with no implication on cuticle formation (triacylglycerol biosynthetic genes (C) and cold-related genes (D)) in stems of Col-0, the *acbp1* mutant and *acbp1-COM* line. Asterisks denote significant differences from the wild type (* P < 0.05, ** P < 0.01). Values are means ± SE (n = 3).

Supplementary Figure S2.



Figure S2. Cuticular wax (A) and cutin monomer (B) composition and amount in leaves of Col-0 and the *acbp1* mutant. Four-week-old leaves were used for wax and cutin analysis by gas chromatography (GC)-flame ionization detector and GC-mass spectrometry. FA, fatty acid; 10,16-diol, C16-10,16-dihydroxyl fatty acids. Asterisks denote significant differences from the wild type (* P < 0.05). Values are means \pm SE (n = 3).

Supplementary Table S1. Sequences of gene-specific primers for qRT-PCR.

ML1124CCCGCTATGTATGTCGCAl3g18780ML1125AAGGTCAAGACGGAGGAT(ACTN2)ML125AGGCAAACATCACCACAATAGGAl1g68530ML1826AGGCACCTTGGATCACCGCTGG(CUT1/KCS6)ML1827AACCGCTGGAATACCGACTG(CER8)ML1828ATTCCACGCGGTTTCCTCTC(Al3g55360)ML1829GGCTTTCCATCTTTCCATCAA(CCR)ML1833GCTTTACCGATCGGTCGTTG(CCR)ML1934GTCGTGATGAGCAGAAAAACC(CCP)ML1919GGCTTTCCAACGCAAAACC(CP)ML1919GGCTTTCCAACGCAAAACC(CP)ML1919GGCTCTTCCAACGCAAAACC(CP)ML1933GATTGTGCCAGTAGCGATGAAAl4g00360ML1934TCTTGCCACCACGGTGAC(CP)ML1935AATGTCGCCAGTAGCGATGAAAl4g00400ML1934TCTTGCCACCACCGTGAC(CP)ML1935AATGTCGCCAGGAGAGAGAGTCCACTAl2g1450ML2026GGAGGGCGAGAGAGAGTCCACTTAl2g1450ML2027CCAATCTCGCAGCGATTCGAGAAAl3g1130ML2030GGTCTTGATCCTGAAGCTGAATCGA(DGAT2)ML2031TCAGTCATCTTCTAGGCTGAGAAl3g51520ML2032TGCTGAACAAAGGAGAGTCTCAAG(DGAT3)ML2033CCTTTCTCCGGTGACCAATCAAG(COR7)ML1716GTCCACGCCGTGGTTGTAl1g20440ML1718TTCCACCAGGGACAAAGG(Al252310)ML1718TTCCACCAGGGACAAGG(Al25490)ML1785GGACTTTCCAAACGCTGAGGAAGTT(CBF1)ML1901GATTTCTGTCTGCTGCGTCACTAl326744ML1902CCATTAGCAGGACATACCAAAACC(CCF)	Name	Sequence $(5^2 \rightarrow 3^2)$	Target gene
ML1125AAGGTCAAGACGGAGGAT(ACTIN2)ML1824AGGCAAACATCACCACAATAGGAlig68530 (CUTI/KCS6)ML1825CTCGTCGATCACCGCTCG(CUTI/KCS6)ML1826AGCACCTTTGAGCCCTGAGAAl2g47240 (CER8)ML1827AACCGCTGGAATACCGACTG(CER8)ML1828ATTCCACGCGGTTCCTCTCAl3g55360 (ECR)ML1829GGCTTTCCATCTTTTCCATCAA(ECR)ML1833GCTTTACCGATCGTCTGTG(KCR1)ML1917TAATACACGTAAGCAAGAAT(Al4g00360) (CPP86A2)ML1918GTCGTGATGAGCCAGAAAAACC(CPP86A2)ML1919GGCTCTTCCAACGCAAAAACC(CPP86A4)ML1933GATTGTGCCAGTAGCGATGAA(Al4g00400) (GPAT8)ML1934TCTTGCCACCACGGTGAC(GPAT8)ML1935AATGTCGCACAGCGCAGTAACC(Al2g19450) (DGAT1)ML2026GGAGGGCGAGAGAGAGTCCACTTAl2g19450 (DGAT1)ML2028CCGAACGCCATATTGCAGCATTGAAC(DGAT1)ML2030GGTCTTGATCCTGAAGGTGGTTGGAl1g43300 (DGAT1)ML2031TCAGTCATCTGCTACGATGGATTGG(DGAT3)ML2032TGCTGAAGCAAAGGGTGTTGCA(Al5g13640 (PDAT1))ML2033CCTTTCTCCGGTGACCAATCAAG(DGAT3)ML1716GTCCACGCCGTTGGTTGT(Al4g25490 (CBF7)ML1718TTCCACCAGGGACAAGGGAl4g25490 (CBF1)ML1901GATTTCTGTCTCTCCTCGGTCACTAl3g26744 (CEI)	ML1124	CCCGCTATGTATGTCGC	At3g18780 (ACTIN2)
ML1824AGGCAAACATCACCACAATAGGA11g68530 (CUTI/KCS6)ML1825CTCGTCGATCACCGCTCG(CUTI/KCS6)ML1826AGCACCTTTGAGCCCTGAGAA12g47240 (CER8)ML1827AACCGCTGGAATACCGACTG(CER8)ML1828ATTCCACGCGGTTTCCTTCA13g55360 (ECR)ML1829GGCTTTCCATCTTTTCCATCAA(CR7)ML1833GCTTTACCGATCGTCTTTG(C7P86A2)ML1917TAATACACGTAAGCAAGAAT(A14g00360) 	ML1125	AAGGTCAAGACGGAGGAT	
ML1825CTCGTCGATCACCGCTCTG $(CUTrKC30)$ ML1826AGCACCTTTGAGCCCTGAGA $A12g47240$ (CER8)ML1827AACCGCTGGAATACCGACTG $(CER8)$ ML1828ATTCCACGCGGTTTCCTCTTC $A13g55360$ (ECR)ML1829GGCTTTCCATCTTTTCCATCAA (ECR) ML1833GCTTTACCGATTCGGTCGTCTGTG (ECR) ML1917TAATACACGTAGCAAGAAAAACC $(CTP86A2)$ ML1918GTCGTGATGAGCCAGAAAAACC $(CTP86A2)$ ML1919GGCTCTTCCAACGCAAAAACC $(CTP86A4)$ ML1920CGAACGCCAATCCGCAAATG $(CTP86A4)$ ML1933GATTGTGCCAGTAGCGATGAA $A14g00400$ ($(CP786A4)$ ML1934TCTTGCCACGACGCAGTAGAC $(IA2S2)$ ML1935AACGCAGCAATTCCGGCAGTATCC $(IACS2)$ ML2026GGAGGCGAGAGAGAGAGTCCACTT $A12g19450$ ($DGATT)$ ML2028CCGAGCTGAGGAACATTCAAATCA $A13g51520$ ($DGAT2$)ML2030GGTCTTGATCCTGCAGGAGGAGAGAGTCGAGA $A11g48300$ ($DGAT2$)ML2031TCAGTCATCTTCTTCAGGGTTTGG $A11g48300$ ($DGAT2$)ML2032TGCTGAAGCAAAAGGATGTTGCA $A13g13640$ ($PDATI$)ML2033CCTTTCTCCGGTGGACCAATCAAGG $A13g2310$ ($LT778$)ML1716GTCCACGCCGTTGGTTGT $A11g20440$ ($CBF1$)ML1719CATCGTGTCCGTAAGAGGGC $A13g25120$ ($CBF1$)ML1786CAAGCCGAGTCAGCGAAGGG $A13g257241$ ((CE))ML1901GATTTCTCTCTCTCCCG $A13g26744$ ((CE))	ML1824	AGGCAAACATCACCACAATAGG	At1g68530 (CUT1/KCS6)
ML1826AGCACCTTTGAGCCCTGAGAAl2g47240 (CER8)ML1827AACCGCTGGAATACCGACTG(CER8)ML1828ATTCCACGCGGTTTCCTCTCAl3g55360 (ECR)ML1829GGCTTTCCATCTTTTCCATCAAAl1g67730 (KCR)ML1831GCTTTACCGATTCGTCTGTTG(KCR)ML1917TAATACACGTAGCAAGAAAAACC(CP86A2)ML1918GTCGTGATGAGCCAGAAAAACC(CP86A2)ML1919GGCTCTTCCAACGCAAAAACC(CP86A4)ML1913GATTGTGCCAGTAGCGATGAAAl4g00360 	ML1825	CTCGTCGATCACCGCTCTG	
ML1827AACCGCTGGAATACCGACTG(CERs)ML1828ATTCCACGCGGTTTCCTCTCAl3g55360 (ECR)ML1829GGCTTTCCATCTTTTCCATCAA(ECR)ML1832ACCCACTTGGCTCCTCATTCTCAl1g67730 (KCR)ML1833GCTTTACCGATTCCGTCTGTG(KCR)ML1917TAATACACGTAAGCAAGAATAl4g00360 (CYP86A2)ML1919GGCTCTTCCAACGCAAAACC(CYP86A2)ML1919GGCTCTTCCAACGCAAAACCAl1g01600 (CYP86A4)ML1920CGAACGCCAATCCGCAAATG(CP86A4)ML1933GATTGTGCCAGTAGCGATGAAAl4g00400 (GPA78)ML1934TCTTGCCACCACGTTGAC(LACS2)ML1938AATGCGCACGCAGTATCC(LACS2)ML2026GGAGGGCGAGAGAGAGTCCACTTAl2g19450 (DGAT1)ML2028CCGAGCTGAGGAACATTCAAATCAAl3g51520 (DGAT1)ML2030GGTCTTGATCCTGAAGCTGGTTTGAl1g48300 (DGAT2)ML2031TCAGTCATCTTCTTCATGGCTTTGGAl1g48300 (DGAT3)ML2032TGCTGAAGCAAAGGATGTTGCAAl1g20440 (COR47)ML2033CCTTTCCCGGTGACCAATCAAGGAl5g13640 (PDATI)ML1716GTCCACGCCGTTGGTTGTAl1g20440 (CBAT3)ML1718TTCCACCAGGGACAAAGGAl5g52310 (LIT78)ML1719CATCGTGTCCGTAAGAGGCAl4g25490 (CBF1)ML1901GATTTCTGTCTGCTGGTCGGTCACTAl3g26744 (CE)	ML1826	AGCACCTTTGAGCCCTGAGA	At2g47240 (CER8)
ML1828ATTCCACGCGGTTTCCTCTCAt3g55360 (ECR)ML1829GGCTTTCCATCTTTTCCATCAA(ECR)ML1832ACCCACTTGGCTCCTCATTCTCAt1g67730 (KCRI)ML1833GCTTTACCGATTCCGTCTGTTGAt4g00360 (CVP86A2)ML1917TAATACACGTAAGCAAGAATAt4g00360 	ML1827	AACCGCTGGAATACCGACTG	
ML1829GGCTTTCCATCTTTTCCATCAA(LCA)ML1832ACCCACTTGGCTCCTATTCTCAt1g67730 (KCR)ML1833GCTTTACCGATTCCGTCGTTGAt1g00360 (CYP86A2)ML1917TAATACACGTAAGCAAGAATAt4g00360 (CYP86A2)ML1918GTCGTGATGAGCCAGAAAACCAt1g01600 (CYP86A4)ML1920CGAACGCCAATCCGCAAAACCAt1g01600 (CYP86A4)ML1933GATTGTCCACGCAGTAGCGATGAAAt4g00400 (GPA78)ML1934TCTTGCCACCACCGTTGAC(LACS2)ML1935AATGTCGCCACGCCAGTATCCAt1g49430 (LACS2)ML2026GGAGGGCGAGAGAGAGTCCACTTAt2g19450 (DGAT1)ML2028CCGAGCTGAGGAGAACATTCAAATCAAt3g51520 (DGAT2)ML2030GGTCTTGATCCTGAAGCTGGTTTGAt1g48300 (DGA73)ML2031TCAGTCATCTTCTCTCAGAGCGATGTGGAAt1g16430 (DGA73)ML1716GTCCACGCGGTGGTGTAt1g20440 (CCR47)ML1718TTCCACCAGGGACAAAGGAt5g2310 (LATS)ML1718TTCCACCAGGGACAAAGGAt5g2310 (LTT78)ML1719CATCGTGTCCGTAAGAGGCAt4g25490 (CBF1)ML1901GATTTCTGGTCGTCGGTGACCAAt6g25490 (CBF1)ML1902CCATTAGCAGGGACTACCAAAACCAt3g26744 (ICE1)	ML1828	ATTCCACGCGGTTTCCTCTTC	At3g55360 (ECR)
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ML1920CGAACGCCAATCCGCAAATG(CFP80A4)ML1933GATTGTGCCAGTAGCGATGAAAt4g00400 (GPAT8)ML1934TCTTGCCACCACCGTTGACAt1g49430 (LACS2)ML1937AACGCAGCAATTCGGTCCAGAt1g49430 (LACS2)ML1938AATGTCGCCACGCCAGTATCCAt2g19450 (DGAT1)ML2026GGAGGGCGAGGAGAGAGAGTCCACTTAt2g19450 (DGAT1)ML2027CCAATCTCGCAGCGATCTTGAACDGAT2)ML2028CCGAGCTGAGGAACATTCAAATCAAt3g51520 (DGAT2)ML2030GGTCTTGATCCTGAAGCTGGTTTGAt1g48300 (DGAT3)ML2031TCAGTCATCTTCTTCATGGCTTTGGDGAT3)ML2032TGCTGAAGCAAAGGATGTTGCAAt1g20440 (COR47)ML1716GTCCACGCCGTTGGTTGTAt1g20440 (COR47)ML1718TTCCACCAGGGACAAAGGAt5g52310 (L1778)ML1718GGACTTTCCAAACGCTGAGAt4g25490 (CBF1)ML1901GATTTCTTGTCTGCTGGTCACTAt3g26744 (ICEI)	ML1919	GGCTCTTCCAACGCAAAACC	At1g01600 (CYP86A4)
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ML2029CGACCATATTTGCTACGATGATCGA(DGAT2)ML2030GGTCTTGATCCTGAAGCTGGTTTGAt1g48300 (DGAT3)ML2031TCAGTCATCTTCTTCATGGCTTTGG(DGAT3)ML2032TGCTGAAGCAAAGGATGTTGCAAt5g13640 (PDAT1)ML2033CCTTTCTCCGGTGACCAATCAAG(DCAT2)ML1716GTCCACGCCGTTGGTTGTAt1g20440 (COR47)ML1717AATCCCCTTCTTCTCCTCCG(COR47)ML1718TTCCACCAGGGACAAAGGAt5g52310 (LTI78)ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 (ICE1)ML1902CCATTAGCAGGACTACCAAAACCAt3g26744 (ICE1)	ML2028	CCGAGCTGAGGAACATTCAAATCA	At3g51520 (DGAT2)
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ML2033CCTTTCTCCGGTGACCAATCAAG(PDAT1)ML1716GTCCACGCCGTTGGTTGTAt1g20440 (COR47)ML1717AATCCCCTTCTTCTCCTCCG(COR47)ML1718TTCCACCAGGGACAAAGGAt5g52310 (LTI78)ML1719CATCGTGTCCGTAAGAGGC(LTI78)ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 ML1902CCATTAGCAGGACTACCAAAACCAt3g26744 (ICE1)	ML2032	TGCTGAAGCAAAGGATGTTGCA	At5g13640 (PDAT1)
ML1716GTCCACGCCGTTGGTTGTAt1g20440 (COR47)ML1717AATCCCCTTCTTCTCCCCG(COR47)ML1718TTCCACCAGGGACAAAGGAt5g52310 (LTI78)ML1719CATCGTGTCCGTAAGAGGC(LTI78)ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 	ML2033	CCTTTCTCCGGTGACCAATCAAG	
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ML1718TTCCACCAGGGACAAAGGAt5g52310 (LTI78)ML1719CATCGTGTCCGTAAGAGGC(LTI78)ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1786CAAGCCGAGTCAGCGAAGTT(CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 (ICE1)ML1902CCATTAGCAGGACTACCAAAACC(ICE1)	ML1717	AATCCCCTTCTTCTCCTCCG	
ML1719CATCGTGTCCGTAAGAGGC(L1178)ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1786CAAGCCGAGTCAGCGAAGTT(CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 (ICE1)ML1902CCATTAGCAGGACTACCAAAACC(ICE1)	ML1718	TTCCACCAGGGACAAAGG	At5g52310 (<i>LTI78</i>)
ML1785GGACTTTCCAAACCGCTGAGAt4g25490 (CBF1)ML1786CAAGCCGAGTCAGCGAAGTT(CBF1)ML1901GATTTCTTGTCTGCTCGGTCACTAt3g26744 (ICE1)ML1902CCATTAGCAGGACTACCAAAACC(ICE1)	ML1719	CATCGTGTCCGTAAGAGGC	
ML1786 CAAGCCGAGTCAGCGAAGTT (CBFT) ML1901 GATTTCTTGTCTGCTCGGTCACT At3g26744 (ICE1) ML1902 CCATTAGCAGGACTACCAAAACC	ML1785	GGACTTTCCAAACCGCTGAG	At4g25490 (CBF1)
ML1901 GATTTCTTGTCTGCTCGGTCACT At3g26744 (ICE1) ML1902 CCATTAGCAGGACTACCAAAACC (ICE1)	ML1786	CAAGCCGAGTCAGCGAAGTT	
ML1902 CCATTAGCAGGACTACCAAAACC (ICE1)	ML1901	GATTTCTTGTCTGCTCGGTCACT	At3g26744 (<i>ICE1</i>)
	ML1902	CCATTAGCAGGACTACCAAAACC	

The genes implicated in wax biosynthesis tested in qRT-PCR were *CUT1/KCS6*, *CER8*, *ECR* and *KCR1*. The genes implicated in cutin biosynthesis tested were

CYP86A2, *CYP86A4*, *GPAT8* and *LACS2*. The genes with no implication in cuticle formation tested included triacylglycerol biosynthetic genes (*DGAT1*, *DGAT2*, *DGAT3* and *PADT1*) and cold-related genes (*COR47*, *LTI78*, *CBF1* and *ICE1*).

Cutin compound	Mass-to-charge ratios (m/z)
C17:0-FA (internal standard)	284
C15:0 cycloketone (internal standard)	282
C18:1-FA	296
C18:2-FA	294
C10,16-dihydroxyl fatty acids	343
C16:0-DCA	314
C18:0-DCA	342
C18:1- DCA	339
C18:2-DCA	337

Supplementary Table S2. Mass-to-charge ratios (m/z) of cutin compounds in mass spectrometry.

FA, fatty acid; DCA, dicarboxylic fatty acid.