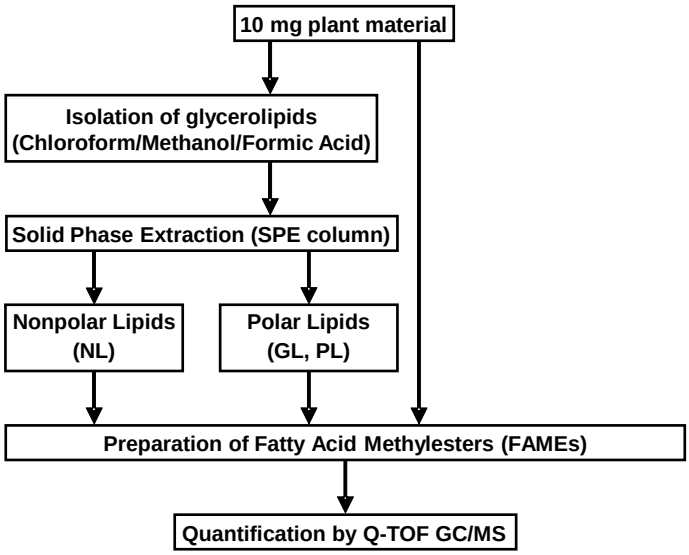
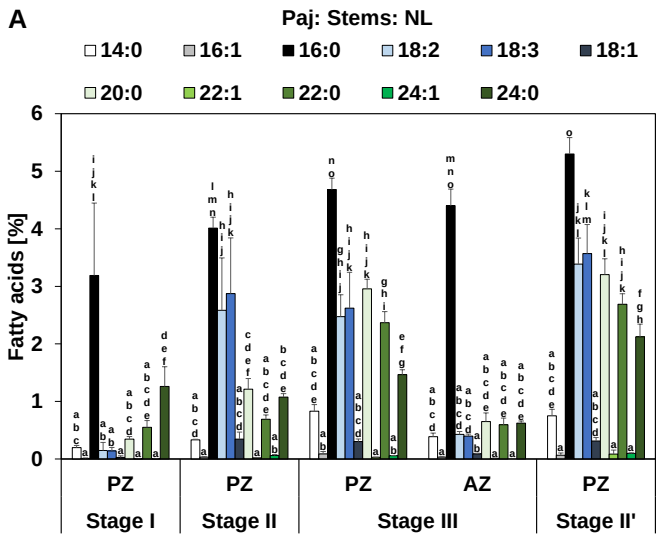
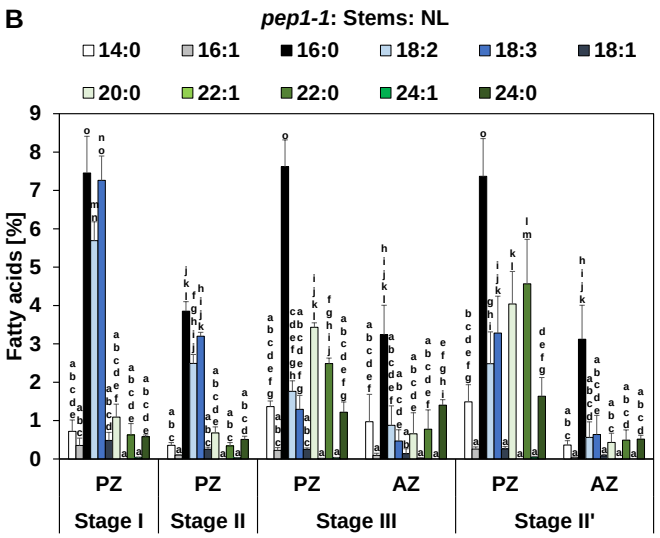
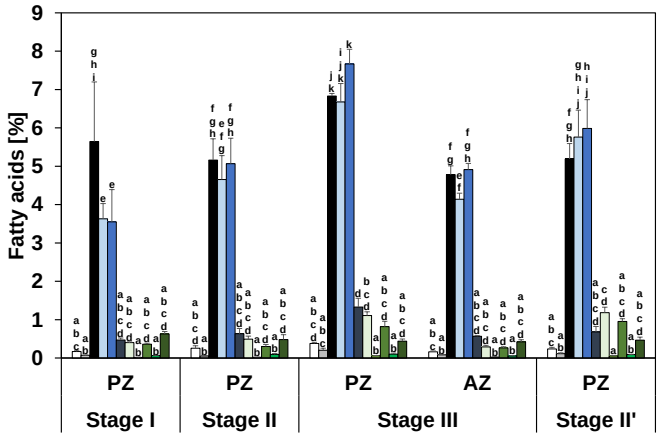
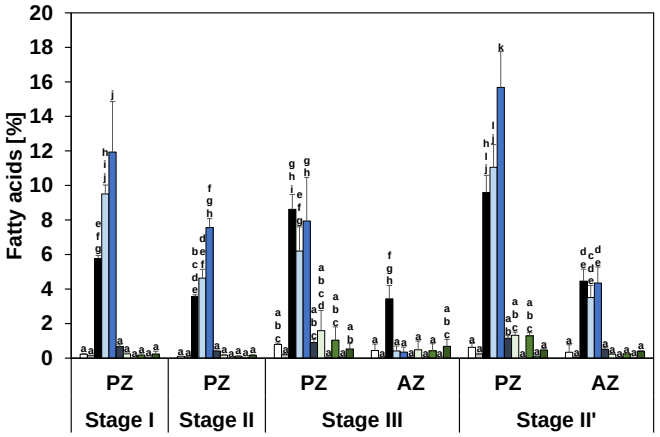


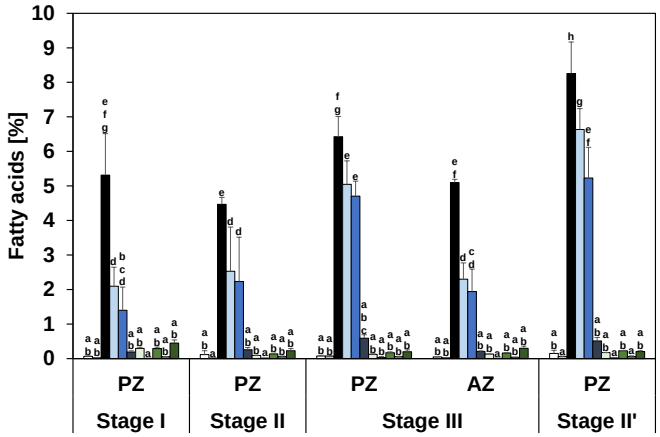
Supplementary Fig. S1. Overview of biochemical lipid analysis. 10 mg of dry starting plant material was either used for preparation of glycerolipids or directly used for preparation of fatty acid methylesters (FAMEs). Extracted glycerolipids were fractionated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) on solid phase extraction (SPE) columns. Fractionated lipids were applied to the preparation of FAMEs. FAMEs were finally quantified by Q-TOF GC/MS analysis.

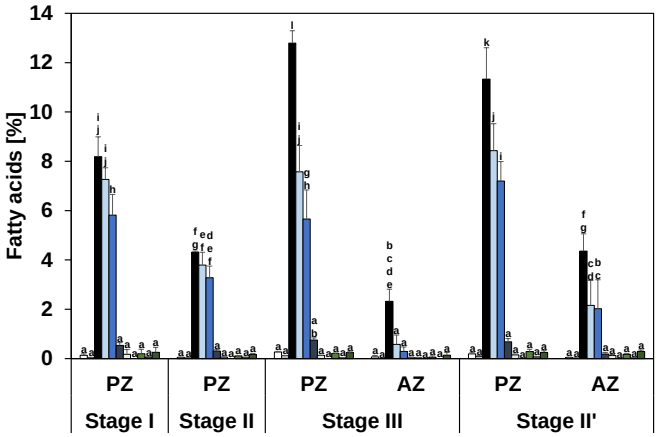

A
Paj: Stems: NL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
Stage I
Stage II
Stage III
Stage II'


B
pep1-1: Stems: NL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
AZ
Stage I
Stage II
Stage III
Stage II'

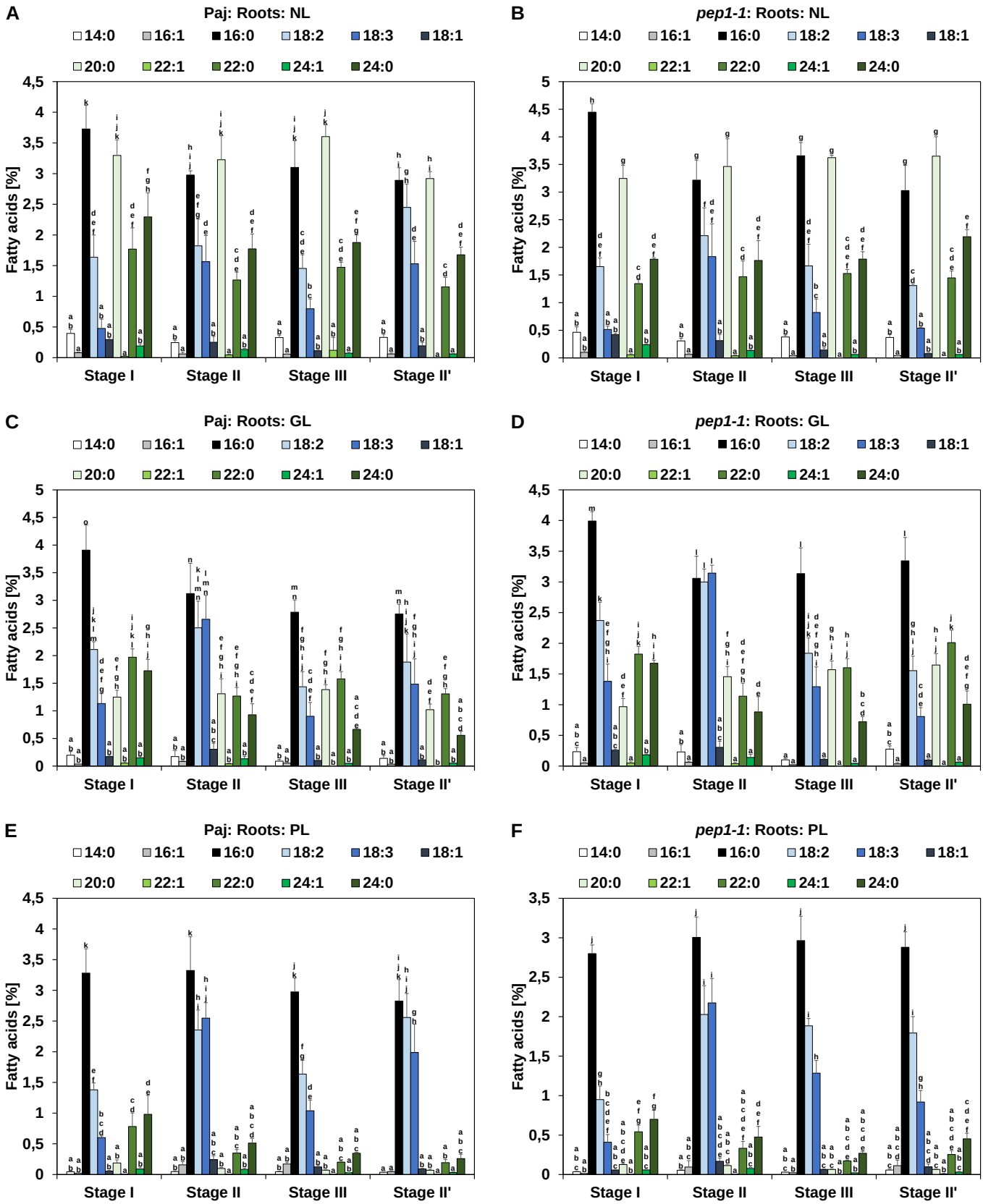

C
Paj: Stems: GL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
Stage I
Stage II
Stage III
Stage II'


D
pep1-1: Stems: GL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
AZ
Stage I
Stage II
Stage III
Stage II'

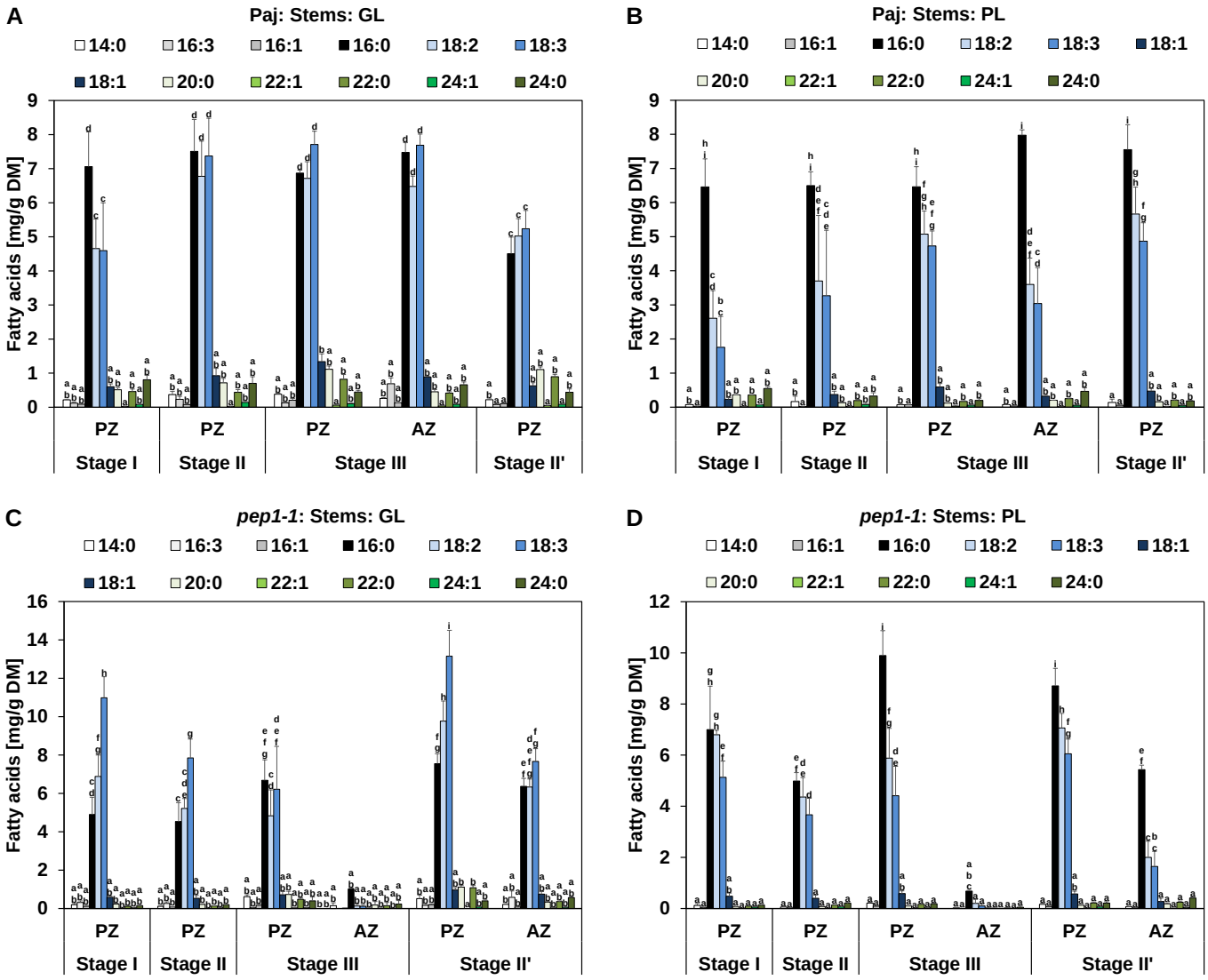

E
Paj: Stems: PL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
Stage I
Stage II
Stage III
Stage II'


F
pep1-1: Stems: PL
14:0
16:1
16:0
18:2
18:3
18:1
20:0
22:1
22:0
24:1
24:0
Fatty acids [%]
PZ
PZ
PZ
AZ
PZ
AZ
Stage I
Stage II
Stage III
Stage II'

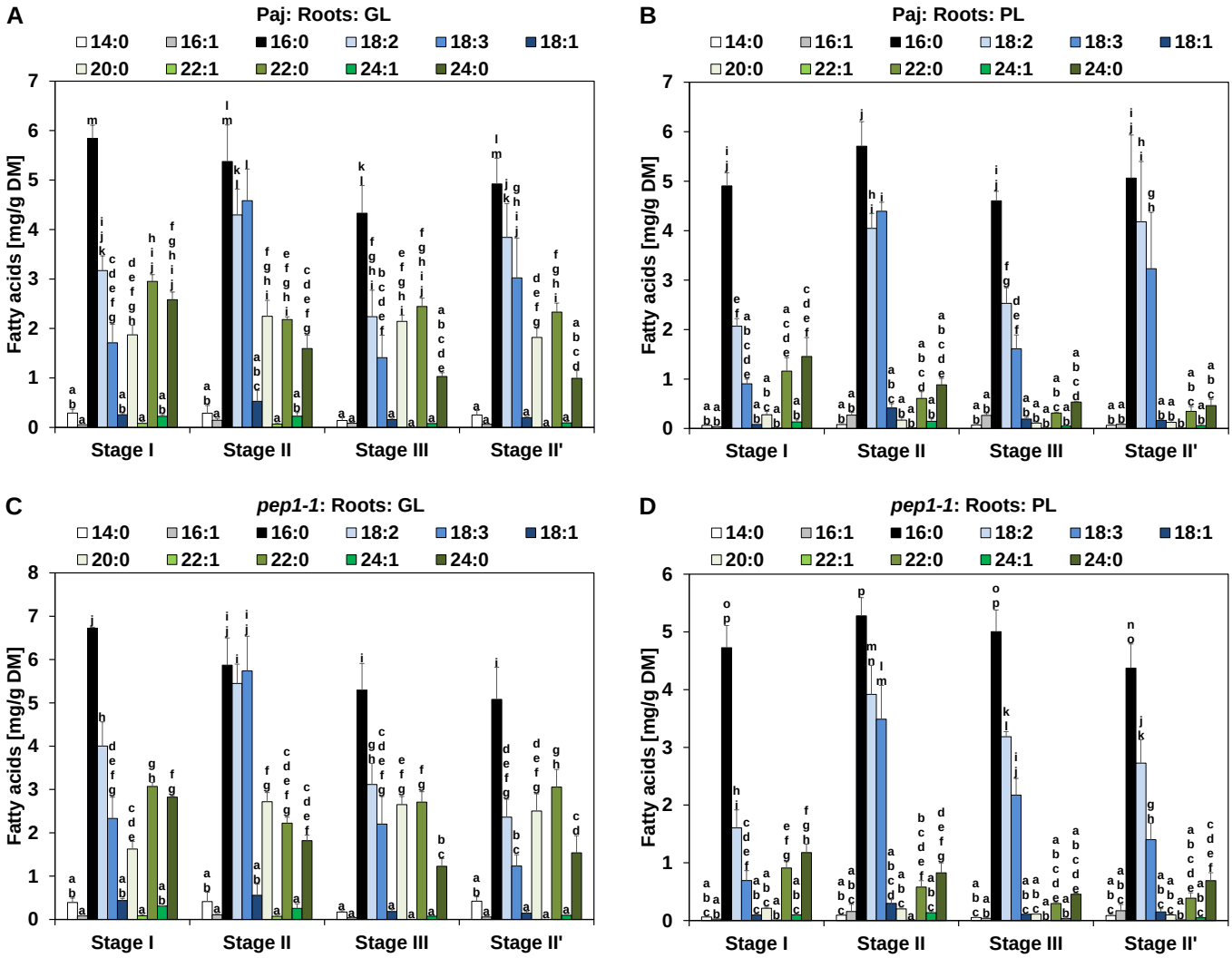
Supplementary Fig. S2. Individual fatty acids (FAs) of the three glycerolipid fractions in stems (s. Fig. 2A, Fig. 2B). The three glycerolipid fractions are neutral lipids (NL), glycolipids (GL) and phospholipids (PL), represented as percentage of (A, B) NL, (C, D) GL and (E, F) PL fraction-derived individual long-chain FAs (14:0, 16:1, 16:0, 18:2, 18:3, 18:1) and individual very long-chain FAs (20:0, 22:1, 22:0, 24:1, 24:0) in relation to total FA contents (as 100 %) of dry matter of perennial (PZ) and annual (AZ) internode stem zones of (A, C, E) *A. alpina* Pajares (Paj, wild type) and (B, D, F) *perpetual flowering 1-1* (*pep1-1*) mutant derivative. Plant material was harvested at four different developmental stages, stage I, II, III and II‘, as indicated in Fig. 1A. In addition, the PZ and AZ of Paj contained low percentage of 16:3 FAs in the GL fraction (compare in C) (0.09 % at stage I, 0.16 % at stage II, 0.13 % at stage III (PZ), 0.44 % at stage III (AZ) and 0.08 % at stage II’). The PZ of *pep1-1* contained low percentage of 16:3 and 20:1 FAs in the NL fraction (compare in B), namely 16:3 FAs at stages I and II (0.04 % and 0.02 %) and 20:1 FAs at stages I and III (0.08 % and 0.69 %). The PZ and AZ of *pep1-1* contained low percentage of 16:3, 16:2 and 20:1 FAs in the GL fraction (compare in D), namely 16:3 FAs with 0.34 % at stage I, 0.21 % at stage II, 0.07 % at stage III (PZ), 0.19 % at stage II’ (PZ) and 0.38 % at stage II’ AZ, 16:2 FAs with 0.02 % at stage I, 0.02 % at stage II and 0.06 % at stage II’ (PZ) and 20:1 FAs with 0.04 % at stage I and 0.4 % at stage III (PZ). For comparable representation, these FAs are not shown in the diagram, but were included in all calculations. Data are represented as mean +/- SD (n = 3-7). Different letters indicate statistically significant differences, determined by one-way ANOVA-Tukey’s HSD test ($P < 0.05$).



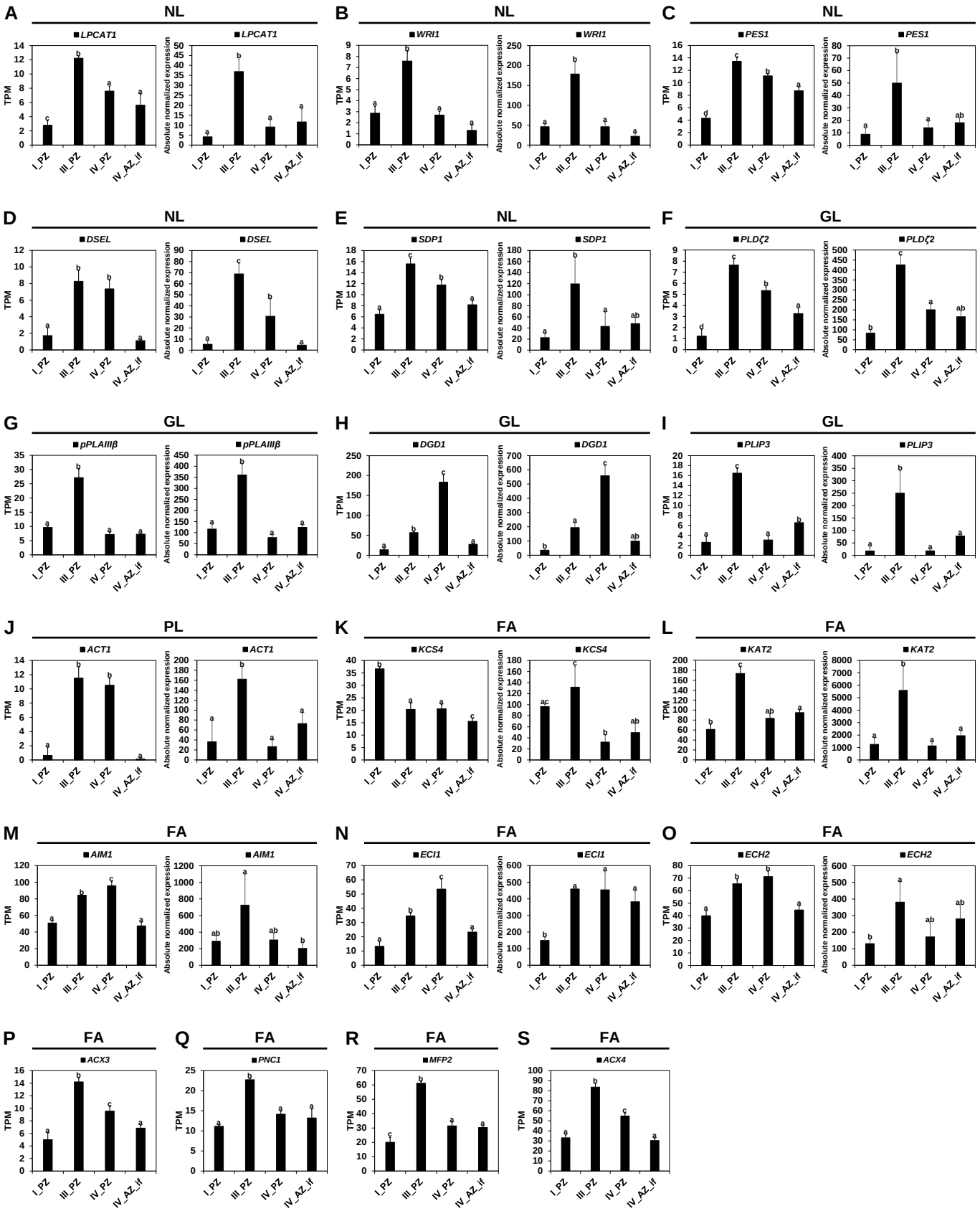
Supplementary Fig. S3. Individual fatty acids (FAs) of the three glycerolipid fractions in roots (s. Fig. 2C, Fig. 2D). The three glycerolipid fractions are neutral lipids (NL), glycolipids (GL) and phospholipids (PL), represented as percentage of (A, B) NL, (C, D) GL and (E, F) PL fraction-derived individual long-chain FAs (14:0, 16:1, 16:0, 18:2, 18:3, 18:1) and individual very long-chain FAs (20:0, 22:1, 22:0, 24:1, 24:0) in relation to total FA contents (as 100 %) of dry matter in roots of (A, C, E) *A. alpina* Pajares (Paj, wild type) and (B, D, F) *perpetual flowering 1-1* (*pep1-1*) mutant derivative. Plant material was harvested at four different developmental stages, stage I, II, III and II‘, as indicated in Fig. 1A. Data are represented as mean +/- SD (n = 3-7). Different letters indicate statistically significant differences, determined by one-way ANOVA-Tukey’s HSD test ($P < 0.05$).



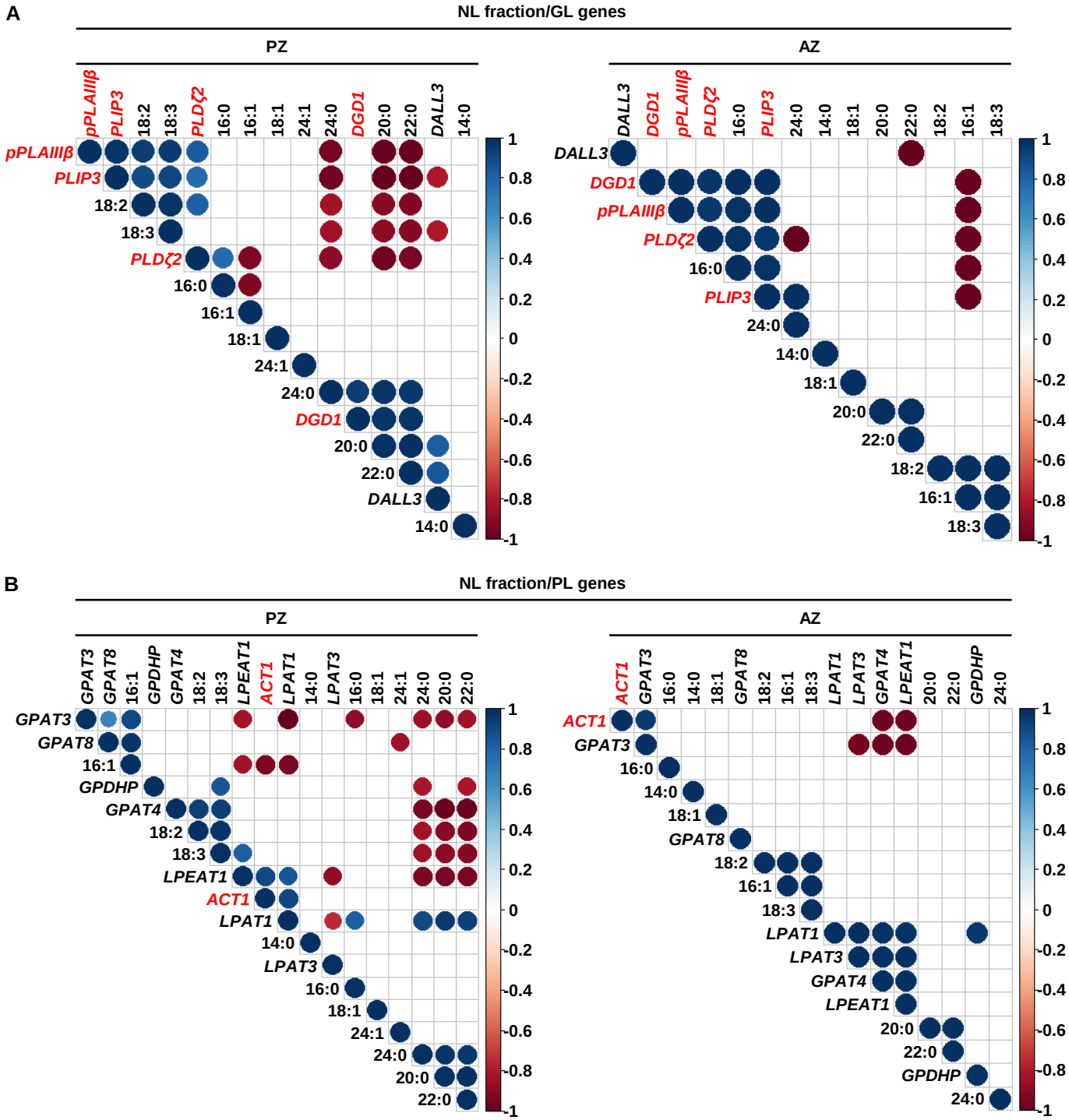
Supplementary Fig. S4. Long-chain and very long-chain fatty acid contents in glycolipid (GL) and phospholipid (PL) fractions in stems. Represented are the contents of (A, C) GL and (B, D) PL fraction-derived individual LCFAs (14:0, 16:3, 16:1, 16:0, 18:2, 18:3, 18:1) and individual VLCFAs (20:0, 22:1, 22:0, 24:1, 24:0) per dry matter (DM) of perennial (PZ) and annual (AZ) internode stem zones of (A, C) *A. alpina* Pajares (Paj, wild type) and (B, D) *perpetual flowering 1-1* (*pep1-1*) mutant derivative. Plant material was harvested at four different developmental stages, stage I, II, III and II‘, as indicated in Fig. 1A. In addition, the PZ of *pep1-1* contained low contents of other FAs in the GL fraction (compare in C), namely 16:2 FAs at stages I, II and II’ (0.02 mg/g DM, 0,02 mg/g DM and 0.05 mg/g DM) and of 20:1 FAs at stages I and II (0.04 mg/g DM and 0.32 mg/g DM). For comparable representation, these FAs are not shown in the diagram, but were included in all calculations. Data are represented as mean +/- SD (n = 3-7). Different letters indicate statistically significant differences, determined by one-way ANOVA-Tukey’s HSD test ($P < 0.05$).



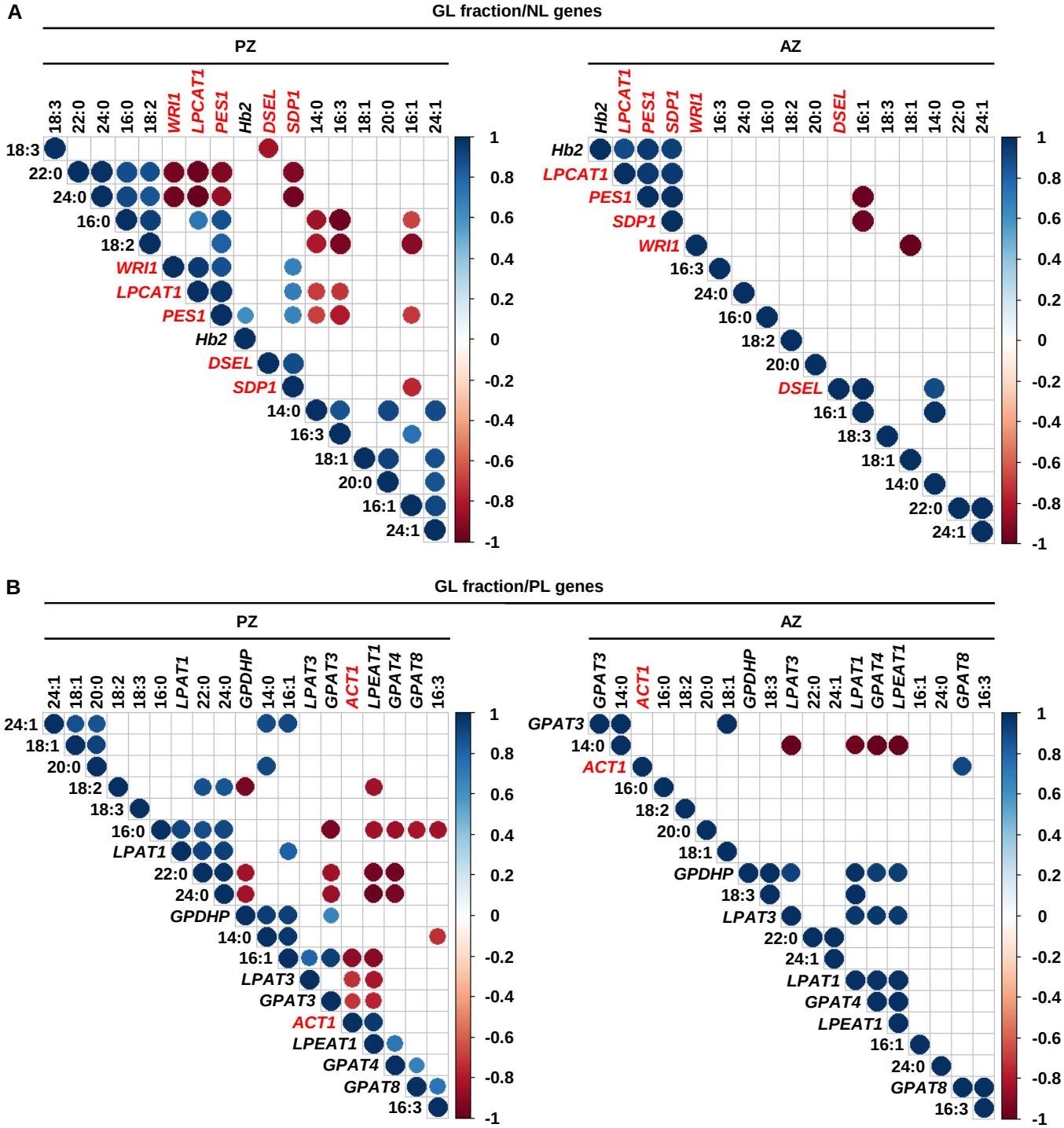
Supplementary Fig. S5. Long-chain and very long-chain fatty acid contents in glycolipid (GL) and phospholipid (PL) fractions in roots. Represented are the contents of (A, C) GL and (B, D) PL fraction-derived individual LCFAs (14:0, 16:1, 16:0, 18:2, 18:3, 18:1) and individual VLCFAs (20:0, 22:1, 22:0, 24:1, 24:0) per dry matter (DM) in roots of (A, C) *A. alpina* Pajares (Paj, wild type) and (B, D) *perpetual flowering 1-1* (*pep1-1*) mutant derivative. Plant material was harvested at four different developmental stages, stage I, II, III and II‘, as indicated in Fig. 1A. Data are represented as mean +/- SD (n = 3-7). Different letters indicate statistically significant differences, determined by one-way ANOVA-Tukey’s HSD test ($P < 0.05$).


A
NL
LPCAT1
TPM
Absolute normalized expression
B
NL
WRI1
C
NL
PES1
D
NL
DSEL
E
NL
SDP1
F
GL
PLDζ2
G
GL
pPLAIIIβ
H
GL
DGD1
I
GL
PLIP3
J
PL
ACT1
K
FA
KCS4
L
FA
KAT2
M
FA
AIM1
N
FA
ECI1
O
FA
ECH2
P
FA
ACX3
Q
FA
PNC1
R
FA
MFP2
S
FA
ACX4
I_PZ
III_PZ
IV_PZ
IV_AZ_if

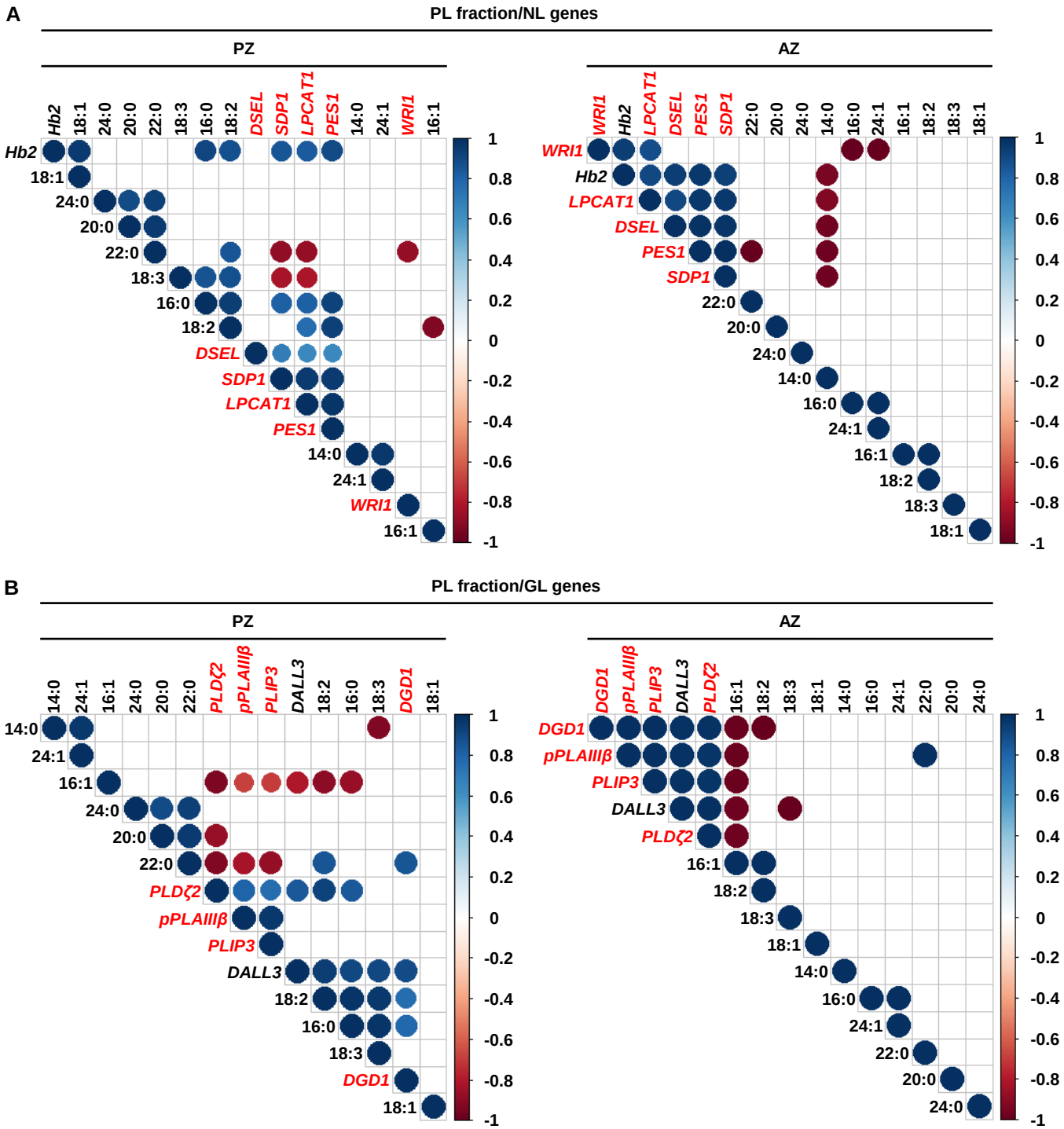
Supplementary Fig. S6. Gene expression of lipid metabolism genes in lateral stem internodes. Represented are RNA-seq data (left; TPM, transcripts per million) and reverse transcription-qPCR data (right; absolute normalized expression) of *A. alpina perpetual flowering 1-1* (*pep1-1*) lateral stem internodes of perennial (PZ) and annual (AZ) zones of RNA-seq at stages I_PZ, III_PZ, IV_PZ, and IV_AZ_if (see Fig. 5A). (A-E) NL genes (A) *LPCAT1*, *LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE 1*, (B) *WRI1*, *WRINKLED 1*, (C) *PES1*, *PHYTYL ESTER SYNTHASE 1,* (D) *DSEL*, *DAD1-LIKE SEEDLING ESTABLISHMENT-RELATED LIPASE*, (E) *SDP1, SUGAR-DEPENDENT1;* (F-I) GL genes (F) *PLDζ2*, *PHOSPHOLIPASE D ζ 2*, (G) *pPLAIIIβ*, *PATATIN-RELATED PHOSPHOLIPASE A IIIβ*, (H) *DGD1*, *DIGALACTOSYL DIACYLGLYCEROL DEFICIENT 1*, (I) *PLIP3*, *PLASTID LIPASE 3;* (J) PL gene *ACT1*, *ACYLTRANSFERASE 1*; (K-S) FA genes (K) *KCS4*, *3-KETOACYL-COA SYNTHASE 4*, (L) *KAT2*, *3-KETOACYL-COA THIOLASE 2*, (M) *AIM1*, *ABNORMAL INFLORESCENCE MERISTEM 1*, (N) *ECI1*, *Δ3, Δ2-ENOYL COA ISOMERASE 1*, (O) *ECH2*, *ENOYL-COA HYDRATASE 2*, (P) *ACX3*, *ACYL-COA OXIDASE 3*, (Q) *PNC1*, *PEROXISOMAL ADENINE NUCLEOTIDE CARRIER 1*, (R) *MFP2*, *MULTIFUNCTIONAL PROTEIN 2*, (S) *ACX4*, *ACYL-COA OXIDASE 4*. Data are represented as mean +/- SD (n = 3). Different letters indicate statistically significant differences, determined by one-way ANOVA-Tukey‘s HSD test (P < 0.05). Reverse transcription-qPCR data for *ACX3*, *PNC1*, *MFP2* and *ACX4* are not shown.



Supplementary Fig. S7. Pearson correlation analysis between gene expression levels of lipid metabolism genes and amounts of single FAs of the NL fraction of *A. alpina perpetual flowering 1-1* (*pep1-1*) lateral stem internodes of perennial (PZ) and annual (AZ) zones. The analysis was performed between stages III_PZ, IV_PZ and I', II' for PZ, and IV_AZ_if and II' for AZ (see Fig. 1 and Fig. 5A). Correlation analysis between NL-derived FAs and (A) GL metabolism-related genes, *DALL3*, *DAD1-LIKE LIPASE 3*, *DGD1*, *DIGALACTOSYL DIACYLGLYCEROL DEFICIENT 1*, *PLDζ2*, *PHOSPHOLIPASE D ζ 2*, *PLIP3*, *PLASTID LIPASE 3*, *pPLAIIIβ*, *PATATIN-RELATED PHOSPHOLIPASE A IIIβ*; and (B) PL metabolism-related genes, *ACT1*, *ACYLTRANSFERASE 1*, *GPAT3*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 3*, *GPAT4*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 4*, *GPAT8*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 8*, *GPDHP*, *GLYCEROL-3-PHOSPHATE DEHYDROGENASE PLASTIDIC*, *LPAT1*, *LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 1*, *LPAT3*, *LYSOPHOSPHATIDYL ACYLTRANSFERASE 3*, *LPEAT1*, *LYSOPHOSPHATIDYLETHANOLAMINE ACYLTRANSFERASE 1*. Only correlation coefficients with significant levels of correlation ($P < 0.05$) are displayed in the diagrams. Putative candidate genes that may regulate PZ development and the delimitation of PZ and AZ are represented in red.



Supplementary Fig. S8. Pearson correlation analysis between gene expression levels of lipid metabolism genes and amounts of single FAs of the GL fraction of *A. alpina perpetual flowering 1-1* (*pep1-1*) lateral stem internodes of perennial (PZ) and annual (AZ) zones. The analysis was performed between stages III_PZ, IV_PZ and I', II' for PZ, and IV_AZ_if and II' for AZ (see Fig. 1 and Fig. 5A). Correlation analysis between GL-derived FAs and (A) NL metabolism-related genes, *DSEL*, *DAD1-LIKE SEEDLING ESTABLISHMENT-RELATED LIPASE*, *Hb2, HAEMOGLOBIN 2*, *LPCAT1*, *LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE 1*, *PES1*, *PHYTYL ESTER SYNTHASE 1, SDP1, SUGAR-DEPENDENT1, WRI1*, *WRINKLED 1*; and (B) PL metabolism-related genes, *ACT1*, *ACYLTRANSFERASE 1*, *GPAT3*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 3*, *GPAT4*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 4*, *GPAT8*, *GLYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 8*, *GPDHP*, *GLYCEROL-3-PHOSPHATE DEHYDROGENASE PLASTIDIC*, *LPAT1*, *LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 1*, *LPAT3*, *LYSOPHOSPHATIDYL ACYLTRANSFERASE 3*, *LPEAT1*, *LYSOPHOSPHATIDYLETHANOLAMINE ACYLTRANSFERASE 1*. Only correlation coefficients with significant levels of correlation ($P < 0.05$) are displayed in the diagrams. Putative candidate genes that may regulate PZ development and the delimitation of PZ and AZ are represented in red.



Supplementary Fig. S9. Pearson correlation analysis between gene expression levels of lipid metabolism genes and amounts of single FAs of the PL fraction of *A. alpina perpetual flowering 1-1* (*pep1-1*) lateral stem internodes of perennial (PZ) and annual (AZ) zones. The analysis was performed between stages III_PZ, IV_PZ and I', II' for PZ, and IV_AZ_if and II' for AZ (see Fig. 1 and Fig. 5A). Correlation analysis between PL-derived FAs and (A) NL metabolism-related genes, *DSEL*, *DAD1-LIKE SEEDLING ESTABLISHMENT-RELATED LIPASE*, *Hb2, HAEMOGLOBIN 2*, *LPCAT1*, *LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE 1*, *PES1*, *PHYTYL ESTER SYNTHASE 1, SDP1, SUGAR-DEPENDENT1, WRI1*, *WRINKLED 1*; and (B) GL metabolism-related genes, *DALL3*, *DAD1-LIKE LIPASE 3*, *DGD1*, *DIGALACTOSYL DIACYLGLYCEROL DEFICIENT 1*, *PLDζ2*, *PHOSPHOLIPASE D ζ 2*, *PLIP3*, *PLASTID LIPASE 3*, *pPLAIIIβ*, *PATATIN-RELATED PHOSPHOLIPASE A IIIβ*. Only correlation coefficients with significant levels of correlation ($P < 0.05$) are displayed in the diagrams. Putative candidate genes that may regulate PZ development and the delimitation of PZ and AZ are represented in red.