Integrated Analysis of Comparative Lipidomics and Proteomics Reveals the Dynamic Changes of Lipid Molecular Species in High-oleic Peanut Seed

Authors

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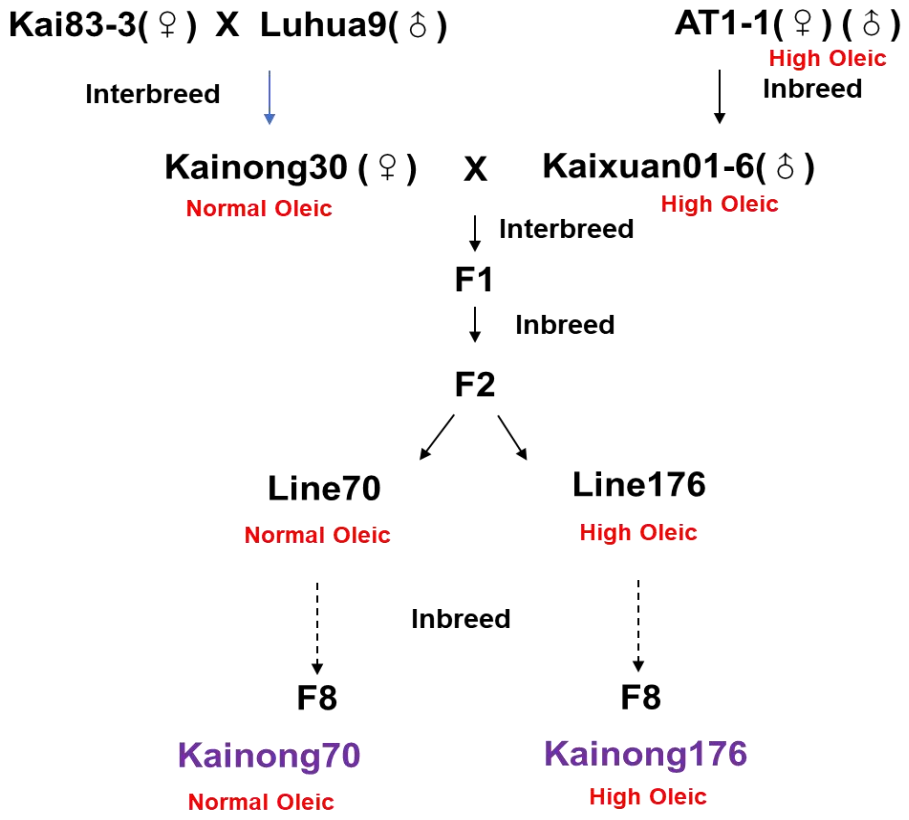
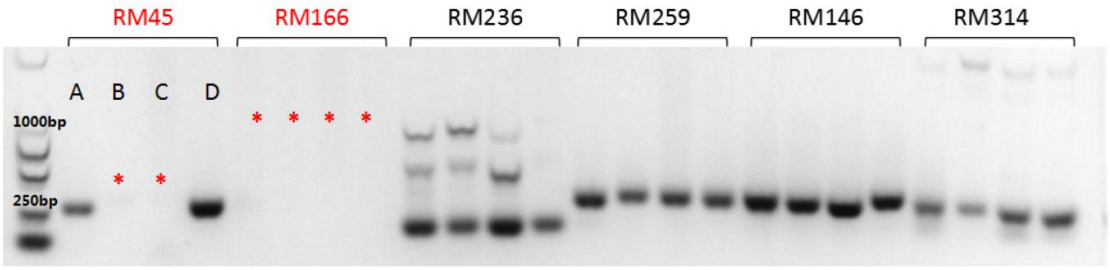
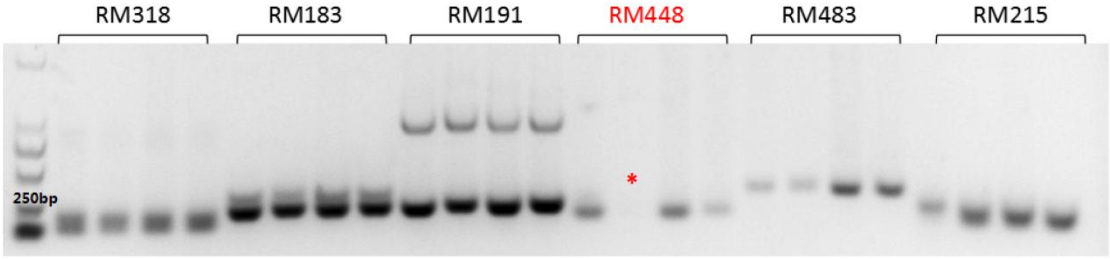
A

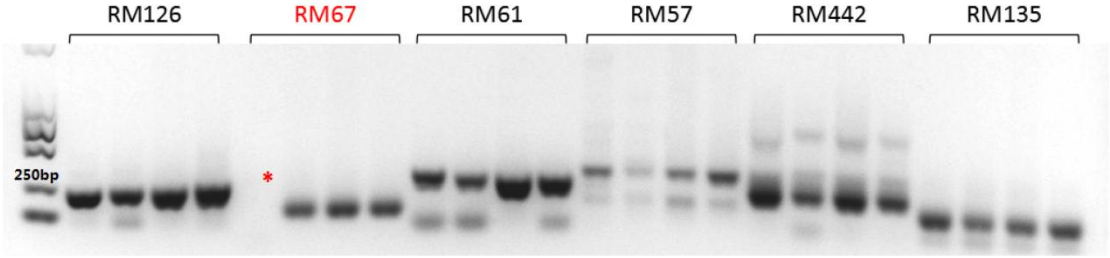

Kai83-3(♀) X Luhua9(♂)
AT1-1(♀)(♂)
High Oleic
Interbreed
Inbreed
Kainong30 (♀)
X
Kaixuan01-6(♂)
Normal Oleic
High Oleic
Interbreed
F1
Inbreed
F2
Line70
Normal Oleic
Line176
High Oleic
Inbreed
F8
F8
Kainong70
Normal Oleic
Kainong176
High Oleic

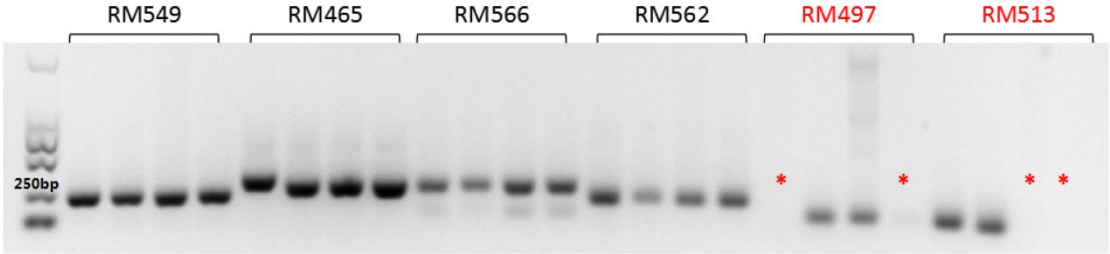
B

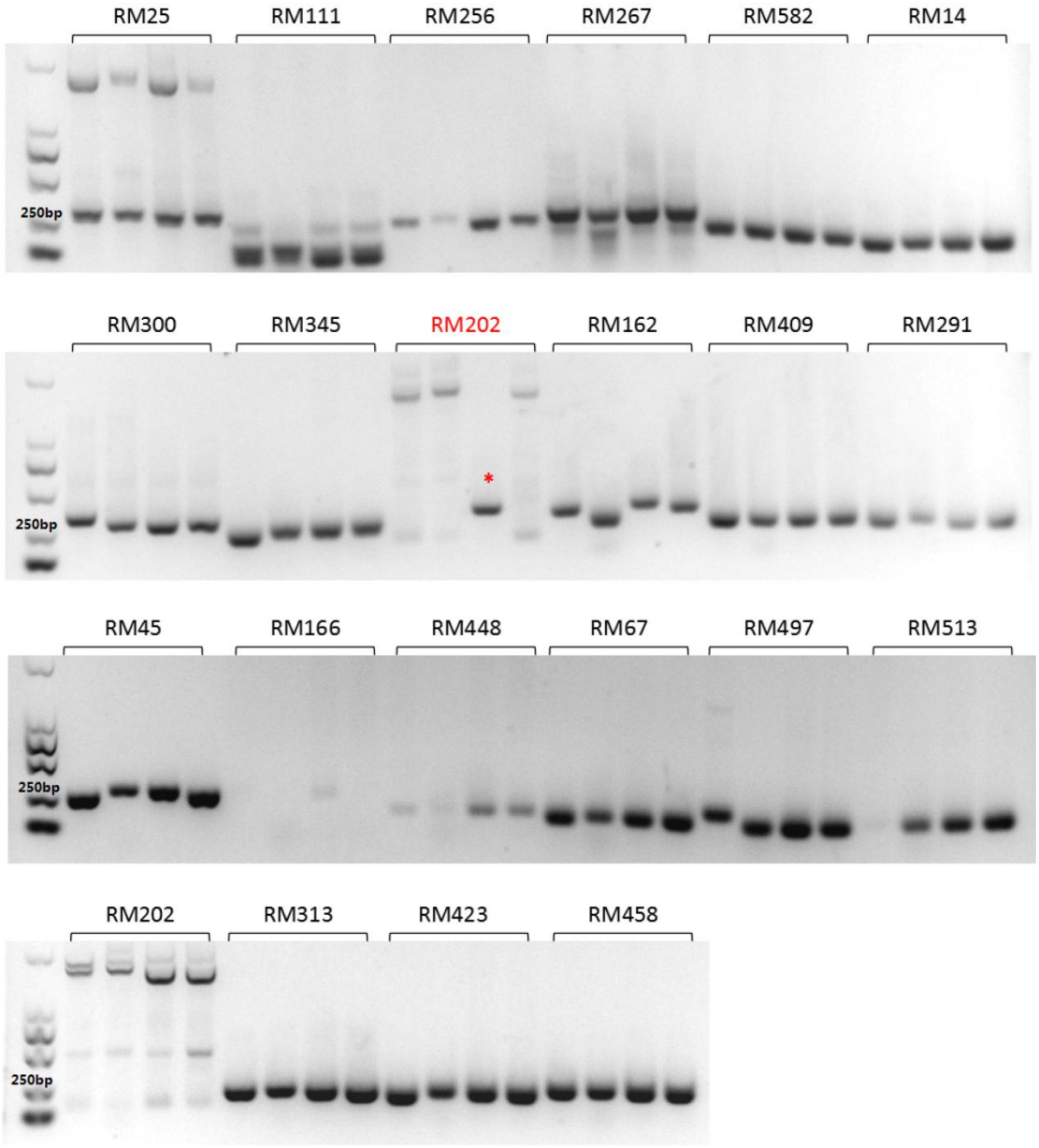


DNA template
A: Kaixuan01-6; B: Kainong30; C: Kainong70 (L70); D: Kainong176 (H176).
*** indicates the no amplification.**








RM25
RM111
RM256
RM267
RM582
RM14
250bp
RM300
RM345
RM202
RM162
RM409
RM291
*
250bp
RM45
RM166
RM448
RM67
RM497
RM513
250bp
RM202
RM313
RM423
RM458
250bp

Name	Chr.	F primer	R primer
RM61	a01	GGAGAACCAGTGACGTGACATA	GGATTAATTCTGATACCATGAAAGG
RM67	a01	TCAGGGTGGGTTACCAACAT	TAATCCACATTGAACCGACG
RM126	a02	GATCTTTCCGCCATTTTCTC	GGTGAATGACAGATGCTCCA
RM166	a03	TTCGGCTGACAGCTCTAAG	GAAAGAAATTATACACTCCAATTATGC
RM45	a03	AATGTGGCGTTCTTCTTCGT	TCCTATCCGTTATCCCCCTC
RM259	a04	ACAATGCAATGACCGTTGTT	TTGTTGCATGAGAACGTGAA
RM236	a04	CAGCGGCAACAGTTTTGATG	GAAAAGTATGCCGCCGTTG
RM146	a05	TATTTGTTTCTGGTTCCGCC	TCTGTTAGAGAAGTCAAGTCTGTTG
RM314	a05	CAGGCTTAAACTCCGTGAGC	ACTTGGATGACCCGGTACAA
RM318	a06	GGCAGGGGAATAAAACTACTAACT	TTTTCCTTCCTTCTCCTTTGTC
RM183	a06	ATGACAAGGCTCTTTCGATCC	TACTTGACCCTCCTCCTCCAT
RM448	a07	TCCTTCCCACAATAACAATGAA	GAGGAGAAAACATGGCCTAAAA
RM191	a07	TGCTTTTGCTCAACATGCTA	ACAGACATTGCAGCTTCACG
RM483	a08	GGGAATAGCGAGATACATGTCAG	CAGGAGAGAAGGATTGTGCC
RM215	a08	TGTCCGAACAACTTGAGACG	GCGTGGTCATACACACTTGG
RM267	a09	ACGTCAGGTTCACAACGACA	ATTTTTCTCCGAGTCAGCCA
RM256	a09	CAATCAAATCAATAATGTCTCTTTCTC	CGCTCGACCAAGAAAAGTTC
RM442	a10	TTCGGTCATGTTTGTCCAGA	CTCGAGTGCTCACCCTTCAT
RM582	a10	TGAGGCCGTCTTGTTTAGAGA	CCTCTTCCATCACCGTTCATA
RM300	b01	TACTACTGTTGCCGCTGTGC	TTTCTCACCATTGGCATTCA
RM111	b01	CTCTCTCTCCCCTTCATCGAC	GGCAGAATGAAAGGTGAGAACT
RM135	b02	CCTCACTTCTTTTTGCATGGT	TGGAAAGGAAATGATTTGGTG
RM345	b02	GCTAAGCTAAATTACCCATTTTGTG	GTTTGAGCTTGTGCAGTGGA
RM162	b03	TGTTGCCCACTGTTCTAATCA	TCAAATGGCATAGTCTCCCC
RM202	b03	CGTTGGGGACAAAAACGATA	TTTTCTTGAAACTCGTTGATATGG
RM291	b04	CCTCCGTTGCTCTTCTGAAC	GATCAAGCACTTCAGACAATGG
RM14	b04	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT
RM423	b05	CTGCATAGTGGCGGTGATAA	CATCGGATTAATTCAACGGT
RM313	b05	GCCCATATCAAGCTCCAAAA	TAGCCAGCGAAGGACTCAAT
RM409	b06	CCGCAGATCTTCTCCTGTGT	CCTCCTCATCCTCTAAACTCTGC
RM458	b06	ATTCCGCGAACTTCATTAGC	GGATTGAATGGCAAAGAGGA
RM25	b07	GCCGAGCTAGTTTGATTTGG	TTGGATTTGAATGGAGGAATG
RM465	b07	TTAGCGACAAAGGATGGTGAG	TAGGGACGAAAATAGGGACTGA
RM497	b08	GTTGTGGTGACGAGCTCAAA	CCACTAACCACCCCATCATC
RM513	b08	TGGGAGTCATGGCAATTTTT	CACCATCTCTCATCCATTTTCA
RM562	b09	TCGGTTTGGGAGACACTCTT	TTGTAAGCAGACGCCACATC
RM566	b09	CAAGCATCAACAACAACGA	GTCCGACCACATACAAGAGTT
RM57	b10	TATTTCTGCTTCGGCGCTAT	TTTCCCCCATCTCACTCAAC
RM549	b10	AATACCCTTCCCCAATCACC	TGCTTCTGCTCGATGTTCTG

Figure S1. The breeding processes of normal- and high- OA cultivars Kainong70 and Kainong176. A, Kai83-3(♀) intercrossed with Luhua9(♂) to cultivate the Kainong30, Kaixuan01-6 selected from the inbreeding population of American introduced high OA peanut variety AT1-1(♀) (♂). Kainong30 (♀) intercrossed with Kaixuan01-6 (♂) to generate the F2 population, normal- and high OA line 70 and 176 isolated from the F2 population, and self-fertilized to generate the normal cultivar Kainong70 and high OA cultivar Kainong176 until the stable F8 population. Information of these varieties was provided by the website of www.peanutdata.cn. B, 39 pairs of SSR markers were

used to detect the polymorphism of genetic background between the L70, H176 and their parents (kaixuan01-6 and Kainong30). This result indicated that the genetic background of L70 and H176 was very similar, only the RM45 and RM146 performed difference amplification in L70 and H176 genome. Five pairs of SSR marker existed difference in Kaixuan01-6 and Kainong30, including the RM45, RM497, RM300, RM345, and RM162. DNA amplification was observed by agarose gel electrophoresis.

>*FAD2-A* coding sequence in high OA cultivar Kainong176

ATGGGAGCTGGAGGGCGTGTCACTAAGATTGAAGCTCAAAAGAAGCCTCT
TTCAAGGGTTCCACATTCAAACCCTCCATTCAGTGTTGGCCAACTCAAGAA
AGCAATTCCACCACATTGCTTTGAACGTTCTCTTTTCATATCATTCTCCTATG
TTGTCTATGATCTCTTAGTGGCCTACTTACTCTTCTACATTGCCACCACTTAT
TTCCACAAGCTTCCATACCCATTTTCCTTCCTTGCTTGGCCAATCTATTGGGC
CATCCAAGGCTGCATTCTCACTGGTGTTTGGGTGATTGCTCATGAGTGTGG
CCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGGTTTGAC
CCTTCACTCTTGA(375bp)CTATTAGTTCCTTATTTCTCATGGAAAATCAGCCA
CCGCCGCCACCACTCCAACACCGGTTCCCTCGACCGCAACGAAGTGTTTG
TCCCAAAACCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATC
CACCAGGGAGGGCTATCTCCCTCTTCATCACACTCACACTAGGATGGCCCT
TGTACTTGGCCTTCAATGTTTCTGGCAGACCCTATGATAGATTTGCAAGCCA
CTATGACCCTTATGCTCCCATATACTCTAACAGGGAAAGGCTTCTAATTTATG
TCTCAGATTCATCTGTCTTTGCTGTAACATATCTGCTATATCACATAGCAACT
TTGAAAGGTTTGGGTTGGGTGGTATGTGTTTATGGGGTGCCATTGCTCATTG
TGAATGGGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCATCATT
GACTCACTATGATTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAAC
AGTGGACAGAGATTATGGGATACTGAATAAGGCATTTCATCATATAACTGAT
ACGCATGTGGCTCATCATTTGTTCTCAACAATGCCTCATTACCATGCAATGG
AAGCAACCAATGCAATAAAGCCAATATTGGGTGATTACTACCAATTTGATGG
CACCCCAGTTTACAAAGCATTGTGGAGAGAAGCCAAAGAGTGCCTCTATGT
GGAGCCAGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGTACAAGAACA
AGTTCTGA

>*FAD2-A* in normal OA cultivar Kainong70

ATGGGAGCTGGAGGGCGTGTCACTAAGATTGAAGCTCAAAAGAAGCCTCT
TTCAAGGGTTCCACATTCAAACCCTCCATTCAGTGTTGGCCAACTCAAGAA
AGCAATTCCACCACATTGCTTTGAACGTTCTCTTTTCATATCATTCTCCTATG
TTGTCTATGATCTCTTAGTGGCCTACTTACTCTTCTACATTGCCACCACTTAT
TTCCACAAGCTTCCATACCCATTTTCCTTCCTTGCTTGGCCAATCTATTGGGC
CATCCAAGGCTGCATTCTCACTGGTGTTTGGGTGATTGCTCATGAGTGTGG
CCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGGTTTGAC
CCTTCACTCTTGTCTATTAGTTCCTTATTTCTCATGGAAAATCAGCCACCGCC
GCCACCACTCCAACACCGGTTCCCTCGACCGCGACGAAGTGTTTGTCCCA
AAACCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCA
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TGGCCTTCAATGTTTCTGGCAGACCCTATGATAGATTTGCAAGCCACTATGA
CCCTTATGCTCCCATATACTCTAACAGGGAAAGGCTTCTAATTTATGTCTCAG
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GGTTTGGGTTGGGTGGTATGTGTTTATGGGGTGCCATTGCTCATTGTGAATG
GGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCATCATTGCCTCA
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CAGAGATTATGGGATACTGAATAAGGCATTTCATCATATAACTGATACGCATG
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CAATGCAATAAAGCCAATATTGGGTGATTACTACCAATTTGATGGCACCCCA
GTTTACAAAGCATTGTGGAGAGAAGCCAAAGAGTGCCTCTATGTGGAGCC
AGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGTACAAGAACAAGTTCTG
A

>*FAD2-B* coding sequence in Kainong176

ATGGGAGCTGGAGGGCGTGTCACTAAGATTGAAGCTCAAAAGAAGCCTCC
TTCAAGGGTTCCACATTCAAACCCTCCATTCAGTGTTGGCCAACTCAAGAA
GGCAATTCCACCACATTGCTTTGAACGTTCTCTTTTCATATCATTCTCATATG
TTGTCTATGATCTCTTAATGGCCTACTTACTCTTCTACATTGCCACCACTTATT
TCCACAAGCTTCCATACTCATTATCCTTCCTTGCTTGGCCAATCTATTGGGCC
ATCCAAGGCTGCATTCTCACCGGTGTTTGGGTGATTGCTCATGAGTGTGGC
CACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGGTTTGACC
CTTCACTCTTGTCTATTAGTTCCTTATTTCTCGTGGAAAATCAGCCACCGCC
GCCACCACTCCAACACAGGTTCCCTCA(442bp)GACCGCGACGAAGTGTTTG
TCCCGAAACCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATC
CACCAGGGAGGGCTATTTCCCTTTTCATCACACCCACACTAGGATGGCCCT
TGTACTTGGCCTTCAATGTTTCTGGCAGACCCTATGATAGATTTGCAAGCCA
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TCTCAGATTCATCTGTCTTTGCTGTAACATATCTGCTATATCACATAGCAACT
TTGAAAGGTTTGGGTTGGGTGGTATGTGTTTATGGGGTGCCATTGCTCATTG
TGAATGGGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCAGCATT
GCCTCACTATGATTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAAC
AGTGGACAGAGATTATGGGATACTGAATAAGGCATTTCATCATATAACTGAT
ACGCATGTGGCTCATCATTTGTTCTCAACAATGCCTCATTACCATGCAATGG
AAGCAACCAATGCAATAAAGCCAATATTGGGTGATTACTACCAATTTGATGG
CACCCCAGTTTACAAAGCATTGTGGAGAGAAGCCAAAGAGTGCCTCTATGT
GGAGCCAGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGTACAAGAACA
AGTTCTGA

>*FAD2-B* coding sequence in Kainong70

ATGGGAGCTGGAGGGCGTGTCACTAAGATTGAAGCTCAAAAGAAGCCTCT
TTCAAGGGTTCCACATTCAAACCCTCCATTCAGTGTTGGCCAACTCAAGAA
AGCAATTCCACCACATTGCTTTGAACGTTCTCTTTTCATATCATTCTCATATG
TTGTCTATGATCTCTTAATGGCCTACTTACTCTTCTACATTGCCACCACTTATT
TCCACAAGCTTCCATACCCATTTTCCTTCCTTGCTTGGCCAATCTATTGGGC
CATCCAAGGCTGCATTCTCACCGGTGTTTGGGTGATTGCTCATGAGTGTGG
CCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGGTTTGAC
CCTTCACTCTTGTCTATTAGTTCCTTATTTCTCATGGAAAATCAGCCACCGCC
GCCACCACTCCAACACAGGTTCCCTCGACCGCGACGAAGTGTTTGTCCCG

AAACCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCA
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TGGCCTTCAATGTTTCTGGCAGACCCTATGATAGATTTGCAAGCCACTATGA
CCCTTATGCTCCCATATACTCTAACAGGGAAAGGCTTCTAATTTATGTCTCAG
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GGTTTGGGTTGGGTGGTATGTGTTTATGGGGTGCCATTGCTCATTGTGAATG
GGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCATCATTGCCTCA
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CAGAGATTATGGGATACTGAATAAGGCATTTCATCATATAACTGATACGCATG
TGGCTCATCATTTGTTCTCAACGATGCCTCATTACCATGCAATGGAAGCAAC
CAATGCAATAAAGCCAATATTGGGTGATTACTACCAATTTGATGGCACCCCA
GTTTACAAAGCATTGTGGAGAGAAGCCAAAGAGTGCCTCTATGTGGAGCC
AGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGTACAAGAACAAGTTCTG
A

Figure S2. Coding sequence of *FAD2-A* and *FAD2-B* in Kainong176 (H176) and Kainong70 (L70).

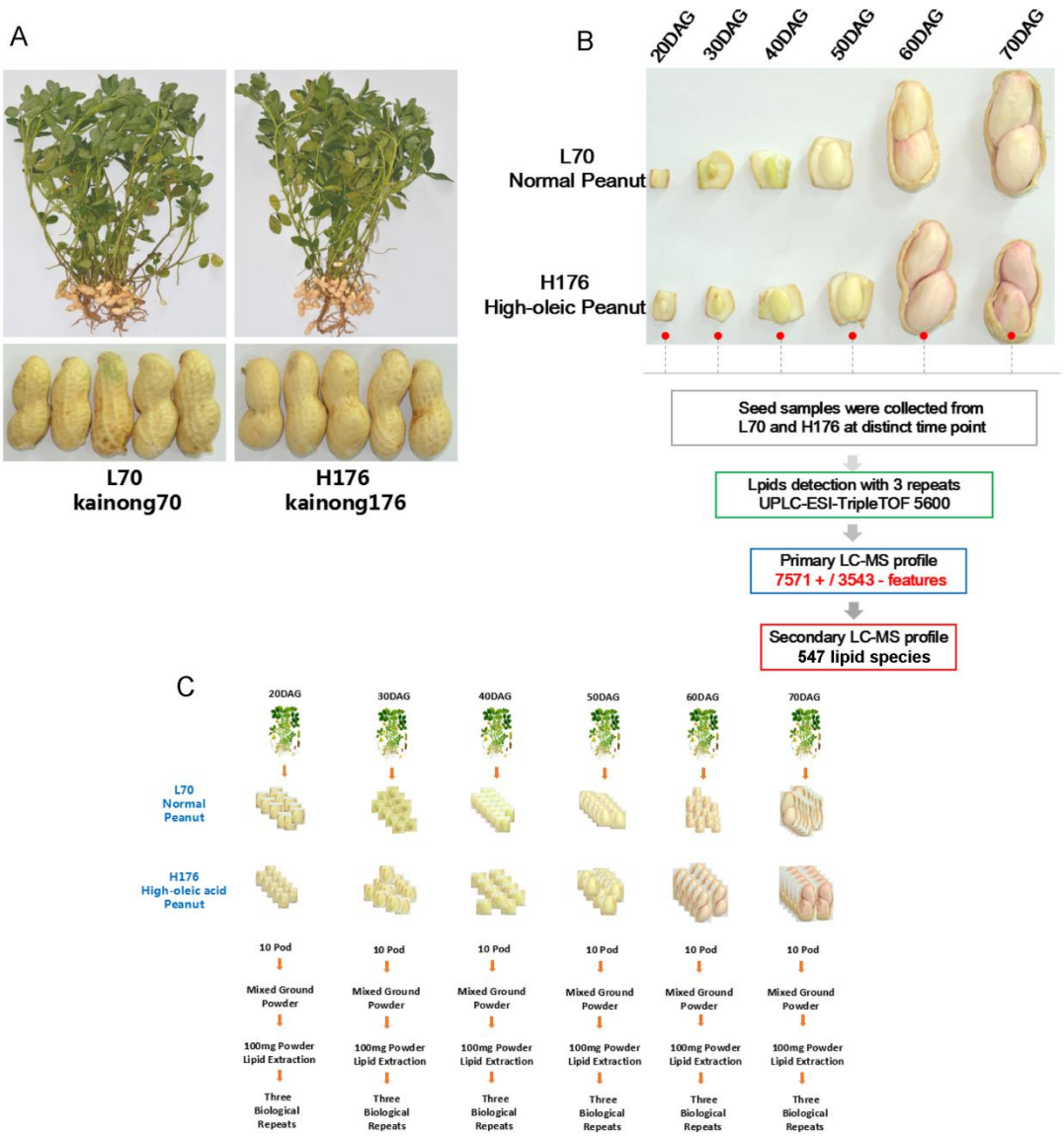


Figure S3. Morphological of normal- (L70, Kainong70) and high- OA (H176, Kainong176) peanut cultivars. A, Phenotypes of L70 and H176 plants. B, Seed samples obtained from six different developmental stages (20-70 DAG) in L70 and H176, respectively. C, The detailed process of lipid sample collection. Each sample contained three biological repeats, and the peanut sample collected from individual plant at each stage. 10 pods were collected from each tree at each stage, and then the seeds were mixed to extract the total lipid. DAG represented the days after gynophores.

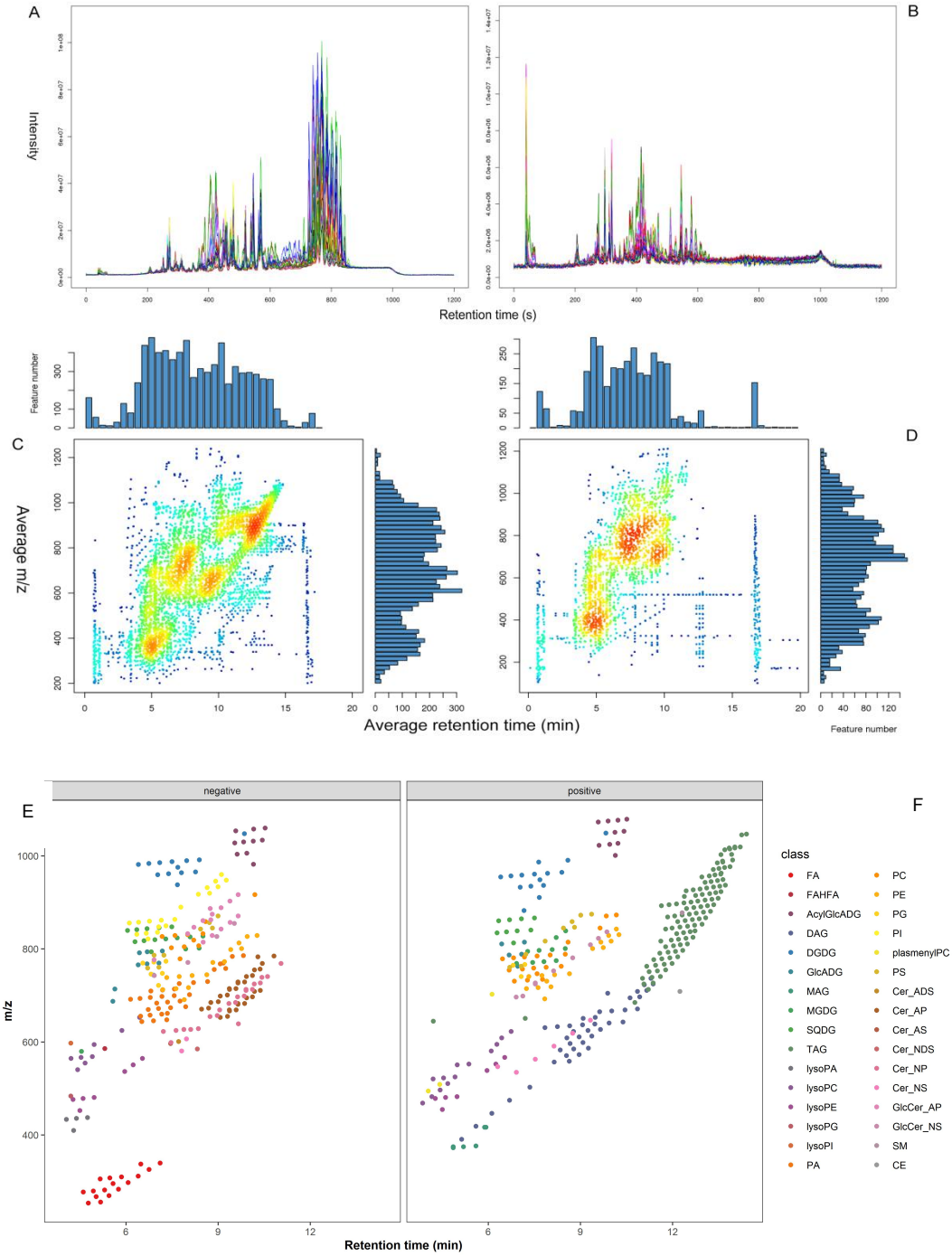


Figure S4. LC-MS identified the lipid features in peanut seed. A-B, LC-MS scan of total ion chromotogram in peanut seed lipid profile under the positive and negative modes, respectively. C-D, Primary LC retention map of total extracted lipid molecular species under the positive and negative scan modes. Y-axis indicated the average m/z of each lipid feature, and X-axis indicated the average RT (retention time, min) of each lipid feature. Numerical value indicated the identical lipid feature number, each

dot represented an individual lipid species, and different colors represented the distribute-intensive situation. E-F, Secondary MS map of total identified lipid classes under the filter pattern with positive and negative modes based on retention time screening, respectively.

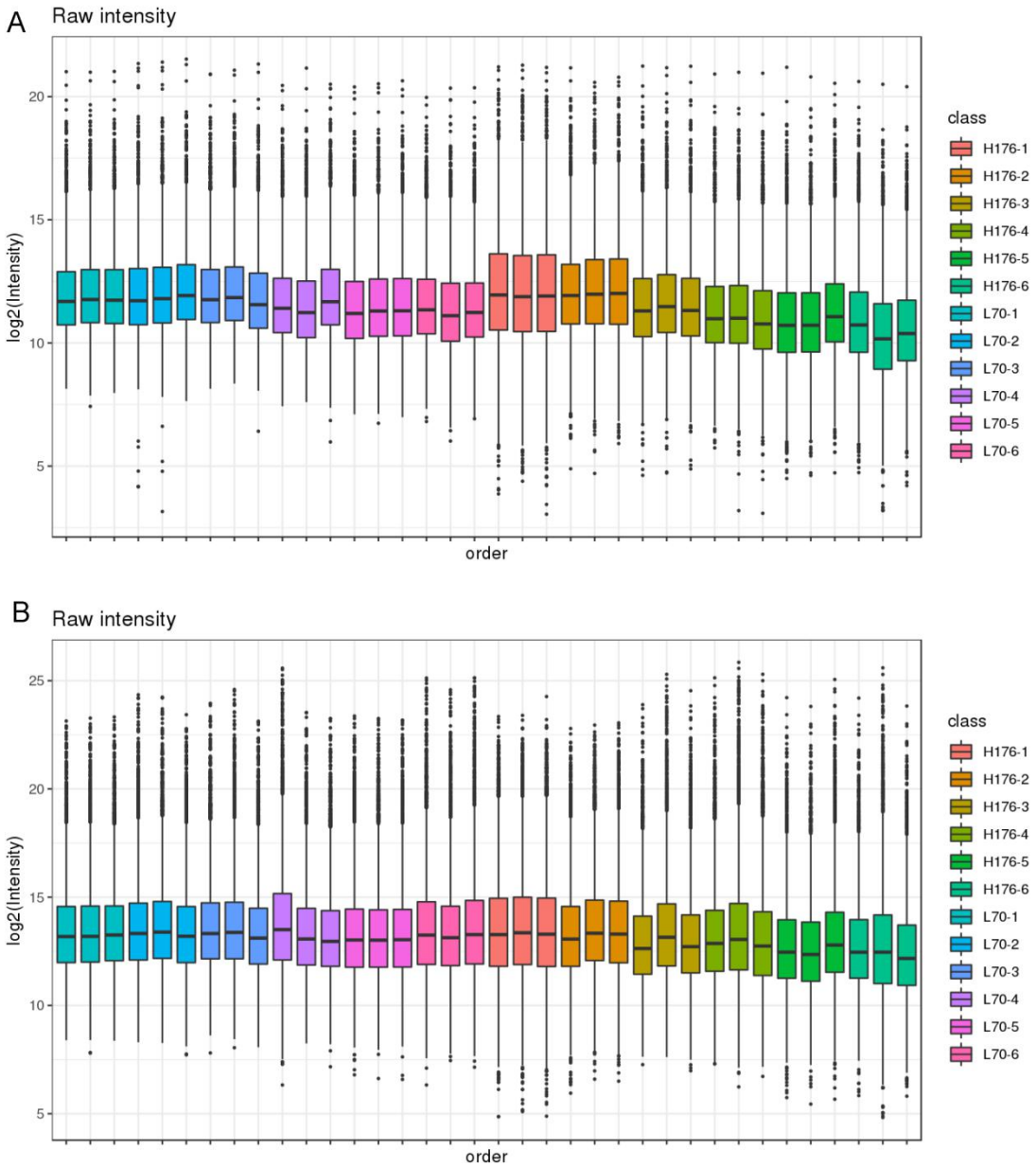


Figure S5. Data normalization analysis of all generated lipidomic datasets under the negative and positive modes, respectively.

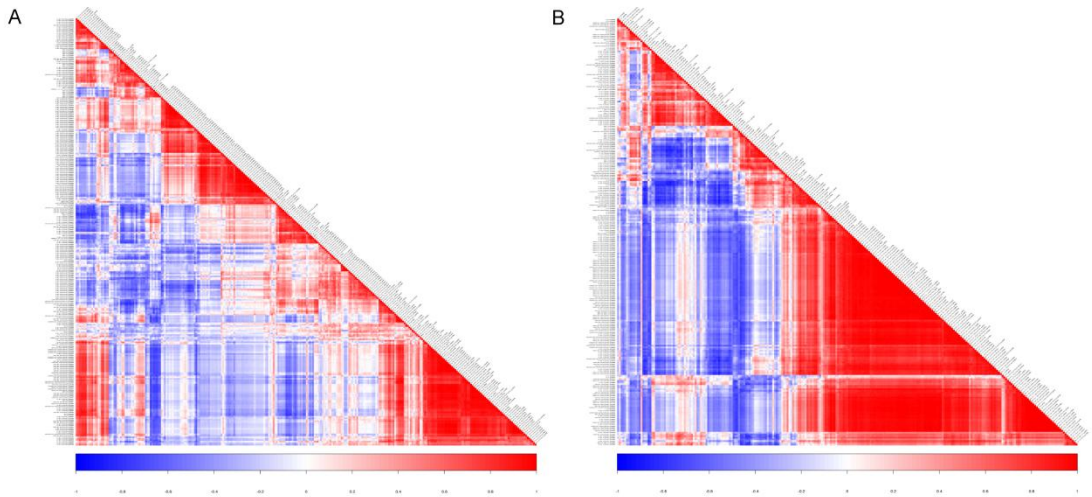


Figure S6. Data correlation analysis of lipid species in tandem mass spectrum profile (MS2). A, Data correlation analysis of all identified lipid molecular species in the positive profile. B, Data correlation analysis of all identified lipid molecular species in the negative profile.

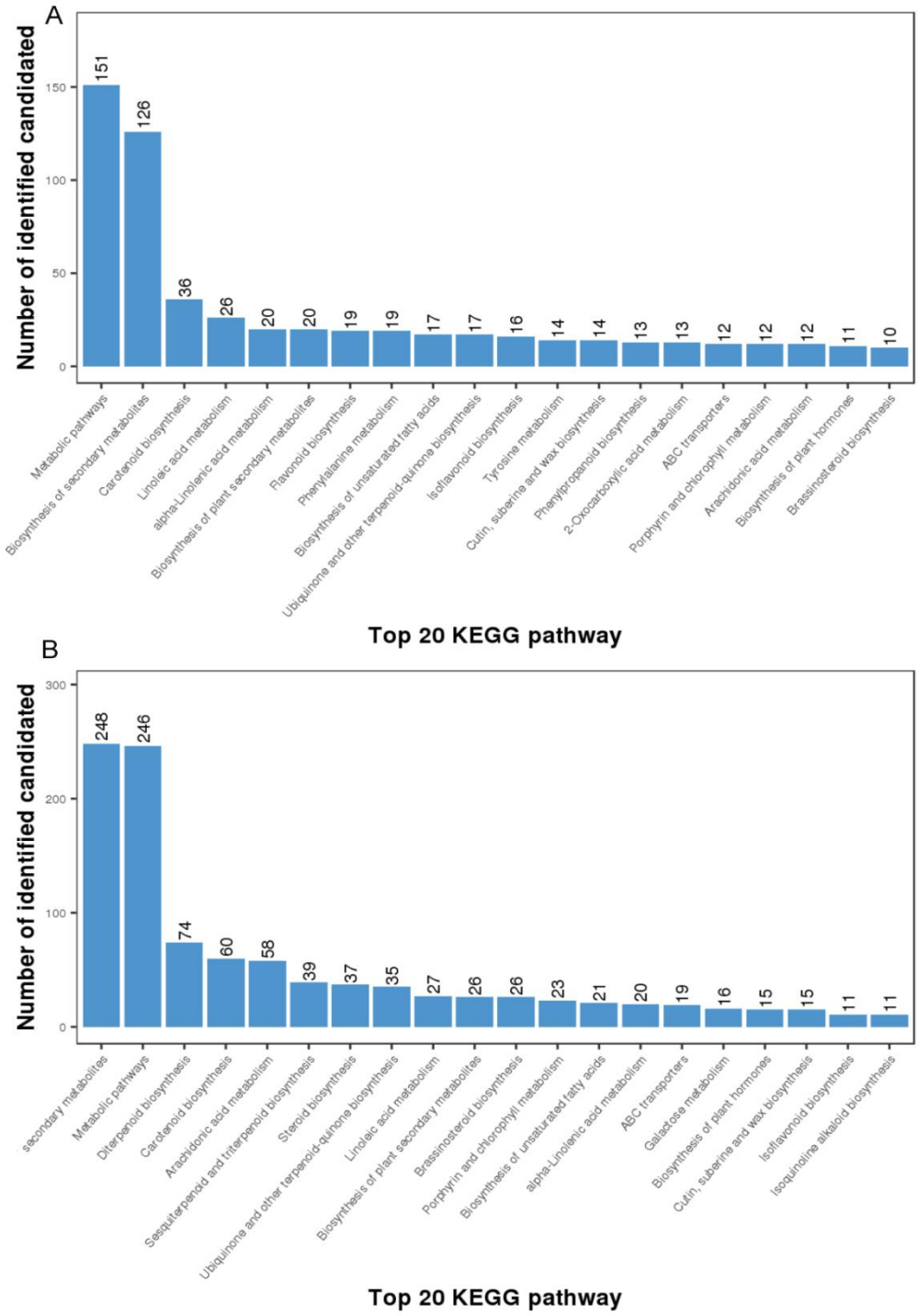
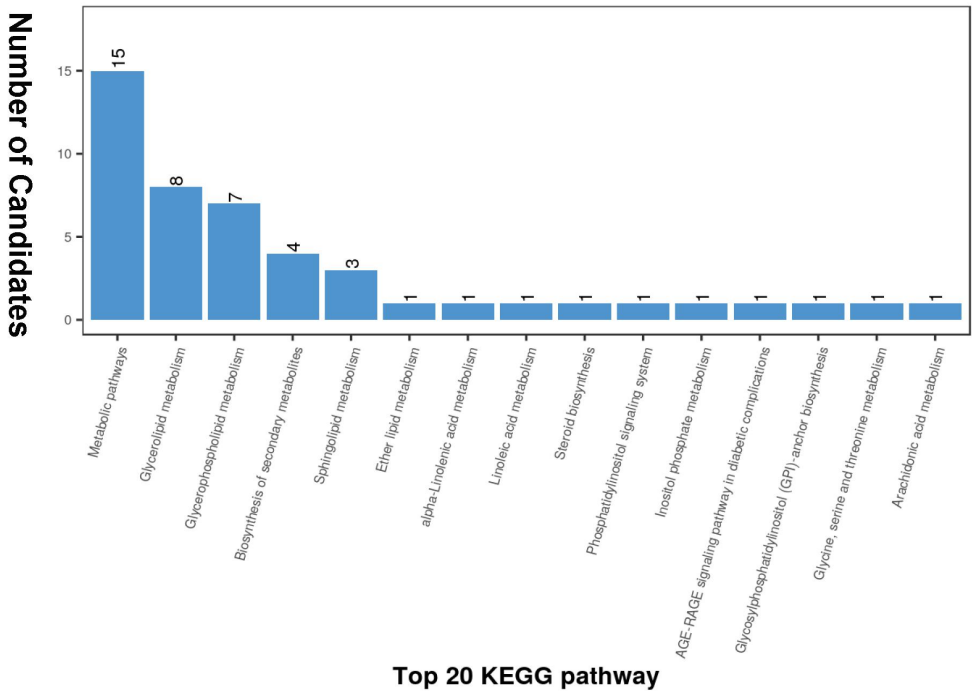


Figure S7. KEGG pathway enrichment analysis of all identified lipid features in primary mass spectrum profile (top 20 terms). A, KEGG enrichment of all lipid features with negative model. B, KEGG enrichment pathway of all lipid features with positive model. KEGG pathways fulfilling the criterion of a cut-off P-value ≤ 0.05 were defined as significantly enriched.



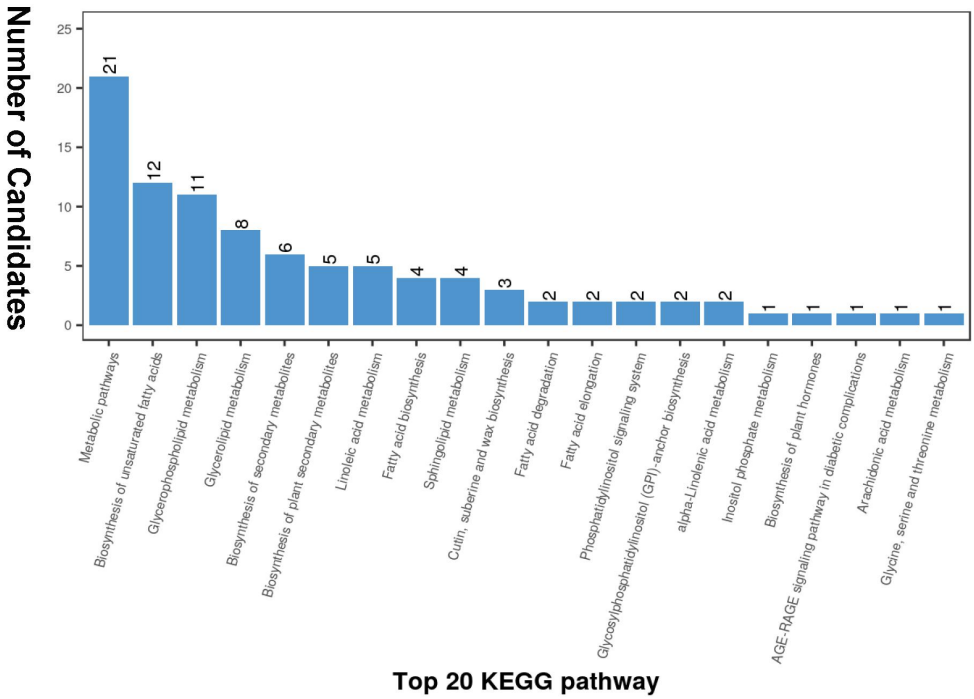


Figure S8. KEGG pathway enrichment analysis of 547 lipid features in secondary MS profile. A, KEGG pathway enrichment of 292 lipid species with positive model. B, KEGG enrichment analysis of 255 lipids with negative model. KEGG pathways fulfilling the criterion of a cut-off P-value $\leqslant 0.05$ were defined as significantly enriched.

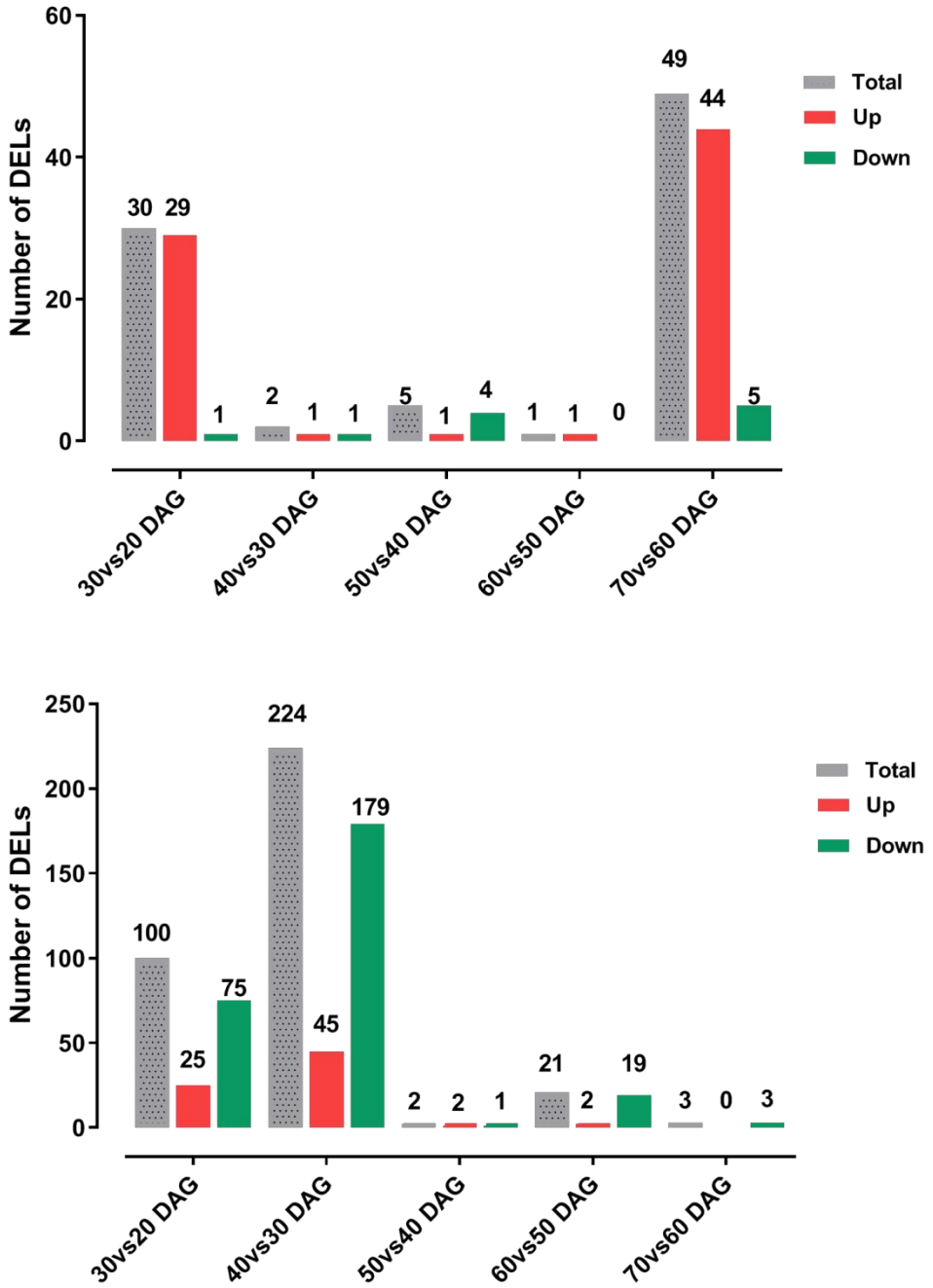


Figure S9. Statistical analysis of the number of DELs distribution in L70 and H176, respectively. A, The number of DELs distribution during the normal peanut seed development (**Table S5**). B, Statistical analysis of DELs' number at different seed developmental stages in H176 (**Table S8**).

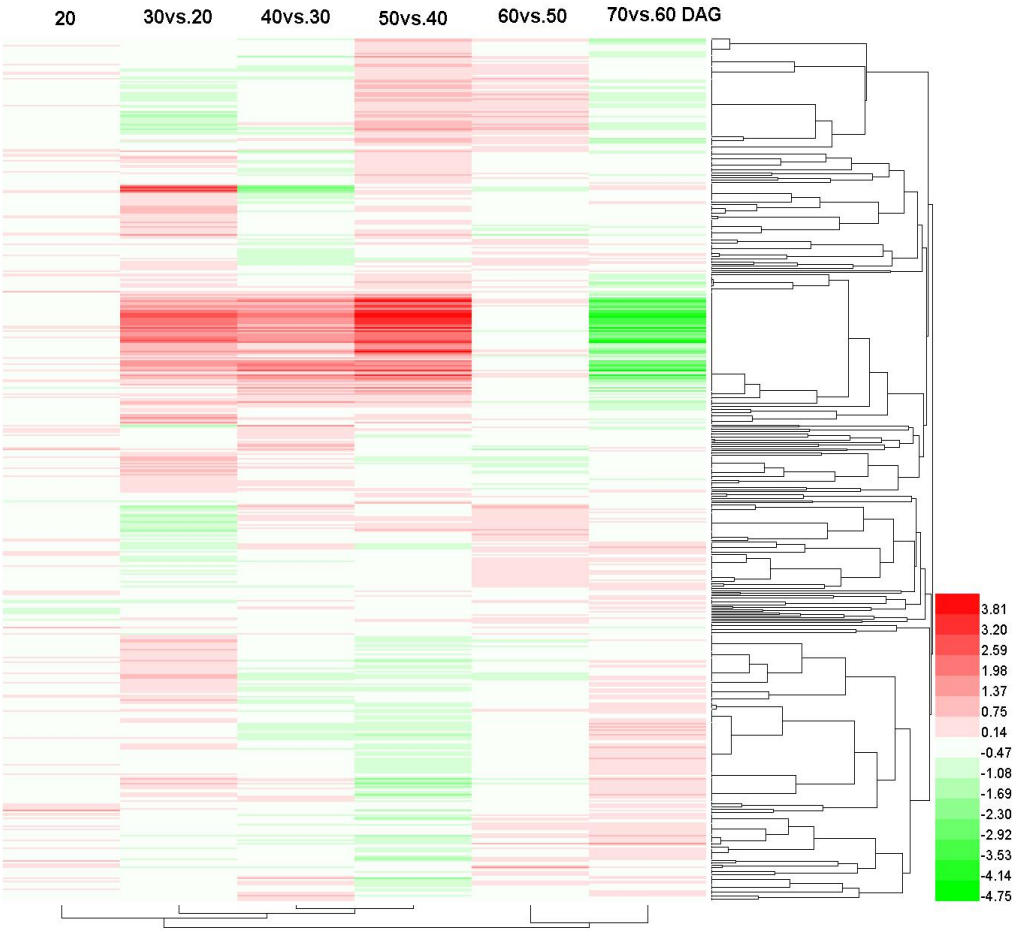


Figure S10. Heatmap (**Table S7**) displayed the variation trend of 547 lipids during the seed development in normal peanut variety (L70). 20 DAG represented the reference sample, each block represented the average relative intensity of lipid compound that came from statistics of three biological replicates (value of $\log^2$ fold change), and the heatmap picture was generated by the software HemI.

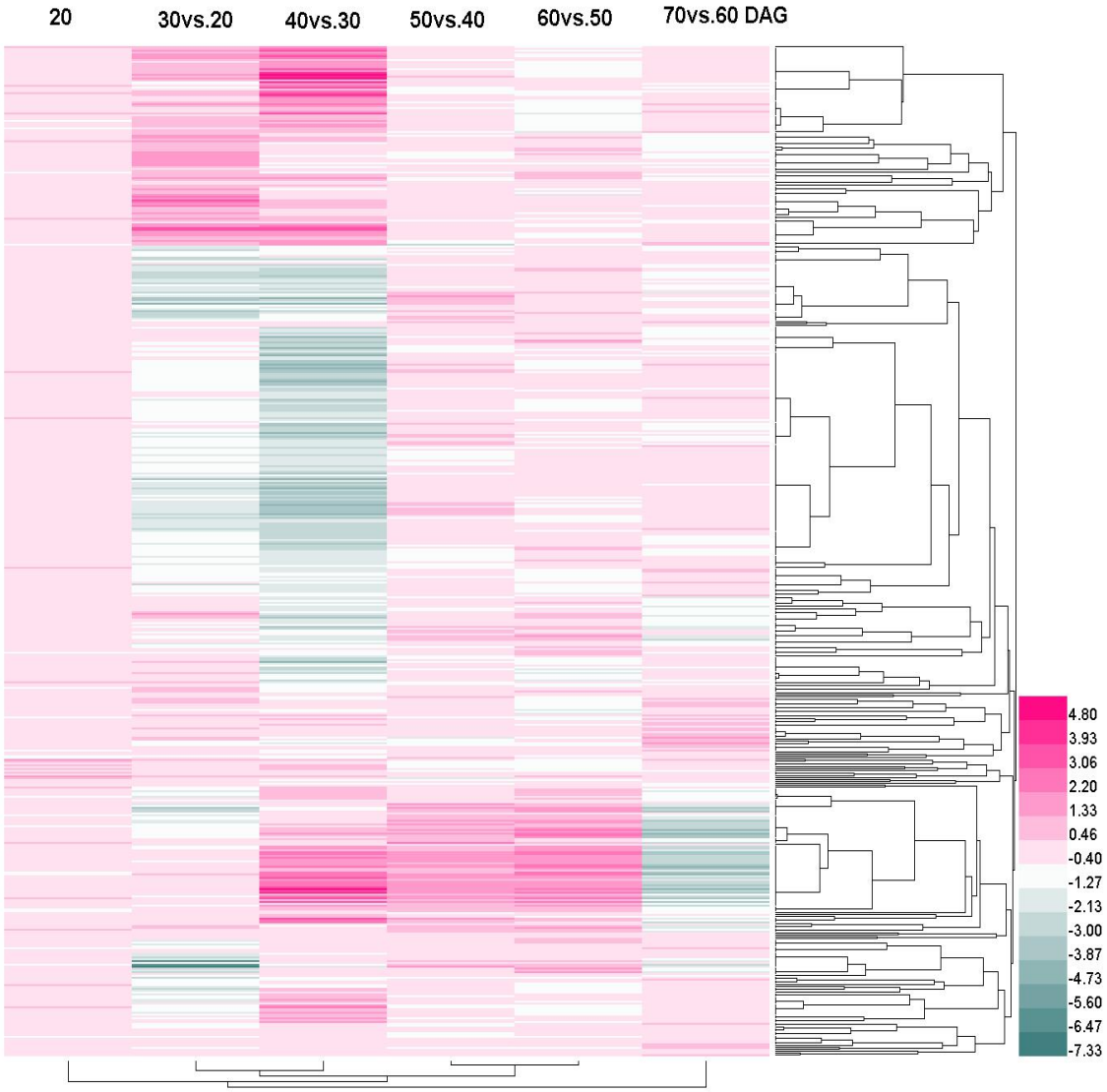


Figure S11. Heatmap (**Table S10**) showed the relative intensity of 547 lipids at each stage in high OA peanut (H176) seed. 20 DAG represented the reference sample, each block represented the average relative intensity of lipid compound that came from statistics of three biological replicates (value of $\log^2$ fold change), and the heatmap picture was generated by the software HemI.

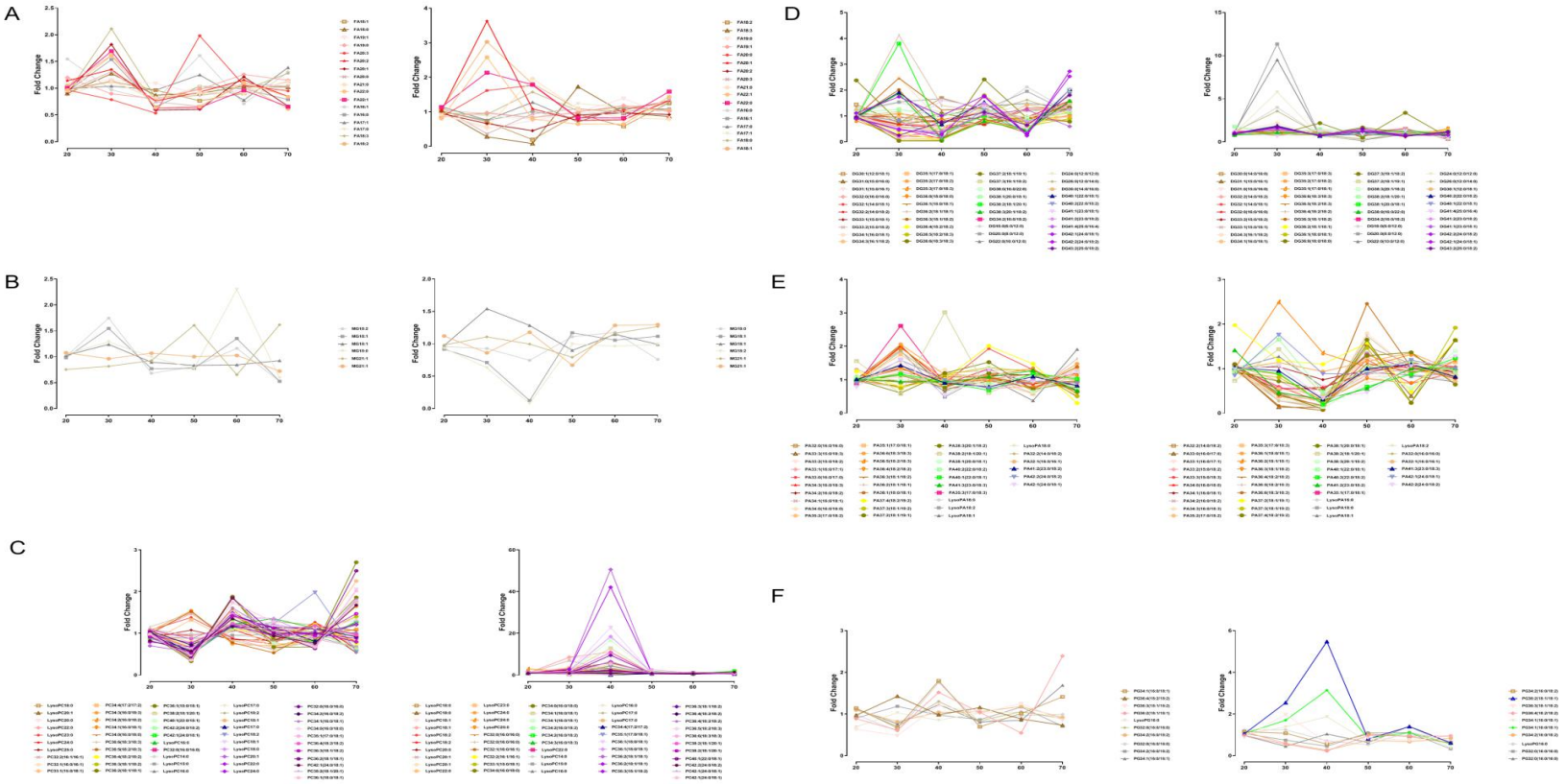


Figure S12. Variation tendency of lipid molecular species regarding of TAGs synthesis during the seed development in normal (L70) and high OA (H176) peanut, respectively. A-F represented the acyl FAs, MG, PC, DG, PA, and PG. These variation curves described by the average fold change in relative intensity of lipid molecular species (**Table S6** and **Table S9**).

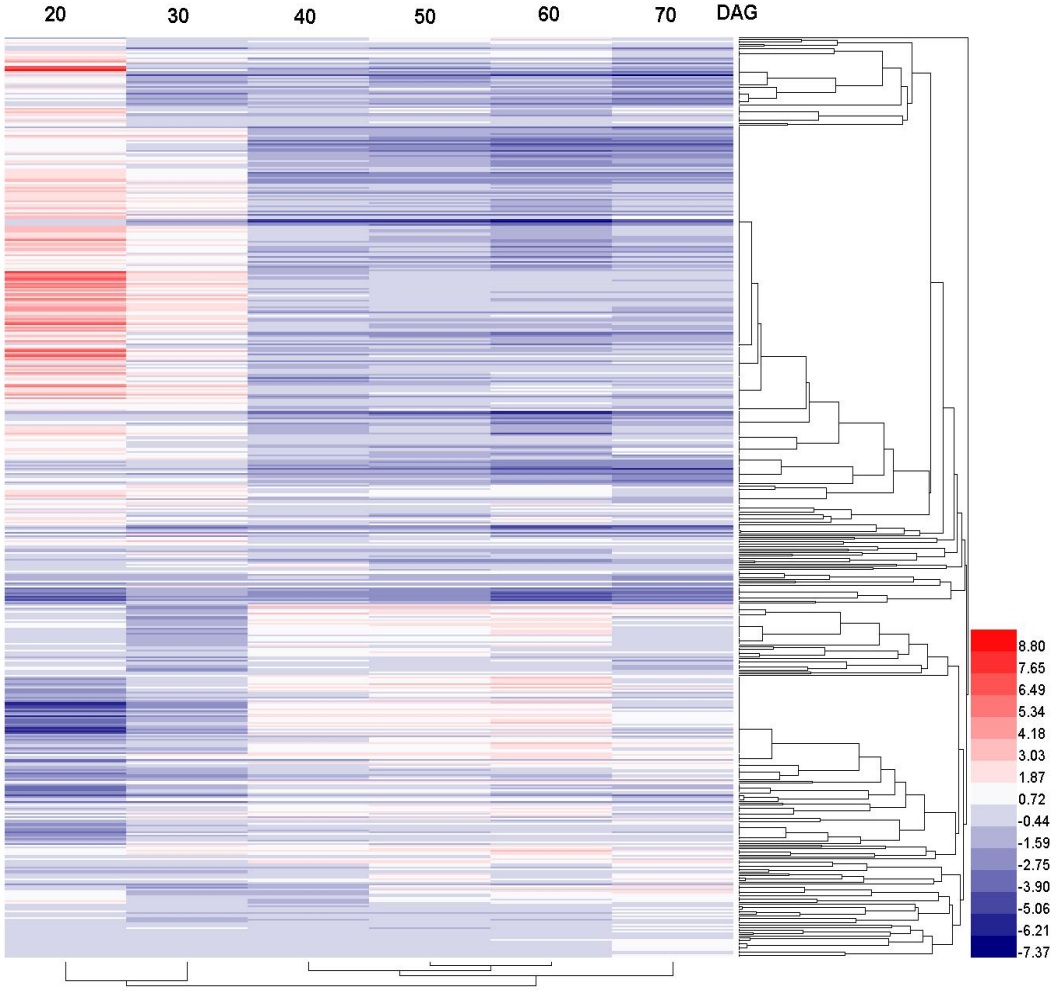


Figure S13. Heatmap (**Table S11**) showed the variation tendency of 547 lipids' relative intensity at each stage in the seed lipid expression profile of high OA (H176) vs. normal (L70) peanut. Each block represented the average relative intensity of lipid compound that came from statistics of three biological replicates (value of $\log^2$ fold change), and the heatmap picture was generated by the software HemI.

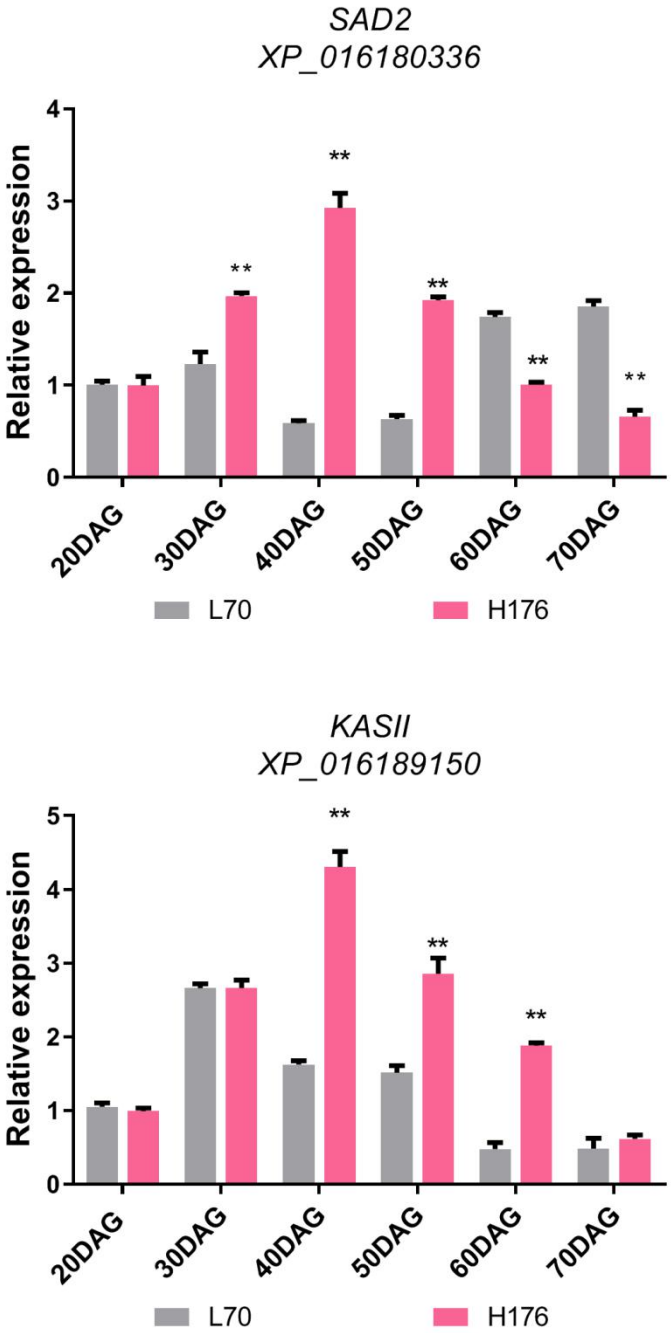


Figure S14. Quantitative real-time PCR validated the relative expression levels of *SAD2* and *KAS II*(**Table 1**) at the transcriptional level during the seed development in L70 and H176, respectively. Each measurement was carried out with three biological replicates, 20 DAG was used as the reference sample, and the values are shown as means±SD (*P<0.05, **P<0.01) compared with normal cultivar L70.

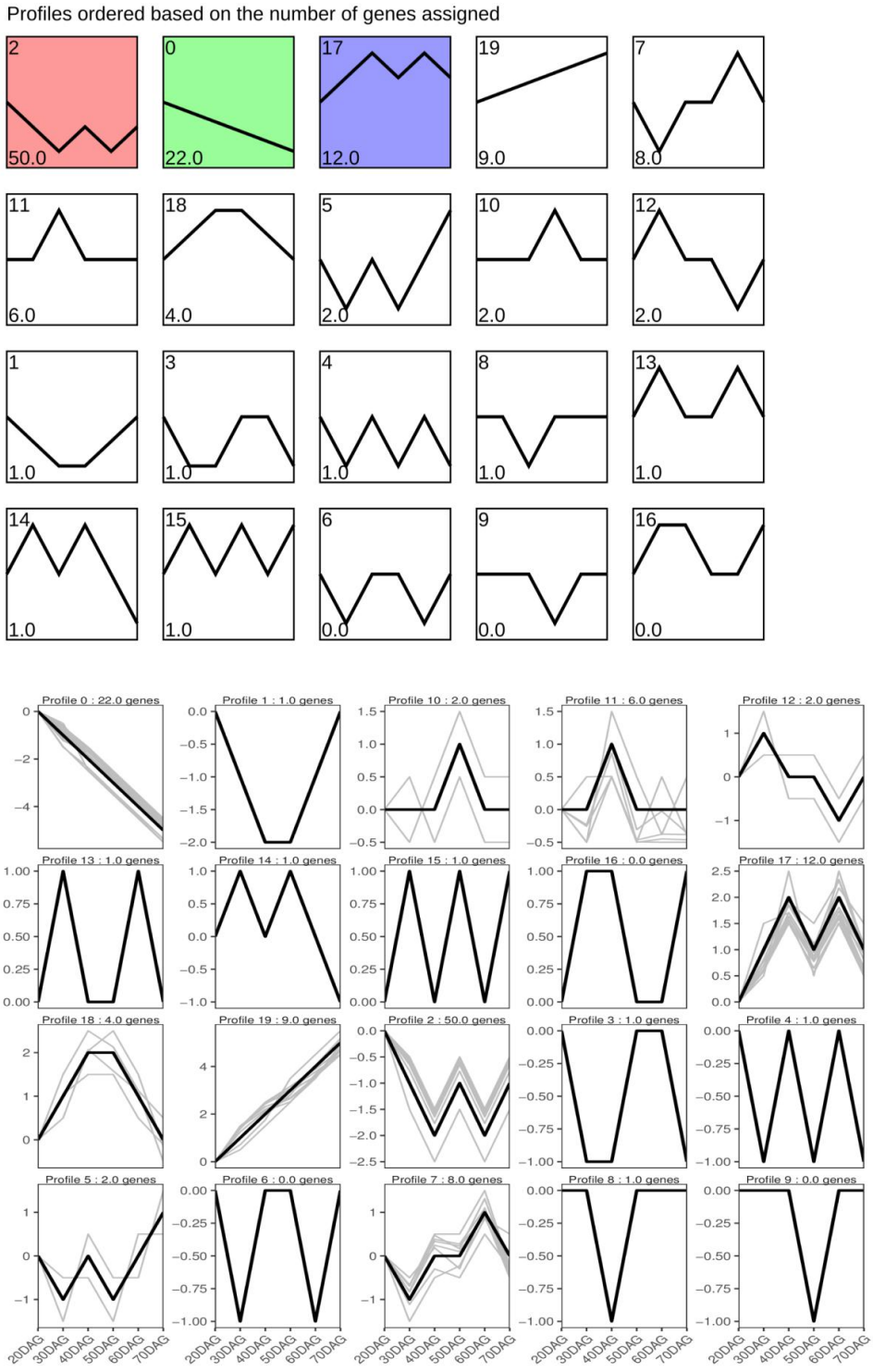


Figure S15. Trend analysis of DELs and DAPs expression profile (20 trends). Colored block trend: significant enrichment trend (P<0.05). Without color trend: the enrichment of significant trends (**Table S17**).

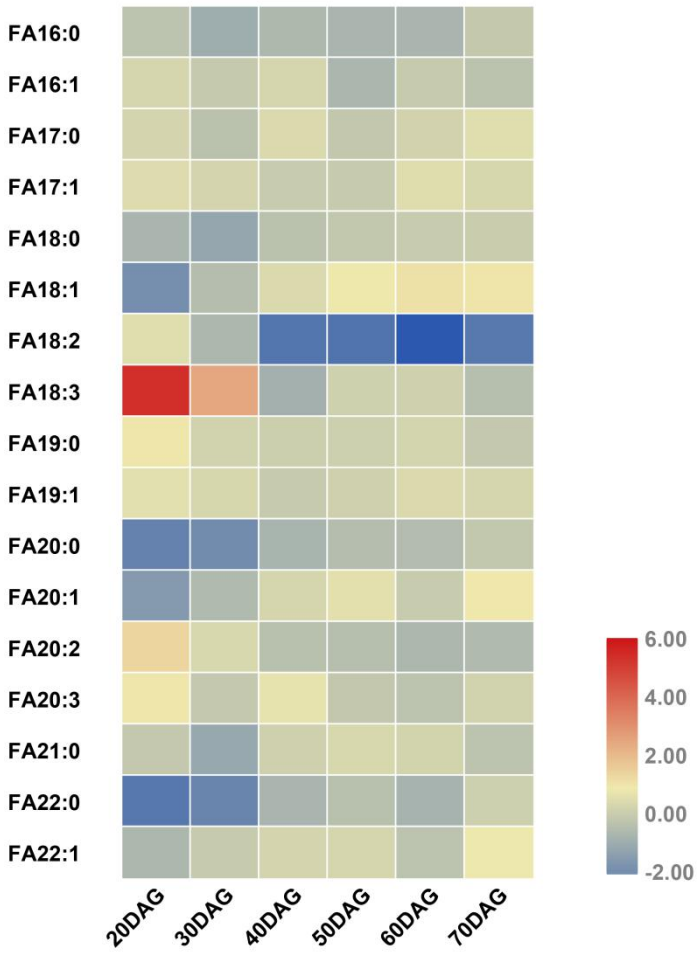


Figure S16. Heatmap showed the relative intensity variation of acyl FAs at each stage in the profile of H176 vs. L70. Each block represented the value of $\log^2$ fold change (**Table S18**), and the heatmap picture was generated by the software TBtool.

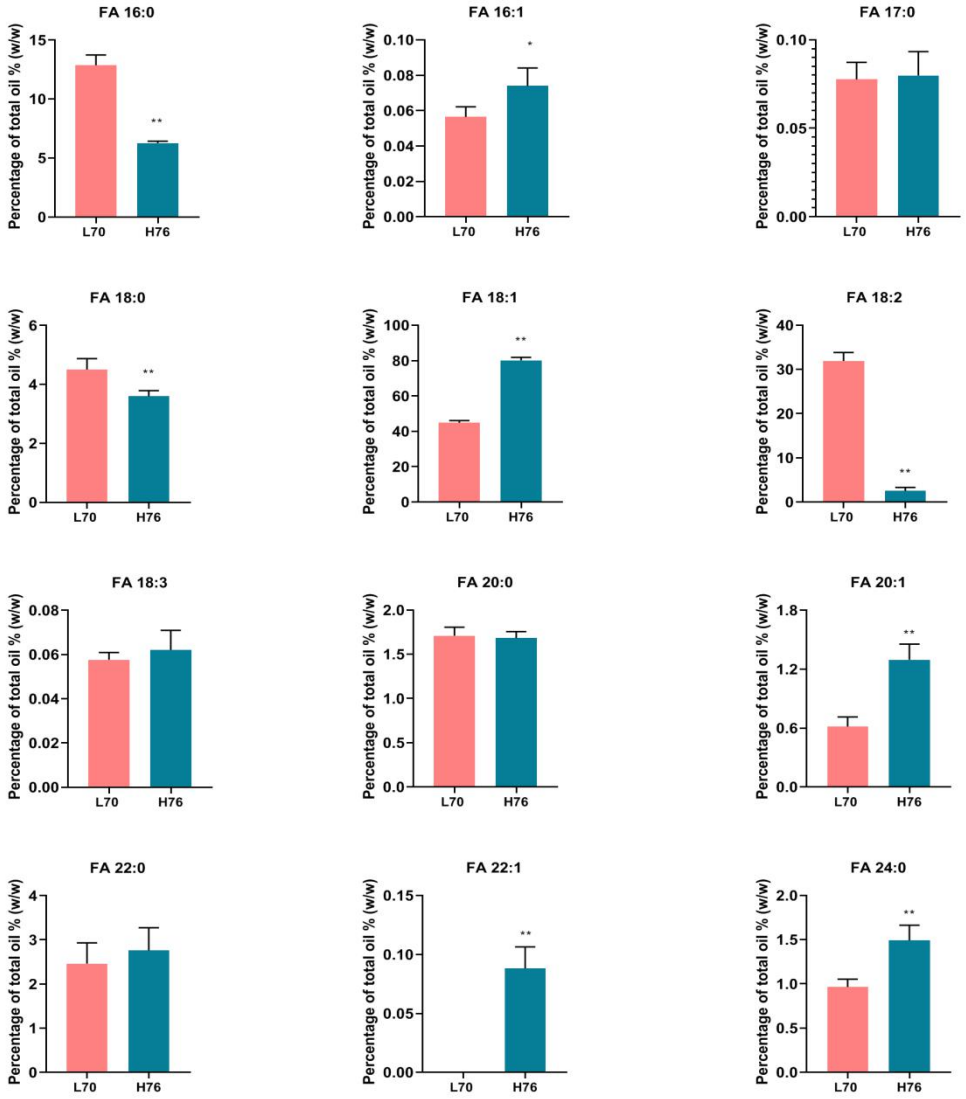


Figure S17. The composition of fatty acids in dehydrated peanut seeds (H176 and L70). Each measurement was carried out with four biological replicates, values are shown as means ± SD (*P < 0.05, **P < 0.01) compared with normal cultivar L70 (**Table S19**). w/w indicates each fatty acid weight compared with total oil weight.