

Title:

**Transcriptomic Analysis Reveals the High-oleic Acid
Feedback Regulating the Homologous Gene Expression of
Stearoyl-ACP Desaturase 2 (*SAD2*) in Peanut**

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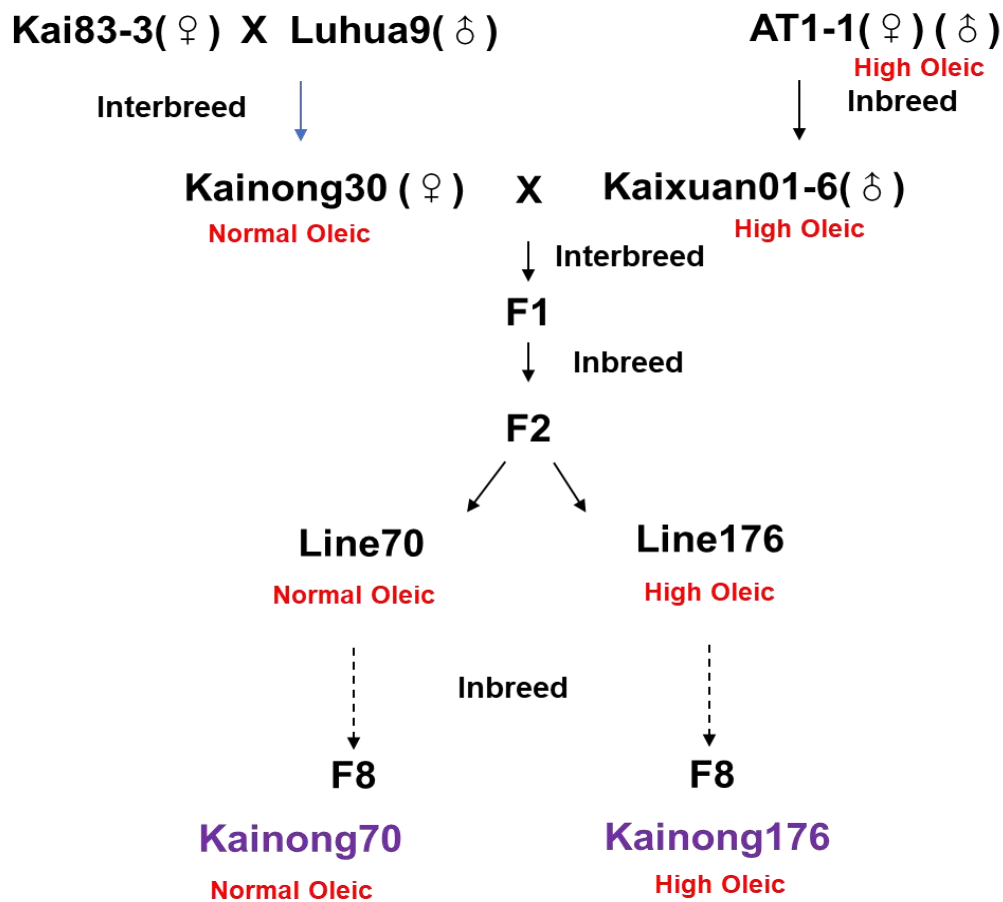


Figure S1. The breeding processes of normal- and high-oleic cultivars Kainong70 and Kainong176. Kai83-3(♀) intercrossed with Luhua9(♂) to cultivate the kainong30, Kaixuan01-6 selected from the inbreeding population of American introduced high-oleic peanut variety AT1-1(♀) (♂). Kainong30 (♀) intercrossed with Kaixuan01-6 (♂) to generate the F2 population, normal- and high-oleic line70 and 176 isolated from the F2 population, and self-fertilized to generate the normal-oleic cultivar Kainong70 and high-oleic cultivar Kainong176 until the stable F8 population. These varieties information provided by the website of www.peanutdata.cn.

>*FAD2-A* coding sequence in high-oleate cultivar kainong176

ATGGGAGCTGGAGGGCGTGCTACTAAGATTGAAGCTCAAAAGAAGCCTCTTTCAAGG
GTTCCACATTCAAACCCCTCCATTTCAGTGTGGCCAACTCAAGAAAGCAATTCACCAC
ATTGCTTTGAACGTTCTCTTTTCATATCATTCTCCTATGTTGTCTATGATCTCTTAGTGCC
CTACTTACTCTTCTACATTGCCACCACTTATTTCCACAAGCTTCCATACCCATTTTCCTTC
CTTGCTTGGCCAATCTATTGGGCCATCCAAGGCTGCATTCTCACTGGTGTGTTGGGTGAT
TGCTCATGAGTGTGGCCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTG
GTTTGACCCTTCACTCTTGA(375bp)CTATTAGTTCCTTATTTCTCATGGAAAATCAGCCA
CCGCCGCCACCACTCCAACACCGGTTCCCTCGACCGCAACGAAGTGTGTTGTCCCAAAA
CCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCAGGGAGGGCTAT
CTCCCTCTTCATCACACTCACACTAGGATGGCCCTTGTAAGTGGCCTTCAATGTTTCTGG
CAGACCCTATGATAGATTTGCAAGCCACTATGACCCTTATGCTCCCATATACTCTAACAG
GGAAAGGCTTCTAATTTATGTCTCAGATTTCATCTGTCTTTGCTGTAAACATATCTGCTATAT
CACATAGCAACTTTGAAAGGTTTGGGTGGGTGGTATGTGTTTATGGGGTGCCATTGCT
CATTGTGAATGGGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCATCATTGAC
TCACTATGATTTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGA
GATTATGGGATACTGAATAAGGCATTTTCATCATATAACTGATACGCATGTGGCTCATCATT
TGTTCTCAACAATGCCTCATTACCATGCAATGGAAGCAACCAATGCAATAAAGCCAATA
TTGGGTGATTACTACCAATTTGATGGCACCCCAGTTTACAAAGCATTGTGGAGAGAAGC
CAAAGAGTGCCTCTATGTGGAGCCAGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGT
ACAAGAACAAGTTCTGA

>*FAD2-A* in normal-oleate cultivar kainong70

ATGGGAGCTGGAGGGCGTGCTACTAAGATTGAAGCTCAAAAGAAGCCTCTTTCAAGG
GTTCCACATTCAAACCCCTCCATTTCAGTGTGGCCAACTCAAGAAAGCAATTCACCAC
ATTGCTTTGAACGTTCTCTTTTCATATCATTCTCCTATGTTGTCTATGATCTCTTAGTGCC
CTACTTACTCTTCTACATTGCCACCACTTATTTCCACAAGCTTCCATACCCATTTTCCTTC
CTTGCTTGGCCAATCTATTGGGCCATCCAAGGCTGCATTCTCACTGGTGTGTTGGGTGAT
TGCTCATGAGTGTGGCCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTG
GTTTGACCCTTCACTCTTGTCTATTAGTTCCTTATTTCTCATGGAAAATCAGCCACCGCC
GCCACCACTCCAACACCGGTTCCCTCGACCGGACGAAGTGTGTTGTCCCAAAACCAA
AATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCAGGGAGGGCTATCTCC
CTCTTCATCACACTCACACTAGGATGGCCCTTGTAAGTGGCCTTCAATGTTTCTGGCAG
ACCCTATGATAGATTTGCAAGCCACTATGACCCTTATGCTCCCATATACTCTAACAGGGA
AAGGCTTCTAATTTATGTCTCAGATTTCATCTGTCTTTGCTGTAAACATATCTGCTATATCAC
ATAGCAACTCTGAAAGGTTTGGGTGGGTGGTATGTGTTTATGGGGTGCCATTGCTCAT
TGTGAATGGGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCATCATTGCCTCA
CTATGATTTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGAT
TATGGGATACTGAATAAGGCATTTTCATCATATAACTGATACGCATGTGGCTCATCATTGT
TCTCAACAATGCCTCATTACCATGCAATGGAAGCAACCAATGCAATAAAGCCAATATTG
GGTGATTACTACCAATTTGATGGCACCCCAGTTTACAAAGCATTGTGGAGAGAAGCCA
AAGAGTGCCTCTATGTGGAGCCAGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGTAC
AAGAACAAGTTCTGA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130			
FAD2-AKM176	ATGGAGCTGGAGGCGGTGTCTACTAGATTGAGCTCAAAAGAGCCCTTTTCAGGGTTCCACATTCARACCCCTCATTCAGTGTGGCCACTCAGAAAGCAATCCACCACATTGCTTTGACGCTT																
FAD2-AKN70	ATGGAGCTGGAGGCGGTGTCTACTAGATTGAGCTCAAAAGAGCCCTTTTCAGGGTTCCACATTCARACCCCTCATTCAGTGTGGCCACTCAGAAAGCAATCCACCACATTGCTTTGACGCTT																
Consensus	ATGGAGCTGGAGGCGGTGTCTACTAGATTGAGCTCAAAAGAGCCCTTTTCAGGGTTCCACATTCARACCCCTCATTCAGTGTGGCCACTCAGAAAGCAATCCACCACATTGCTTTGACGCTT																
	131	140	150	160	170	180	190	200	210	220	230	240	250	260			
FAD2-AKM176	CTCTTTTCATATCAATTCCTATGTGTCTATGATCTCTTAGTGGCCACTTACTCTTACATTTGCCACCACTTATTTCCACAGCTTCATACCCATTTCTCTCTTGGCTTGGCCACTTATGGGC																
FAD2-AKN70	CTCTTTTCATATCAATTCCTATGTGTCTATGATCTCTTAGTGGCCACTTACTCTTACATTTGCCACCACTTATTTCCACAGCTTCATACCCATTTCTCTCTTGGCTTGGCCACTTATGGGC																
Consensus	CTCTTTTCATATCAATTCCTATGTGTCTATGATCTCTTAGTGGCCACTTACTCTTACATTTGCCACCACTTATTTCCACAGCTTCATACCCATTTCTCTCTTGGCTTGGCCACTTATGGGC																
	261	270	280	290	300	310	320	330	340	350	360	370	380	390			
FAD2-AKM176	CATCAGGCTGCATTTCTACTGGTGTGGGTGATTGCTCATGAGTGTGGCCACCATGCTTCAGCAGTACCACTTGTGATGACATGGTTGGTTTGACCTTCACTCTTGACTATAGTTCCTTAT																
FAD2-AKN70	CATCAGGCTGCATTTCTACTGGTGTGGGTGATTGCTCATGAGTGTGGCCACCATGCTTCAGCAGTACCACTTGTGATGACATGGTTGGTTTGACCTTCACTCTTGACTATAGTTCCTTAT																
Consensus	CATCAGGCTGCATTTCTACTGGTGTGGGTGATTGCTCATGAGTGTGGCCACCATGCTTCAGCAGTACCACTTGTGATGACATGGTTGGTTTGACCTTCACTCTTGACTATAGTTCCTTAT																
	391	400	410	420	430	440	450	460	470	480	490	500	510	520			
FAD2-AKM176	TTCTCATGAAATACGACCGCCGCCACCACTCCACACCGGTTCCCTCGACCGCAGCAGGTGTTTGTCCAAACCAAAATCAGAGTATCATGGTATACAGTACATGACAAATCCACCAGGGA																
FAD2-AKN70	TTCTCATGAAATACGACCGCCGCCACCACTCCACACCGGTTCCCTCGACCGCAGCAGGTGTTTGTCCAAACCAAAATCAGAGTATCATGGTATACAGTACATGACAAATCCACCAGGGA																
Consensus	TTCTCATGAAATACGACCGCCGCCACCACTCCACACCGGTTCCCTCGACCGCAGCAGGTGTTTGTCCAAACCAAAATCAGAGTATCATGGTATACAGTACATGACAAATCCACCAGGGA																
	521	530	540	550	560	570	580	590	600	610	620	630	640	650			
FAD2-AKM176	GGGCTATCTCCCTTTCATCACTCACTAGGATGGCCCTTGACTTGGCTTCATGTTTCTGGACGCCATGATAGATTGGACGCCATATGACCCCTATGCTCCCATATCTCTACAGGGA																
FAD2-AKN70	GGGCTATCTCCCTTTCATCACTCACTAGGATGGCCCTTGACTTGGCTTCATGTTTCTGGACGCCATGATAGATTGGACGCCATATGACCCCTATGCTCCCATATCTCTACAGGGA																
Consensus	GGGCTATCTCCCTTTCATCACTCACTAGGATGGCCCTTGACTTGGCTTCATGTTTCTGGACGCCATGATAGATTGGACGCCATATGACCCCTATGCTCCCATATCTCTACAGGGA																
	651	660	670	680	690	700	710	720	730	740	750	760	770	780			
FAD2-AKM176	AAGGCTTCTAATTTATGTCTCAGATTCATCTGCTTTGCTGTACATATCTGCTATATCAGATGACACTTGAAGGTTGGGTGGGTGATGTGTTTATGGGGTGCATTGCTCATTTGATGAGG																
FAD2-AKN70	AAGGCTTCTAATTTATGTCTCAGATTCATCTGCTTTGCTGTACATATCTGCTATATCAGATGACACTTGAAGGTTGGGTGGGTGATGTGTTTATGGGGTGCATTGCTCATTTGATGAGG																
Consensus	AAGGCTTCTAATTTATGTCTCAGATTCATCTGCTTTGCTGTACATATCTGCTATATCAGATGACACTTGAAGGTTGGGTGGGTGATGTGTTTATGGGGTGCATTGCTCATTTGATGAGG																
	781	790	800	810	820	830	840	850	860	870	880	890	900	910			
FAD2-AKM176	TTTCTAGTTACCATACCTATTTGCACACACATGATCATTTGCTCACTATGATTCATCCGATGGGACTGGTTAAGAGGAGCATTGGCAGCTGGACAGAGATTATGGGATCTGATAGGCAT																
FAD2-AKN70	TTTCTAGTTACCATACCTATTTGCACACACATGATCATTTGCTCACTATGATTCATCCGATGGGACTGGTTAAGAGGAGCATTGGCAGCTGGACAGAGATTATGGGATCTGATAGGCAT																
Consensus	TTTCTAGTTACCATACCTATTTGCACACACATGATCATTTGCTCACTATGATTCATCCGATGGGACTGGTTAAGAGGAGCATTGGCAGCTGGACAGAGATTATGGGATCTGATAGGCAT																
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040			
FAD2-AKM176	TTCTCATATATCTGATACGATGTGGCTCATCTTTGTTCTCAACATGCTTCATACCATGATGAGGACCACTGATATAGGCCATATGGGTGATTACTACATTTGATGGCCGCCAGT																
FAD2-AKN70	TTCTCATATATCTGATACGATGTGGCTCATCTTTGTTCTCAACATGCTTCATACCATGATGAGGACCACTGATATAGGCCATATGGGTGATTACTACATTTGATGGCCGCCAGT																
Consensus	TTCTCATATATCTGATACGATGTGGCTCATCTTTGTTCTCAACATGCTTCATACCATGATGAGGACCACTGATATAGGCCATATGGGTGATTACTACATTTGATGGCCGCCAGT																
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140						
FAD2-AKM176	TTTCAAGCATTTGGGAGAGGCCAAGAGTGCCTCTATGTGGGCGAGATGATGAGCTTCTCAGAGGGTGTATTATGGTACAGAACAGTTCTGA																
FAD2-AKN70	TTTCAAGCATTTGGGAGAGGCCAAGAGTGCCTCTATGTGGGCGAGATGATGAGCTTCTCAGAGGGTGTATTATGGTACAGAACAGTTCTGA																
Consensus	TTTCAAGCATTTGGGAGAGGCCAAGAGTGCCTCTATGTGGGCGAGATGATGAGCTTCTCAGAGGGTGTATTATGGTACAGAACAGTTCTGA																

>FAD2-B coding sequence in kainong176

ATGGGAGCTGGAGGCGTGTCACTAAGATTGAAGCTCAAAGAAGCCTCCTTCAAGG
GTTCCACATTCAAACCCTCCATTTCAGTGTGGCCAACTCAAGAAGGCAATTCACCAC
ATTGCTTTGAACGTTCTCTTTTCATATCATTCTCATATGTTGTCTATGATCTCTTAATGGCC
TACTTACTCTTCTACATTGCCACCACCTTATTTCCACAAGCTTCCATACTCATTATCCTTCC
TTGCTTGGCCAATCTATTGGGCCATCCAAGGCTGCACTTCTCACCGGTGTTTGGGTGATT
GCTCATGAGTGTGGCCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGG
TTTGACCCCTTCACTCTTGTCTATTAGTTCCCTTATTTCTCGTGGAAAATCAGCCACCGCCG
CCACCACTCCAACACAGGTTCCC**TCA**(442bp)GACCGCGACGAAGTGTTTGTCCCGAAA
CCAAAATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCAGGGAGGGCTAT
TTCCCTTTTCATCACACCCACACTAGGATGGCCCTTGTACTTGGCCTTCAATGTTTCTGG
CAGACCCTATGATAGATTTGCAAGCCACTATGACCCCTTATGCTCCCATATACTCTAACAG
GGAAAGGCTTCTAATTTATGTCTCAGATTTCATCTGTCTTTGCTGTAACATATCTGCTATAT
CACATAGCAACTTTGAAAGGTTTGGGTGGGTGGTATGTGTTTATGGGGTGCCATTGCT
CATTGTGAATGGGTTTCTAGTTACCATAACCTATTTGCAGCACACACATGCAGCATTTGCC
TCACTATGATTTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGA
GATTATGGGATACTGAATAAGGCATTTTCATCATATAACTGATACGCATGTGGCTCATCATT
TGTTCTCAACAATGCCTCATTACCATGCAATGGAAGCAACCAATGCAATAAAGCCAATA
TTGGGTGATTACTACCAATTTGATGGCACCCCAAGTTTACAAAGCATTGTGGAGAGAAGC
CAAAGAGTGCCTCTATGTGGAGCCAGATGATGGAGCTTCTCAGAAGGGTGTTTATTGGT
ACAAGAACAAGTTCTGA

>FAD2-B coding sequence in kainong70

ATGGGAGCTGGAGGCGTGTCACTAAGATTGAAGCTCAAAGAAGCCTCCTTCAAGG
GTTCCACATTCAAACCCTCCATTTCAGTGTGGCCAACTCAAGAAGGCAATTCACCAC

ATTGCTTTGAACGTTCTCTTTTCATATCATTCTCATATGTTGTCTATGATCTCTTAATGGCC
TACTTACTCTTCTACATTGCCACCACTTATTTCCACAAGCTTCCATACCCATTTTCCTTCC
TTGCTTGGCCAATCTATTGGGCCATCCAAGGCTGCATTCTCACCGGTGTTTGGGTGATT
GCTCATGAGTGTGGCCACCATGCCTTCAGCAAGTACCAACTTGTTGATGACATGGTTGG
TTTGACCCCTTCACTCTTGTCTATTAGTTCCTTATTTCTCATGGAAAATCAGCCACCGCCG
CCACCACTCCAACACAGGTTCCCTCGACCGCGACGAAGTGTTTGTCCCGAAACCAAA
ATCAAAGGTATCATGGTATAACAAGTACATGAACAATCCACCAGGGAGGGCTATTTCTC
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TATGATTTCATCCGAATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATT
ATGGGATACTGAATAAGGCATTTTCATCATATAACTGATACGCATGTGGCTCATCATTTGTT
CTCAACGATGCCTCATTACCATGCAATGGAAGCAACCAATGCAATAAAGCCAATATTGG
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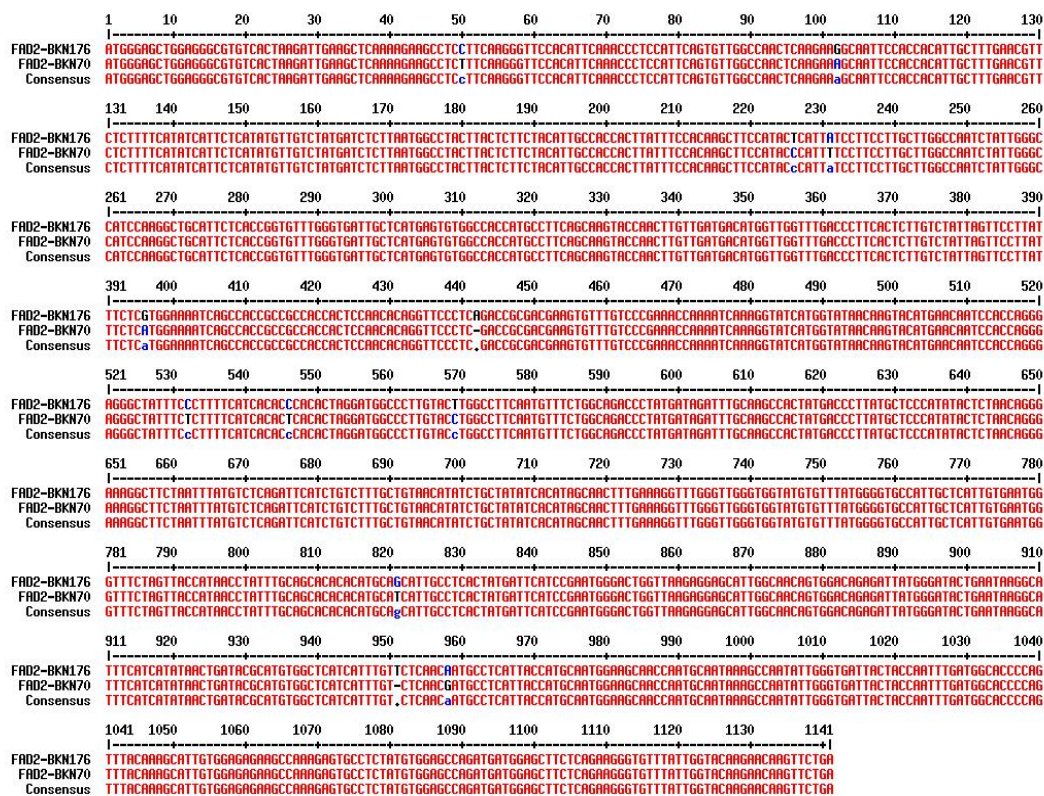


Figure S2. Coding sequence of *FAD2-A* and *FAD2-B* in kainong176 and kainong70.

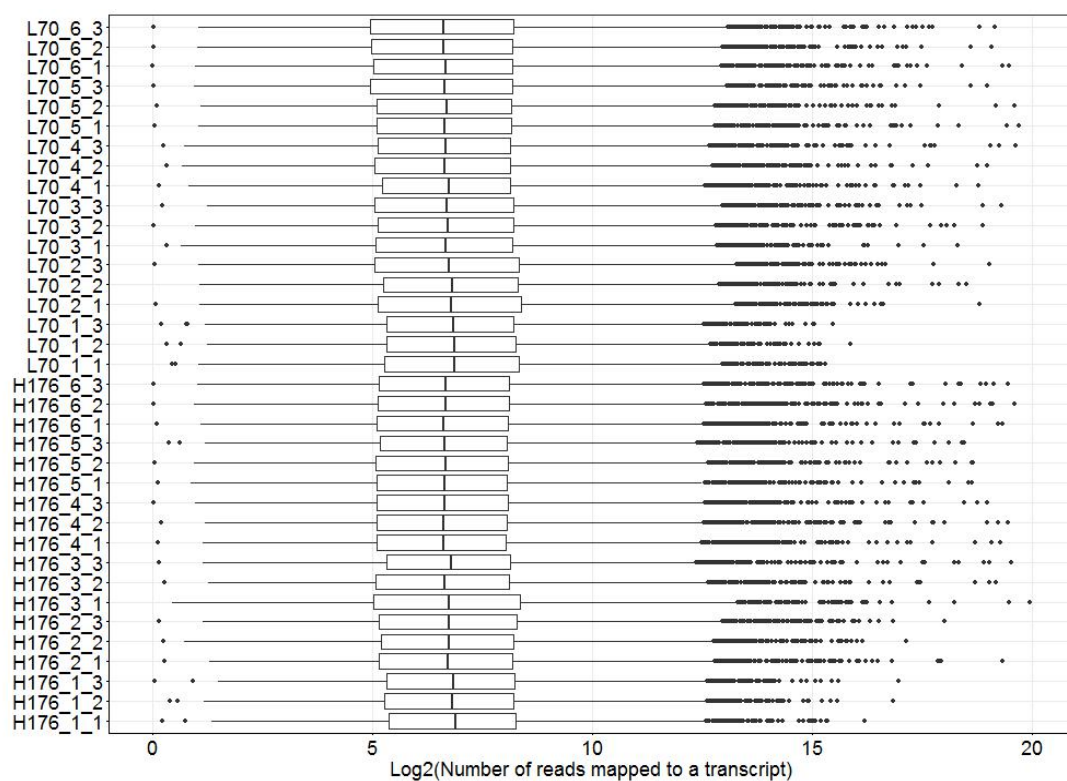


Figure S3. Statistical analysis of assembly reads mapped to the transcript.

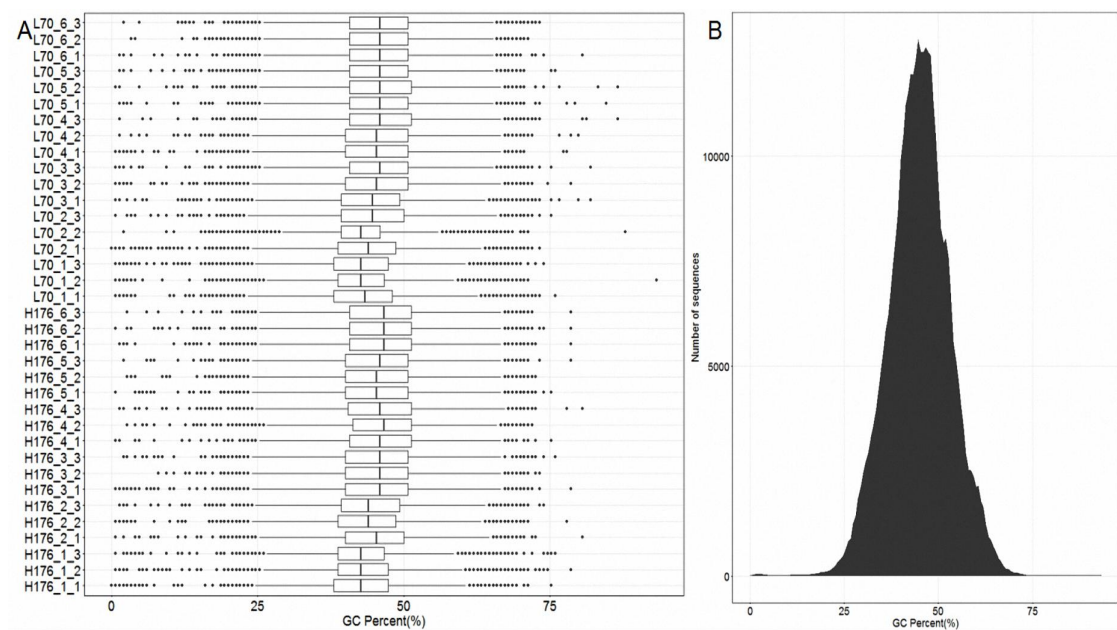


Figure S4. GC content analysis of peanut transcripts. A, Frequency of GC content of peanut transcripts. B, Distribution of GC content of transcripts for peanut transcriptome-seq.

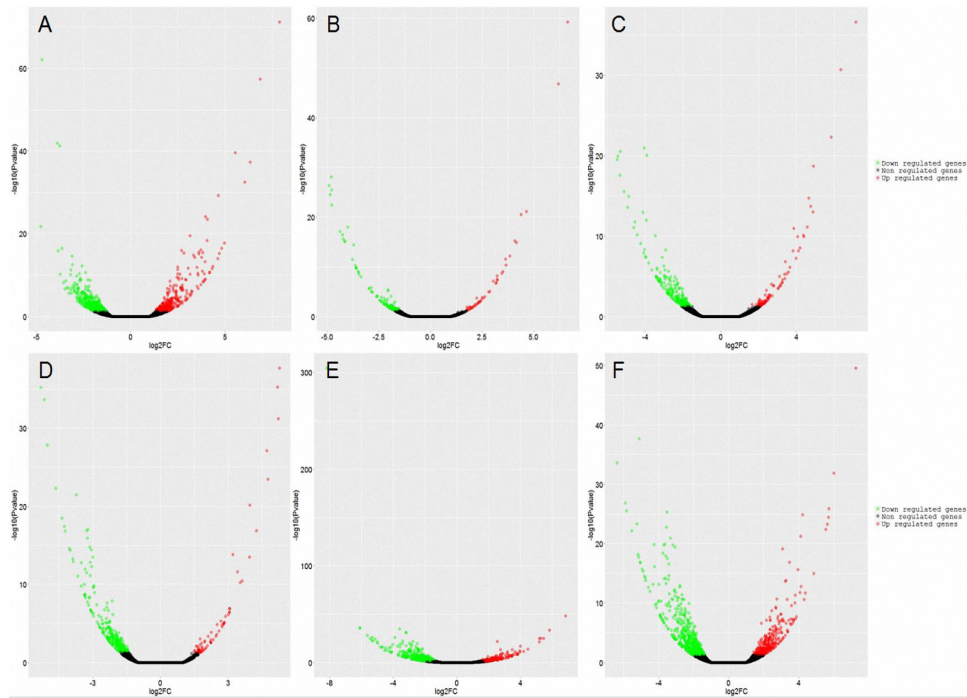


Figure S5. Volcano plot displayed the screening criteria of total DEGs at different seed development stages. A-F represented from 20DAF to 70DAF, respectively.

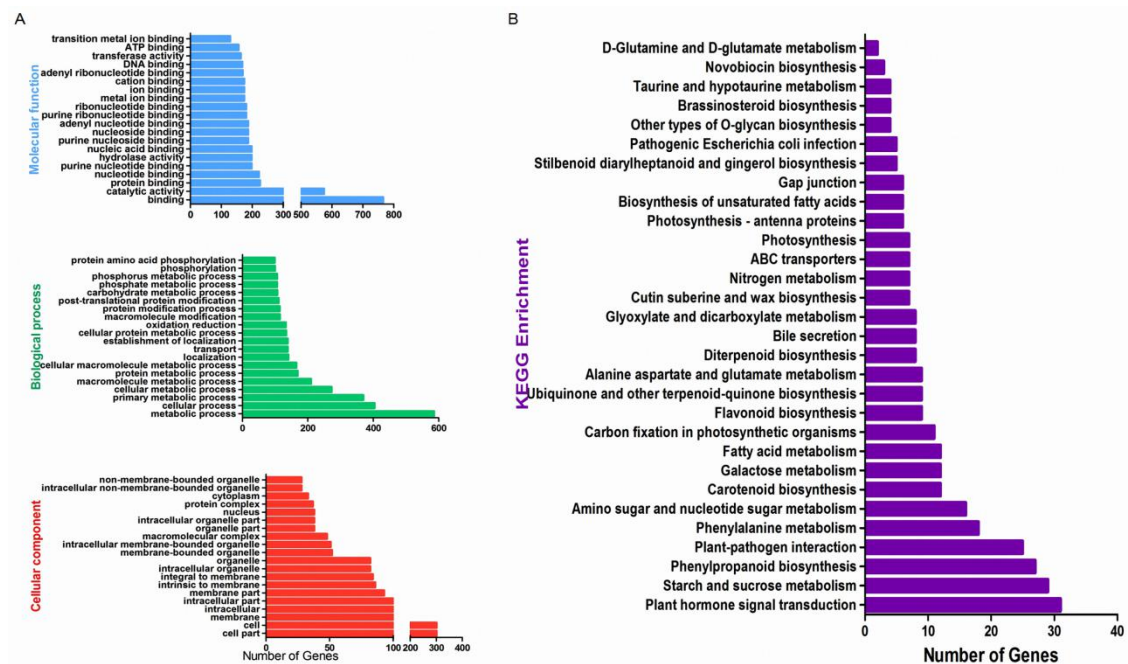


Figure S6. Bioinformatics and statistical analyses of differentially expressed genes. A, GO analysis of the total DEGs in molecular function, biological process, and cellular component, respectively (Top 20 terms). B, KEGG pathway enrichment analysis of DEGs at different seed developmental stages in H176 vs. L70 (Top 30 terms).

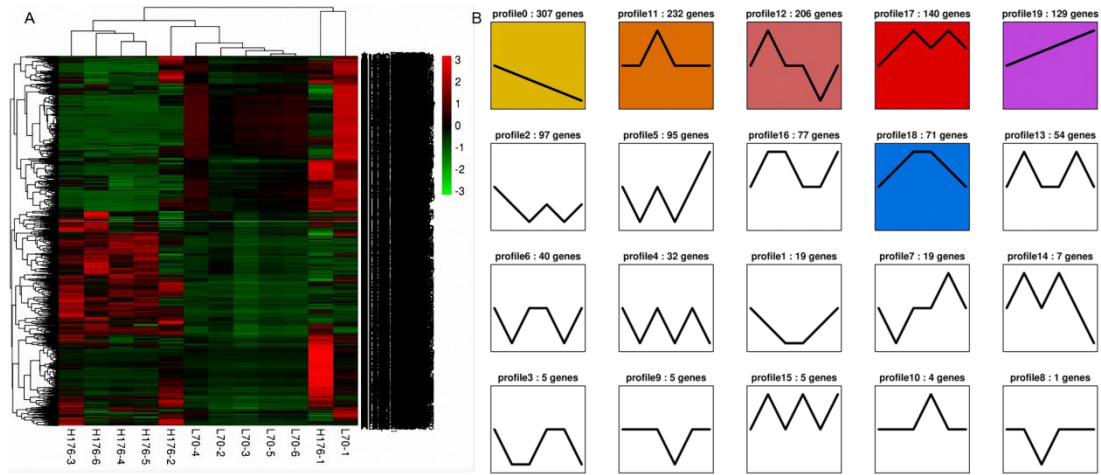


Figure S7. Expression analysis of DEGs between six developmental stages. A, Heatmap showing the relative expression of total DEGs at each stage in L70 and H176. B, Trend analysis of DEGs expression (20 trends). Colored block trend: significant enrichment trend ($P < 0.05$). Without color trend: the enrichment of significant trends.

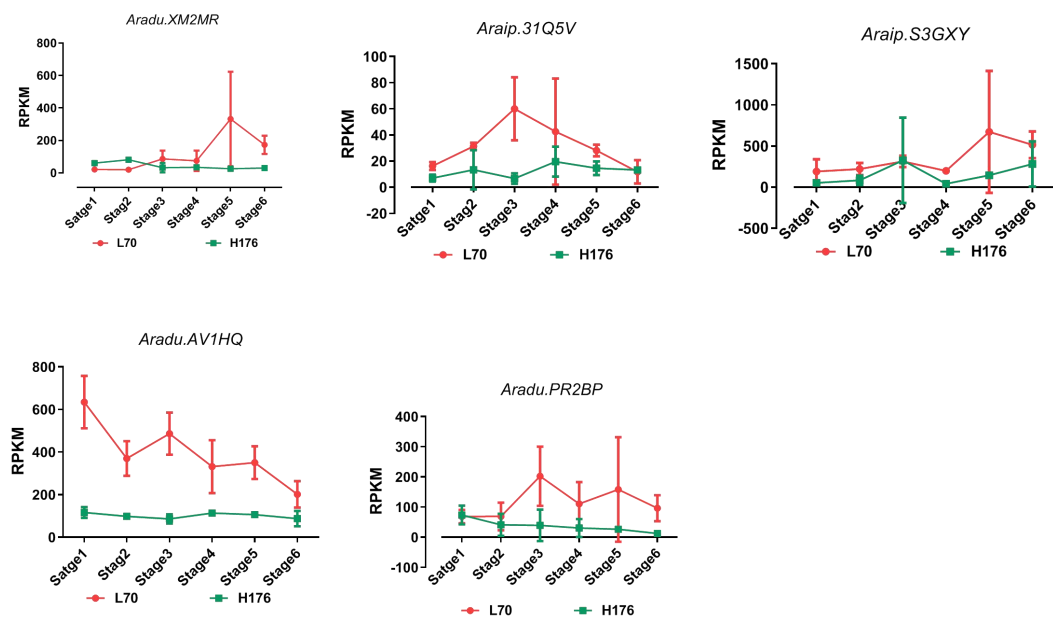


Figure S8. Relative expression levels of lipid DEGs measured by RNAseq.

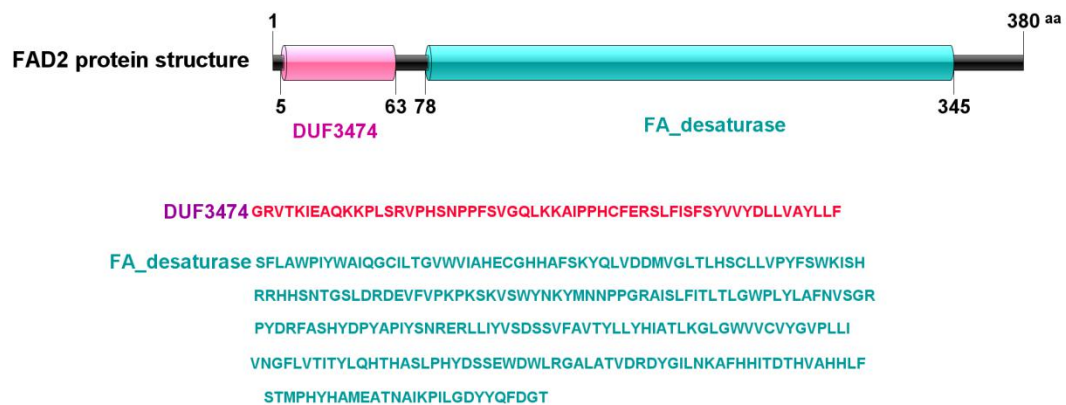


Figure S9. FAD2 protein structure in peanut. FAD2 protein structure predicted by the online database SMART (<http://smart.embl-heidelberg.de>). The FAD2 protein contains two major conserved domains DUF3474 (5-63aa) and FA-desaturase (78-345aa). DUF3474 indicated the domain of unknown function 3474, this domain is found in fatty acid desaturases (FADs), mainly from plants and fungi. FA indicated the fatty acid, and aa indicated the amino acid.

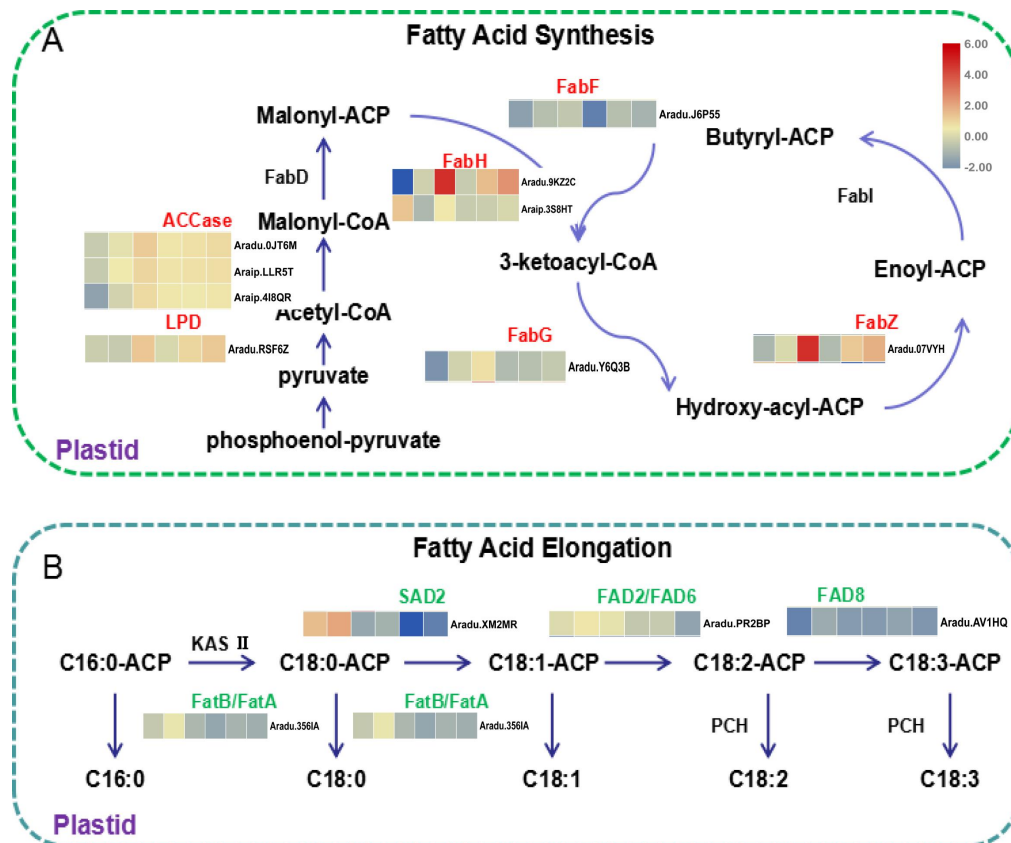


Figure S10. The contrapuntal patterns of lipid DEGs in developing high-oleic peanut seeds. A, identified lipid DEGs are annotated into fatty acid synthesis pathway, the icon is consisted of six blocks that represented six time points of seed development, the icons under the enzyme name show the number of RPKM corresponding to the relative expression of enzyme during high-oleic peanut seed development when compared with L70. B, lipid DEGs are annotated into fatty acid elongation pathway, the icons under the enzyme name show the number of RPKM corresponding to the relative expression of enzyme in H176. Enzyme name with green color indicates the down-regulated gene, and enzyme name with red color represents the up-regulated gene.

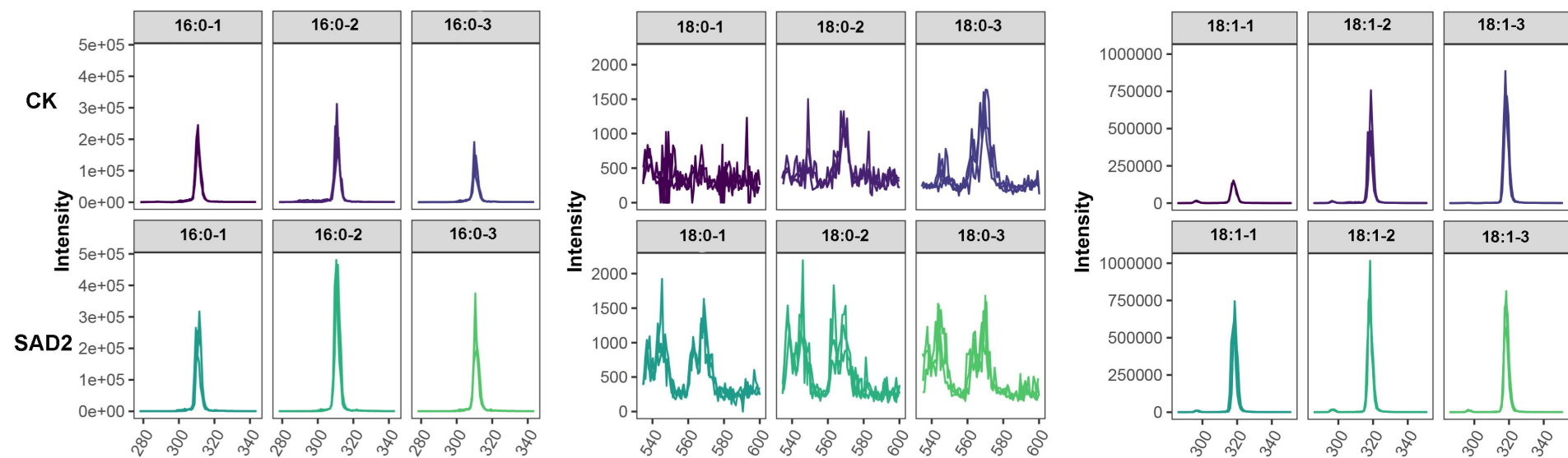


Figure S11. Mass spectrum of C16:0, C18:0, and C18:1 in ectopic expressed yeast cells of *SAD2* (*Aradu.XM2MR*), respectively.