

## Supplementary Information

### **Spatio-temporal expression pattern of Raffinose Synthase genes determine the levels of Raffinose Family Oligosaccharides in peanut (*Arachis hypogaea* L.) seed**

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**Table S1. Complete library QC status of the peanut samples**

Sl. No.	Well	Sample ID	Qubit conc. (ng/ $\mu$ l)	Size (bp)	Conc. (nmol)
1	B2	TG37A_S1_R1	13.1	530	37.45
2	C1	TG37A_S1_R2	11.8	498	35.90
3	A2	TG37A_S1_R3	8	541	22.41
4	B1	TG37A_S2_R1	8.72	493	26.80
5	C3	TG37A_S2_R2	3.82	505	11.46
6	H1	TG37A_S2_R3	7.64	536	21.60
7	G2	Girnar2_S1_R1	4.36	548	12.05
8	B3	Girnar2_S1_R2	29.2	503	87.96
9	E1	Girnar2_S1_R3	11	536	31.09
10	H2	Girnar2_S2_R1	7.26	509	21.61
11	A3	Girnar2_S2_R2	18.9	540	53.03
12	D1	Girnar2_S2_R3	9.5	509	28.28
13	F2	GG7_S1_R1	43.4	512	128.43
14	D2	GG7_S1_R2	23.4	547	64.82
15	C2	GG7_S1_R3	7.38	522	21.42
16	E2	GG7_S2_R1	19.9	503	59.94
17	G1	GG7_S2_R2	20.2	547	55.95
18	F1	GG7_S2_R3	18.2	527	52.33

Qubit concentrations above 2ng/ $\mu$ l or 10nmol signifies the permissible range. Data was analyzed using TapeStation Analysis Software 3.2 in Agilent 4150 TapeStation System. R1, R2 and R3 indicates the three replications in each sample.

**Table S2. Details of the primers selected for reference gene identification and qPCR analysis**

Sl. No.	Gene name	Tentative annotation	Primer sequence	Amplicon length (bp)
1	<i>ADH3</i>	Alcohol dehydrogenase class III	F: GACGCTTGCGAGATCAACA R: AACCGGACAACCACCACATG	140
2	<i>ACTIN11</i>	Actin 11	F: ATGCTAGTGGTCGTACAACCTGG R: CTAGACGAAGGATAGCATGTGG	108
3	<i>ELF1B</i>	Elongation factor1-beta	F: AAGCTTCCCTGGCAAAGCTCAA R: TTCCTCAGCTGCCTTCTTATCC	154
4	<i>G6PD</i>	Glucose 6-phosphate-dehydrogenase	F: ACCATTCCAGAGGCTTATGAGC R: AAGGGAGTGACTTGAACCTCTCC	151
5	<i>ACTIN1</i>	Actin1	F: TGGTCTCGGTTTCCTGAGTT R: AATACCACTCCAAAGCAAACG	114
6	<i>UBI1</i>	Polyubiquitin	F: TCTTGTCCTCCGTCTTAGGG R: AGCAAGGGTCCCTTCCATCTT	196
7	<i>UBC1</i>	Ubiquitin C	F: AAGCCGAAGAAGATCAAGCAC R: GGTTAGCCATGAAGGTTCCA	145

8	<i>AhGolS4</i>	Galactinol Synthase (arahy.KNQB38)	F: TTGGGAGTTTGTGGAGTATAGC R: CCTTAGGCCATTGAACCTTATCA	203
9	<i>AhGolS6</i>	Galactinol Synthase (arahy.T7DX97)	F: TGCCTTCGTGACTTTCCTTG R: GATGTTGCGGTGTTCTTGAG	142
10	<i>AhRS14</i>	Raffinose Synthase (arahy.1YYJ7Z)	F: GTGTACATGGTGAAAGACCAC R: GGATTGAATTGAGCCTCCAGA	186
11	<i>AhRS4</i>	Raffinose Synthase (arahy.WB441U)	F: AGTGTACATGGTGAAAAACCAA R: GGATTGAATTGAGCCTCCAGA	187
12	<i>AhRS6</i>	Raffinose Synthase (arahy.82P426)	F: GATGGTGTAAGGTAGAGAAGAGG R: TATCACCGGAACTGAAGCAC	194
13	<i>AhRSV</i>	Raffinose Synthase (arahy.AY7U99)	F: TCAACTCACAGGGCCTATTTC R: CACCTGAAAGCTGGTTCTTATTG	104
14	<i>AhRS8</i>	Raffinose Synthase (arahy.PF1JUH)	F: GAACATGCTCAACTCTGGC R: TCACACCCAACCCATTAATCT	149
15	<i>AhRS3</i>	Raffinose Synthase (arahy.SCX26U)	F: TGAACATGCTTAACTCCGGT R: CCACATCCACCCCATTAATCT	150
16	<i>AhSS7</i>	Stachyose Synthase (arahy.T832D6)	F: TGGGATCCTAAAGAACACACG R: ACTGCCACAAAGCTGAAGA	242

**Table S3. Ranking of genes according to their stability value or standard deviation by integration of different ranking methods**

Genes	Comprehensive Ranking	Delta Ct	NormFinder	GeNorm	BestKeeper	Overall Ranking
	Geomean of ranking values	Average of SD	Stability value	Stability value	SD	
<i>AhUBI1</i>	1.41	1.26	0.128	0.256	0.87	1
<i>AhADH3</i>	1.78	1.30	0.128	0.256	0.89	2
<i>AhActin1</i>	2.28	1.34	0.378	0.428	0.83	3
<i>AhG6PD</i>	3.94	1.57	0.963	0.703	0.89	4
<i>AhUBC1</i>	4.47	1.61	0.868	0.883	0.89	5
<i>AhActin11</i>	6.24	2.54	2.316	1.350	2.25	6
<i>AhELF1B</i>	6.74	2.87	2.693	1.784	2.17	7

Different ranking tools were integratively used in RefFinder software to select the best reference gene as endogenous control. *Ah* stands for *Arachis hypogaea*, UBI1 = Polyubiquitin, ADH3 = Alcohol dehydrogenase class III, ELF1B = Elongation factor1-beta, G6PD = Glucose 6-phosphatedehydrogenase, UBC1 = Ubiquitin C, SD = Standard Deviation.

**Table S4. Distribution of genes involved in biosynthesis of Raffinose Family Oligosaccharides in *Arachis hypogaea* chromosome and size of each chromosome.**

Family	Gene Name	Gene ID		Chromosome	Start (bp)	End (bp)
		Peanut Genome Resources	PeanutBase			
Galactinol Synthase	<i>AhGolS1</i>	AH06G04780	arahy.N6N04K	A6	7372956	7374154
	<i>AhGolS2</i>	AH09G11340	arahy.9HBV2U	A9	19359461	19361880
	<i>AhGolS3</i>	AH09G11360	arahy.FK4NNE	A9	19369230	19373832

	<i>AhGols4</i>	AH09G12530	arahy.KNQB38	A9	23295792	23297552
	<i>AhGols5</i>	AH16G08210	arahy.HZ3A81	B6	13919203	13921118
	<i>AhGols6</i>	AH16G09600	arahy.T7DX97	B6	15938464	15940252
	<i>AhGols7</i>	AH19G14790	arahy.96F7ZV	B9	24050668	24052744
	<i>AhGols8</i>	AH19G14820	arahy.96F7ZV	B9	24062000	24065052
	<i>AhGols9</i>	AH19G16350	arahy.B1GHBV	B9	29335476	29337107
<b>Raffinose Synthase</b>	<i>AhRS1</i>	AH01G25000	arahy.UR837P	A1	97414835	97417909
	<i>AhRS2</i>	AH03G22950	arahy.7F6VM6	A3	43550202	43555938
	<i>AhRS3</i>	AH03G23470	arahy.SCX26U	A3	44115868	44122524
	<i>AhRS4</i>	AH03G23920	arahy.WB441U	A3	46419360	46431663
	<i>AhRS5</i>	AH05G24120	arahy.B3XDAM	A5	91409141	91415144
	<i>AhRS6</i>	AH06G01900	arahy.82P426	A6	3781168	3785408
	<i>AhRS7</i>	AH07G00130	arahy.SCX26U	A7	363064	365523
	<i>AhRS8</i>	AH07G00140	arahy.PF1JUH	A7	365572	368992
	<i>AhRS9</i>	AH07G01370	arahy.C3FF4U	A7	1289070	1292937
	<i>AhRS10</i>	AH07G17720	No hits	A7	51980576	51981067
	<i>AhRS11</i>	AH09G16320	arahy.UR837P	A9	53685525	53685821
	<i>AhRS12</i>	AH11G32960	arahy.SM3TC1	B1	145745289	145748844
	<i>AhRS13</i>	AH13G01440	arahy.4X3ZEJ	B3	1834889	1840567
	<i>AhRS14</i>	AH13G26910	arahy.1YYJ7Z	B3	48501704	48514269
	<i>AhRS15</i>	AH15G31330	arahy.DBZB80	B5	145581591	145586985
	<i>AhRS16</i>	AH16G04370	arahy.3HRB74	B6	8188588	8192653
	<i>AhRS17</i>	AH17G01490	arahy.C3FF4U	B7	1941587	1945308
<b>Stachyose Synthase</b>	<i>AhSS1</i>	AH01G31080	arahy.T832D6	A1	105403482	105403784
	<i>AhSS2</i>	AH06G04600	arahy.RWK4UY	A6	7153076	7156467
	<i>AhSS3</i>	AH06G04610	arahy.3E1AF2	A6	7161436	7164458
	<i>AhSS4</i>	AH07G07050	arahy.3E1AF2	A7	7460657	7460887
	<i>AhSS5</i>	AH13G25550	arahy.TR51Z4	B3	42936278	42937422
	<i>AhSS6</i>	AH15G16580	arahy.Y2LGUI	B5	74218184	74218342
	<i>AhSS7</i>	AH16G08010	arahy.T832D6	B6	13618714	13622241
	<i>AhSS8</i>	AH16G08020	arahy.Y2LGUI	B6	13636266	13639347

<b>Chromosome</b>	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>A4</b>	<b>A5</b>	<b>A6</b>	<b>A7</b>	<b>A8</b>	<b>A9</b>	<b>A10</b>
<b>Length (Mb)</b>	111.624	99.792	143.207	122.065	116.167	109.265	83.117	51.955	121.059	114.520
<b>Chromosome</b>	<b>B1</b>	<b>B2</b>	<b>B3</b>	<b>B4</b>	<b>B5</b>	<b>B6</b>	<b>B7</b>	<b>B8</b>	<b>B9</b>	<b>B10</b>
<b>Length (Mb)</b>	149.380	122.214	150.766	135.086	153.119	153.789	132.999	136.777	159.155	140.659

**Table S5. Sugar profile and content of RFOs (g 100 g<sup>-1</sup>) in the dry seeds of peanut**

Genotypes	Agronomic Group	Inositol (g 100 g <sup>-1</sup> )	Glucose (g 100 g <sup>-1</sup> )	Sucrose (g 100 g <sup>-1</sup> )	Raffinose (g 100 g <sup>-1</sup> )	Stachyose (g 100 g <sup>-1</sup> )	Total RFOs (g 100 g <sup>-1</sup> )
GG 20	VB	0.06 cd	0.003 b	5.27 e	0.06 b	0.49 b	0.55 bc
GG 5	SB	0.08 ab	0.006 a	7.08 a	0.09 a	0.43 c	0.52 c
GG 7	SB	0.09 a	0.001 b	6.32 bc	0.06 b	0.34 d	0.40 d
Girnar 2	VB	0.04 e	0.001 b	6.26 c	0.07 ab	0.55 ab	0.62 ab
Girnar 3	SB	0.04 de	0.008 a	6.06 d	0.05 b	0.52 b	0.58 bc
TG37A	SB	0.07 bc	0.001 b	6.36 b	0.07 ab	0.60 a	0.67 a
C.V. (%)		15.48	32.61	0.82	16.65	6.60	7.14
SEm±		0.01	0.001	0.01	0.01	0.02	0.02
S.E.D.		0.01	0.001	0.04	0.01	0.03	0.03
LSD (p<0.05)		0.02	0.002	0.09	0.02	0.06	0.07
ProbF		0.000**	0.000**	0.000**	0.028*	0.000**	0.000**

All values are mean of three replications. Mean values with different letters within the same column are statistically different (p-value < 0.05). VB = Virginia Bunch, SB = Spanish Bunch C.V. (%) = Coefficient of Variation, S.E.M = Standard Error of the Mean, S.E.D. = Standard Error of Difference, LSD = Least Significant Difference, ProbF = Probability from an *F* distribution (\* denotes significant and \*\* denotes highly significant).

**Table S6. Glucose, Sucrose and RFOs content (g 100 g<sup>-1</sup>) in the maturing stage (S1) and matured stage (S2) of seeds in contrast peanut genotypes.**

Variety	Seed Stage	D-Glucose (g 100g <sup>-1</sup> )	Sucrose (g 100g <sup>-1</sup> )	Total RFOs (g 100g <sup>-1</sup> )
TG37A	S1	0.05 ± 0.002	10.23 ± 0.023	0.54 ± 0.023
	S2	0.04 ± 0.002	6.09 ± 0.030	0.72 ± 0.011
GG7	S1	0.06 ± 0.002	9.55 ± 0.030	0.26 ± 0.013
	S2	0.06 ± 0.001	6.09 ± 0.011	0.47 ± 0.013
Girnar 2	S1	0.12 ± 0.013	10.23 ± 0.026	0.49 ± 0.016
	S2	0.09 ± 0.003	6.34 ± 0.027	0.64 ± 0.018
C.V. (%)		14.39	0.54	5.42
SEm±		0.01	0.03	0.02
S.E.D.		0.01	0.04	0.02
LSD (p<0.05)		0.02	0.08	0.05
ProbF		0.000**	0.000**	0.000**

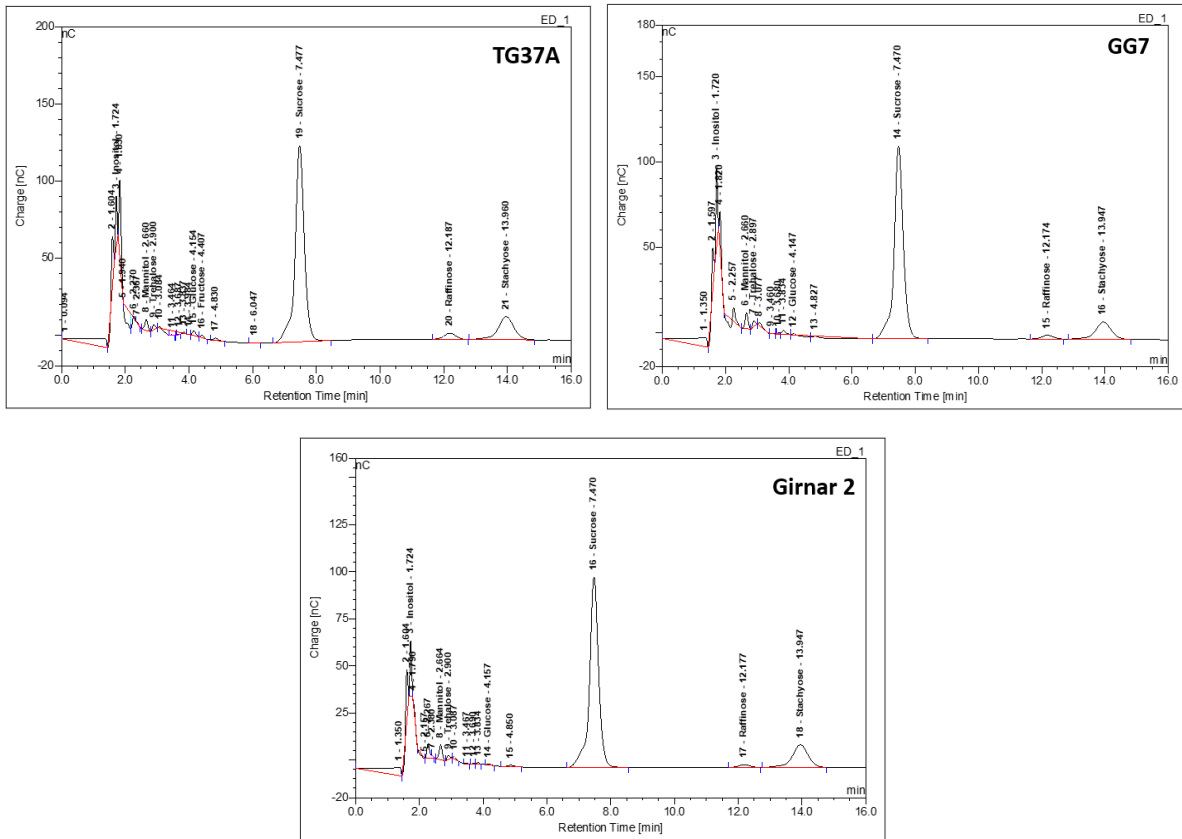
All values are mean of three replications ± Standard Error. S1 = maturing seed stage, S2 = matured seed stage, C.V. (%) = Coefficient of Variation, S.E.M = Standard Error of the Mean, S.E.D. = Standard Error of Difference, LSD = Least Significant Difference, ProbF = Probability from an *F* distribution (\*\* denotes highly significant).

**Table S7. Number of differentially expressed genes.**

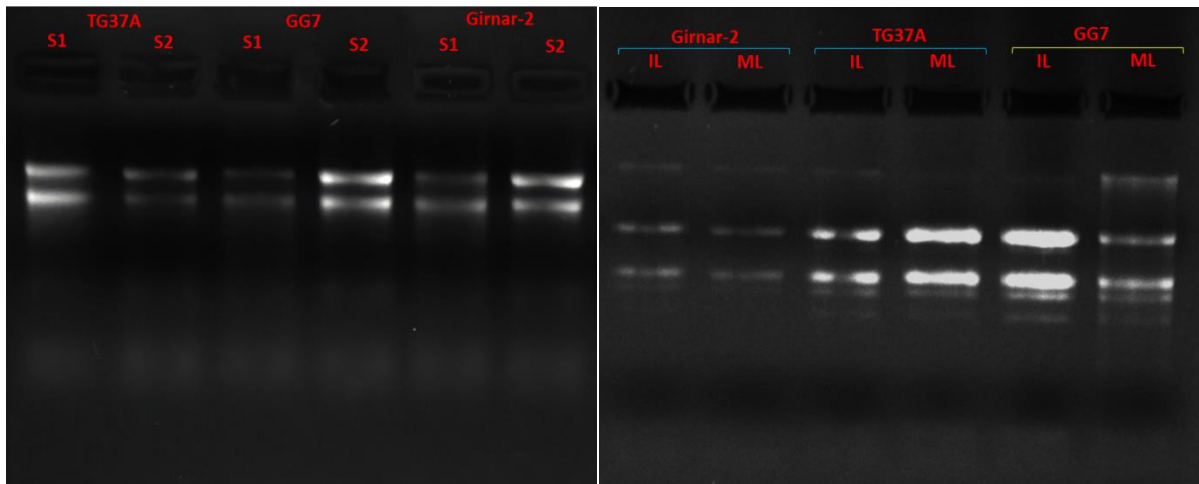
Condition	Significantly Expressed Genes	Up Regulated Genes	Down Regulated genes
GG7_S2 vs GG7_S1	2,321	622	1699
TG37A_S1 vs GG7_S1	6,341	2,690	3,651
TG37A_S2 vs GG7_S2	8,149	2,951	5,198
Girnar2_S2 vs Girnar2_S1	2,629	416	2,213
TG37A_S1 vs Girnar2_S1	5,015	1,775	3,240

TG37A_S2 vs Girnar2_S2	4,461	1,379	3,082
TG37A_S2 vs TG37A_S1	2,247	301	1,946

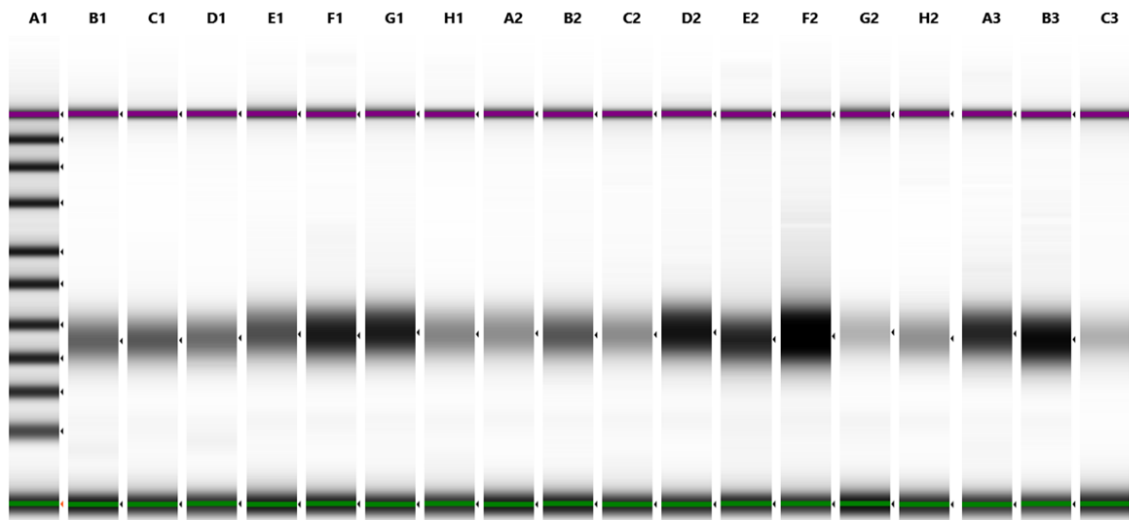
Seed maturity stages are denoted by S1 (maturing stage) and S2 (matured stage). Total number of tested genes were 62,987. The data is the average of three biological replicates.



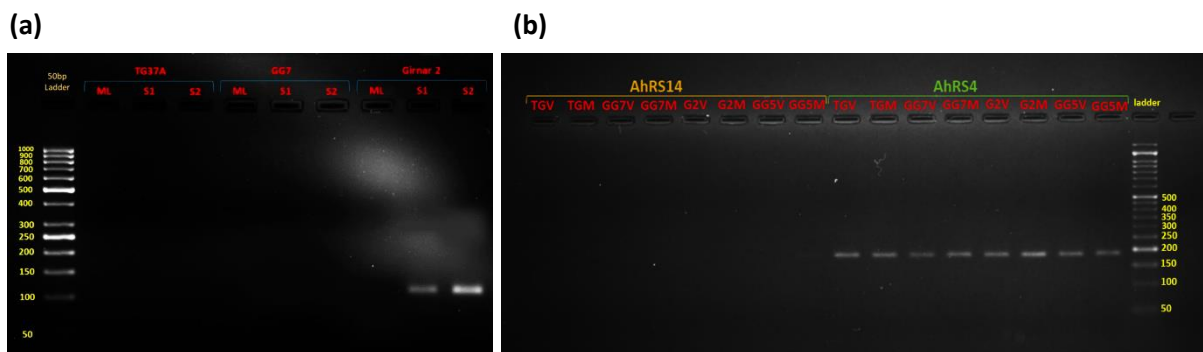
**Fig. S1. Ion Chromatogram of peanut seed samples of contrast genotypes.** Each peak represents the detected compounds and the values indicate the retention time. Data integration was done using Chormeleon software.



**Fig. S2. Qualitative assessment of total RNA.** The eluted RNA samples were run on 1.3% denaturing agarose gel electrophoresis and 28S, 18S, and 5S rRNA bands were separated, revealing the integrity of the RNA. **S1** = maturing seed, **S2** = matured seed, **IL** = immature leaf, **ML** = mature leaf.



**Fig. S3. Gel profile of cDNA libraries.** cDNA libraries of three peanut genotypes at two seed stages qualified the criteria based on the presence of a broad peak in the range of 300bp to 1000bp, with an average size of 400 – 600 bp in the Agilent 4150 TapeStation system **A1** = Ladder, **B1** to **C3** are the sample wells.



**Fig. S4. Agarose gel electrophoresis images of different genes across peanut tissue samples.** (a) *AY7U99* or *AhRSV* primer band pattern in Matured Leaf (ML), Maturing (S1) seed, Matured (S2) seed stage of Spanish genotypes (TG37A and GG7) and Virginia genotype (Girnar 2). Spanish genotypes do not show any expression while Virginia genotype shows seed-specific expression when run on 3% denaturing agarose gel. (b) *AhRS4* and *AhRS14* gene present on chromosome A3/B3 shows a differential expression pattern. *AhRS4* shows expression in the leaf while *AhRS14* does not. Leaf samples at two different stages, vegetative (V) and mature (M) in four peanut genotypes - TG37A (TG), GG7, Girnar 2 (G2), and GG5 are shown here. Samples were run on 3% denaturing agarose gel. A 50bp DNA ladder was used.



TG37A vs GG7 (S1)					TG37A vs GG7 (S2)					TG37A vs Girnar2 (S1)				
Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change	
			qPCR	RNA-Seq				qPCR	RNA-Seq				qPCR	RNA-Seq
AhGolS4	-2.065	4.185	2.065	2.848	AhGolS4	-0.251	1.190	0.251	-0.048	AhGolS4	-8.702	416.556	8.702	6.021
AhGolS6	-6.015	64.657	6.015	3.801	AhGolS6	-1.432	2.699	1.432	0.488	AhGolS6	-3.185	9.093	3.185	2.211
AhRS14	-7.257	152.973	7.257	6.340	AhRS14	-7.341	162.078	7.341	4.575	AhRS14	-1.758	3.383	1.758	5.897
AhRS4	-2.114	4.329	2.114	7.165	AhRS4	-4.217	18.592	4.217	6.229	AhRS4	0.680	0.624	-0.680	5.909
AhRS6	-0.648	1.567	0.648	1.168	AhRS6	0.976	0.508	-0.976	-0.918	AhRS6	-7.009	128.816	7.009	7.482
AY7U99	-2.546	5.840	2.546	0.000	AY7U99	-3.577	11.930	3.577	1.896	AY7U99	5.961	0.016	-5.961	-10.466
AhRS8	-3.699	12.984	3.699	1.467	AhRS8	-1.743	3.347	1.743	-3.546	AhRS8	0.242	0.845	-0.242	-0.341
AhRS3	-5.220	37.269	5.220	3.087	AhRS3	-1.180	2.266	1.180	-2.524	AhRS3	0.162	0.894	-0.162	-0.067
AhSS7	-3.834	14.264	3.834	0.803	AhSS7	0.888	0.540	-0.888	-2.187	AhSS7	1.082	0.472	-1.082	-0.101

**A** **B** **C**

TG37A vs Girnar2 (S2)					Girnar2 vs GG7 (S1)					Girnar2 vs GG7 (S2)				
Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change	
			qPCR	RNA-Seq				qPCR	RNA-Seq				qPCR	RNA-Seq
AhGolS4	-2.954	7.748	2.954	1.582	AhGolS4	6.637	0.010	-6.637	-3.176	AhGolS4	2.703	0.154	-2.703	-1.634
AhGolS6	0.254	0.839	-0.254	-0.997	AhGolS6	-2.830	7.111	2.830	1.594	AhGolS6	-1.686	3.217	1.686	1.485
AhRS14	1.684	0.311	-1.684	0.397	AhRS14	-5.499	45.219	5.499	0.585	AhRS14	-9.025	520.779	9.025	4.184
AhRS4	2.440	0.184	-2.440	0.501	AhRS4	-2.794	6.937	2.794	1.585	AhRS4	-6.657	100.915	6.657	5.769
AhRS6	-6.703	104.187	6.703	5.969	AhRS6	6.361	0.012	-6.361	-6.316	AhRS6	7.679	0.005	-7.679	-6.888
AY7U99	8.657	0.002	-8.657	-8.981	AY7U99	-8.507	363.742	8.507	7.462	AY7U99	-12.234	4816.121	12.234	7.831
AhRS8	0.813	0.569	-0.813	-4.072	AhRS8	-3.941	15.357	3.941	1.825	AhRS8	-2.555	5.878	2.555	0.531
AhRS3	2.101	0.233	-2.101	-3.444	AhRS3	-5.382	41.701	5.382	3.156	AhRS3	-3.282	9.725	3.282	0.920
AhSS7	3.494	0.089	-3.494	-2.563	AhSS7	-4.917	30.202	4.917	0.905	AhSS7	-2.606	6.090	2.606	0.376

**D** **E** **F**

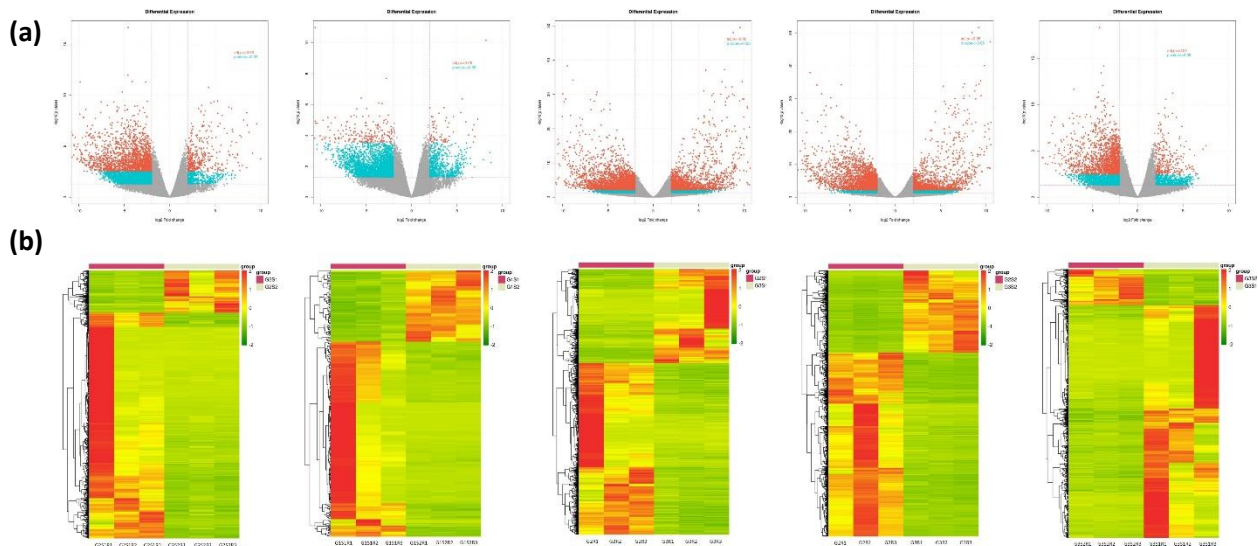
TG37A (S2 vs S1)					TG37A (Leaf vs Seed)					GG7 (S2 vs S1)				
Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change	
			qPCR	RNA-Seq				qPCR	RNA-Seq				qPCR	RNA-Seq
AhGolS4	4.429	0.046	-4.429	-4.225	AhGolS4	2.880	0.136	-2.880		AhGolS4	2.614	0.163	-2.614	-1.329
AhGolS6	1.232	0.426	-1.232	-0.591	AhGolS6	5.596	0.021	-5.596		AhGolS6	-3.350	10.198	3.350	2.722
AhRS14	-2.552	5.863	2.552	1.375	AhRS14	7.312	0.006	-7.312		AhRS14	-2.468	5.533	2.468	3.140
AhRS4	-3.060	8.341	3.060	1.399	AhRS4	-1.257	2.390	1.257		AhRS4	-0.958	1.942	0.958	2.336
AhRS6	-0.070	1.050	0.070	-0.547	AhRS6	14.025	0.000	-14.025		AhRS6	-1.694	3.236	1.694	1.540
AY7U99	0.430	0.742	-0.430	1.896	AY7U99	0.548	0.684	-0.548		AY7U99	1.460	0.363	-1.460	0.000
AhRS8	-1.517	2.862	1.517	-2.674	AhRS8	2.220	0.215	-2.220		AhRS8	-3.473	11.101	3.473	2.339
AhRS3	0.548	0.684	-0.548	-2.608	AhRS3	2.217	0.215	-2.217		AhRS3	-3.491	11.244	3.491	3.004
AhSS7	2.383	0.192	-2.383	-1.792	AhSS7	3.566	0.084	-3.566		AhSS7	-2.339	5.060	2.339	1.199

**G** **H** **I**

GG7 (Leaf vs Seed)					Girnar2 (S2 vs S1)					Girnar2 (Leaf vs Seed)				
Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change		Gene	$\Delta\Delta C_T$	Fold Change ( $2^{-\Delta\Delta C_T}$ )	log 2 Fold Change	
			qPCR	RNA-Seq				qPCR	RNA-Seq				qPCR	RNA-Seq
AhGolS4	2.152	0.225	-2.152		AhGolS4	-1.320	2.496	1.320	0.214	AhGolS4	0.500	0.707	-0.500	
AhGolS6	4.949	0.032	-4.949		AhGolS6	-2.206	4.614	2.206	2.616	AhGolS6	6.724	0.009	-6.724	
AhRS14	2.243	0.211	-2.243		AhRS14	-5.994	63.726	5.994	6.876	AhRS14	11.710	0.000	-11.710	
AhRS4	-2.165	4.485	2.165		AhRS4	-4.820	28.254	4.820	6.807	AhRS4	2.583	0.167	-2.583	
AhRS6	14.878	0.000	-14.878		AhRS6	-0.377	1.298	0.377	0.966	AhRS6	6.946	0.008	-6.946	
AY7U99	-5.225	37.409	5.225		AY7U99	-2.267	4.812	2.267	0.410	AY7U99	8.415	0.003	-8.415	
AhRS8	0.293	0.816	-0.293		AhRS8	-2.087	4.249	2.087	1.058	AhRS8	3.003	0.125	-3.003	
AhRS3	0.723	0.606	-0.723		AhRS3	-1.391	2.622	1.391	0.769	AhRS3	4.230	0.053	-4.230	
AhSS7	10.517	0.001	-10.517		AhSS7	-0.029	1.020	0.029	0.670	AhSS7	9.385	0.001	-9.385	

**J** **K** **L**

**Fig. S5. Analysis of peanut tissue samples under different combinations of contrast genotype for determination of expression. A.** Maturing seed stage (S1) of High RFOs variety (TG37A) vs Low RFOs variety (GG7), **B.** Matured seed stage (S2) of High RFOs variety (TG37A) vs Low RFOs variety (GG7), **C.** High RFOs variety of Spanish group (TG37A) vs Virginia group (Girnar 2) at maturing seed (S1) stage, **D.** High RFOs variety of Spanish group (TG37A) vs Virginia group (Girnar 2) at matured seed (S2) stage, **E.** High RFOs Virginia variety (Girnar 2) vs Low RFOs Spanish variety (GG7) at maturing seed (S1) stage, **F.** High RFOs Virginia variety (Girnar 2) vs Low RFOs Spanish variety (GG7) at matured seed (S2) stage, **G.** High RFO cultivar (TG37A) matured seed (S2) vs maturing seed (S1) stage, **H.** High RFO Spanish cultivar (TG37A) leaf vs seed, **I.** low RFO cultivar (GG7) matured seed (S2) vs maturing seed (S1) stage, **J.** Low RFO Spanish cultivar (GG7) leaf vs seed, **K.** High RFO Virginia Cultivar (Girnar 2) matured seed (S2) vs maturing seed (S1) stage, **L.** High RFO Virginia Cultivar (Girnar 2) leaf vs seed.



**Fig. S6. Expression profile of the significant differentially expressed genes across the contrast genotypes.** (a) Volcano plot showing differential expression profile of genes. Blue indicates  $\log_2$  fold change  $\geq 2$  and  $p$  value  $\leq 0.05$ . “Red” dots indicate absolute  $\log_2$  fold change  $\geq 2$  and FDR/adjusted  $p$  value  $\leq 0.05$ . From left to right, Girmar 2 (S2 vs S1), GG7 (S2 vs S1), Girmar 2 vs TG37A (S1), Girmar 2 vs TG37A (S2), TG37A (S2 vs S1). (b) Heatmap showing the DEGs in Girmar 2 (S2 vs S1), GG7 (S2 vs S1), Girmar 2 vs TG37A (S1), Girmar 2 vs TG37A (S2), TG37A (S2 vs S1) (*left to right*). R1, R2, R3 represent the three replications.