

[10.1071/FP21197](https://doi.org/10.1071/FP21197)

Functional Plant Biology

Supplementary Material

iTRAQ and RNA-seq analyses provide an insight into mechanisms of recalcitrance in a medicinal plant *Panax notoginseng* seeds during the after-ripening process

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Legend for Figures

Figure S1. SDS-PAGE electrophoresis for extraction proteins in *P. notoginseng* seeds.

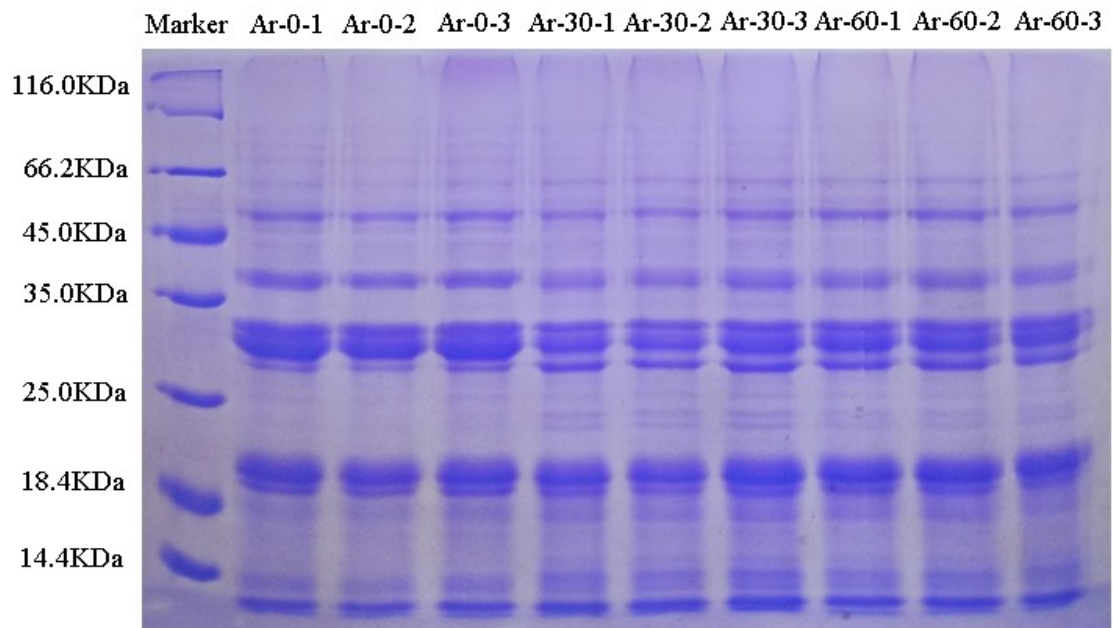


Figure S2. The distribution of all the identified unique proteins. The abscissa is the number of unique peptides contained in the protein, the left ordinate is the number of proteins corresponding to the abscissa, and the right ordinate is the cumulative protein ratio corresponding to the abscissa.

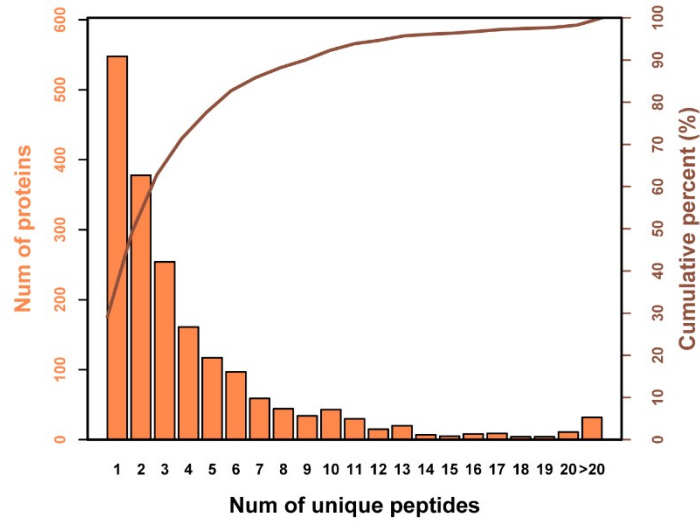


Figure S3. Venn diagram illustrating the number of proteins detected in all three biological replicates. Each circle represents a set containing three samples. Bio. Replicate 1: Ar.0.1, Ar.30.1 and Ar.60.1; Bio. Replicate 2: Ar.0.2, Ar.30.2 and Ar.60.2; Bio. Replicate 3: Ar.0.3, Ar.30.3 and Ar.60.3. Ar: after-ripening.

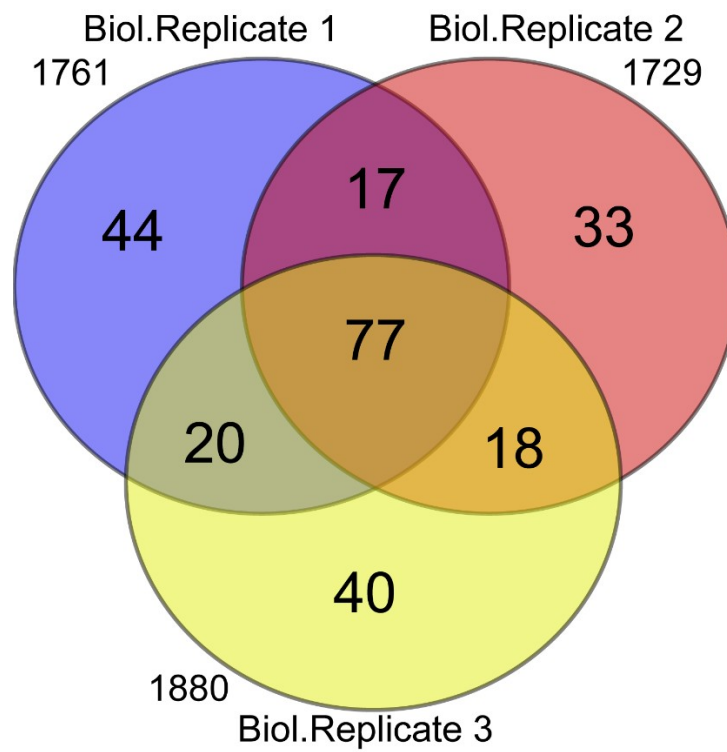


Figure S4. COG classification of all ID sequence. The abscissa is COG entry and the ordinate is the protein quantity. The graph shows the statistics of proteins with different functions in the sample.

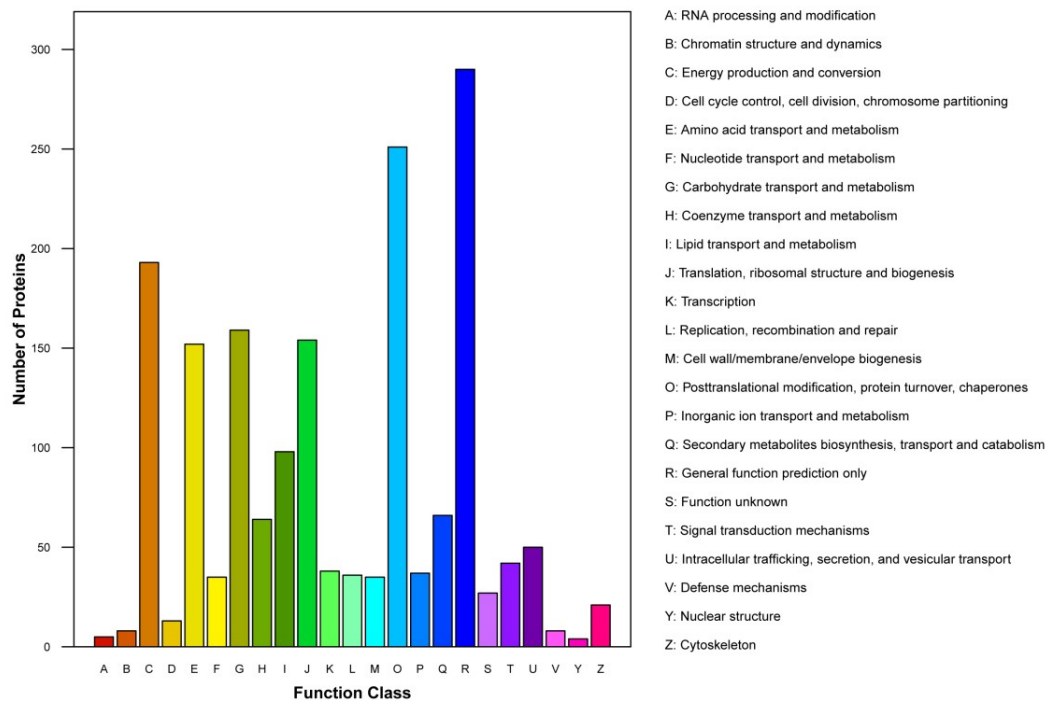


Figure S5. Protein abundance distribution and the distribution of differential

multiples of all quantifiable proteins, in which abscissa represents the logarithmic transformation of the fold change with a base of 2. Up-regulated >0 , down-regulated <0 .

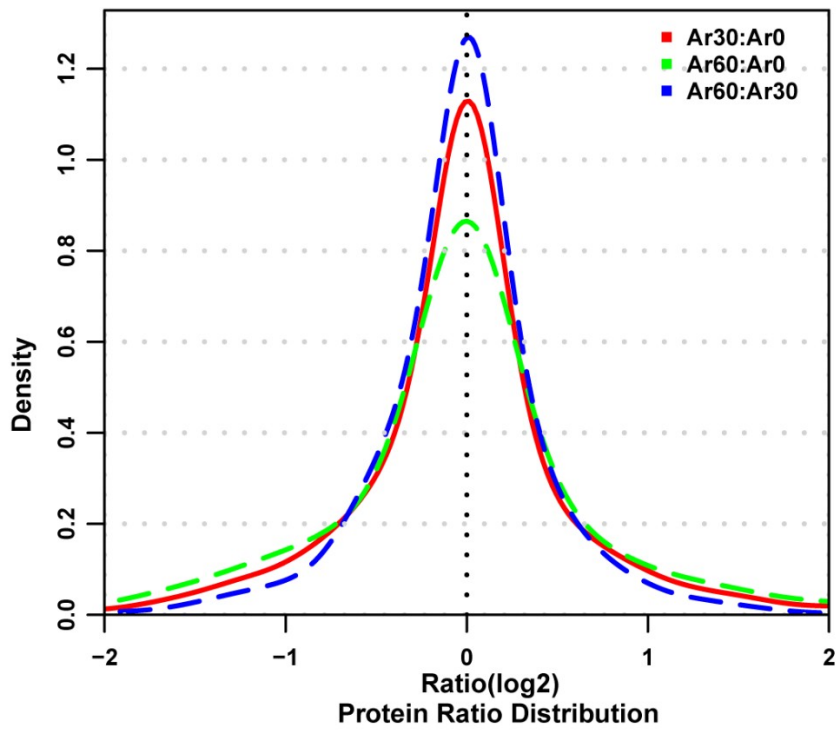


Figure S6. Pathway comparisons of up- or down-regulated differentially expressed proteins in three after-ripening process. (a) Ar30-Ar0 pathway compare; (b) Ar60-Ar0 pathway compare; (c) Ar60-Ar30 pathway compare.

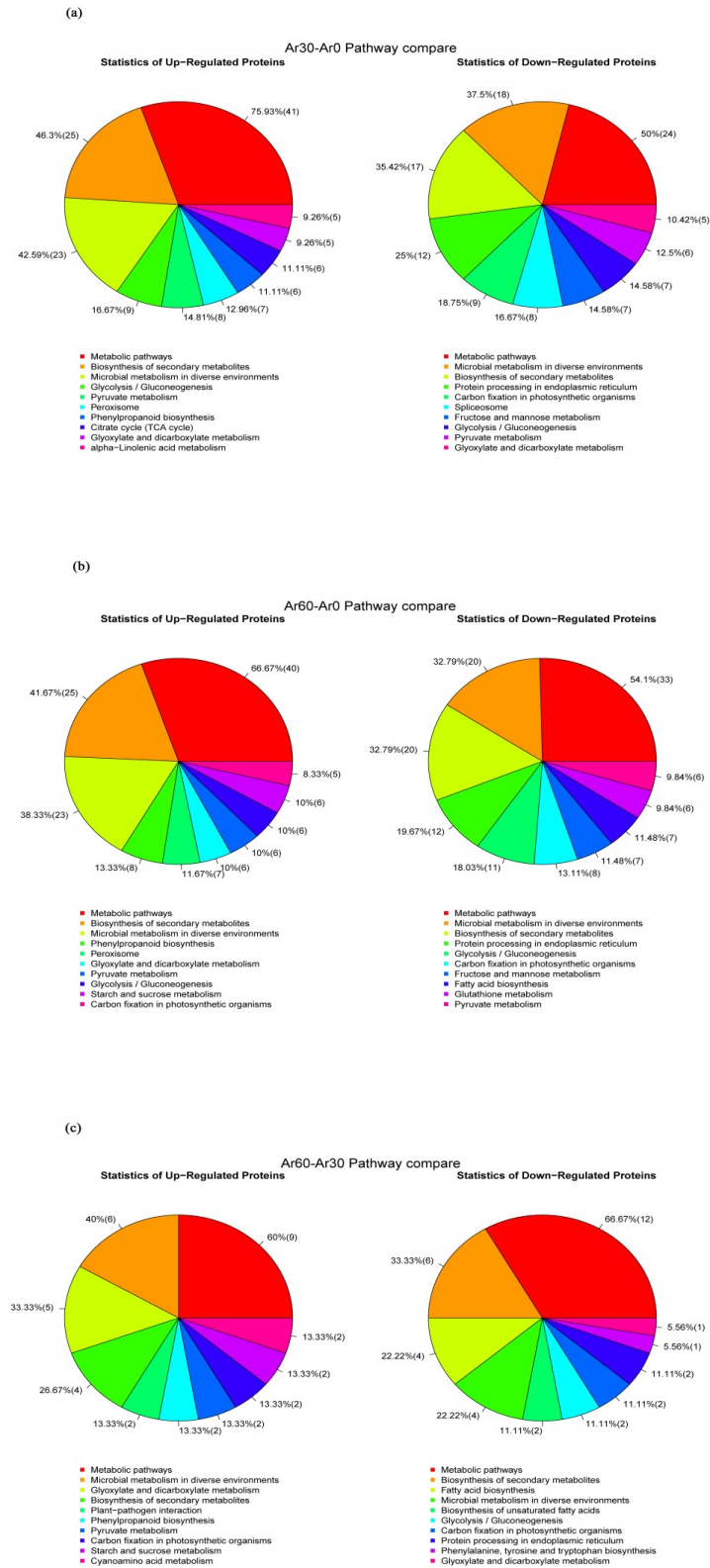


Figure S7. Statistical histogram of GO functional annotation of differentially expressed genes (DEGs). z-score value: the differences between the number of up-regulated genes and the number of down-regulated genes as a proportion of the total differential genes. The yellow line represents the threshold value of Q value = 0.05. The list is the top 20 GO terms. The different colors represent different Ontology. (a) CK-T1 in embryo compare; (b) CK-T2 in embryo compare; (c) CK-T1 in endosperm compare; (d) CK-T2 in endosperm compare;

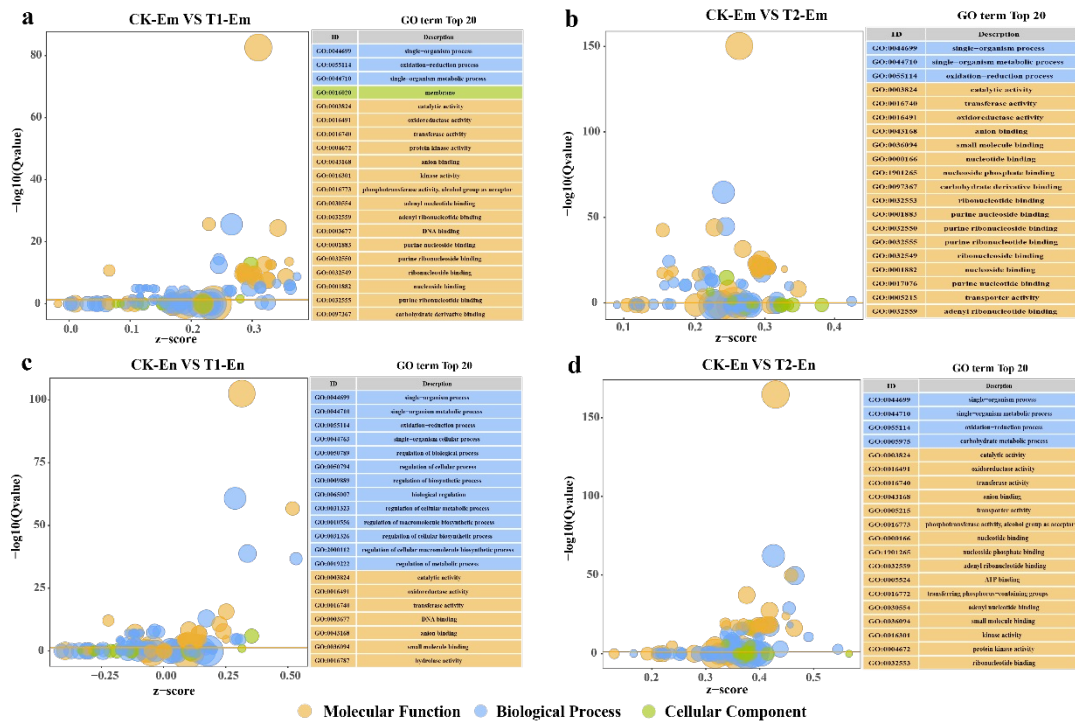


Figure S8. Analysis of energy metabolism pathways in *P.notoginseng* seeds at the 0 DAR, 30 DAR and 60 DAR. PFK: 6-phosphofructokinase; FBPA: Fructose-1,6-bisphosphate aldolase; TPI: Triosephosphate isomerase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase/erythrose-4-phosphate dehydrogenase; PGK: 3-phosphoglycerate kinase; PGM: Phosphoglyceromutase; PK: Pyruvate kinase; PEPCK(ATP): Phosphoenolpyruvate carboxykinase (ATP); AKR: Aldo/keto reductases, related to diketogulonate reductase; DHs: Dehydrogenases with different specificities; eIF-2Bgamma/eIF-2Bepsilon: Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide biosynthesis/translation initiation factor 2B, gamma/epsilon subunits; GMD: GDP-D-mannose dehydratase; CS: Citrate synthase; ACO: Aconitase; IDH: Isocitrate dehydrogenase; SCS: Succinyl-CoA synthetase; SDH: Succinic dehydrogenase; G6PD: Glucose-6-phosphate 1-dehydrogenase; 6PGDH: 6-phosphogluconate dehydrogenase; TKT: Transketolase; TAL: Transaldolase; PPE: Pentose-5-phosphate-3-epimerase. Red represents an up-expression, and Blue represents a down-expression.

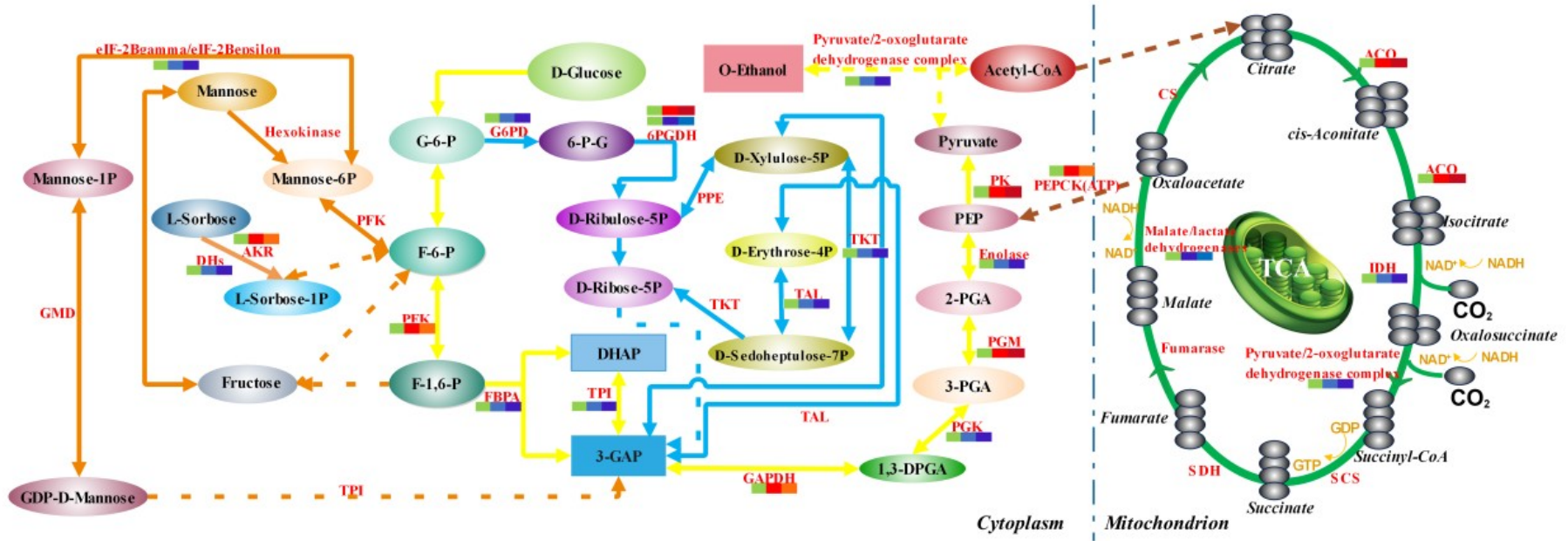


Table S1 KEGG pathways of all the identified proteins of *P. notogensing* during the after-ripening process (DAR).

Different after-ripening process	Pathways	Pathway DEPs	Pathway proteins
30 vs 0 DAR	54	93	1366
60 vs 0 DAR	70	115	1366
60 vs 30 DAR	24	24	1366

Table S2 RNA-Seq data filtering statistics in embryo and endosperm tissues of *P. notogensing*

Sample	RawData s	CleanData(%)	Adapter(%)	LowQuality(%)	polyA(%))	N(%)
CK-Em-1	48287880	48190920 (99.80%)	15452 (0.03%)	76756 (0.16%)	0 (0.00%)	4752 (0.01%)
CK-Em-2	50072374	49999528 (99.85%)	10578 (0.02%)	57402 (0.11%)	0 (0.00%)	4866 (0.01%)
CK-Em-3	44740146	44668302 (99.84%)	11304 (0.03%)	56052 (0.13%)	0 (0.00%)	4488 (0.01%)
T1-Em-1	50427482	50376458 (99.90%)	10256 (0.02%)	40658 (0.08%)	0 (0.00%)	110 (0.00%)
T1-Em-2	45333468	45284702 (99.89%)	9758 (0.02%)	38904 (0.09%)	0 (0.00%)	104 (0.00%)
T1-Em-3	45061160	45010718 (99.89%)	8574 (0.02%)	41804 (0.09%)	0 (0.00%)	64 (0.00%)
T2-Em-1	38590164	38520466 (99.82%)	10772 (0.03%)	55098 (0.14%)	0 (0.00%)	3828 (0.01%)
T2-Em-2	42559816	42487626 (99.83%)	11264 (0.03%)	56764 (0.13%)	0 (0.00%)	4162 (0.01%)
T2-Em-3	46094190	46011462 (99.82%)	13184 (0.03%)	65080 (0.14%)	0 (0.00%)	4464 (0.01%)
CK-En-1	52251604	52180660 (99.86%)	12740 (0.02%)	52608 (0.10%)	0 (0.00%)	5596 (0.01%)
CK-En-2	44271282	44191226 (99.82%)	13442 (0.03%)	62622 (0.14%)	0 (0.00%)	3992 (0.01%)
CK-En-3	51839496	51746724 (99.82%)	15922 (0.03%)	71838 (0.14%)	0 (0.00%)	5012 (0.01%)
T1-En-1	39429226	39381124 (99.88%)	7610 (0.02%)	36620 (0.09%)	0 (0.00%)	3872 (0.01%)
T1-En-2	40539842	40467962 (99.82%)	10462 (0.03%)	57566 (0.14%)	0 (0.00%)	3852 (0.01%)
T1-En-3	44258620	44183180 (99.83%)	11280 (0.03%)	59656 (0.13%)	0 (0.00%)	4504 (0.01%)
T2-En-1	43979184	43911200 (99.85%)	10054 (0.02%)	53762 (0.12%)	0 (0.00%)	4168 (0.01%)
T2-En-2	43813004	43734094 (99.82%)	11768 (0.03%)	62830 (0.14%)	0 (0.00%)	4312 (0.01%)
T2-En-3	45387646	45310116 (99.83%)	11460 (0.03%)	61740 (0.14%)	0 (0.00%)	4330 (0.01%)

Table S3. A list of primers used in qRT-PCR analysis.

Accession	Description	Primer Sequence 5' - 3'		Size/ bp
		Forward	Reverse	
c61526_g2_1	chitinase	TAATTGCCAAAGCCAGTGCG	ATTGCATTTCCAGCTTGCCC	20
c55010_g1_2	Transketolase	TTGGGCAGGGTGTTGCTAAT	AACATGCCAACCGAGTCCTT	20
c23921_g1_4	catalase (CAT)	TCCACCGTTATCCATGAGCG	ACACCAGAGCCTTCCATGTG	20
c50225_g2_4	Malate synthase	TTGTTGCCGGATTGCAAAGG	TCCCAGTTTGGTGAGAGTGC	20
c62059_g1_5	Pectinesterase 2-like	CCATAACTGCTCAAGGCCGA	GAATTTAGCCGCATCCGTCG	20
c43663_g1_1	epoxide hydrolase 2 (EH2)	ATCTTGCAGCCTATGGAGCC	ATTCACCTGGTCCGGCATT	20
c11406_g1_2	superoxide dismutase[Fe-Mn] (SOD)	TTGCAGACGACTTTCTCGCT	ACCACCCCCTGCATTAACAG	20
c52270_g1_2	dehydrin 9[<i>Panax ginseng</i>] (Dhn9)	AGCTAACTGATCAACACGGCA	TCTCTGGTAAACCCCCTCCA	20
c60777_g2_2	CDPK-related protein kinase	ACAGAGGTCAACAGACAGGC	TTTGCAAACCGAGCACCTTG	20
c54908_g1_1	Late embryogenesis abundant (LEA) group 1	ATGGCATCAGAGCAAGAACG	ACTGGTCGATCCCTTGTTCC	20

6 c62297_g1_ 3	Glycosyltransferase (GT)	CACACAGTGCAACATCGCTC	ACATGCTCGTATCTGCTCCG	20
c61389_g2_ 1	pyruvate dehydrogenase E1 (PDHB)	CTGAGCATTCAACGCCTC	CACGTGATACCTCATCCGGG	20
c57687_g1_ 2	malate dehydrogenase(MDH)	CCGATCAGCCTGTGATCCTC	AGCAGAAGCTTGGGACTTGT	20
c31083_g1_ 6	dehydrin 7[<i>Panax ginseng</i>] (Dhn7)	GACCGGTACCGAAGCCTATG	CTCCCCCTCCTTATTGTGCC	20
c58735_g1_ 2	Triosephosphate isomerase (TPI)	ATCTGGGCATTCCTTGGGTC	TGCGACCTTTCCTGTTCCAA	20
c58636_g1_ 2	eIF-2Bgamma/eIF-2Bepsilon	CGGCCTGAGACTTTACCTCG	CAGTTGAGTGCCAACCGATG	20
c62743_g2_ 5	GAPDH	GGCGCAGCAGAGTATTTTGG	TGAGCAGCCATGACTTCCAAT	20

Table S4. Selected Late embryogenesis abundant (LEA) group proteins in *P. notoginseng* seeds during the after-ripening (Ar) process.

Accession	Description	Ar30/		Ar60/		Ar60/		Protein Length
		Ar0	<i>P</i> -value	Ar0	<i>P</i> -value	Ar30	<i>P</i> -value	
c48474_g1_6	Late embryogenesis abundant (LEA) group 1	0.4289	0.0090	0.1862	0.0090	0.4125	0.0177	334
c54908_g1_6	Late embryogenesis abundant (LEA) group 1	0.5664	0.2662	0.4938	0.3701	0.8018	0.4254	334
c55395_g1_1	Late embryogenesis abundant protein	0.9963	0.9125	0.9660	0.5173	0.9788	0.0728	324
c55866_g2_6	Late embryogenesis abundant (LEA) group 1	0.5228	0.3267	0.5361	0.3802	0.6854	0.2048	370
c57902_g3_4	late embryogenesis abundant protein group 9 protein	-	-	-	-	-	-	294
c6422_g1_2	Late embryogenesis abundant protein Dc3	0.4427	0.0314	0.2744	0.0115	0.6338	0.2001	305
c37169_g1_4	late embryogenesis abundant protein B19.3	-	-	-	-	-	-	225
c40293_g1_2	Late embryogenesis abundant protein (LEA) family protein	-	-	-	-	-	-	365
c52270_g1_2	Dehydrin 9 [Panax ginseng]	0.5246	0.1257	0.3517	0.1533	0.6246	0.3217	267
c52270_g2_2	Dehydrin 9 [Panax ginseng]	0.8649	0.5448	0.8053	0.4538	1.0388	0.8855	456
c6480_g1_1	Dehydrin 2 [Panax ginseng]	-	-	-	-	-	-	292
c56778_g1_1	Dehydrin 4 [Panax ginseng]	1.0423	0.4157	0.5843	0.1174	0.5703	0.0910	474
c31083_g1_3	Dehydrin	15.0774	0.1707	20.7995	0.1717	1.3081	0.1670	507
c58930_g4_5	Dehydrin	1.0010	0.9493	0.7886	0.2859	0.8308	0.4242	464
c31083_g1_6	Dehydrin	0.3674	0.0600	0.3077	0.0291	0.8658	0.2959	507

The P -value \leq 0.05 is regarded as a significant difference.

Table S5. Selected antioxidant enzymes related to glutathione metabolism in *P. notoginseng* seeds during the after-ripening (Ar) process.

Accession	Description	Ar30/		Ar60/		Ar60/		Protein Length
		Ar0	P-value	Ar0	P-value	Ar30	P-value	
c58750_g2_5	5-oxoprolinase	1.0187	0.8109	0.9674	0.6090	0.9685	0.7385	1441
c53404_g1_3	Glucose-6-phosphate 1-dehydrogenase	0.4825	0.0035	0.4484	0.0175	0.9500	0.6579	764
c62212_g2_3	Glucose-6-phosphate 1-dehydrogenase	1.8536	0.0585	1.6638	0.0695	0.9042	0.0533	783
c47493_g1_6	Glutaredoxin	0.9526	0.5743	0.8337	0.4582	0.8522	0.4261	286
c47493_g2_5	Glutaredoxin	1.3753	0.2144	1.4873	0.2296	1.0922	0.6090	249
c27579_g1_2	Glutaredoxin-C1	0.7841	0.6023	0.7017	0.3608	0.8016	0.3145	250
c53105_g1_5	Glutaredoxin-C4	1.0439	0.0051	0.9336	0.2518	0.9050	0.1184	321
c22567_g1_3	glutathione peroxidase	0.7442	0.0264	0.4051	0.0491	0.5431	0.0947	103
c63559_g2_1	Glutathione peroxidase	0.9434	0.6560	1.0146	0.8110	1.0413	0.9499	1319
c28911_g1_5	glutathione peroxidase 1	1.0357	0.6896	0.8045	0.2562	0.7762	0.1264	200
c48679_g1_2	glutathione peroxidase 2 [<i>Panax ginseng</i>]	0.6764	0.0758	0.3625	0.0327	0.5308	0.0261	470
c43430_g1_2	Glutathione reductase	1.8664	0.0182	2.1922	0.0390	1.1953	0.2728	637
c52494_g1_5	Glutathionyl-hydroquinone reductase	1.0198	0.6501	1.0328	0.7234	1.0234	0.6469	451
c52019_g1_4	Glutathione reductase	0.9859	0.6974	0.9406	0.0310	0.9620	0.3634	837
c115839_g1_5	Glutathione S-transferase	0.6228	0.6807	0.5964	0.5236	0.6426	0.1560	131
c24449_g1_1	Glutathione S-transferase	0.5975	0.2513	0.6565	0.8834	0.7335	0.4665	388
c28803_g1_6	glutathione S-transferase	1.0144	0.8089	0.9463	0.0351	0.9474	0.2539	390
c47297_g1_6	Glutathione S-transferase	1.3869	0.1429	1.8793	0.1977	1.3453	0.2470	313

c50251_g1_2	glutathione S-transferase	0.6857	0.1582	1.9209	0.9864	3.4531	0.6391	252
c51262_g1_1	Glutathione S-transferase	0.9566	0.3830	1.1154	0.3667	1.1884	0.3351	443
c51262_g2_1	Glutathione S-transferase	1.4521	0.1431	1.3684	0.1380	0.9585	0.2222	388
c62402_g1_3	Glutathione S-transferase	1.1944	0.2161	0.7867	0.1727	0.7070	0.2014	421
c63700_g1_1	Glutathione S-transferase	0.6898	0.0009	0.4552	0.0265	0.6492	0.0617	1208
c38862_g1_1	Glutathione S-transferase	1.2536	0.3650	1.0416	0.6163	0.8720	0.3319	363
c38862_g1_2	glutathione S-transferase	0.8053	0.0465	1.0191	0.0552	0.8439	0.1112	363
c44223_g1_5	Lactoylglutathione lyase	1.6715	0.2044	1.9030	0.1754	1.1585	0.1154	353
c56838_g1_1	lactoylglutathione lyase	1.2771	0.3968	0.9885	0.6914	0.8525	0.4480	883
c56877_g1_3	lactoylglutathione lyase	0.8106	0.2048	0.8998	0.2108	0.7487	0.2200	1199
c101836_g1_5	S-formylglutathione hydrolase	1.0641	0.7486	1.2987	0.1634	1.2511	0.2931	411

The P -value ≤ 0.05 is regarded as a significant difference.

Table S6. Selected proteins related to Glycolysis in *P. notoginseng* seeds during the after-ripening (Ar) process

Accession	Description	Ar30/Ar0	<i>P</i> -value	Ar60/Ar0	<i>P</i> -value	Ar60/Ar30	<i>P</i> -value	Protein Length
c17802_g1_5	triosephosphate isomerase	0.5966	0.0089	0.5583	0.0435	0.9261	0.4061	499
c53083_g1_2	Fructose-bisphosphate aldolase	0.6166	0.0305	0.4532	0.0196	0.7355	0.0790	120
c58735_g1_2	triosephosphate isomerase	0.5991	0.0126	0.5101	0.0245	0.8741	0.4467	459
c58926_g1_4	Enolase 1 (ENO)	0.5634	0.0356	0.4351	0.0295	0.8004	0.3299	739
c60777_g2_2	CDPK-related protein kinase-like	0.6314	0.0036	0.5187	0.0523	0.8204	0.3011	1268
c62157_g1_3	dihydrolipoyl dehydrogenase (DLD)	0.4993	0.0048	0.3070	0.0201	0.6019	0.0639	756
c37259_g1_5	Phosphoenolpyruvate carboxylase	2.1509	0.0339	1.4458	0.1209	0.6945	0.1897	1576
c49079_g1_4	phosphofructokinase	2.9854	0.0199	2.7828	0.0101	0.9495	0.5472	689
c55721_g1_6	pyruvate kinase	2.7075	0.0139	3.5150	0.0716	1.2376	0.5342	901
c57340_g2_4	glyceraldehyde-3-phosphate dehydrogenase	4.5101	0.0482	5.1019	0.0496	1.1702	0.0234	359
c58682_g1_5	phosphoglyceromutase	2.5265	0.0107	2.8539	0.0104	1.1519	0.0075	900
c63323_g3_1	Dihydrolipoyllysine-residue acetyltransferase	1.7205	0.0398	1.6502	0.0256	0.9659	0.6376	839
c64611_g1_5	glyceraldehyde-3-phosphate dehydrogenase	2.3386	0.0196	1.6481	0.1036	0.7018	0.0923	153
c47906_g1_3	hexokinase 2 (HK)	1.4200	0.4950	1.1138	0.5595	0.6008	0.6813	638
c60145_g1_5	Glyceraldehyde-3-phosphate dehydrogenase	0.8317	0.2922	0.5209	0.0312	0.6826	0.2352	689
c62743_g1_6	Glyceraldehyde-3-phosphate dehydrogenase	0.8548	0.4531	0.3973	0.0222	0.4895	0.0196	1844
c28939_g1_5	NAD-dependent aldehyde dehydrogenases	3.0372	0.1022	4.7442	0.0451	1.7361	0.1423	679
c61389_g2_1	Pyruvate/2-oxoglutarate dehydrogenase complex	0.5757	0.0634	0.4481	0.0381	0.7835	0.0258	571

The P -value \leq 0.05 is regarded as a significant difference.

Table S7. Selected proteins related to Pentose phosphate pathway in *P. notoginseng* seeds during the after-ripening (Ar) process

Accession	Description	Ar30/Ar0	<i>P</i> -value	Ar60/Ar0	<i>P</i> -value	Ar60/Ar30	<i>P</i> -value	Protein Length
c62212_g2_3	glucose-6-phosphate dehydrogenase 2 (G6PD)	1.8536	0.0585	1.6638	0.0695	0.9042	0.0533	783
c26400_g1_6	glucose-6-phosphate isomerase 1	0.8166	0.2190	0.7884	0.1068	1.0202	0.9328	650
c47300_g1_5	glucose-6-phosphate isomerase	0.7884	0.3798	0.8054	0.3745	1.0556	0.3514	874
c49079_g1_4	phosphofructokinase	2.9854	0.0199	2.7828	0.0101	0.9495	0.5472	689
c49908_g1_2	6-phosphogluconate dehydrogenase	0.4969	0.0075	0.5417	0.0256	1.1299	0.5837	250
c51096_g1_6	ribulose-phosphate 4-epimerase	0.7304	0.5000	0.4051	0.1587	0.7737	0.9757	652
c53083_g1_2	Fructose-biphosphate aldolase	0.6166	0.0305	0.4532	0.0196	0.7355	0.0790	120
c53083_g2_6	Fructose-biphosphate aldolase	0.4730	0.0540	0.4053	0.0032	0.9247	0.6293	484
c53404_g1_3	glucose-6-phosphate dehydrogenase	0.4825	0.0035	0.4484	0.0175	0.9500	0.6579	764
c55010_g1_2	Transketolase	0.7133	0.0460	0.4738	0.0038	0.6685	0.0645	928
c56006_g1_2	6-phosphofructokinase 5	1.3696	0.1895	1.2183	0.0242	0.9309	0.5534	826
c58544_g1_4	6-phosphogluconate dehydrogenase	2.1946	0.0384	2.2363	0.0329	1.0191	0.5101	1049
c60453_g1_5	Phosphoglucomutase	0.8077	0.1586	0.6975	0.2268	0.8538	0.4142	784
c60614_g1_4	Ribose-phosphate pyrophosphokinase 4	0.8978	0.5331	0.9933	0.8845	1.1531	0.3200	568
c60632_g1_3	6-phosphogluconolactonase 4	0.9652	0.7234	0.8891	0.2361	1.0260	0.9568	446
c61942_g1_5	transketolase	1.5771	0.1808	1.3518	0.1794	0.8762	0.2198	905

The P -value \leq 0.05 is regarded as a significant difference.