

Supplementary Materials: Proteomic Analysis of Tung Tree (*Vernicia fordii*) Oilseeds during the Developmental Stages

Zhiyong Zhan, Yicun Chen, Jay Shockey, Xiaojiao Han and Yangdong Wang

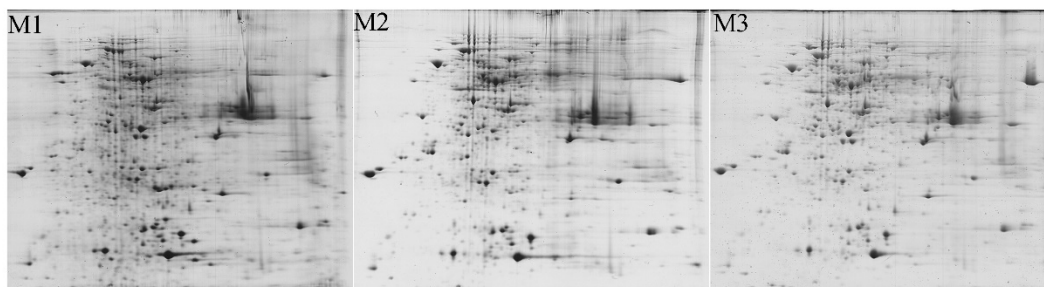


Figure S1. Images from 2-DE gel samples prepared using different protein extraction methods in three time-point. (M1) TCA-acetone extraction; (M2) Phenol extraction; (M3) TCA-acetone combined with phenol extraction.

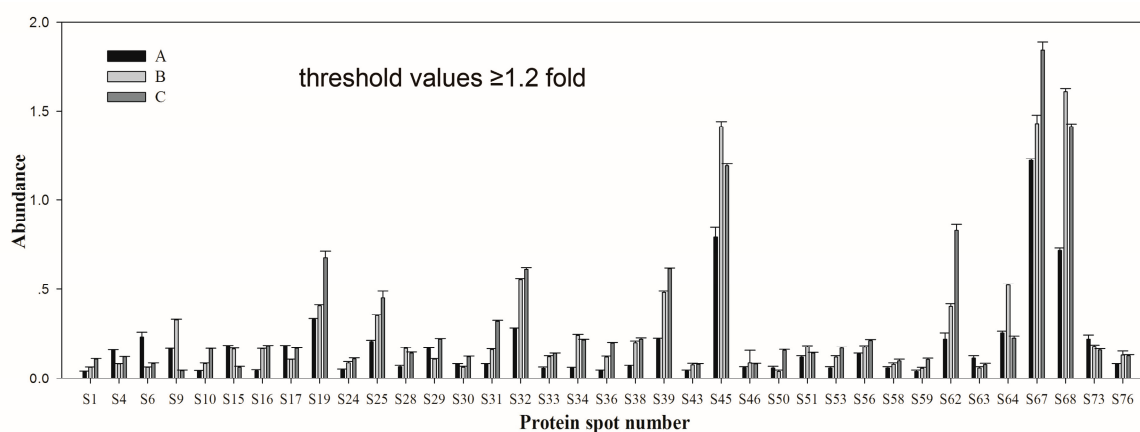


Figure S2. Abundance of 37 differentially expressed proteins. For each spot, each value is the mean of three biological replicates ($\pm$ SD). Spot numbers correspond to the numbers described in Table 1. A, B and C represent the seeds harvested on 25 August, 9 September and 26 September.

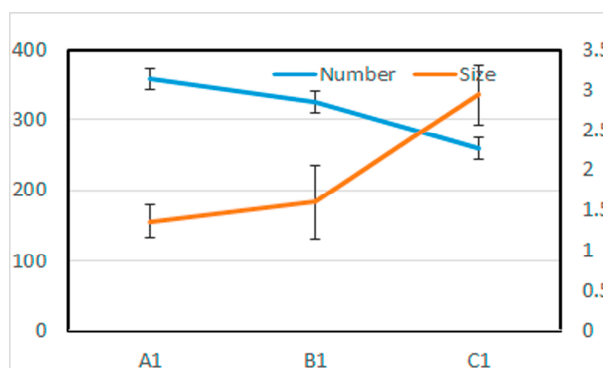


Figure S3. The volumes of oil bodies decreased and the numbers increased through progressive developmental stages. A1, B1 and C1 represent the seeds harvested on 25 August, 9 September and 26 September.

Table S1. Tung tree seed proteins identified by MALDI-TOF MS.

SN2 ¹	Protein Name	Accession Number.	Homology to Species	Sequences	Mr/pI	PS3 ²	PC4 ³	Comparison ⁴		
								α	β	γ
Energy metabolism										
S11	Rubisco subunit binding-protein alpha subunit	gi 255587664	<i>Ricinus communis</i>	AIELPDPMENAGAALIR	53,280.5/5.25	192	100	U	U	U
S18	6-phosphogluconate dehydrogenase	gi 255537671	<i>Ricinus communis</i>	DLFGAHTYER	54,510.8/6.25	190	100	D	D	-
S19	Caffeoyl-CoA O-methyltransferase	gi 120561153	<i>Brassica rapa</i>	VEISQISVGDGVTLCR	19,595.2/4.64	85	99.6	U	U	U
S28	Lactylgutathione lyase	gi 255554865	<i>Ricinus communis</i>	DPDGYIFELIQR	31,641.3/7.63	295	100	U	D	U
S29	Malate dehydrogenase, cytosolic	gi 225438145	<i>Vitis vinifera</i>	VLVTGAAGQIGYALVPMIAR	35,881.4/6.18	107	99.9	D	U	U
S30	Cinnamoyl-CoA reductase	gi 255544904	<i>Ricinus communis</i>	DVANAHIQAFEIPSASGR	45,052/6.39	76	97.5	D	U	U
S33	Glutamine synthetase	gi 2213877	<i>Hevea brasiliensis</i>	GNNILVMCDAYTPAGEPIPTNKR	39,503.7/5.81	241	100	U	U	U
S43	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	gi 255549601	<i>Ricinus communis</i>	FLFAGVVDGR	90,473.4/6.45	158	100	U	U	-
S45	Enolase	gi 356559925	<i>Glycine max</i>	AAVPSGASTGVYEALRL	48,230.8/6.06	234	100	U	D	U
S54	Succinate semialdehyde dehydrogenase	gi 255577875	<i>Ricinus communis</i>	EEVFGPVAPILR	65,727.4/8.84	97	99.9	U	U	D
S55	S-adenosylmethionine synthase	gi 356565143	<i>Glycine max</i>	FVIGGPHGDAGLTGR	43,448.9/5.65	120	100	U	U	U
S56	Pyruvate dehydrogenase	gi 255557267	<i>Ricinus communis</i>	SENPVILFEHVLLYNLK	44,901.7/6.09	199	100	U	U	U
S62	Cytosolic, 3-phosphoglycerate kinase	gi 28172915	<i>Zea mays</i>	LASVADLYVNDAFGTAHR	31,662.8/5.01	207	100	U	U	U
S63	Malate dehydrogenase, putative	gi 255541140	<i>Ricinus communis</i>	VLVTGAAGQIGYAIIVPMIAR	36,058.6/6.19	112	100	D	U	D
S68	Triosephosphate isomerase, cytosolic	gi 226495391	<i>Zea mays</i>	VIACVGETLEQR	27,236.1/5.52	167	100	U	D	U
S70	Ribulose-1,5-bisphosphate carboxylase/oxygenase	gi 18140587	<i>Laplacea fruticosa</i>	VTPQGPVPPEEAGAAVAESSTGTWTTVWTDGLTSLDR	52,058.2/6.34	604	100	D	U	U
S71	Legumin A precursor	gi 255567248	<i>Ricinus communis</i>	AMPIEVVANAFQVSVDEA	57,872.6/8.03	81	99.1	D	D	D
S72	UTP-glucose-1-phosphate uridylyltransferase	gi 255571289	<i>Ricinus communis</i>	VQLLEIAQVPDEHVNEFK	51,770.4/5.71	234	100	U	U	-
S74	Diaminopimelate epimerase	gi 255584553	<i>Ricinus communis</i>	YQGLGNDFILVDNR	40,540.1/6.07	152	100	U	U	U
Fatty acid metabolism										
S4	NAD(P)-binding rosmann-fold-containing protein	gi 18404496	<i>Arabidopsis thaliana</i>	KAEQYLADSGIPYTIIR	34,972.2/8.37	276	100	D	U	D
S9	Methionine adenosyltransferase	gi 75297911	<i>Arabidopsis thaliana</i>	FVIGGPHGDAGLTGR	43,333.8/5.67	74	95.3	U	D	D
S17	Esterase precursor	gi 255560956	<i>Ricinus communis</i>	ALYTFDIGQNDLSVGFR	46,194.2/6.6	513	100	D	U	D
S31	Stearoyl-acyl-carrier protein desaturase	gi 110741382	<i>Arabidopsis thaliana</i>	TENNPYLGFIYTSFQER	32,330.3/5.94	191	100	U	U	U
S40	Plastid-lipid-Associated protein	gi 2632088	<i>Nicotiana tabacum</i>	QLVDSFYGTNR	28,385.4/4.83	90	99.8	U	U	D
S46	HXXXD-type acyl-transferase-like protein	gi 15229017	<i>Arabidopsis thaliana</i>	RLVELVTDGFMR	48,315.9/5.51	74	96.2	U	U	-
S50	Pyruvate dehydrogenase E1 component subunit alpha-like	gi 356520231	<i>Glycine max</i>	SQLENVFADPK	48,315.1/6.41	147	100	D	U	U
S51	3-hydroxybutyryl-CoA dehydratase	gi 255549046	<i>Ricinus communis</i>	NDFAEGVR	45,616.1/5.97	142	100	U	U	D
S57	Stearoyl-acyl carrier protein desaturase	gi 3355632	<i>Linum usitatissimum</i>	TENNPYLGFIYTSFQER	44,999.7/5.81	220	100	U	U	U
S58	Plastid 3-ketoacyl-ACP synthase I	gi 74475410	<i>Cuphea avigera</i> var. <i>pulcherrima</i>	GAPIIAEYLGGAINDAYHMTDPR	49,912.2/6.86	88	99.8	U	U	U
S64	Acetyl-CoA carboxylase	gi 347662503		MILAGYEHDIK	259,206.8/5.99	71	91.2	U	D	D
S76	3-oxoacyl-[acyl-carrier-protein] reductase	gi 356540173	<i>Glycine max</i>	ILETIPLGR	33,862.7/9.17	302	100	U	U	-
Defense-related										
S3	Nucleoside diphosphate kinase 2	gi 145712785	<i>Arabidopsis lyrata</i> subsp. <i>petraea</i>	GLVGEIISR	22,522.7/9.27	78	98.5	D	-	U

S13	Heat shock protein, putative	gi 255554571	<i>Ricinus communis</i>	AVITVPAYFNDAQR	71,304/6.1	129	100	U	U	-
S14	Heat shock protein, putative	gi 255575054	<i>Ricinus communis</i>	EQVFSTYSDNQPGVLIQV YEGER	71,470.3/5.07	299	100	U	U	-
S23	Chloroplast Cu/Zn superoxide dismutase	gi 86920323	<i>Arabidopsis thaliana</i>	AFVVHELEDDLGKGGHE LSLTTGNAGGR	6977.5/4.41	199	100	U	-	D
S32	17.5KDa class I heat shock protein-like	gi 356521398	<i>Glycine max</i>	ETPEAHVFEADIPGLKK	17,534.9/5.33	200	100	U	U	U
S34	18.8KDa class II heat shock protein-like	gi 225429618	<i>Vitis vinifera</i>	EYPNAYVFLVDMPLGK	18,463.6/8.46	87	99.7	U	D	U
S37	Manganese superoxide dismutase	gi 9929159	<i>Digitalis lanata</i>	HHQAYITNYNK	24,978.9/8.01	135	100	U	U	U
S47	Heat shock 70KDa protein	gi 357120971	<i>Brachypodium distachyon</i>	SKFESLVNNLIER	121,337.9/5.4	124	100	U	U	D
S59	Cysteine protease inhibitor	gi 1638842	<i>Ricinus communis</i>	QVVAGTLHHLTIEAIEAGK	23,289.8/6.17	72	93.9	U	U	U
S61	Alcohol dehydrogenase	gi 255570416	<i>Ricinus communis</i>	GQSVAFGLGAVGLAAA EGAR	42,038.1/5.91	207	100	U	U	-
S69	Glycine-rich protein	gi 171451986	<i>Bruguiera gymnorhiza</i>	NITVNEAQRS	17,036.1/9.81	83	99.4	D	D	U
Unknown										
S1	Hypothetical protein OsI_34099	gi 218184783	<i>Oryza sativa Indica Group</i>	AAAGSSIHTMLTGR	62,243.7/5.83	84	99.6	U	U	U
S7	Os01g0964400	gi 297720793	<i>Oryza sativa Japonica Group</i>	AGFAGDDAPR	24,702.4/6.08	172	100	U	U	U
S8	Conserved hypothetical protein	gi 255568303	<i>Ricinus communis</i>	ELVLGQDQPR	60,659.1/5.89	89	99.8	U	U	D
S27	Conserved hypothetical protein	gi 255568303	<i>Ricinus communis</i>	EQPSLEEISKLK	60,659.1/5.89	79	98.6	D	D	-
S38	OSIGBa0140J09.5	gi 116310837	<i>Oryza sativa Indica Group</i>	DLCEDFGIK	160,858.4/9.3	73	95.0	U	U	U
S39	Hypothetical protein SELMODRAFT_410993	gi 302773530	<i>Selaginella moellendorffii</i>	ELELLTPER	37,543.8/6.34	80	98.8	U	U	U
S41	Predicted protein	gi 224138130	<i>Populus trichocarpa</i>	DLFEILMDENR	39,787.6/5.77	182	100	U	U	D
Peroxidase										
S5	Peroxiredoxin, putative	gi 255556526	<i>Ricinus communis</i>	LSFLYPASTGR	24,321.4/5.64	133	100	U	U	U
S24	Peroxiredoxin	gi 300078580	<i>Jatropha curcas</i>	SYGVLPDQGIALR	25,054.9/8.37	505	100	U	U	U
S26	Peroxiredoxin	gi 255556526	<i>Ricinus communis</i>	LSFLYPASTGR	24,321.4/5.64	174	100	D	D	-
S60	Peroxiredoxin	gi 2555575353	<i>Arabidopsis thaliana</i>	EQPSLEEISKLK	23,943.7/7.63	186	100	U	U	-
S66	Glutathione peroxidase	gi 255537447	<i>Ricinus communis</i>	FLVDKDGNNVDR	18,717.5/6.58	183	100	U	U	U
Protease										
S12	Proteasome subunit alpha type, putative	gi 255538698	<i>Ricinus communis</i>	NQYDTDVTTWSPAGR	30,638.1/4.89	395	100	D	D	U
S20	Proteasome subunit alpha type-5-A	gi 15220961	<i>Arabidopsis thaliana</i>	FSYGEPMTVESTTQALC DLALR	26,102/4.7	342	100	U	U	U
S25	Proteasome subunit	gi 255541320	<i>Ricinus communis</i>	GCVYTYDAVGSYER	24,880.2/6.08	216	100	U	U	U
S35	26S protease regulatory subunit 6A	gi 195635679	<i>Zea mays</i>	DSYLILDTLPSEYDSR	47,886.6/4.98	102	99.9	U	U	-
S48	Aspartic proteinase precursor	gi 255578112	<i>Ricinus communis</i>	EPVFSWFENR	56,595.9/5.19	91	99.9	U	-	D
S49	26S protease regulatory subunit 6a, putative	gi 255542742	<i>Ricinus communis</i>	DSYLILDTLPSEYDSR	48,271/5.05	201	100	U	U	D
S53	Peptidase	gi 255571742	<i>Ricinus communis</i>	QPGVLQVPSR	13,853.3/8.04	96	99.9	U	U	U
S52	Proteasome subunit beta type	gi 255558626	<i>Ricinus communis</i>	LFLGLSGLATDAQTLYQR	23,126.6/5.17	598	100	D	D	U
S65	Nascent polypeptide associated complex alpha subunit	gi 255569201	<i>Ricinus communis</i>	IEDLSSQLQTQAAEQFK	14,221.1/4.24	308	100	D	D	D
Signal transduction										
S2	Nucleoside diphosphate kinase	gi 255537805	<i>Ricinus communis</i>	TFIAIKPDGVQR	25,627/9.16	143	100	U	U	D
S10	Glycine-rich RNA-binding protein	gi 255571692	<i>Arabidopsis thaliana</i>	CDEPAMPKTMCLR	16,562.6/7.79	193	100	U	U	U
S44	Profilin	gi 30841324	<i>Gossypium hirsutum</i>	FMVIQGEPPGAVIR	14,924.4/5.38	128	100	D	-	U
S75	Nucleoside diphosphate kinase	gi 284433792	<i>Jatropha curcas</i>	IIGATNPAESAPGTIR	16,339.5/6.32	499	100	D	D	U

Cell construction										
S6	Actin	gi 50660331	<i>Vitis vinifera</i>	NYELPDGQVITIGAER	19,185.8/4.86	605	100	D	U	D
S15	Actin-like protein	gi 294884347	<i>Populus tremula</i> x <i>Populus alba</i>	NYELPDGQVITIGAER	16,084.2/4.78	559	100	D	D	D
S16	Myosin class II heavy chain (ISS)	gi 308801757	<i>Ostreococcus tauri</i>	ELEHQMSLLEEQR	30,4318.4/5.0	79	98.6	U	U	U
S36	Actin	gi 296881978	<i>Jatropha curcas</i>	NYELPDGQVITIGAER	41,973.1/5.31	767	100	U	U	U
Transcription-related										
S21	Peptidyl-prolyl cis-trans isomerase	gi 359480227	<i>Vitis vinifera</i>	CFFDVDIGGEPVGR	23,214.2/8.25	123	100	U	-	D
S22	Nuclear transport factor 2 isoform 1	gi 225425388	<i>Vitis vinifera</i>	AFVEHYSTFDANR	13,699.7/5.67	119	100	U	U	U
Protein modification										
S42	Ubiquitin 1, putative	gi 255572315	<i>Ricinus communis</i>	TLTGKEIEIDIEPTDTIDR	13,525.3/6.6	639	100	D	D	-
S73	Ubiquitin	gi 111218906	<i>Arabidopsis thaliana</i>	IQDKGIPPDQQR	24,638.2/6.4	382	100	D	D	D
Storage										
S67	Nutrient reservoir	gi 255570801	<i>Ricinus communis</i>	REGEDWFLQDSK	52,414.5/6.4	80	98.9	U	U	U

Proteins identified in the table with a false discovery rate (FDR) < 0.05. Mr/pI indicates the protein molecular weight and isoelectric point. Accession no. is the NCBI database identifier. ¹. (Spot no.) indicates the different expression protein spots which selected by the software during the process of 2-DE gels analysis. ² and ³ are the thresholds. Generally, PS (Protein Score) > 60 and PC (Protein Score C.I. %) > 95 mean that the protein identification analysis results from MALDI TOF/MS were credible. ⁴. U and D represent up-regulated and down-regulated protein spots, respectively. The protein spots marked by “-” indicated these spots were only undetectable in the group by the methods used in this report. (α) the sample from 25 August was compared with 9 September period (β) the sample of 9 September compared with 26 September. (γ) the sample of 25 August compared with 26 September.