

Supplementary Materials: Digital Gene Expression Profiling to Explore Differentially Expressed Genes Associated with Terpenoid Biosynthesis during Fruit Development in *Litsea cubeba*

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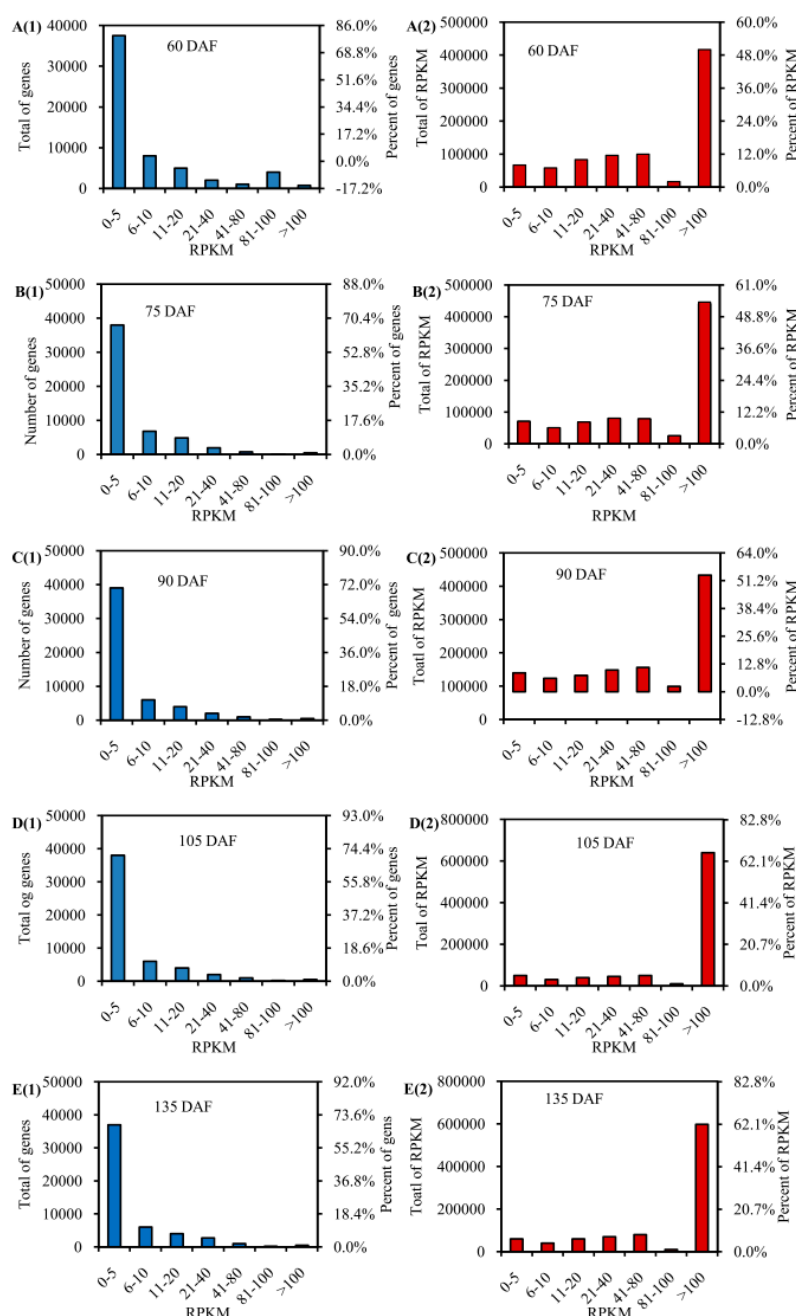


Figure S1. RPKM distribution of all genes. (A)–(E) represents 60 DAF, 75 DAF, 90 DAF, 105 DAF and 135 DAF, respectively. (1): The x-axis represents the range of RPKMs, whereas the y-axis denotes the number of genes. (2): The x-axis represents the range of RPKMs, whereas the y-axis denotes the total RPKM values of all the genes in this range. RPKM means the reads per kilo base transcriptome per million mapped reads.

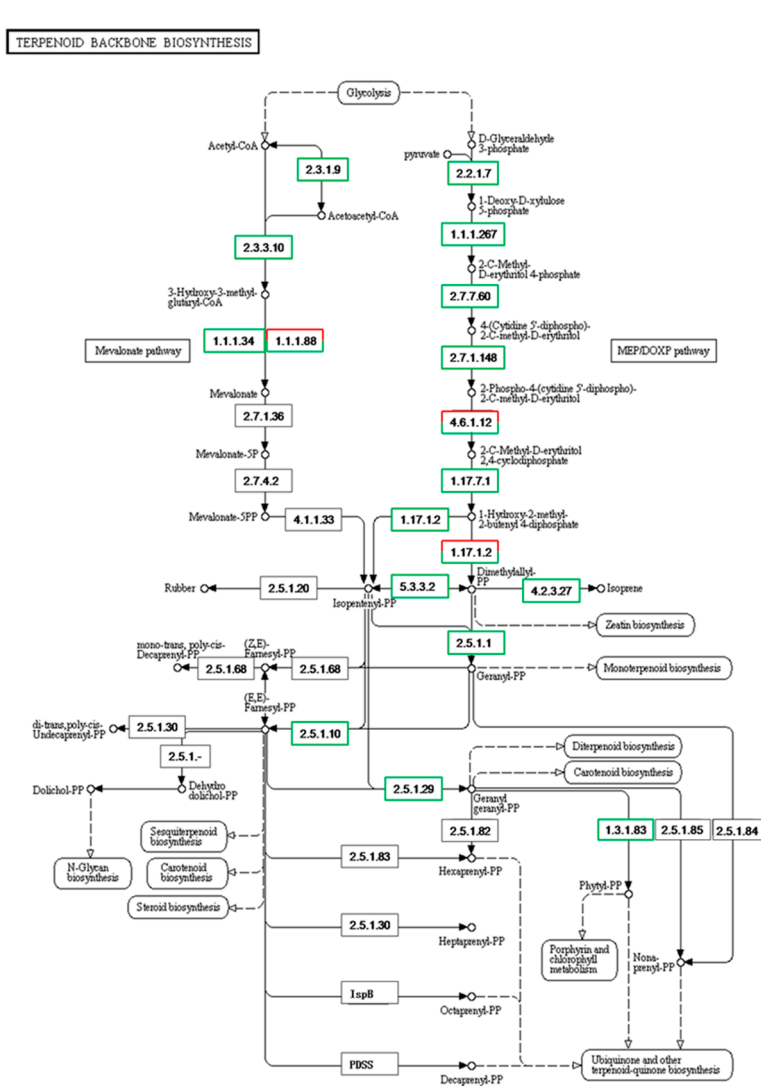


Figure S2. Terpenoid backbone biosynthesis pathway in *L. cubeba*. Red or green rectangles indicate up-regulated or down-regulated genes, respectively.

Table S1. DEGs identity and Gene Ontology annotations.

GO	GO Annotation	Gene ID	
Biological Process	monoterpenoid biosynthetic process (GO:0016099)	5970	
	terpenoid biosynthetic process (GO:0016114)	62474; 56508; 43974; 34118; 55836; 53869	
	isoprenoid biosynthetic process (GO:0008299)	4005; 4493; 43919; 50359; 59387; 57667; 25404; 59227	
	pentacyclic triterpenoid biosynthetic process (GO:0019745)	4768; 45261; 19146; 49096; 56497	
	diterpene phytoalexin metabolic process (GO:0051501)	50359	
	isopentenyl diphosphate biosynthetic process (GO:0019288)	60959; 62474; 49100; 56508; 32294; 7256; 55747; 39745; 52697; 25934; 58721; 38494; 46352; 62457; 4268; 57427; 25757; 51212	
	glyceraldehyde-3-phosphate biosynthetic process (GO:0046166);	51212	
	coenzyme biosynthetic process (GO:0009108)	60959; 55747; 4268; 57427; 25757;	
	coenzyme A metabolic process (GO:0015936)	43919	
	aromatic amino acid family metabolic process (GO:0009072)	60959; 4268; 57427; 25757;	
	carotenoid biosynthetic process (GO:0016117)	58937; 32294; 60959; 60296; 4268; 57427; 54564; 39745; 60544; 51265; 53845; 25757; 54565	
	gibberellin biosynthetic process (GO:0009686)	60333; 61838; 61351; 43050; 22310; 22311; 20979; 32523; 41339; 57133	
	regulation of abscisic acid biosynthetic process (GO:0010115)	32347; 32346	
	abscisic acid biosynthetic process (GO:0009688)	40547; 54485	
	Molecular Function	myrcene synthase activity (GO:0050551)	5970
		(R)-limonene synthase activity (GO:0034002)	36997
		terpene synthase activity (GO:0010333)	7718; 59387; 25404; 33560; 59227; 44865
		4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase activity (GO:0046429)	60959; 7256; 56508
		hydroxymethylglutaryl-CoA synthase activity (GO:0004421)	4005; 57667
myrcene synthase activity (GO:0050551)		5970	
hydroxymethylglutaryl-CoA reductase (NADPH) activity (GO:0004420)		43919	
1-deoxy-D-xylulose-5-phosphate reductoisomerase activity (GO:0030604)		57427	
4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase activity (GO:0051745)		56508; 7256	
acetoacetyl-CoA reductase activity (GO:0018454)		62945	
oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor (GO:0016652)		59809	
oxidoreductase activity, acting on NADH or NADPH, quinone or similar compound as acceptor (GO:0016655)		54302	
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NADH or NADPH as one donor, and incorporation of one atom of oxygen (GO:0016709)		43509; 54506; 5294	
NADPH dehydrogenase activity (GO:0003959)		57822	
NADPH binding (GO:0070402)		57427	
carotenoid isomerase activity (GO:0046608)		51265; 60296	
C-19 gibberellin 2-beta-dioxygenase activity (GO:0052634)		38935; 58941	
gibberellin 3-beta-dioxygenase activity (GO:0016707)		38935; 29280	
abscisic acid glucosyltransferase activity (GO:0010294)		41418; 40881; 42180; 14037	
(+)-abscisic acid 8'-hydroxylase activity (GO:0010295)		45239; 59012	
farnesyltranstransferase activity (GO:0004311)		50359	

Table S2. Real-time PCR confirmation of differential gene expression.

Name	Forward Primer	Reverse Primer
acetoacetyl-CoA thiolase (AACT)	5'-ACTTCTGCTGACGCTTCGTT-3'	5'-AGCTGGAGCCATGAGATGAT-3'
HMG-CoA synthase (HMGS)	5'-TTCTGAGGAATGCCAGCTCT-3'	5'-TGTTGCCAACTTGTTTTGGT-3'
HMG-CoA reductase (HMGR)	5'-AAAGTTCCCACTGCATCACC-3'	5'-CAAGAGCCTGGAATTTGCTC-3'
4-diphosphocytidyl-2C-methyl-D-erythritol kinase (CMK)	5'-GGCCTTCAAAGGCTCTCTCT-3'	5'-GGACTCCAGGCACATTGTT-3'
1-Hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate Synthase (HDS)	5'-AATCCGTGTTTCCCTCACAG-3'	5'-GGTGCAAGACTCCTCGGTAG-3'

Table S3. Chemical Composition (%) of the Essential Oil at different stages of fruit development.

Chemistry Content	60 DAF	75 DAF	90 DAF	105 DAF	135 DAF
1R- α -Pinene	3.1	1.7	1.41	0.64	0.53
Camphene	1.61	1.28	0.82	0.38	0.31
β -Pinene	2.81	2.18	1.31	0.68	0.62
β -Myrcene25273	1.98	1.91	1.84	1.28	1.57
d-Limonene	31.64	13.48	13.81	9.31	8.96
Geraniol 22040	3.15	1.64	0.85	0.7	0.63
Caryophyllene	1.29	0.76	1.58	1.85	1.98

Table S4. RPKM change of DEG related with some compounds during the development of *L. cubeba* fruit.

Gene ID	Compounds	\log_2 ratio ¹			
		75 DAF	90 DAF	105 DAF	135 DAF
25404	D-Limonene	-2.91	-10.33	-	-
36997	Geraniol	-16.0102	-0.23704	-3.72247	-0.83494
59227	Caryophyllene	-1.86464	-9.12171	-5.38755	-10.1983
59387	β -Myrcene	-0.70294	-6.87505	-0.97369	-3.65008
1025	β -Myrcene	-2.04123	1.270621	-0.55216	-0.19302
57447	β -Myrcene	-1.09638	-1.62044	-0.46047	1.516021
33560	β -Myrcene	-2.38178	-6.22777	-4.37814	-3.86699

¹ \log_2 ratio of 75 DAF, 90 DAF, 105 DAF and 135 DAF RPKM value to 60 DAF RPKM value, respectively.