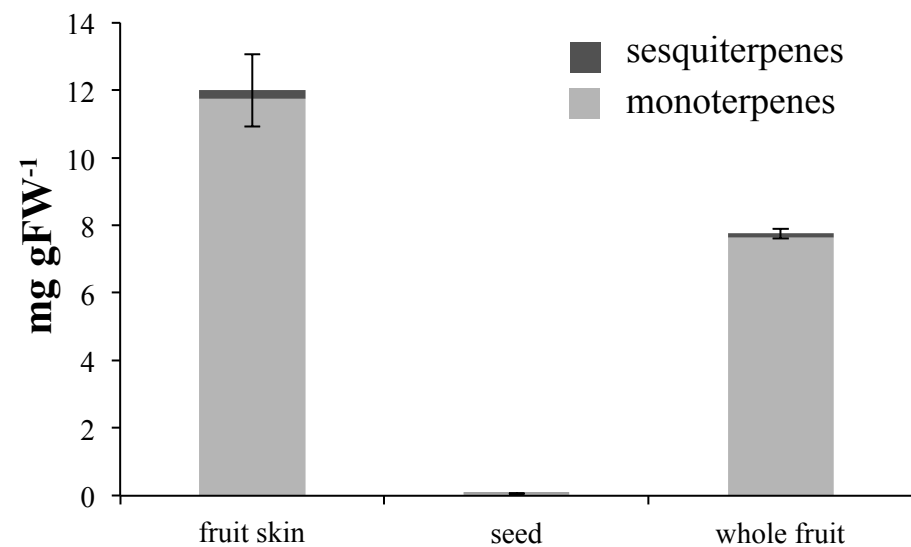
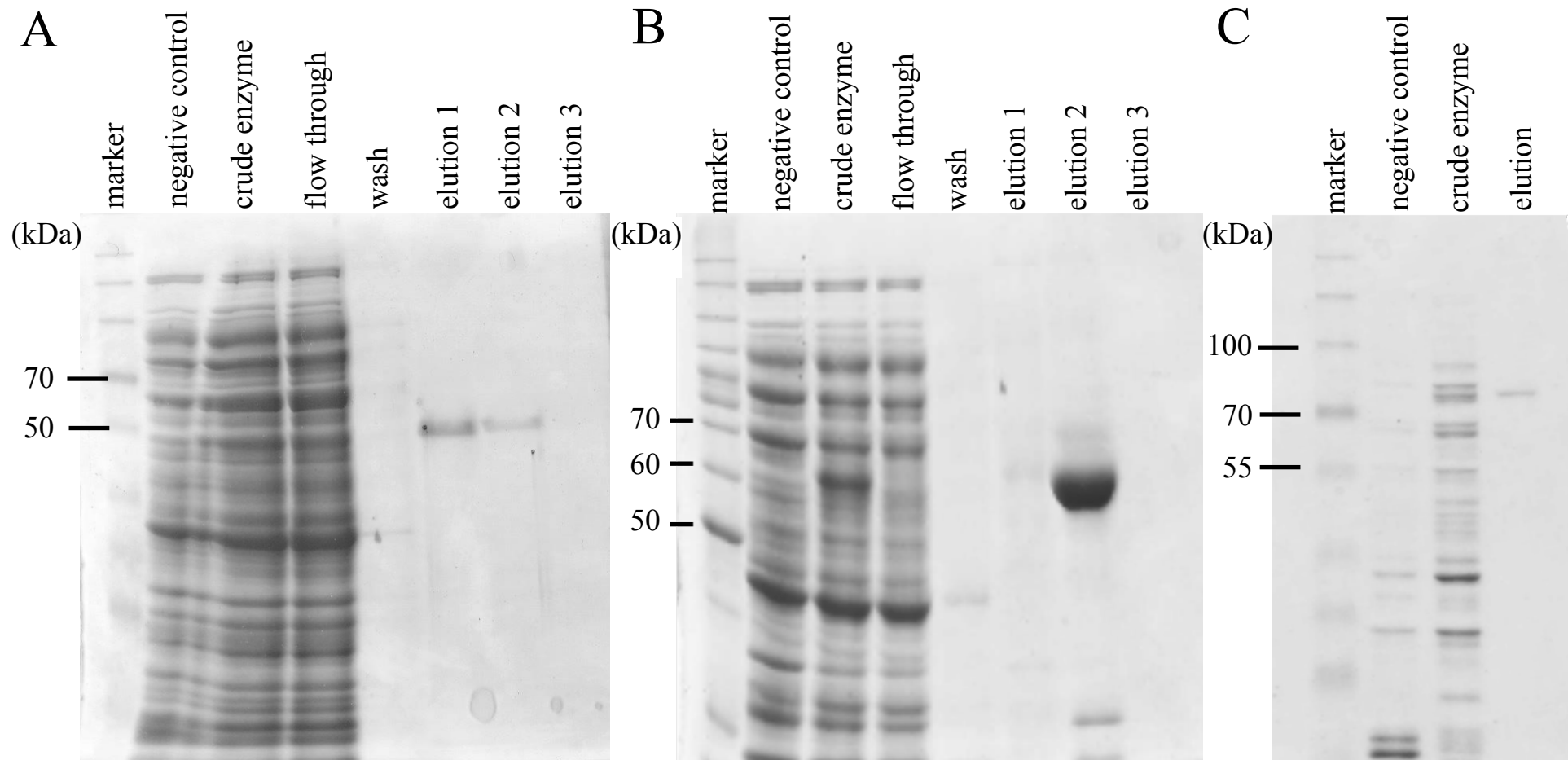
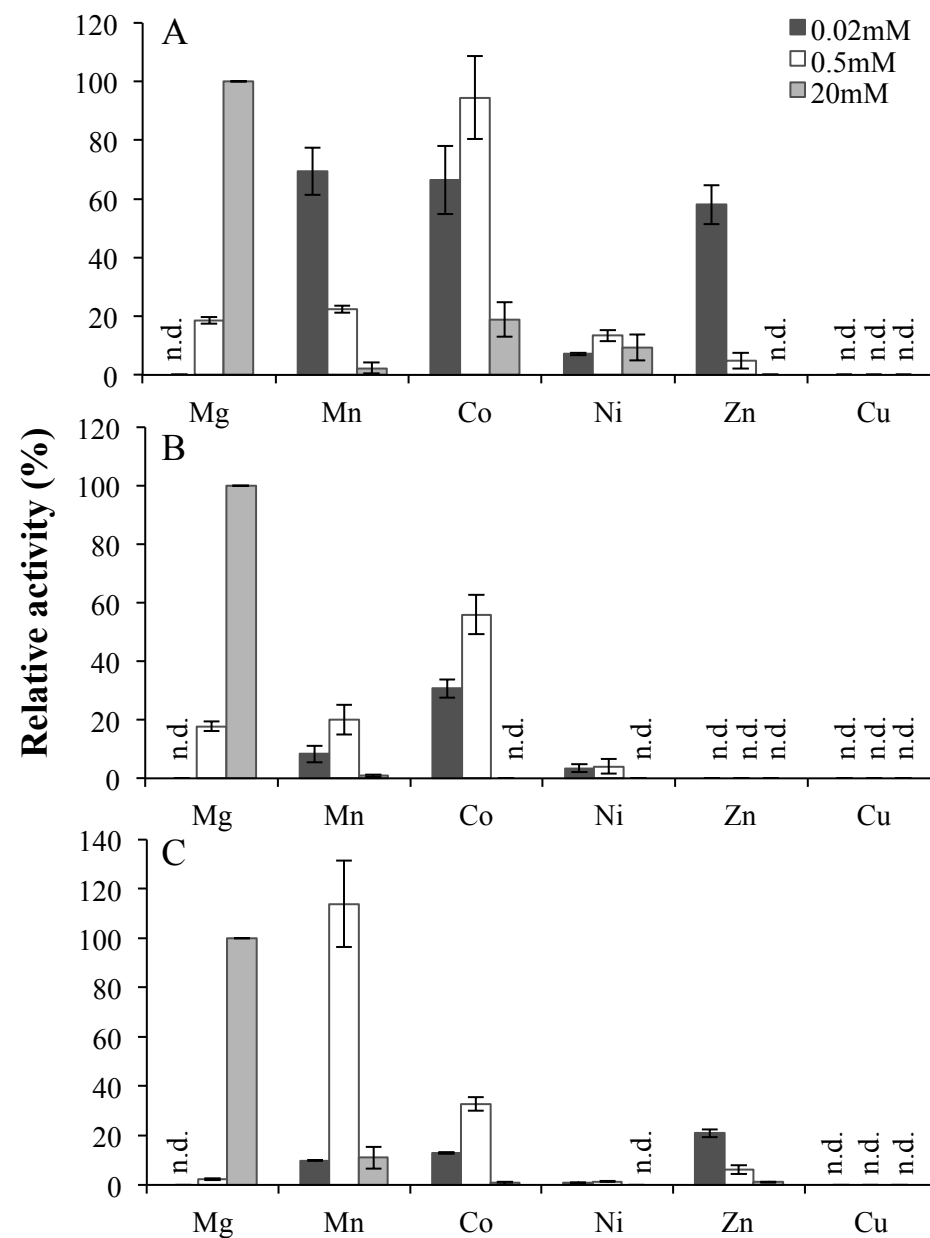




Supplementary Figure S1. Terpene amount in fruit skin, seeds, and whole fruits of Japanese pepper. Monoterpenes (β -phellandrene, D-limonene, and citronellal) and sesquiterpenes (β -caryophyllene and germacrene D) are indicated by gray and black, respectively. Mean values $\pm$ SE are shown ($n=3$).



Supplementary Figure S2. SDS-PAGE analysis of ZpTPSs. ZpTPS1 (A) and ZpTPS2 (B) were produced as His-tagged proteins and purified using Ni²⁺ affinity chromatography. ZpTPS3 (C) was produced as a GST-fusion protein and purified using Glutathione Sepharose 4B.



Supplementary Figure S3. Effects of divalent metal ions of varying concentration on the enzymatic activity of ZpTPSs. (A) ZpTPS1, (B) ZpTPS2, and (C) ZpTPS3 activity in the presence of different divalent cations. Mean values $\pm$ SE are shown ($n=3$).

ZpTPS3	1	-MAMSSCIIPSTFITSVNGLKCLFATNRATIRICVHKSIOQVATTASTHLDNSTVARRSCGYOFTIWDNDYQSLSSHYTGETYRROVEKLEGEVKTMINNVAKPLDQL
CILIMS	1	---MSSCINPSTLIVTSVNAFKCLPLATNKAIRI--MAKYKPVQCLISAKYDNLTVDRRSANYQPSIWDHDFLQSLNSNYTDEAYKRAEELRGKVKTAIKDVIEPLDQL
CtLIMS	1	---MSSCINPSTLIVTSVNGFKYLPLATNRAAIRI--TAKNKPVQCLVSAKYDNLTVDRRSANYQPSIWDHDFLQSLNSDYTDETYRRRAEELKGVKTAIEDVTEPLDQL
CjGES	1	---MSSSINPSTLIVTSVNGFKCLPLITNKAIRI--MAKNKPLQCLVSAKYDNLTVDRRSANYQPSIWDHDFLQSLNSKYTDEAYKRAEELKGVKTAIKDVIEPLDQL
MsLIMS	1	MALKVFSVATQMAIFSKLTTCLQPSHLKSSPKLFPSTNSSGRSRLRYCSSSLTERRSCTNYPNPSRWDFEVIQSLHSDYKKDKHAIRASELVTIVKMELEKETDQIRQL
		RRxxxxxxxxW
ZpTPS3	110	ELIDTLQRLGLAYHEDTEISNIIQNIYNNKDD-E---WKKENLYATSLEFRLRLQHGYPVSOEVFNSFKDKKGSFKLCLCDDIKAMLSFYEASYSTEEESIMEEAWQF
CILIMS	106	ELIDNLQRLGLAHRFETEIRNIIINNIYNNNKDYN---WRKENLYATSLEFRLRLQHGYPVSOEVFNGFKDDQGGF---ICDDFKGILSLHEASYSLLEGESIMEEAWQF
CtLIMS	106	ELIDNLQRLGLAYHFETEIRNIIHNIYNNNKDYI---WRKENLYATSLEFRLRLQHGYPVSOEVSTGFKEDKGVF---ICDDFMGILSLHEASYSLLEGESIMEEAWQF
CjGES	106	ELIDNLQRLGLAHRFETEIRNIIINNIYNNNKDYN---WRKENLYATSLEFRLRLQHGYPVSOEVFNGLKDGQGGF---ICDDFKGILSLHEASYSLLEGESIMEEAWQF
MsLIMS	111	ELIDDLQRMGLSDHEQNEFKEIISIIYLDHHYKPNFPKEERDLYSTSLAFRLRLRHGFCVAQAEVFDSEFKNEEGEFKESLSDDTRGLIQLIYASFLLTEGETTLESAREF
		324
ZpTPS3	215	TSKNLKDVN---NKCVDLNVAAAEVRDALELPLHWKTPRLETFRFINFYERKDKNNMLLQLAKIDENIVQGIYLEELKEMSSWWKNTGLGEKLSFARDRLVASFYVWSIG
CILIMS	209	TSKHLKEVMT-SKNMEEDVFVAEQAKRALELPLHWKVPMLERWFIHITYERREDKNHLLLELAKMEFNTLQAIYQEELKEISGWWKDTGLGEKLSFARNRLVASFLWSMG
CtLIMS	209	TSKHLKEMMIISNSKEEDVFVAEQAKRALELPLHWKVPMLERWFIHIVYEKREDKNHLLLELAKLEFNVLQAIYQEELKDVSRWWKDIGLGEKLNFAARSLVASFLWSMG
CjGES	209	TSKHLKEVMT-SKSEEFVFVAEQAKRALELPLHWKVPMLERWFIHIVYEKREDKNHLLLELAKLEFNVLQAIYQEELKDISGWWKETGLGEKLSFARDRLVASFLWSMG
MsLIMS	221	ATKFLFERV---NEGGDGDLLTRIAYSIDIPLHWRIKRPNAPVWIEWYRKRPNNPAVLELAILDLNIVQAQFQEELKESFRWWNTGFVEKLEFARDRLVECYTWNTG
		345
ZpTPS3	322	TAFKPQLSFCRKMLTEVFALITVIDDIYDVYGTIPELELFTFAVERWDIK-AMKELPDYMKICFLITLENVAVNEMAFDILKEQGSQVVMNIKETWVAILLOAYMV EAKWYHN
CILIMS	318	IAFEPQFAYCRRVLTISTALITVIDDIYDVYGTLDLELEIFTDFAVERWDINYALKHLECYMKMCFLALYNFVNEFAYYVLKQDFDILLSIKNAWIGLLOAYLVEAKWYHS
CtLIMS	319	IVFEPQFAYCRRILTITFALISVIDDIYDVYGTLDLELELFAFAVERWDINYALNHLPDYMKICFLALYNLVNEFTYYVLKQDFDILRSIKNAWIRNIQAYLVEAKWYHG
CjGES	318	IGSEPFQFAYCRRIVTITATALITVIDDIYDVYGTLDLELELFTAFAVARWDIHYALNHLPDYMKLCFFALYNFVNEFAYYVLKQDFDILRSIKNSWIGLLOACLVEAKWYHT
MsLIMS	328	IIEPROHASARIMMGYNALITVIDDIYDVYGTLEELEQFTDLIRWDIN-SIDQLPDYMQLCFLALNNFVDDTSYDVMKEKGVNVIPLYRQSWDLADKYMVEARWYFG
		RxR
ZpTPS3	431	QHTPTMEEYMNNGWITVCAITVALNAYLSATNPPIEKELEFLETYPDILQLLOKIVRLQDDLGTSSDELKRGDVPKSIQCYMNETGVSEESREHIKELMRQMWKKNLY
CILIMS	428	KYTPKLEEYLENGLVSTIGELIITISYLSGTNPPIEKELEFLESNPDIHWSKIFRLQDDLGTSSDEIQRGDVPKSIQCYMHETGASEEVARQHIKDMMRQMWKKNAY
CtLIMS	429	KYTPTLGEFLENGLVSIGGPMVTMTAYLSGTNPPIEKELEFLESNODIHWSEKIFRLQDDLGTSSDEIQRGDVPKSIQCYMHETGASEEVARQHIKDMMRQMWKKNAY
CjGES	428	KYTPTLGEFLENGLVSIGGPMGIMTAYLSGTNPPIEKELEFLESNODIHWSEKIFRLQDDLGTSSDEIQRGDVPKSIQCYMHETGASEEVARQHIKDMMRQMWKKNAY
MsLIMS	437	GHKPSLEEYLENSWQSISGECMLTHIFFRVTDSFTKETVDSLYKYHDLVRWSSFVLRLADDLGTSSVEEVSRGDVPKSLQCYMSDYNASEAEARKHVKWLIAEVWKKMNAE
		458
ZpTPS3	541	IANKDSDPLSQTTIELISNLVRS SHFMYLNGDGHGIREKETMDVPLSLIFQPIPLEEKEMVFTSSRGITNY-----
CILIMS	538	TADKDS-PLTGTTFEFLNLVVRMSHFMYLHGDSVQONQETIDVGFLLFQPIPLEDKHMAFTASPGTKG-----
CtLIMS	539	RADKDS-PLSQTTVEFILNVVRVSHFMYLHGDSGAQONQETMDVVFLLFQPIPLDKHIVATISSPVTKG-----
CjGES	538	RADKDS-PLSQNTVDFMLNLVVRMSHFMYLRGDGHGAQONQETMDVASTWLFQPIPLEDKHMAFTAPKADEFPEYSFS
MsLIMS	547	RVSKDS-PFGKDFIGCAVDLGRMAQLMYHNGDGHGTQHPIIHQQMTRTLFEPFA-----
		579
		DDxxTxxxE

Supplementary Figure S4. Amino acid sequence alignment of ZpTPS3, limonene synthase from *Citrus limon* (CILIMS; AAM53944.1), *Citrus trifoliate* (CtLIMS; BAG74774.1) and *Mentha spicata* (MsLIMS; AGN90912.1), and geraniol synthase from *Citrus jambhiri* (CjGES; BAM29049.1). The program GENETYX-MAC Ver.12.2.0 was used for the alignment. Black highlighting indicates those residues with more than 50% conservation among all sequences. The canonical highly conserved regions are labeled with RR(x)8W, RxR, DDxxD and DDxx(S,T)xxxE (Huang et al. 2013). The important residues (W324, N345, M458 and H579) for catalytic activity of MsLIMS (Srividya et al. 2015) are indicated by red squares.

Supplementary Table S1. Accession numbers of protein sequences used for phylogenetic analysis of plant TPSs.

Protein name	Accession number
<i>Antirrhinum majus</i> myrcene synthase	AAO41727.1
<i>Antirrhinum majus</i> β -ocimene synthase	AAO42614.1
<i>Arabidopsis lyrata</i> linalool synthase	XP_002886523.1
<i>Arabidopsis thaliana</i> β -caryophyllene synthase	AAO85539
<i>Arabidopsis thaliana</i> cdp synthase	Q38802.1
<i>Arabidopsis thaliana</i> kaurene synthase	AAC39443.1
<i>Cannabis sativa</i> α -pinene synthase	ABI21838
<i>Citrus hystrix</i> germacrene D synthase	ADX01384
<i>Citrus jambhiri</i> δ -elemene synthase	BAP74389
<i>Citrus jambhiri</i> geraniol synthase	BAM29049.1
<i>Citrus limon</i> limonene synthase	AAM53944.1
<i>Citrus trifoliata</i> limonene synthase	BAG74774.1
<i>Citrus unshiu</i> β -ocimene synthase	BAD91046.1
<i>Citrus unshiu</i> β -pinene synthase	BAD27260
<i>Clarkia breweri</i> linalool synthase	AAD19840.1
<i>Clarkia concinna</i> linalool synthase	AAD19839.1
<i>Cucurbita maxima</i> kaurene synthase	AAB39482.1
<i>Glycine soja</i> linalool synthase	KHN36571.1
<i>Gossypium arboreum</i> δ -cadinene synthase	CAA76223.1
<i>Gossypium arboreum</i> myrcene synthase	KHG20211.1
<i>Lavandula angustifolia</i> β -phellandrene synthase	ADQ73631.1
<i>Matricaria recutita</i> β -caryophyllene synthase	AFM43734
<i>Mentha aquatica</i> linalool synthase	AAL99381.1
<i>Mentha spicata</i> limonene synthase	AGN90912.1
<i>Mikania micrantha</i> β -caryophyllene synthase	ACN67535
<i>Morus notabilis</i> myrcene synthase	EXB31237.1
<i>Ocimum basilicum</i> geraniol synthase	AAR11765.1
<i>Ocimum basilicum</i> germacrene D synthase	AAV63786
<i>Oryza sativa Japonica Group</i> β -caryophyllene synthase	ABJ16553
<i>Oryza sativa Japonica Group</i> farnesol synthase	ABJ16554
<i>Phylla dulcis</i> β -caryophyllene synthase	AFR23370
<i>Picea sitchensis</i> limonene synthase	ABA86248.1
<i>Pinus taeda</i> α -farnesene synthase	AAO61226.1
<i>Pinus taeda</i> α -pinene synthase	AAO61228.1
<i>Pogostemon cablin</i> germacrene D synthase	AAS86322
<i>Populus trichocarpa</i> cdp synthase	EEE93773.1
<i>Populus trichocarpa</i> kaurene synthase	XP_002311286.1
<i>Solanum lycopersicum</i> β -caryophyllene synthase	ADD96698
<i>Solanum lycopersicum</i> cineole synthase	AEM05857.1
<i>Solanum lycopersicum</i> linalool synthase	AEM05855.1
<i>Thymus caespitosus</i> α -terpineol synthase	AGK88257.1
<i>Vitis vinifera</i> β -caryophyllene synthase	ADR74221
<i>Vitis vinifera</i> geraniol synthase	NP_001267920.1
<i>Vitis vinifera</i> germacrene D synthase	AAS66357.1
<i>Vitis vinifera</i> α -pinene synthase	ADR74203.1
<i>Zea mays</i> cdp synthase	AAT70084.1
<i>Zingiber officinale</i> germacrene D synthase	AAX40665
<i>Zingiber zerumbet</i> α -humulene synthase	BAG12315.1

Supplementary Table S2. RPKM of biosynthetic genes for volatile terpene formation.

Feature ID	Gene length	Green fruit	Ripe fruit
sesquiterpene synthase-like gene			
Zanthoxylum21_contig00027846 (ZpTPS1)	1793	13.7	0.8
Zanthoxylum21_contig00006748 (ZpTPS2)	1915	112.2	1.0
Zanthoxylum21_contig00011403	731	21.4	30.0
Zanthoxylum21_contig00011404	349	14.4	1.4
Zanthoxylum21_contig00014497	670	15.0	6.7
monoterpene synthase-like gene			
Zanthoxylum21_contig00008921 (ZpTPS3)	2293	10.2	3.7
Zanthoxylum21_contig00000439	2284	41.8	45.3
Zanthoxylum21_contig00007231	644	19.1	12.4
Zanthoxylum21_contig00008263	1906	15.8	13.1
Zanthoxylum21_contig00009768	642	27.0	36.4
Zanthoxylum21_contig00020015	227	4.9	4.4
geranyl diphosphate synthase-like gene			
Zanthoxylum21_contig00010188	1726	23.0	42.1
Zanthoxylum21_contig00011418	1133	17.7	17.6
farnesyl diphosphate synthase-like gene			
Zanthoxylum21_contig00008866	1401	29.9	16.3
Zanthoxylum21_contig00010526	1424	6.7	3.5
Zanthoxylum21_contig00013264	482	20.8	32.0