

Expressed Sequence Tags from Citrus Albedo at the Initiation Stage of Rind Peeling

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Abstract

The gene expression and regulation associated with the initial stage of rind peeling of the satsuma mandarin (*Citrus unshiu* cv. 'Miyagawa-wase') was characterized through the generation of 626 expressed sequence tags (ESTs) derived from a cDNA library prepared from albedo tissues collected 60 days after anthesis. A Total of 451 ESTs was found to have significant similarity to the known sequences. Using our in-house database, a catalogue of 222 ESTs annotated with their putative functions was prepared and compared to the previously prepared catalogue of 442 ESTs derived from mature albedo tissues. In consequence, gene homologues associated with cell wall events were preferably found in this library.

Key words: *Citrus unshiu*; ESTs; cDNA library, gene catalogue

Introduction

The analysis of expressed sequence tags (ESTs) has become a part of the genomic approach to plant science for many plant species (Yamamoto and Sasaki, 1997). The cataloguing of ESTs has emerged as a powerful tool capable of revealing gene expression patterns, gene regulation and sequence diversity (Brandle et al., 2002) as well as assisting in the generation of DNA markers for genome mapping (Kuhara et al., 1994). In most cases it has also given an indispensable aid to cDNA microarray analysis. Furthermore, EST databases have recently contributed to the discovery of the specific gene repertoires associated with the fragrances of roses (Guterman et al., 2002) and terpenoids in *Stevia rebaudiana* (Brandle et al., 2002).

In order to understand gene expression and regulation associated with the development and ripening of citrus

fruits, we have completed the EST analysis of several cDNA libraries derived from sweet orange young seeds (Hisada et al., 1996), juice sac of satsuma mandarin during rapid cell development phase (Hisada et al., 1997), satsuma mandarin fruit at mature phase (Moriguchi et al., 1998), albedo of satsuma mandarin at mature phase (Kita et al., 2000) and ovary at the day of anthesis (Shimada et al., in press). We are particularly interested in the traits that contribute to the quality and commercial value of citrus fruits. It is considered that easy-peeling, which is one of the important traits for commercial value in citrus, is related with the relaxation of cell wall in particular (Brummell and Harpster, 2001). Therefore, we have catalogued EST derived from albedo of satsuma mandarin fruits at the initiation stage of peeling of the rind in which the genes related to cell wall relaxation should be highly expressed. In this report, we list 222 cDNA clones, which were identified by their putative functions by database searches and discuss the characteristic features of the

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The catalogued cDNA clones have been submitted to DDBJ under accession numbers AU300309-AU300928.

catalogue.

Materials and Methods

1. Construction of cDNA library

'Miyagawa-wase' variety of satsuma mandarin (*Citrus unshiu* Marc.), which was cultivated at the National Institute of Fruit Tree Science, Okitsu (Shimizu, Shizuoka, Japan), was used as all fruit sample in this study. Samples were collected 60 days after anthesis (July 16, 1999) when the albedo (inner spongy portion) was capable of being peeled from the segment membrane. The average fruit size was 26.2 mm in diameter with a fresh weight of 9.0 g. Albedo tissue was peeled from the segment membrane using a razor, immediately frozen with liquid nitrogen, and stored at -80°C . The construction of the cDNA library and the subsequent sequencing were conducted following the method of Kita et al. (2000). The cDNA library derived from pre-mature albedo tissue was designated as the ALP cDNA library.

2. Data processing and cataloguing

A total of 626 cDNA clones were randomly selected and sequenced from both the 5' and 3' ends of the inserts. Each sequence was compared with the nucleic acid sequences in the DDBJ (<http://www.ddbj.nig.ac.jp/>; release 51, Sep. 2002) and also with our in-house citrus EST database (Fujii et al., 2003) using the FASTA algorithm (Pearson and Lipman, 1988). A significant match was declared when the optimized similarity score (OPT) was greater than 400. ESTs information including sequence data and annotations were entered into our in-house citrus EST database. Following annotation, a catalogue of 222 ESTs based on function was prepared. Preparation of the cDNA library and catalogue derived from mature albedo tissue designated as ALM was previously reported (Kita et al., 2000). Data analysis, including the comparison of the ALP and ALM catalogues was carried out using the in-house database.

Results and Discussion

1. Sequence Analysis

A total of 626 cDNA clones from the ALP cDNA library derived from albedo tissue of satsuma mandarin ('Miyagawa-

wase') were sequenced. The 626 ESTs generated from the library were clustered into 541 independent groups. The average insert size of the resulting ESTs was on ca. 440bps. Comparison of the sequences with ESTs in the DDBJ and our in-house citrus EST database resulted in the identification of 451 sequences with significant homology to known sequences. Among them, 336 ESTs (53.7%) could be functionally identified, 90 ESTs (14.3%) had homology to EST from other organisms and 25 ESTs (4.0%) shared homology with only putative open reading frame of *Arabidopsis thaliana* genomic sequences. There were only 10 ESTs with homology to genes of other citrus species (Table 1). Significant similarities were not detected for the remaining 175 ESTs (28.0%). As a cause of no similarity, it was considered that either the sequences of 3' untranslated region were obtained, or ESTs were derived from unknown genes which had never registered in the DDBJ. The ALP EST data will be registered in the DDBJ for the public use. Two hundred and twenty two ESTs were catalogued and the resulting ESTs are shown in Table 1 with their putative functions and homology search results. Even in case when isozymes of the same function were found among the above 336 ESTs identified the functions, a single example was entered into the catalogue.

2. Gene repertoires of ALP cDNA library

ESTs in ALP cDNA library were classified according to putative function (Table 2). In this library, 20 gene homologues associated with cell wall events were found. Changes in the cell wall including expansion of the cell wall may possibly be related to the easy-peeling characteristic of the satsuma mandarin. Since morphological changes in albedo would be related to the reduction of pectic substances (Kuraoka and Kikuchi, 1961), homologues of pectate lyase (MAP9U30), pectin acetyltransferase (MAPAE60), pectin methylesterase (MAPAE10), polygalacturonase (MAP9U96), arabinogalactan protein (MAPAE91: Shultz et al., 2000) were found and they might be related to such an event. Homologue of glycine-rich protein (MAPAE88: Uthappa et al., 2001) and proline-rich protein (MAPAE26: Salts et al., 1992) are possibly related to cell structure and cell adhesion in the cell wall. Homologues associated to cellulose, which is a major component of cell wall structure, were also detected (MAPF194, MAPH163:

Favery et al., 2001). The above-mentioned MAPEM60 and MAP9U96 had two redundant ESTs each in total of 626 ESTs and were constructed contig sequences respectively.

When the ALP gene catalogue was compared to a catalogue ALM derived from mature albedo tissue, only 44 kinds (80 clones) of 336 gene homologues in ALP were overlapped. Several examples of them were dynamin-like protein (MAPAE24), ubiquitin SMT3 protein (MAPAE81), cystein proteinase (MAPAT76), actin (MAPDD12) and thioredoxin (MAPFF53). Since the percentage of the overlapped clones is low, the ALP library is suitable for the exploration of important genes associated with the maturation of albedo.

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Table 1. Inventory of *C. unshiu* ESTs with significant similarity to deduced genes from other organisms

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPGL13	AU300674	13-lipoxygenase	Responsive	stress	X96405	Solanum melongena	1057	58.4	1013
MAPEF48	AU300517	14-3-3 protein	Unclassified		AF039709	Maackia amurensis	1353	81.0	411
MAPH109	AU300763	21kD cell wall protein	Cell structure	cell wall	Y11553	Medicago sativa	571	66.0	353
MAPEM44	AU300848	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MECS)	Metabolism	secondary	AF250236	Catharanthus roseus	1345	66.9	755
MAPDD68	AU300899	3-deoxy-D-manno-octulosonic acid transferase-like protein (MED24.5)	Metabolism	lipid/membrane formation	AY065115	Arabidopsis thaliana	846	67.8	423
MAPFI02	AU300900	4-methyl-5-(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein putative	Metabolism	vitamin	AF326856	Arabidopsis thaliana	453	72.3	177
MAPDD12	AU300432	actin	Cell structure	skelton	X68649	Pisum sativum	711	84.1	201
MAPGL96	AU300718	actin depolymerizing factor	Cell structure	skelton	AF183904	Petunia x hybrida	1469	81.9	442
MAPDD32	AU300839	adenine nucleotide translocator 1 (CANT1)	Metabolism	nucleic acid	AF006489	Gossypium hirsutum	500	71.2	208
MAPBG79	AU300812	adenylosuccinate synthase	Metabolism	nucleic acid synthesis	U49389	Arabidopsis thaliana	613	74.0	227
MAPDR28	AU300473	ADP-ribosylation factor	Protein regulation	trafficking/G-protein	M95166	Arabidopsis thaliana	704	87.3	181
MAPFF16	AU300632	aldolase (SCA1) cytosolic	Metabolism	sugar	AF308587	Fragaria x ananassa	1234	81.5	368
MAPDD41	AU300914	-cpn60 precursor (alphacpn60)	Protein regulation	folding	U21105	Pisum sativum	705	82.8	204
MAPH120	AU300765	aquaporin PIP1-1 (PIP1-1) putative	Membrane	membrane protein	AF141643	Vitis berlandieri x Vitis rupestris	1230	78.9	408
MAPBG63	AU300421	aquaporin PIP1-3 (PIP1-3) putative	Membrane	membrane protein	AF141899	Vitis berlandieri x Vitis rupestris	1142	81.0	352
MAPAE91	AU300917	arabinogalactan protein (AGP16)	Cell structure	cell wall	AF195897	Arabidopsis thaliana	479	64.7	286
MAP9U70	AU300348	arbutin synthase	Metabolism	secondary	AJ310148	Rauwolfia serpentina	567	69.8	255
MAPGL32	AU300927	aspartic protease precursor	Protein regulation	protease	L46681	Lycopersicon esculentum	647	66.5	337

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPAE07	AU300880	-1,3-glucanase putative	Cell structure	cell wall	AY065273	Arabidopsis thaliana	856	71.8	341
MAPGL79	AU300710	-hydroxybutyryl-CoA dehydrogenase (hbdA)	Metabolism	lipid	U32229	Bradyrhizobium japonicum	520	62.4	338
MAPAT93	AU300403	-tubulin 4	Cell structure	skelton	D63136	Zinnia elegans	806	71.3	373
MAPFF37	AU300642	-tubulin 5 (Tubb5)	Cell structure	skelton	U76896	Triticum aestivum	1734	85.4	471
MAPEF36	AU300512	biotin carboxyl carrier protein subunit precursor (accB-2), of acetyl CoA carboxylase	Metabolism	lipid	AF271071	Glycine max	607	79.2	197
MAPGR85	AU300846	Ca ²⁺ /H ⁺ exchanger	Membrane	transport	AB012932	Vigna radiata	918	70.9	381
MAP9U41	AU300338	calcium binding protein	Signal	calcium	AJ309072	Sesbania rostrata	425	70.9	172
MAPGR59	AU300743	calmodulin	Signal	calcium	X60738	Malus domestica	732	84.3	204
MAPEF07	AU300501	carboxypeptidase II, serine-type putative	Protein regulation	proteinase	AF370244	Arabidopsis thaliana	570	72.0	225
MAPFF21	AU300635	catalase	Responsive	stress	AF151368	Hevea brasiliensis	703	74.2	275
MAP9U22	AU300856	catechol O-methyl transferase 5	Cell structure	lignin	AF064695	Thalictrum tuberosum	445	61.9	320
MAPH180	AU300820	CBL-interacting protein kinase 3 (CIPK3)	Signal	calcium	AF286051	Arabidopsis thaliana	1210	75.2	431
MAPF194	AU300624	cellulose synthase catalytic subunit (celA3)	Cell structure	cell wall	AF150630	Gossypium hirsutum	1564	88.1	395
MAPH163	AU300811	cellulose synthase like CSLD3 (CSLD3) involved in biosynthesis of non-cellulosic beta-glucan-containing polysaccharide	Cell structure	cell wall	AF232907	Arabidopsis thaliana	869	73.1	334
MAPFF60	AU300842	centrin	Responsive	defense/cytoskelton	AJ009672	Arabidopsis thaliana	1235	77.8	427
MAPGL63	AU300845	chaperonin 60	Protein regulation	folding	X70867	Cucurbita sp.	1403	80.9	430
MAPEI58	AU300537	chitinase-like protein 1 (CTL1)	Responsive	defense	AF422179	Arabidopsis thaliana	706	74.0	281
MAPEI91	AU300853	chlorophyll a/b-binding protein	Metabolism	photosystem	M85150	Arabidopsis thaliana	778	75.5	278

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPFF75	AU300660	chloroplast nucleoid DNA-binding protein putative	Chloroplast	nuclear protein	AY080874	Arabidopsis thaliana	799	65.1	455
MAPEM08	AU300550	chorismate mutase, cytosolic CM2	Metabolism	secondary	AJ242648	Arabidopsis thaliana	460	69.4	209
MAPGR29	AU300731	chromatin protein (SUVH1) with SET domain	Cell structure	chromatin	AF344444	Arabidopsis thaliana	807	67.7	393
MAPEM35	AU300557	cinnamate 4-hydroxylase	Metabolism	secondary	AF255014	Citrus sinensis	1735	98.1	359
MAPH142	AU300769	cinnamyl alcohol dehydrogenase	Metabolism	secondary	U63534	Fragaria x ananassa	1493	68.9	730
MAPEI93	AU300547	circadian clock coupling factor ZGT	Growth regulation	light & circadian	AF368237	Nicotiana tabacum	482	69.7	208
MAP9U91	AU300818	clathrin binding protein putative	Protein regulation	trafficking	AY074304	Arabidopsis thaliana	682	73.1	353
MAPEM96	AU300588	coatmer putative	Protein regulation	trafficking	AY090955	Arabidopsis thaliana	1458	82.9	420
MAPAE52	AU300380	coatmer epsilon subunit protein putative	Protein regulation	trafficking	AF348588	Arabidopsis thaliana	1044	75.3	384
MAPEM06	AU300815	copper chaperone homolog	Protein regulation	folding	AF198627	Glycine max	673	75.5	245
MAPGR94	AU300823	cp protein IM30 protein	Chloroplast		M73744	Pisum sativum	561	76.0	204
MAPEM59	AU300569	cystatin-like protein	Protein regulation	proteinase	AF283536	Citrus x paradisi	2192	98.0	454
MAPAT76	AU300915	cysteine proteinase putative	Protein regulation	proteinase	Z47793	Citrus sinensis	3006	96.0	651
MAPAT95	AU300404	cysteine proteinase precursor	Protein regulation	proteinase	AF417109	Carica candamarcensis	857	73.2	340
MAPBG47	AU300416	cytochrome P450 (At2g23180; T20D16.19) putative	Responsive	defense	AF370589	Arabidopsis thaliana	592	68.3	293
MAP9U84	AU300352	cytochrome P-450, n-alkane inducible ALK6-A (CYP52C2)	Responsive	defense	D12718	Candida maltosa	414	69.5	117
MAPGL58	AU300863	dehydrin (DHN1 gene) putative	Responsive	stress	AJ300524	Populus euramericana	751	73.1	323
MAPEF53	AU300518	dihydroflavonol reductase	Metabolism	secondary	AY042880	Arabidopsis thaliana	988	81.1	302

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPGR75	AU300813	disease resistance-like protein GS0-1	Responsive	defense	AF403250	Glycine max	420	67.1	213
MAPAT72	AU300840	disease responsive SOR1 putative	Responsive	defense	AF370296	Arabidopsis thaliana	960	78.9	327
MAPEF04	AU300499	DnaJ-1	Protein regulation	folding	X67695	Cucumis sativus	996	78.6	336
MAPDR18	AU300468	dormancy associated protein	Growth regulation	dormancy	AF053746	Arabidopsis thaliana	803	70.0	373
MAPBG29	AU300411	dTDP-glucose 4-6-dehydratases putative	Metabolism	carbohydrate	Z49239	Arabidopsis thaliana	551	75.5	208
MAPAE24	AU300370	dynammin-like protein SDL12	Cell structure	skelton	U25547	Glycine max	776	81.5	232
MAPEM43	AU300816	early nodulin ENOD18	Growth regulation	nodulation	AJ271816	Vicia faba	1152	70.7	501
MAPEM18	AU300554	elongation factor 1 alpha subunit	Translation	elongation	AJ223969	Malus domestica	1721	86.1	460
MAPEM71	AU300576	elongation factor fusA	Translation	elongation	X71439	Glycine max	1450	81.9	425
MAP9C52	AU300800	endomembrane protein EMP70 precursor isolog putative	Membrane	membrane protein	AY063907	Arabidopsis thaliana	1360	80.9	413
MAPF111	AU300591	enolase	Metabolism	sugar	Z28386	Ricinus communis	1232	77.5	427
MAPH112	AU300764	ent-kaurenoic acid hydroxylase (KA02)	Signal	hormone(GA)	AF318501	Arabidopsis thaliana	532	62.7	332
MAPF171	AU300614	expansin 2	Growth regulation	expansin	AF297522	Prunus avium	1093	78.3	359
MAPGR18	AU300728	expansin 3	Growth regulation	expansin	AF527800	Malus x domestica	1733	79.7	713
MAPBG04	AU300909	-adaptin 1	Protein regulation	trafficking	AF061286	Arabidopsis thaliana	1239	78.8	402
MAPEF40	AU300514	germin putative	Responsive	stress	AF417299	Castanea sativa	700	70.9	302
MAPBG88	AU300429	glutamine synthetase	Metabolism	amino acid	AF003197	Hevea brasiliensis	1067	80.4	328
MAPEI54	AU300826	glutaredoxin (Cl31)	Metabolism	energy	AF406809	Tilia platyphyllos	675	77.3	291

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPGL46	AU300691	glutathione S-transferase/ peroxidase (BI-GST/GPX)	Responsive	defense	AF193439	Lycopersicon esculentum	1707	73.8	660
MAPF136	AU300600	glutathione-conjugate transporter	Responsive	defense	AJ002584	Arabidopsis thaliana	567	71.5	228
MAPDD53	AU300912	glyceraldehyde 3-phosphate dehydrogenase protein putative	Metabolism	energy	AF348583	Arabidopsis thaliana	579	64.6	381
MAPGL66	AU300702	glycine decarboxylase P protein, a part of glycine cleavage complex	Metabolism	amino acid	X59773	Pisum sativum	1403	83.1	403
MAPAE88	AU300913	glycine-rich protein LeGRP1	Cell structure	cell wall	AY026037	Lycopersicon esculentum	459	65.8	231
MAPDR74	AU300898	glyoxalase I (lactoyl-glutathione lyase)	Responsive	defense/detox	AJ224520	Cicer arietinum	1796	75.5	664
MAP9C03	AU300311	GTP-binding protein	Transcription	G-protein	D12542	Pisum sativum	636	77.7	216
MAPGL01	AU300881	guanine nucleotide regulatory protein (ran-related GTP-binding protein)	Transcription	G-protein	Z24678	Vicia faba	1643	84.4	469
MAPGR92	AU300757	heat shock protein 70 kD	Responsive	stress	X99197	Medicago sativa	818	77.7	283
MAPDD13	AU300433	heterochromatin protein LHP1	Cell structure	chromatin	AF387639	Arabidopsis thaliana	450	78.2	147
MAPFF71	AU300659	histidine kinase receptor	Signal	protein receptor	AJ298990	Fagus sylvatica	1216	78.3	410
MAP9C12	AU300850	histone acetyltransferase	Cell structure	chromatin	AY099684	Arabidopsis thaliana	875	78.3	286
MAPAE25	AU300371	histone H1 putative	Cell structure	chromatin	AY045797	Arabidopsis thaliana	695	62.4	481
MAPF179	AU300618	histone H4	Cell structure	chromatin	Z79638	Sesbania rostrata	1066	78.9	374
MAPF109	AU300590	HMG beta 1 protein	Protein regulation	folding	Y14072	Arabidopsis thaliana	1119	74.5	345
MAPEM95	AU300907	hormone responsive CS-IAA2	Responsive	hormone (auxin)	AB026822	Cucumis sativus	618	64.2	402
MAPF155	AU300893	hormone responsive Nr-iaa28 deduced protein	Responsive	hormone (auxin)	AF123508	Nicotiana tabacum	563	92.5	133
MAPF147	AU300810	hormone responsive-ADR11	Responsive	hormone (auxin)	X69640	Glycine max	1028	68.8	535

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPAT85	AU300921	hormone responsive GAI-like protein 1 (GAI1)	Responsive	hormone	AF378125	Vitis vinifera	407	67.8	199
MAPH165	AU300779	IAA-amino acid hydrolase	Signal	hormone	AY074380	Arabidopsis thaliana	537	69.5	249
MAP9U75	AU300350	imbibition protein putative	Responsive	stress	AJ271668	Cicer arietinum	620	67.1	301
MAPEM79	AU300821	importin alpha (LeKAP alpha)	Membrane	transport (nuclear) disease response	AF017252	Lycopersicon esculentum	719	77.3	238
MAP9C10	AU300885	laminin receptor-like protein (40S ribosomal protein SA)	Translation	ribosomal	U01955	Arabidopsis thaliana	1113	77.0	381
MAPEM68	AU300574	Lea5 protein	Responsive	stress	Z46824	Citrus sinensis	1845	95.4	417
MAPF173	AU300616	leucine rich repeat	Signal	protein receptor	Y07748	Oryza sativa	1505	75.6	553
MAPEI53	AU300814	lipoxigenase(loxC)	Responsive	defence	U37839	Lycopersicon esculentum	529	61.0	419
MAPH151	AU300832	membrane protein RER1 related to ER retrieval	Protein regulation	trafficking	AB010946	Arabidopsis thaliana	776	73.2	295
MAPGL03	AU300669	metallothionein-like protein	Responsive	stress	AF320905	Citrus unshiu	1916	98.0	400
MAPEI88	AU300544	methionine synthase	Metabolism	amino acid	X83499	Catharanthus roseus	723	77.1	258
MAPH157	AU300776	miraculin-like protein	Protein regulation	proteinase	AF283533	Citrus x paradisi	3678	93.4	843
MAPDD55	AU300448	mitochondrial F0 ATP synthase D chain	Membrane	ATPase	AJ271469	Arabidopsis thaliana	1370	77.3	481
MAPF133	AU300597	monodehydroascorbate reductase	Metabolism	vitamin	U06461	Pisum sativum	1312	78.3	461
MAPBG16	AU300847	myb-like DNA-binding protein	Transcription	myb	AJ251686	Catharanthus roseus	522	70.0	243
MAPAE74	AU300388	myb-related protein putative	Transcription	myb	AY063912	Arabidopsis thaliana	1006	74.1	401
MAPF134	AU300598	myb-related transcription factor	Transcription	myb	AF161711	Pimpinella brachycarpa	1332	67.6	735
MAPGR02	AU300720	nitrilase associated protein NAP16kDa	Signal	hormone (IAA)	Z96936	Arabidopsis thaliana	476	66.4	265

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPFF09	AU300829	nuclease (nucZe2) bifunctional	Growth regulation	senescence	U90266	Zinnia elegans	718	72.6	299
MAPEM15	AU300553	nucleoside diphosphate kinase	Metabolism	nucleic acid	U50150	Glycine max	1451	80.7	460
MAPF183	AU300619	nucleotide sugar epimerase-like protein (F14O13.1)	Metabolism	nucleic acid	AY062625	Arabidopsis thaliana	496	71.0	224
MAP9C02	AU300310	O-acetylserine (thiol) lyase B	Metabolism	amino acid (cystein)	AJ271728	Arabidopsis thaliana	579	72.6	249
MAPAT89	AU300905	pathogenesis-related protein 5	Responsive	defense	AF154636	Nicotiana tabacum	1462	79.1	470
MAPFF81	AU300664	pathogenesis-related protein PR1 (Ypr1)	Responsive	defense	U64806	Brassica napus	401	60.5	352
MAP9U30	AU300336	pectate lyase	Cell structure	cell wall	AB048260	Salix gligiana	653	79.7	217
MAPAE10	AU300361	pectin methyltransferase	Cell structure	cell wall	Z71753	Nicotiana plumbaginifolia	634	75.8	227
MAPEM60	AU300570	pectin acetyltransferase	Cell structure	cell wall	X99348	Vigna radiata	717	70.5	312
MAPGR46	AU300738	pectinesterase (PECS-1.1)	Cell structure	cell wall	U82973	Citrus sinensis	1992	96.5	423
MAPEM84	AU300584	permease, possibly related to nucleobase-ascorbate transporter	Membrane	transporter	AY093137	Arabidopsis thaliana	566	74.4	211
MAPFF65	AU300656	peroxidase	Responsive	stress	L08199	Gossypium hirsutum	1577	84.9	430
MAPGL16	AU300676	peroxidase ascorbate cytosolic	Responsive	stress	AF022213	Fragaria x ananassa	679	73.7	266
MAPEM25	AU300825	peroxidase isozyme 40K precursor cationic	Responsive	stress	D42065	Nicotiana tabacum	475	60.0	380
MAPGL85	AU300712	phosphate/triose-phosphate translocator TPT precursor, non-green plastid	Metabolism	sugar	U13632	Brassica oleracea	409	63.2	359
MAPEI74	AU300541	phosphoenolpyruvate carboxylase (pepc)	Metabolism	energy	AF512995	Citrus junos	1520	98.1	313
MAPDR88	AU300492	photosystem II type I chlorophyll a/b binding protein	Metabolism	photosystem	X64459	Arabidopsis thaliana	432	69.8	222
MAPFF85	AU300665	plasma membrane intrinsic protein (SIMIP)	Membrane	membrane protein	AF003728	Arabidopsis thaliana	2787	78.2	930

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPFF47	AU300851	plastid-specific ribosomal protein 3 precursor (Psrp-3)	Translation	ribosomal	AF239218	Spinacia oleracea	898	66.4	533
MAPFF19	AU300802	poly(A)-binding protein (PABP)	Translation	mRNA stability	AF190655	Nicotiana tabacum	1327	79.1	431
MAP9U96	AU300356	polygalacturonase isoenzyme 1 beta subunit	Cell structure	cell wall	U63373	Arabidopsis thaliana	585	59.8	423
MAPAE05	AU300894	polyubiquitin (ubq4)	Protein regulation	proteolysis	U33014	Arabidopsis thaliana	1511	83.4	430
MAPF167	AU300612	polyubiquitin (ubq8)	Protein regulation	proteolysis	L05917	Arabidopsis thaliana	840	64.8	486
MAPGR01	AU300719	polyubiquitin rub1	Protein regulation	proteolysis	X76064	Oryza sativa	1633	85.7	434
MAPAE26	AU300857	proline rich protein, TPRP-F1	Cell structure	cell wall	X61395	Lycopersicon esculentum	739	64.1	473
MAPDR59	AU300484	proteasome (26S) beta subunit	Protein regulation	proteolysis	D78172	Spinacia oleracea	591	70.8	243
MAPDD40	AU300791	proteasome (26S) subunit 4-like protein SPH	Protein regulation	proteolysis	AJ291500	Brassica napus	949	75.6	356
MAPGL88	AU300714	protein kinase (fl-E6)	Protein regulation	PK	AF218378	Gossypium hirsutum	416	59.3	376
MAPFF11	AU300630	protein kinase Ndr-kinase	Protein regulation	PK	AB047278	Arabidopsis thaliana	496	67.4	239
MAPGR17	AU300727	protein kinase Pti1 kinase-like protein (Pti1a)	Responsive	defense	AF249317	Glycine max	923	80.8	286
MAPGL48	AU300692	protein kinase-like protein	Protein regulation	PK	AY099731	Arabidopsis thaliana	433	65.0	254
MAPAE85	AU300392	protein phosphatase 2C putative	Protein regulation	phosphatase	AY050873	Arabidopsis thaliana	906	73.0	348
MAPDD90	AU300827	proteinase nClpP4 (nuclear encoded ClpP4)	Protein regulation	proteinase	AB022329	Arabidopsis thaliana	674	64.7	394
MAPDD35	AU300441	psaG Photosystem I subunit V precursor	Metabolism	photosystem	AJ245630	Arabidopsis thaliana	1202	70.3	583
MAPGR71	AU300843	psbO mRNA for oxygen evolving enhancer protein 1 precursor	Metabolism	photosystem	AB043980	Brugiera gymnorrhiza	797	78.5	265
MAPGR11	AU300726	pyruvate decarboxylase	Metabolism	energy	AF193791	Fragaria x ananassa	1866	81.9	570

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPEM48	AU300803	QM family gene (60S ribosomal protein L10)	Translation	ribosomal	AB001891	Solanum melongena	2124	80.6	654
MAPEI72	AU300540	raffinose synthase	Metabolism	sugar	E25448	Glycine max	984	76.4	348
MAP9U94	AU300355	ran GTPase activating protein	Transcription	nuclear import	AF215731	Medicago sativa	770	68.7	364
MAPFF33	AU300908	RD2 protein, responsive to desiccation	Responsive	stress	AB039925	Arabidopsis thaliana	544	72.4	221
MAPDR38	AU300478	receptor-kinase isolog putative	Signal	protein receptor	AY056128	Arabidopsis thaliana	854	73.9	322
MAPEM04	AU300849	receptor-like LRR protein kinase	Signal	protein receptor	M84660	Arabidopsis thaliana	630	62.3	477
MAPAT67	AU300397	receptor-like protein kinase (RLK)	Signal	protein receptor	AF149037	Prunus dulcis	861	79.2	288
MAPGR89	AU300790	receptor-like protein kinase (WRK)	Responsive	defense	AB073628	Nicotiana tabacum	1762	71.5	758
MAPGR49	AU300740	ribonucleoprotein-like	Group protein		AY136466	Arabidopsis thaliana	749	63.9	451
MAPEF47	AU300516	ribosomal protein small subunit 4e	Translation	ribosomal	X79300	Gossypium hirsutum	1404	84.0	393
MAPFF27	AU300638	ribosomal (40S) protein	Translation	ribosomal	AY054622	Arabidopsis thaliana	718	79.6	230
MAPF166	AU300611	ribosomal (40S) protein S15 (Sb23) probable	Translation	ribosomal	AF051217	Picea mariana	1319	80.7	436
MAPFF29	AU300639	ribosomal (40S) protein S5 putative	Translation	ribosomal	AY045846	Arabidopsis thaliana	960	72.1	408
MAPAE78	AU300390	ribosomal (50S) protein L28 chloroplast putative	Translation	ribosomal	AY072373	Arabidopsis thaliana	595	68.6	296
MAPAE13	AU300363	ribosomal (50S) protein L29 putative	Translation	ribosomal	AY045966	Arabidopsis thaliana	960	62.8	651
MAPDR10	AU300465	ribosomal (60S) protein (rib 60S)	Translation	ribosomal	AJ278460	Juglans regia	994	71.2	425
MAPFF80	AU300663	ribosomal (60S) protein L2	Translation	ribosomal	X62500	Nicotiana tabacum	1154	77.7	382
MAPFF50	AU300649	ribosomal (60S) protein L37 putative	Translation	ribosomal	AF370216	Arabidopsis thaliana	847	81.4	296

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPGR28	AU300730	ribosomal protein L17 (rpl17)	Translation	ribosomal	AF034948	Zea mays	805	77.3	269
MAP9C49	AU300326	ribosomal protein L17-1	Translation	ribosomal	AF264022	Poa secunda	1447	69.5	656
MAPGL62	AU300700	ribosomal protein L19	Translation	ribosomal	Z31720	Nicotiana tabacum	2095	76.5	761
MAPBG72	AU300424	ribosomal protein L26 like	Translation	ribosomal	D78495	Brassica rapa	1295	72.5	523
MAPGR47	AU300739	ribosomal protein L5 putative	Translation	ribosomal	AY056194	Arabidopsis thaliana	441	72.5	171
MAPAE16	AU300365	ribosomal protein S14 (rps14)	Translation	ribosomal	AF026079	Lupinus luteus	1423	78.3	425
MAPEM51	AU300563	ribosomal protein S24 40S	Translation	ribosomal	AF298769	Zea mays	1147	69.7	554
MAP9U44	AU300340	ribosomal protein S6 (rps6 gene)	Translation	ribosomal	AJ277533	Asparagus officinalis	2395	76.6	824
MAP9U10	AU300332	ribosomal protein S8	Translation	ribosomal	U64436	Zea mays	827	70.6	361
MAPEM64	AU300571	ribosomal protein L23	Translation	ribosomal	AF349961	Daucus carota	900	74.9	351
MAPF107	AU300805	RING zinc finger ankyrin protein putative	Transcription	zinc finger	AY042842	Arabidopsis thaliana	540	67.4	258
MAPF139	AU300601	RNA helicase DRH1	Transcription	RNA helicase	AY062591	Arabidopsis thaliana	983	76.3	334
MAPFF15	AU300631	RNA helicase ATP-dependent putative	Transcription	RNA helicase	AY091050	Arabidopsis thaliana	614	68.5	289
MAP9C42	AU300793	RSP ribosome sedimenting protein	Translation	ribosomal	AB021873	Pisum sativum	482	61.9	391
MAP9C55	AU300819	S-adenosyl-homocysteine hydrolase (SHH)	Metabolism	energy	M81885	Petroselinum crispum	555	78.3	198
MAPH176	AU300782	S-adenosyl-L-methionine decarboxylase proenzyme	Metabolism	energy	U12573	Catharanthus roseus	629	84.3	178
MAPGL26	AU300683	S-adenosyl-L-methionine synthetase	Metabolism	energy	AJ277206	Camellia sinensis	2486	82.6	748
MAPAE80	AU300391	serine carboxypeptidase precursor (SCP) putative	Protein regulation	proteinase	AY072822	Gossypium hirsutum	948	71.6	405

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAP9U71	AU300806	serine palmitoyltransferase	Metabolism	amino acid	AJ242659	Solanum tuberosum	878	71.3	391
MAPFF87	AU300667	serine protease	Protein regulation	proteinase	X98929	Lycopersicon esculentum	1913	69.1	895
MAPF178	AU300920	serine/threonine protein kinase-like protein	Protein regulation	proteinase	AY062765	Arabidopsis thaliana	641	73.1	253
MAPDD42	AU300901	SF16 pollen specific gene putative	Morphogenesis	pollen	AY099822	Arabidopsis thaliana	680	78.0	223
MAPFF73	AU300844	factor SigB	Transcription	TF	AB019943	Arabidopsis thaliana	532	67.3	272
MAPGL92	AU300717	sinapyl alcohol dehydrogenase	Cell structure	lignin	AF273256	Populus tremuloidea	1189	79.8	371
MAPAE58	AU300384	small GTP-binding protein	Transcription	G-protein	Z49902	Pisum sativum	909	73.0	381
MAPGL10	AU300673	small GTP-binding protein rab-type	Transcription	G-protein	AB024994	Cicer arietinum	895	76.1	326
MAP9C07	AU300313	splicing factor (ORF1) putative	Transcription	splicing	AJ299058	Cicer arietinum	590	80.6	181
MAP9C48	AU300325	starch synthase granule-bound	Metabolism	carbohydrate	AF097922	Astragalus membranaceus	1129	75.1	410
MAPFF18	AU300836	stearyl-acyl-carrier protein desaturase	Metabolism	lipid	M59857	Ricinus communis	1287	80.2	405
MAPAT92	AU300402	stress responsive phi-1 protein putative	Responsive	stress	AF326891	Arabidopsis thaliana	435	76.7	150
MAPF104	AU300903	stress responsive-SRG1	Responsive	stress	X79052	Arabidopsis thaliana	437	66.0	244
MAPFF42	AU300645	subtilisin serine protease putative	Protein regulation	proteinase	AY051009	Arabidopsis thaliana	533	69.3	238
MAPGL64	AU300701	sucrose synthase CitUSA	Metabolism	sugar	AB022091	Citrus unshiu	1373	94.7	304
MAPF176	AU300617	suppressor of K+ transport growth defect-like protein (SKD1)	Membrane	transport	AF165422	Mesembryanthemum crystallinum	1408	85.3	380
MAPH194	AU300789	synaptobrevin-related protein (SAR1)	Protein regulation	trafficking	M90418	Arabidopsis thaliana	983	70.7	427
MAPGR74	AU300750	TFIIIH p44/SSLI-like protein	Transcription	TF	AF499443	Arabidopsis thaliana	862	76.4	296

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identity (%)	Over lap
			first	second					
MAPFF53	AU300651	thioredoxin	Metabolism	energy	Z70677	Ricinus communis	1267	80.9	404
MAPEI40	AU300532	threonine synthase	Metabolism	amino acid	AF082894	Solanum tuberosum	740	69.9	346
MAPH168	AU300780	transaldolase (TAL)	Metabolism	sugar	AF184164	Lycopersicon esculentum	465	73.9	180
MAPGR34	AU300733	transcription factor KAP2	Transcription	TF / CHS	AF293343	Medicago truncatula	1037	77.6	340
MAPGR60	AU300744	translation initiation factor	Translation	initiation	AF098672	Brassica oleracea	1184	81.6	358
MAPGL73	AU300707	translational elongation factor Tu (tufA) chloroplast	Translation	elongation	AF234537	Pelargonium graveolens	1008	77.0	378
MAPEF57	AU300521	translationally controlled tumor protein (TCTP)	Growth regulation	cell cycle	AF091455	Hevea brasiliensis	1907	83.1	568
MAPFF10	AU300906	transporter MATE efflux family protein putative	Membrane	transporter	AF360254	Arabidopsis thaliana	592	64.9	353
MAPEF14	AU300928	triosphosphate isomerase	Metabolism	sugar	J04121	Coptis japonica	1161	82.7	336
MAPFF62	AU300655	ubiquitin	Protein regulation	proteolysis	X67957	Arabidopsis thaliana	1150	80.3	356
MAPF115	AU300593	ubiquitin activating enzyme E1 (ECR1) putative	Protein regulation	proteolysis	AF051135	Arabidopsis thaliana	1222	78.6	392
MAPGR03	AU300721	ubiquitin conjugating enzyme (UBC4)	Protein regulation	proteolysis	L29077	Pisum sativum	1770	78.1	603
MAPFF44	AU300647	ubiquitin extension protein UBQ1putative	Protein regulation	proteolysis	AY035148	Arabidopsis thaliana	1298	85.1	397
MAPAE81	AU300879	ubiquitin SMT3 protein	Protein regulation	proteolysis	X99609	Arabidopsis thaliana	818	82.6	235
MAPF149	AU300604	ubiquitin/ribosomal protein 27a (Ubi)	Protein regulation	proteolysis	AF298826	Prunus avium	1787	89.9	436
MAPEI33	AU300530	UDP-glucose dehydrogenase	Metabolism	sugar	U53418	Glycine max	1200	83.8	339
MAPGL77	AU300824	UMP synthase (pyr5-6)	Metabolism	energy	U22260	Nicotiana tabacum	541	72.9	218
MAPDD60	AU300452	vacuolar H ⁺ -ATPase subunit E	Membrane	ATPase	AF165939	Citrus limon	1717	97.7	356

Table 1. Continued

Clone	Accession	Putative identity / Homolog	Category of Homolog		Accession of Homolog	Organism	opt	Identify (%)	Over lap
			first	second					
MAPGL06	AU300671	vacuolar H ⁺ -pyrophosphatase (vp1)	Membrane	transporter	AF367446	Prunus persica	1495	85.4	431
MAPGL74	AU300708	vitronectin-like adhesion protein (elongation factor-1 alpha)	Translation	elongation	U04632	Nicotiana tabacum	734	71.9	334
MAPEM39	AU300558	water channel protein	Membrane	membrane protein	U73467	Mesembryanthemum crystallinum	1220	77.8	427
MAP9C39	AU300320	xyloglucan endotransglycosylase-related protein XTR-7 putative	Cell structure	cell wall	U43489	Arabidopsis thaliana	530	65.8	310
MAPAE93	AU300919	zinc-finger C2H2 protein SERRATE (SE)	Transcription	zinc finger	AF311221	Arabidopsis thaliana	605	69.0	277
MAPAE17	AU300817	zinc-finger protein Lsd1 negative regulator to cell death	Transcription	zinc finger	U87833	Arabidopsis thaliana	552	63.8	345

Table 2. Functional classification of *C. unshiu* fruit ESTs from albedo at initiation stage of rind peeling. The ESTs that had sequence similarity to known genes were classified based on their biological functions.

Classification based on biological function		Number of ESTs with matches (non-redundant)	Number of ESTs with matches (redundant)	Classification based on biological function		Number of ESTs with matches (non-redundant)	Number of ESTs with matches (redundant)
Metabolism		55	59	Responsive		68	95
	Photo/electron	16	16		Defense	14	15
	Sugar/carbohydrate	14	16		Hormone	5	7
	Amino acid	6	6		Stress	49	73
	Nucleic acid	4	4	Transcription		19	19
	Vitamin	3	3		Zinc finger	4	4
	Lipid	4	4		G-protein	5	5
	Secondary	8	10		Myb	3	3
Cell structure		36	39		TF	3	3
	Cell wall	20	21		Helicase	2	2
	Skelton	5	5		Nuclear transport	1	1
	Lignin	3	4		Splicing	1	1
	Chromatin	8	9	Translation		36	43
Membrane		21	26		Initiation factor	2	3
	Transport	7	7		Elongation	6	8
	ATPases	2	2		Ribosomal proteins	27	30
	Membrane protein	12	17		mRNA stability	1	2
Growth regulation		25	38	Protein regulation		53	61
	Cell cycle	6	10		Folding	7	8
	Nodulation	1	1		PK	3	3
	Expansin	7	9		Phosphatase	1	1
	Dormancy	9	16		Proteinase	17	21
	Light & circadian	1	1		Proteolysis	15	16
	Senescence	1	1		Trafficking	10	12
Morphogenesis		1	1				
	Pollen	1	1	Chloroplast protein		2	2
Signaling		12	12	unclassified		8	8
	Calcium	4	4				
	Hormone synthesis	3	3				
	Protein receptor	5	5	Total		336	403

ウンシュウミカン果実の剥皮開始期における アルベドに由来する EST の解析

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摘 要

ウンシュウミカン果実の剥皮開始期アルベドで発現した遺伝子から作成した cDNA ライブラリーについて、ランダムに cDNA を選択してシーケンスし、626 の cDNA クローンの EST を得た。相同性検索による EST の機能解析の結果、336 クローン(53.7%)で遺伝子機能が推定された。残りのクローンのうち、90 クローン(14.3%)

では他の生物の EST と相同性があり 25 クローン(4.0%)についてはシロイヌナズナのゲノム塩基配列とのみ相同性があった。残りの 175 クローン(28.0%)については他の生物の塩基配列との相同性は見られなかった。機能が推定されたクローンのうち 222 クローンについて、機能推定の結果を一覧表に示した。この cDNA ライブラリーの特徴として、細胞壁にかかわる遺伝子が多くみられた。

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