Plant Cell Rep (2008) 27:931–938
DOI 10.1007/s00299-008-0518-9

GENETICS AND GENOMICS

Expressed sequence tags from persimmon at different developmental stages

T. Nakagawa · A. Nakatsuka · K. Yano · S. Yasugahira · R. Nakamura · N. Sun · A. Itai · T. Suzuki · H. Itamura

Received: 17 December 2007 / Revised: 28 January 2008 / Accepted: 10 February 2008 / Published online: 27 February 2008

Abstract Persimmon (*Diospyros kaki* Thunb.) is an important fruit in Asian countries, where it is eaten as a fresh fruit and is also used for many other purposes. To understand the molecular mechanism of fruit development and ripening in persimmon, we generated a total of 9,952 expressed sequence tags (ESTs) from randomly selected clones of two different cDNA libraries. One cDNA library was derived from fruit of "Saijo" persimmon at an early stage of development, and the other from ripening fruit. These ESTs were clustered into 6,700 non-redundant sequences. Of the 6,700 non-redundant sequences evaluated, the deduced amino acid sequences of 4,356 (65%) showed significant homology to known proteins, and 2,344 (35%) showed no significant similarity to any known proteins in *Arabidopsis* databases. We report comparison of genes identified in the two cDNA libraries and describe some putative genes involved in proanthocyanidin and carotenoid synthesis. This study provides the first global overview of a set of genes that are expressed during fruit development and ripening in persimmon.

Keywords Carotenoid · *Diospyros kaki* · Fruit · Proanthocyanidin · Ripening

Communicated by P. Puigdomenech.

T. Nakagawa
Department of Molecular and Functional Genomics,
Center for Integrated Research in Science, Shimane University,
Matsue 690-8504, Japan

A. Nakatsuka (✉) · S. Yasugahira · R. Nakamura · N. Sun · H. Itamura
Faculty of Life and Environmental Sciences,
Shimane University, Matsue 690-8504, Japan
e-mail: nakira@life.shimane-u.ac.jp

K. Yano
Graduate School of Agricultural and Life Sciences,
The University of Tokyo, Tokyo 113-8657, Japan

A. Itai
Faculty of Agriculture, Tottori University,
Tottori 680-8533, Japan

T. Suzuki
Graduate School of Science, Nagoya University,
Nagoya 464-8601, Japan

Introduction

Persimmons (*Diospyros* sp.) are distributed in tropical, subtropical and temperate regions of the world (Yonemori 1997). Oriental persimmon or kaki (*Diospyros kaki* Thunb.) is an important fruit in Asian countries such as Japan, China and Korea. In Japan, persimmon ranks fourth in production after citrus (satsuma mandarin and other citrus), apple, and Japanese pear (Yonemori 1997; Kanayama 2006).

Persimmon fruits are rich in vitamins. The vitamin A and C contents are almost equal to those of satsuma mandarin and strawberry, respectively. The vitamin A is derived from carotenoids, the pigments found in skin and flesh. Quantitative analysis of carotenoids shows that the main pigment in yellow- to orange-colored fruit is beta-cryptoxanthin, and that lycopene is the major pigment in red-colored fruit. These colors give the fruit an attractive appearance, which is preferred by consumers. Polyphenols (tannins) are abundant in persimmon fruit, and have an astringent flavor. Carbon dioxide and ethanol treatments are needed for the removal of astringency (Kitagawa and Glucina 1984; Taira 1996). Epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate are

components of soluble tannin, which occurs at 1% in the ripening astringent persimmon fruit (Matsuo and Ito 1978). Tannins have been shown to function as inhibitors of cancer (Fujiki 1999, 2005), heart disease (Chang et al. 2001), hypertension (Sasakawa1955; Mekhfi et al. 2006) and can ameliorate the symptoms of a hangover (Ogata 1976; Okuma et al. 1995). They are therefore in the spotlight as components of functional foods. Persimmon has been used not only as a fresh fruit, but also for many other purposes. For example, a tannin extract known as "kaki-shibu" has been used as water-proofing for fishing nets, paper and umbrellas, and as a fining agent to remove proteins in the production of Japanese sake (rice wine). Also it has also been found to have an antimutagenic effect (Matsuo et al. 1991).

Persimmon is a climacteric fruit that has a short shelf-life due to accelerated fruit softening. Ethylene plays a critical role in fruit softening of persimmon (Itamura et al. 1991). Ethylene may alter the gene expression of cell wall-degrading enzymes (Gray et al. 1992). However there is little information concerning molecular mechanisms controlling gene regulation. This softening process is accelerated after the removal of astringency, and significantly influences the acceptability of the fruit. Among persimmon cultivars, "Saijo" has the shortest shelf-life and begins to soften 4 days after removal of astringency (Itamura et al. 1997). This is the major problem in marketing 'Saijo' persimmon.

Analyses using ESTs have been used for a number of fruits to discover genes and reveal gene expression patterns. Through such studies, a set of ripening-related genes associated with cell walls, pigments, aroma, and ethylene, were identified in grape berry (Terrier et al. 2001; Moser et al. 2005; da Silva et al. 2005), tomato (Fei et al. 2004), pineapple (Moyle et al. 2005), apricot (Grimplet et al. 2005), apple (Newcomb et al. 2006), water melon (Levi et al. 2006) and papaya (Devitt et al. 2006). However, there is little information on genes associated with fruit development and ripening in persimmon. We report distribution of genes identified from two persimmon fruit cDNA libraries using EST sequencing and describe putative genes involved in proanthocyanidin and carotenoid synthesis.

Materials and methods

Plant materials

Ovaries and young fruit of persimmon (*D. kaki* Thunb. cv. Saijo) grown in Tsukisaka's orchard at Matsue, Shimane prefecture, Japan were harvested at six different early stages of development: 1 week before flower opening (May 20, 2005); flowers just opened (May 27, 2005); 1 week after flower opening (June 1, 2005); 2 weeks after flower opening (June 8, 2005); 3 weeks after flower opening (June 15, 2005) and 4 weeks after flower opening (June 22, 2005) (Fig. 1a). These samples (each about 1 g FW) were mixed and used to construct the KA cDNA library as described below. For construction of the KC library, fruit were prepared at six different stages of ripening: stage 1 (October 7, 2005); stage 2 (October 20, 2005, commercial harvest time); stage 3 (November 3, 2005); stage 4 (November 17, 2005); stage 5 (December 1, 2005); and stage 6 (December 15, 2005) (Fig. 1b). These samples (each about 2 g FW) were mixed and used as described below.

Preparation of cDNA libraries

Isolation of poly $(A)^+$ RNA from persimmon samples, and construction of cDNA libraries using the Uni-ZAP XR system (Stratagene, La Jolla, CA) were carried out by a commercial service provider (Eugentech, Taejon, Korea). In brief, total RNA was prepared from ovaries and young fruit (for KA cDNA library) by the hot phenol method, and total RNA from fruit (for KC cDNA library) was prepared by the pine tree method (Chang et al. 1993). Poly $(A)^+$ RNA was purified using a PolyATtract mRNA isolation system (Promega, Madison, WI, USA). Double stranded cDNA was produced from approximately 5 μg of poly $(A)^+$ RNA using a cDNA synthesis kit (Stratagene). The cDNAs larger than 0.5 kb were selected by gel filtration and unidirectionally ligated into the *Eco*RI–*Xho*I sites of the UNI-ZAP XR vector. Libraries were packaged using Giga-PackIII Gold packaging extract (Stratagene). The titers of the primary cDNA library were 2×10^7 pfu/ml for KA, and 1.5×10^6 pfu/ml for KC. The percentage of recombinants was approximately 90% in each library. Because the cDNA inserts were inserted in the same direction, sequencing from the 5′-end was carried out with T3 primer.

PCR and sequencing

Primary phage libraries were plated using *E. coli* strain XL1-Blue MRF' as a host. Each plaque was suspended in 50 μl of SM (100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl pH 7.5, 0.01% gelatin) in a 96-well microplate (stock plate). A 2 μl aliquot of each phage solution was transferred to the corresponding position of new 96-well microplate (reaction plate) compatible with the Genetic Analyzer 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA) and used as a template for amplification of cDNA inserts. The primers used for PCR were T3 (5′-GAATTAACCCTCACTAAAGG-3′) and T7 (5′-TAATACGACTCACTATAGGG-3′). Cycling parameters were as follows: (1) 95°C for 5 min; (2) 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 3.5 min; (3) 72°C for

Fig. 1 Development and ripening of persimmon (*Diospyros kaki* Thunb. cv. Saijo) fruit used for construction of cDNA libraries. **a** Early developmental stage for KA cDNA library: *1* 1 week before flower opening (May 20, 2005); *2* just opened flower (May 27, 2005); *3* 1 week after flower opening (June 1, 2005); *4* 4 weeks after flower opening (June 22, 2005). **b** Ripening stage for KC cDNA library: *1* stage 1 (October 7, 2005); *2* stage 2 (October 20, 2005, commercial harvest time); *3* stage 3 (November 3, 2005); *4* stage 4 (November 17, 2005); *5* stage 5 (December 1, 2005); *6* stage 6 (December 15, 2005)

7 min. Taq DNA polymerase with Thermal Pol Buffer (New England Biolabs, Ipswich, MA, USA) was used for amplification. After PCR, an equal volume of polyethylene glycol solution (13% PEG 6,000 and 1.6 M NaCl) was added and stored at 4°C for at least 3 h. Amplified products were precipitated by centrifugation at 2000*g* for 30 min, washed with 70% ethanol and used for sequence reactions. PCR products were sequenced with the T3 primer, the BigDye Terminator ver 3.1 Cycle Sequencing kit (Applied Biosystems) and an ABI PRISM Genetic Analyzer 3100 (Applied Biosystems). All the reactions were carried out using the reaction microplate described above. In total, 6,000 ESTs were obtained from KA and 6,100 ESTs from KC.

Sequence analysis

Vector-derived and ambiguous sequences (PHRED quality value <20) were eliminated using a combination of the Phred program (Ewing et al. 1998) and cross-match software (http://www.phrap.org). Poly (A) tail sequences in the ESTs processed by our Perl script were at most 10 bases. Subsequently, ESTs with sequences of less than 20 bp were omitted from the final data set. The ESTs were assembled into non-redundant sequences using the phrap program (http://www.phrap.org). Using the BLAST program (Altschul et al. 1990), the sequences of the non-redundant sequences were searched against the *Arabidopsis* protein database in TAIR and non-redundant protein database obtained from NCBI with a search threshold of 1e-10. Results from the BLAST program were parsed with PHP and stored in a PostgreSQL database management system. A publicly accessible web-based database (http://bio14.ipc.shimane-u.ac.jp/kakiest/) was also built on PHP and PostgreSQL. Gene ontology (GO) terms for each non-redundant sequence were also assigned according to those for the *Arabidopsis* genes (Rhee et al. 2003; Berardini et al. 2004) with sequence similarities. ESTs greater than or equal to 50 bp were submitted to the DDBJ database (accession numbers DC583395–DC592851).

Results and discussion

EST analysis

Using the Phrap program (http://www.phrap.org), we assembled 9,952 ESTs into a set of 6,700 non-redundant sequence groups that included 5,211 singletons. The length of the EST sequences ranges from 20 to 637 bp, with an average of 394 bp, and the length of non-redundant sequences ranges from 20 to 1945 bp, with an average of 420 bp. These average lengths of sequences are shorter than those of *Vitis vinifera* (463 bp: Moser et al. 2005; 527 bp: da Silva et al. 2005). Most of the contigs obtained (96.1%) were made up by 1–5 ESTs, and the remained contigs contained 6–10 (2.8%), 11–15 (0.5%) and more 15 ESTs (0.6%). Average number of ESTs per contig was 1.48. Of the 6,700 non-redundant sequences evaluated, the deduced amino acid sequences of 4,356 (65%) showed significant homology to known proteins and 2,344 (35%) showed no significant similarity to any known proteins in *Arabidopsis* database. When non-redundant sequences were assigned GO terms of the homologous *Arabidopsis* genes in the TAIR database, the molecular function for KA and KC libraries, respectively, were classified as follows: hydrolase activity (10.8 and 9.7%), transferase activity (8.1 and 7.5%), protein binding (7.6 and 7.6%), nucleotide binding (5.5 and 4.6%), transporter activity (5.3 and 5.9%), DNA or RNA binding (5.3 and 5.3%), kinase activity (4.2 and 3.9%) (Table 1). There was no significant difference in the population of each molecular function between KA and KC libraries.

Table 1 Distribution of GO-based molecular function for each library

	Library	
	KA (%)	KC (%)
Other molecular functions	20.4	21.9
Other enzyme activity	13.5	13.6
Hydrolase activity	10.8	9.7
Other binding	8.7	8.5
Transferase activity	8.1	7.5
Protein binding	7.6	7.6
Nucleotide binding	5.5	4.6
Transporter activity	5.3	5.9
DNA or RNA binding	5.3	5.3
Kinase activity	4.2	3.9
Structural molecule activity	3.8	4.6
Transcription factor activity	3.4	3.8
Nucleic acid binding	2.8	2.9
Receptor binding or activity	0.7	0.3

Table 2 Assembled clusters that contain more than ten ESTs at early stages in persimmon

Gene function	AGI_code	KA
Copper/zinc superoxide dismutase 1	AT1G08830	27
Polyubiquitin 3	AT5G03240	26
Elongation factor 1-alpha	AT5G60390	24
Aspartyl protease family protein	AT1G11910	20
Vitamin C defective 2	AT4G26850	20
Mutase family protein	AT1G77060	20
Water channel (PIP1C)	AT1G01620	14
Histone H3.2	AT4G40030	13
Chalcone synthase	AT5G13930	13
Glyceraldehyde-3-phosphate dehydrogenase	AT3G04120	12
Fructose-bisphosphate aldolase	AT5G03690	11
Anthocyanidin synthase	AT4G22880	11

Distribution of highly expressed genes in each library

Non-redundant sequences which were assembled with more than 10 ESTs (cluster) in early and late stages are shown in Tables 2 and 3. Of the classes of ESTs with assigned functions, elongation factor 1-alpha, copper/zinc superoxide dismutase, glyceraldehyde-3-phosphate dehydrogenase and aspartyl protease family protein, were present abundantly at both stages. The copper/zinc superoxide dismutase that is known to be a reactive oxygen scavenger was abundant in both early and late stages.

Table 3 Assembled clusters that contain more than ten ESTs at late stages in persimmon

Gene function	AGI_code	
Basic chitinase	AT3G12500	220
Osmotin 34	AT4G11650	104
Glutaredoxin	AT5G40370	49
Protease inhibitor	AT2G02100	39
Elongation factor 1-alpha	AT5G60390	33
Beta-fructofuranosidase	AT3G13790	30
Invertase/pectin methylesterase inhibitor	AT5G62360	28
Lactoylglutathione lyase	AT1G80160	22
Glyceraldehyde-3-phosphate dehydrogenase	AT3G04120	21
Homology-dependent gene silencing 1	AT4G13940	20
Tonoplast monosaccharide transporter 2	AT4G35300	15
Acidic endochitinase	AT5G24090	15
Pathogenesis-related 4	AT3G04720	14
Copper/zinc superoxide dismutase 1	AT1G08830	13
Aspartyl protease family protein	AT1G11910	13
Ubiquitin-protein ligase	AT2G02760	12
Tubulin alpha-2 chain	AT1G50010	12
Fiblillin	AT4G04020	11

Sequences with similarity to the reactive oxygen scavengers catalase and glutathione S-transferase were also found abundantly in ripening fruit cDNA libraries of papaya (Devitt et al. 2006). In early stages, 12 different types of ESTs were found in multiple copies; the most abundant was polyubiquitin with 26 copies, except common ESTs between KA and KC library (Table 2). Other highly abundant sequences in early stages included the vitamin C defective protein, mutase family protein, water channel, histone H3.2, chalcone synthase (CHS), fructose-bisphosphate aldolase and anthocyanidin synthase (ANS). CHS and ANS were genes of proanthocyanidin synthesis pathway as mentioned below. There were 18 different types of EST found in multiple copies in late stages, with 220 basic chitinase ESTs as the most abundant clone (Table 3). At the late stages there were abundant sequences of basic endochitinase, osmotin-like protein, glutaredoxin, protease inhibitor, beta-fructofuranosidase, invertase/pectin methylesterase inhibitor, lactoylglutathione lyase, homology-dependent gene silencing 1, tonoplast monosaccharice transporter 2, pathogenesis related protein, acidic endochitinase, ubiquitin-protein ligase, tubulin alpha-2 chain and fibrillin except common ESTs between KA and KC library. Four plant defense-like proteins, basic endochitinase, osmotin, acidic endochitinase and pathogen-related protein were highly represented in late stages with 220, 104, 15 and 14 ESTs, respectively. Basic endochitinase was also reported to be the most abundant EST in ripening fruit cDNA libraries of papaya (Devitt et al. 2006). A sequence similar to the invertase/pectin methylesterase inhibitor that was abundant in the late stage cDNA library was also found in cDNA libraries of half-ripe and fully ripe apricot (Grimplet et al. 2005). This protein inhibits pectin methylesterases and invertases through the formation of a non-covalent complex (Giovane et al. 1995). It is thought to be involved in the regulation of carbohydrate metabolism, cell wall extension and susceptibility to pathogen attack (Camardella et al. 2000; Chen et al. 2000).

Table 4 *Diospyros kaki* ESTs of proanthocyanidin and carotenoid synthesis

	Gene name	Representative EST name			
		KA		KC	
			Total number		Total number
Proanthocyanidin synthesis pathway					
Phenylalanine ammonialyase	DK-PAL1	KA014_F10	6		
	DK-PAL2	KA009_H08	2		
Cinnamate 4-hydroxylase	DK-C4H	KA048_F03	7		
4-coumarate:CoA ligase	DK-4CL1	KA064_C03	1	KC018_F06	1
	DK-4CL2	KA019_B02	1		
Chalcone synthase	DK-CHS	KA050_D11	13	KC015_H11	1
Chalcone isomerase	DK-CHI1	KA058_H05	1		
	DK-CHI2	KA040_B06	6		
Flavanone 3-hydroxylase	DK-F3H	KA053_F12	8	KC036_C02	1
Dihydroflavonol 4-reductase	DK-DFR	KA041_H02	4		
Anthocyanidin synthase	DK-ANS	KA063_H02	11		
Flavonoid 3′-hydroxylase	DK-F3′5′H[a]	KA060_A02	6		
Leucoanthocyanidin reductase	DK-LCR[b]	KA053_G08	7		
Anthocyanidin reductase	DK-ANR	KA066_D08	1		
Carotenoid synthesis pathway					
Geranylgeranyl pyrophosphate synthase	DK-GGPS			KC026_G03	1
Phytoene desaturase	DK-PDS			KC015_B11	1
Zeta-carotene desaturase	DK-ZDS			KC021_G08	5
Beta-carotene hydroxylase	DK-HYB			KC052_B12	3
9-cis-epoxycarotenoid-dioxygenase	DK-NCED			KC051_E05	1

[a] When these ESTs were compared with other plant ig *Vitis vinifera*, all clones were similar to flavnoid 3′,5′-hydroxylase (F3′5′H) because *Arabidopsis* F3′5′H gene is not found

[b] DK-LCR was similar to BANYULS (NP_176365), which is identical to leucoanthocyanidin reductase

At the late stage, 30 ESTs encoded the beta-fructofuranosidase protein, which converts sucrose consistent with increased contents of glucose and fructose during ripening in "Saijo" fruit (Zheng and Sugiura 1990). Fibrillin, encoded by 11 ESTs at the late stage, is a plastid-localized lipid-binding protein that is regulated by abscisic acid (ABA) and involved in photoprotection (Yang et al. 2006). The ABA content of persimmon fruit increases during ripening (Hirano et al. 1995), therefore fibrillin gene expression may be correlated with elevated ABA content in persimmon fruit. Interestingly, the KC library contained many ESTs that show no homology to genes of *Arabidopsis* (data not shown). These ESTs may be specific to fruit ripening in persimmon. Gene expression during development and ripening in persimmon fruit should be investigated in future studies.

Genes controlling softening pigmentation, and astringency in persimmon fruit

Both the KA and KC libraries from persimmon contained ripening-related EST clones, including 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase, both of which are associated with the ethylene biosynthesis (data not shown). They also both contained cellulose synthase, expansin, pectinesterase, polygalacturonase, xyloglucan:xyloglucosyl transferase and beta-galactosidase, which are all implicated in cell wall metabolism (biogenesis, modification, loosening, etc.). However, each EST differed in its make up of clones related to proanthocyanidin (tannin) and carotenoid synthesis. In "Saijo" persimmon the ovary and young fruit produce high amounts of tannins at the early stage (KA), and the pulp accumulates beta-cryptoxanthin and lycopene at the late stage (KC). The ESTs related to proanthocyanidin and carotenoid pathways in persimmon are shown in Table 4, and the biosynthetic pathways are shown in Figs. 2 and 3. These ESTs related to proanthocyanidin synthesis, including clones with similarity to two phenylalanine ammonia-lyases (PAL), one cinnamate 4-hydroxylase (C4H), two 4-coumarate: CoA ligases (4CL), one CHS, two chalcone isomerases (CHI), one flavone 3-hydroxylase (F3H), one dihydroflavonol 4-reductase (DFR), one ANS, one flavonoid 3′,5′ hydroxylase (F3′5′H), one leucoanthocyanidin reductase (LAR, BANYULS homolog), and one anthocyanidin reductase (ANR). These ESTs also contained clones related to carotenoid biosynthesis with similarity to one geranylgeranyl pyrophosphate synthase (GGPS), one phytoene desaturase (PDS), one zeta-carotene desaturase (ZDS), one beta-carotene hydroxylase (HYD-B), and one 9-cis-epoxycarotenoid dioxygenase (NCED).

Eleven genes of the flavonoid pathway were included in the KA library but in the mature persimmon library (KC) only 4CL, CHS and F3H were present. Recently, seven genes (PAL, C4H, CHI, F3H, F3′H, ANS and ANR) have been isolated from an astringent-type cultivar using suppression subtractive hybridization (Ikegami et al. 2007). Ikegami et al. (2005a, b) reported that transcription of *PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H*, *F3′H*, *F3′5′H*, *DFR* and *ANR* genes is high until the middle of August, and declined in October compared to transcript levels in June and July in the astringent-type cultivar. These results correspond to the relative abundance of proanthocyanidin-related genes between the KA and KC libraries. Clones with similarity to PDS, ZDS HYD-B and NCED were isolated from yellowish and orange pulp in persimmon (Table 4). Niikawa et al. (2007) isolated partial fragments encoding seven genes by RT-PCR technique; phytoene synthase, PDS, ZDS, lycopene beta cyclase, HYD-B, lycopene epsilon cyclase (LCY-E) and zeaxanthin epoxidase (ZEP) from "Fuyu" persimmon. They reported that expression of all genes except ZEP and LCY-E increased during fruit ripening. This result also corresponds to differences in the number of carotenoid-related genes from the KA and KC libraries. Also, library from papaya ripened fruit, which produce beta-cryptoxanthin, contained orthologs of GGPS, ZDS and HYD-B (Devitt et al. 2006).

We successfully isolated unique ESTs from persimmon fruit, including two new genes, LAR and NCED. We are now constructing a microarray set that includes the cDNAs

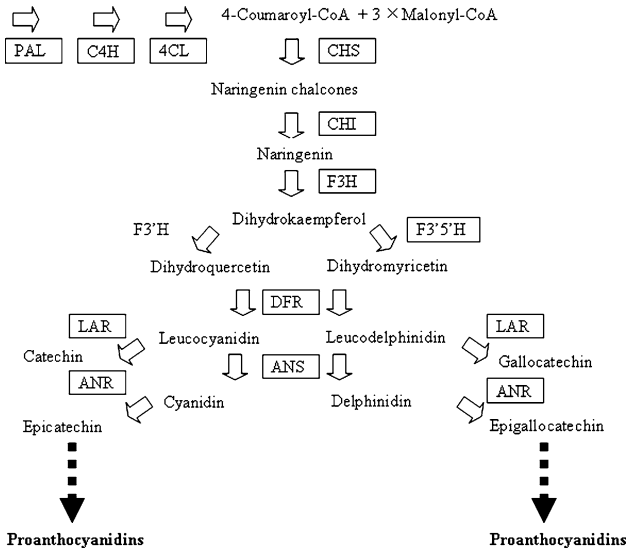

Fig. 2 Proanthocyanidin biosynthesis pathway. *PAL* Phenylalanine ammonia-lyase; *C4H* cinnamate 4-hydroxylase; *4CL* 4-coumarate: CoA ligase; *CHS* chalcone synthase; *CHI* chalcone isomerase; *F3H* flavanone 3-hydroxylase; *DFR* dihydroflavonol 4-reductase; *ANS* anthocyanidin synthase; *F3′H* flavonoid 3′-hydroxylase; *F3′5′H* flavonoid- 3′,5′-hydroxylase; *LAR* leucoanthocyanidin reductase; *ANR* anthocyanidin reductase. *Closed boxes* show genes encoding enzymes that were isolated from persimmon ESTs in this study

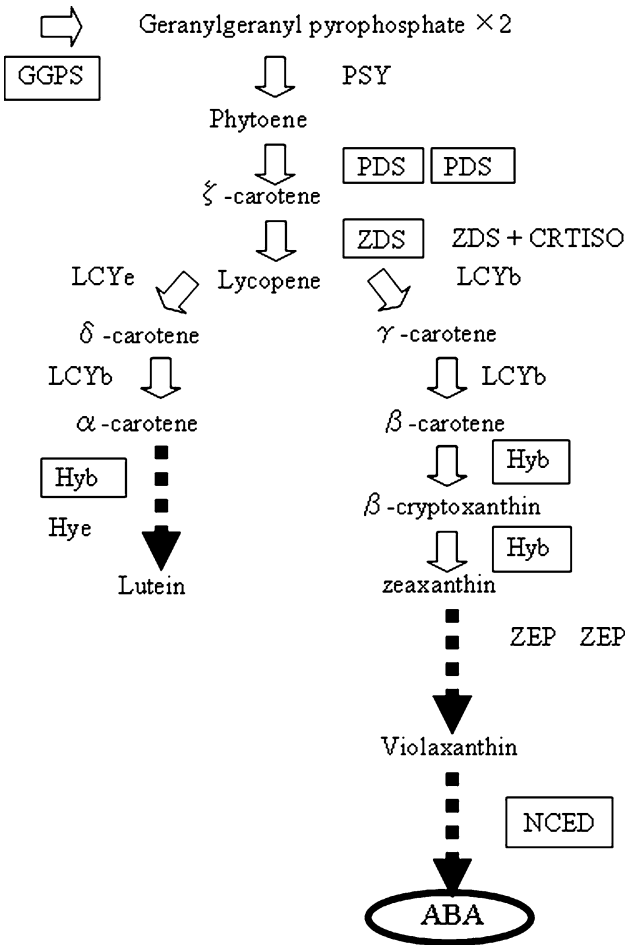

Fig. 3 Carotenoid biosynthesis pathway. *GGPS* Geranylgeranyl pyrophosphate synthase; *PSY* phytoene synthase; *PDS* phytoene desaturase; *ZDS* ζ-carotene desaturase; *CRTISO* carotenoid isomerase; *LCYb* lycopene β-cyclase; *LCYe* lycopene ε-cyclase; *Hyb* β-ring hydroxylase; *Hye* ε-ring hydroxylase; *ZEP* zeaxanthin epoxidase; *NCED* 9-cis-epoxycarotenoid-dioxygenase. *Closed boxes* show genes encoding enzymes that were isolated from persimmon ESTs in this study

from the KA and KC cDNA libraries. These persimmon microarrays for the fruit ESTs will enhance our knowledge of fruit development and ripening.

Acknowledgments This work was supported in part by Grant-in-Aid for Scientific Research (No 17380023) and Grant-in-Aid for Exploratory Research (No 19651084) from the Ministry of Education, Science and Culture, Japan.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Berardini TZ, Mundodi S, Reiser L, Huala E, Garcia-Hernandez M, Zhang P, Mueller LA, Yoon J, Doyle A, Lander G, Moseyko N, Yoo D, Xu I, Zoeckler B, Montoya M, Miller N, Weems D, Rhee SY (2004) Functional annotation of the Arabidopsis genome using controlled vocabularies. Plant Physiol 135:745–755. doi:10.1104/pp.104.040071

Camardella L, Carratore V, Ciardiello MA, Servillo L, Balestrieri C, Giovane A (2000) Kiwi protein inhibitor of pectin methylesterase. Amino-acid sequence and structural importance of two disulfide bridges. Eur J Biochem 267:4561–4565. doi:10.1046/j.1432-1327.2000.01510.x

Chang JJ, Chen TH, Chan P, Chen YJ, Hsu FL, Lo MY, Lin JY (2001) The in vitro inhibitory effect of tannin derivatives on 3-hydroxy-3-methylglutaryl- coenzyme A reductase on Vero cells. Pharmacology 62:224–228. doi:10.1159/000056099

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116. doi:10.1007/BF02670468

Chen MH, Sheng J, Hind G, Handa AK, Citovsky V (2000) Interaction between the tobacco mosaic virus movement protein and host cell pectin methylesterase is required for viral cell-to-cell movement. EMBO J 19:913–920. doi:10.1093/emboj/19.5.913

Da Silva FG, Iandolino A, Al-Kayal F, Bohlmann MC, Cushman MA, Lim H, Ergul A, Figueroa R, Kabuloglu EK, Osborne C, Rowe J, Tattersall E, Leslie A, Xu J, Baek J, Cramer GR, Cushman JC, Cook DR (2005) Characterizing the grape transcriptome. Analysis of expressed sequence tags from multiple *Vitis* species and development of a compendium of gene expression during berry development. Plant Physiol 139:574–597. doi:10.1104/pp.105.065748

Devitt LC, Sawbridge T, Holton TA, Mitchelson K, Dietzgen RG (2006) Discovery of genes associated with fruit ripening in *Carica papaya* using expressed sequence tags. Plant Sci 170:356–363. doi:10.1016/j.plantsci.2005.09.003

Ewing B, Hillier L, Wendl MC, Green P (1998) Base-calling of automated sequencer traces using phred. I Accuracy assessment. Genome Res 8:175–185. doi:10.1101/gr.8.3.175

Fei Z, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni JJ (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. Plant J 40:47–59. doi:10.1111/j.1365-313X.2004.02188.x

Fujiki H (1999) Two stages of cancer prevention with green tea. J Cancer Res Clin Oncol 125:589–597. doi:10.1007/s004320050321

Fujiki H (2005) Green tea: health benefits as cancer preventive for humans. Chem Rec 5:119–132

Giovane A, Balestrieri C, Quagliuolo L, Castaldo D, Servillo L (1995) A glycoprotein inhibitor of pectin methylesterase in kiwi fruit. Purification by affinity chromatography and evidence of a ripening-related precursor. Eur J Biochem 233:926–929. doi:10.1111/j.1432-1033.1995.926_3.x

Gray J, Picton S, Shabbeer J, Schuch W, Grierson D (1992) Molecular biology of fruit ripening and its manipulation with antisense genes. Plant Mol Biol 19:69–87. doi:10.1007/BF00015607

Grimplet J, Romieu C, Audergon JM, Marty I, Albagnac G, Lambert P, Bouchet JP, Terrier N (2005) Transcriptomic study of apricot fruit (*Prunus armeniaca*) ripening among 13,006 expressed sequence tags. Physiol Plant 125:281–292. doi:10.1111/j.1399-3054.2005.00563.x

Hirano K, Yonemori K, Sugiura A (1995) Involvement of sugar metabolism in persimmon growth-inhibition by calyx lobe removal. J Am Soc Hortic Sci 120:75–77

Ikegami A, Kitajima A, Yonemori K (2005a) Inhibition of flavonoid biosynthetic gene expression coincides with loss of astringency in pollination-constant, non-astringent (PCNA)-type persimmon fruit. J Hortic Sci Biotechnol 80:225–228

Ikegami A, Yonemori K, Kitajima A, Sato A, Yamada M (2005b) Expression of genes involved in proanthocyanidin biosynthesis during fruit development in a Chinese pollination-constant,

nonastringent (PCNA) persimmon, 'Luo Tian Tian Shi'. J Am Soc Hortic Sci 130:830–835

Ikegami A, Eguchi S, Kitajima A, Inoue K, Yonemori K (2007) Identificaiton of genes involved in proanthocyanidin biosynthesis of persimmon (*Diospyros kaki*) fruit. Plant Sci 172:1037–1047. doi:10.1016/j.plantsci.2007.02.010

Itamura H, Kitamura T, Taira S, Harada H, Ito N, Takahashi Y, Fukushima T (1991) Relationship between fruit softening, ethylene production and respiration in Japanese persimmon 'Hiratanenashi'. J Japan Soc Hortic Sci 60:695–701. (Japanese with English summary)

Itamura H, Ohno Y, Yamamura H (1997) Characteristics of fruit softening in Japanese persimmon. Acta Hortic 436:179–188

Kanayama Y (2006) Horticultural statistics of Japan. In: The Japanese society of horticultural science (ed) Horticulture in Japan 2006. Nakanishi Printing Co. Ltd, Kyoto, pp 15–20

Kitagawa H, Glucina PG (1984) Cause and removal astringency. In: Ruscoe QW (eds) Persimmon culture in New Zealand. Science Information Publishing Centre, Wellington, pp 55–61

Levi A, Davis A, Hernandez A, Wechter P, Thimmapuram J, Trebitsh T, Tadmor Y, Katzir N, Portnoy V, King S (2006) Genes expressed during the development and ripening of watermelon fruit. Plant Cell Rep 25:1233–1245. doi:10.1007/s00299-006-0163-0

Matsuo T, Ito S (1978) The chemical structure of kaki-tannin from immature fruit of the persimmon *(Diospyros kaki* L.). Agric Biol Chem 42:1637–1643

Matsuo T, Kinoshita M, Itoo S, Shimoi K, Okada Y, Tomita I (1991) Antimutagenic effect of Kaki-shibu and its tannin degraded products on UV-induced mutagenesis in *Escherichia coli*. J Japan Soc Hortic Sci 60:429–435, (Japanese with English summary)

Mekhfi H, ElHaouari M, Bnouham M, Aziz M, Ziyyat A, Legssyer A (2006) A Effects of extracts and tannins from *Arbutus unedo* leaves on rat platelet aggregation. Phytother Res 20:135–139. doi:10.1002/ptr.1822

Moser C, Segala C, Fontana P, Salakhudtinov I, Gatto P, Pindo M, Zyprian E, Toepfer R, Grando MS, Velasco R (2005) Comparative analysis of expressed sequence tags from different organs of *Vitis vinifera* L. Funct Integr Genomics 5:208–217. doi:10.1007/s10142-005-0143-4

Moyle R, Fairbairn DJ, Ripi J, Crowe M, Botella JR (2005) Developing pineapple fruit has a small transcriptome dominated by metallothionein. J Exp Bot 56:101–112. doi:10.1093/jxb/eri015

Newcomb RD, Crowhurst RN, Gleave AP, Rikkerink EHA, Allan AC, Beuning LL, Bowen JH, Gera E, Jamieson KR, Janssen BJ, Laing WA, McArtney S, Nain B, Ross GS, Snowden KC, Souleyre EJF, Walton EF, Yauk Y-K (2006) Analyses of expressed sequence tags from apple. Plant Physiol 141:147–166. doi:10.1104/pp.105.076208

Niikawa T, Suzuki T, Ozeki T, Kato M, Ikoma Y (2007) Characteristics of carotenoid accumulation during maturation of the Japanese persimmon 'Fuyu'. Hort Res (Japan) 6:251–256, (Japanese with English summary)

Ogata S (1976) Waruyoi to futsukayoi no hatsugenkijo kara mita kakinomi no alcohol-meitei e no kouka. Jyo-kyo 71:488–495, (Japanese)

Okuma Y, Ishikawa H, Ito Y, Hayashi Y, Endo A, Watanabe T (1995) Effect of extracts from *Hovenia dulcis* Thunb. on alcohol concentration in rats and men administered alcohol. J Jpn Soc Nutr Food Sci 48:167–172, (Japanese with English summary)

Rhee SY, Beavis W, Berardini TZ, Chen G, Dixon D, Doyle A, Garcia-Hernandez M, Huala E, Lander G, Montoya M, Miller N, Mueller LA, Mundodi S, Reiser L, Tacklind J, Weems DC, Wu Y, Xu I, Yoo D, Yoon J, Zhang P (2003) The *Arabidopsis* information resource (TAIR): a model organism database providing a centralized, curated gateway to *Arabidopsis* biology, research materials and community. Nucleic Acids Res 31:224–228. doi:10.1093/nar/gkg076

Sasakawa Z (1955) Clinical studies on the depressor effects of SHIBU against hypertension. J Jpn Soc Intern Med 43:858–866, (Japanese with English summary)

Taira S (1996) Astringency in persimmon. In: Linskens HF, Jackson JF (eds) Fruit analysis. Modern methods of plant analysis. vol 18, Springer, Berlin, pp 97–110

Terrier N, Ageorges A, Abbal P, Romieu C (2001) Generation of ESTs from grape berry at various developmental stages. J Plant Physiol 158:1575–1583. doi:10.1078/0176-1617-00566

Yang Y, Sulpice R, Himmelbach A, Meinhard M, Christmann A, Grill E (2006) Fibrillin expression is regulated by abscisic acid response regulators and is involved in abscisic acid-mediated photoprotection. Proc Natl Acad Sci USA 103:6061–6066. doi:10.1073/pnas.0501720103

Yonemori K (1997) Persimmon industry and research activities in Japan. Acta Hortic 436:21–32

Zheng GH, Sugiura A (1990) Changes in sugar composition in relation to invertase activity in the growth and ripening of persimmon (*Diospyros kaki*) fruit. J Japan Soc Hortic Sci 59:281–287, (Japanese with English summary)