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MINI REVIEW

A Genomics Approach Using Expressed Sequence Tags and Microarrays in Ripening Apple Fruit (*Malus domestica* Borkh.)

Young Sam Seo · Woo Taek Kim

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Abstract Apple (*Malus domestica* Borkh.), an important horticultural crop, produces human health-promoting metabolites during fruit ripening. Because that process, which involves complex biochemical and physiological changes, is genetically programmed, molecular and genetic approaches have been taken to understand the associated cellular mechanisms. The release of 151,687 apple expressed sequence tags (ESTs) into a public database has made possible large-scale studies of expression. Analysis of apple ESTs allows for the identification and characterization of genes with potential roles in fruit development, particularly those related to aroma production and protein degradation during ripening. Apple cDNA and oligonucleotide microarrays have been generated for more comprehensive examinations. Such tools are powerful means for elucidating the molecular events involved in metabolite biosynthesis and physiological changes and will also enable researchers to understand how to control that ripening process.

Keywords Apple · cDNA microarray · Expressed sequence tags (ESTs) · Fruit ripening

Fruit development and maturation is a unique biological process in the plant kingdom that yields subsequent generations of viable and competitive progeny. Because regulation of ripening is commercially important for the horticultural industry and is tightly linked to human health and diet, research has been critical for furthering our understanding of the molecular and cellular mechanisms within that process [22, 31]. Fruit ripening includes an enhancement of respiration, carotenoid biosynthesis, conversion of chloroplast to chromoplast, increased activity of cell wall-degrading enzymes, production of flavor components, conversion of starch to sugar, and autocatalytic ethylene production [22, 31, 33]. Because these dramatic changes are genetically programmed, molecular and genetic approaches have been taken to understand the cellular and physiological mechanisms associated with fruit ripening, resulting in identification of several genes expressed then [4, 13, 14, 32]. In particular, genome-wide analysis of gene expression using expressed sequence tags (ESTs) and cDNA microarrays has accelerated the accumulation of information about fruit ripening-related genes [4, 7, 10].

Apple (*Malus domestica* Borkh.) is one of the most valuable horticultural fruit crops in the world, especially in East Asia. Apple belongs to the family Rosaceae, which includes economically important fruit and ornamental trees, such as pear (*Pyrus communis*), peach (*Prunus persica*), cherry (*Prunus avium*), strawberry (*Fragaria* spp.), apricot (*Prunus armeniaca*), almond (*Prunus amygdalus*), and rose (*Rosa hybrida*). Because ripe fruits in that family contain numerous secondary metabolites that promote human health and nutrition, investigations of Rosaceous fruits have been focused on the ripening process. Apple fruit especially has a climacteric ripening character that is accompanied by a peak in respiration and a burst of ethylene production [33]. For this reason, investigation is needed into ethylene-regulated mechanisms and the production of volatile compounds during that process.

Despite this importance, genome-wide research resources are limited for the apple. Only recently has it become possible to establish a genome-wide EST database and perform large-scale expression studies with microarrays [21, 24, 27]. Those

Y. S. Seo · W. T. Kim (✉)
Department of Biology, College of Life Science and Biotechnology, Yonsei University,
Seoul 120-749, South Korea
e-mail: wtkim@yonsei.ac.kr

results can be used to elucidate the molecular mechanisms for aroma production, red coloration, and protein degradation in ripening fruits [8, 16, 27, 30]. Here, we review recent progress in the genomics approach to understanding apple fruit development and also discuss future directions in research with reference to other fruit species such as the tomato.

Analysis of Expressed Sequence Tags from Apple

ESTs are established by partial sequences, for which transcripts are isolated randomly from cDNA libraries [1]. ESTs have been used for finding new genes and their family members, elucidating phylogenetic relationships, and analyzing large-scale gene expression in various developmental stages and tissues [9, 25, 26, 29]. Because of recent advances in DNA sequencing technology, EST sequences have become exponentially abundant, and nearly 60 million ESTs can be accessed in the NCBI public database (http://www.ncbi.nlm.nih.gov/dbEST). However, until recently, only a limited number of apple ESTs have been available because cDNA synthesis is very difficult due to extremely small amounts of mRNAs and the large quantity of phenolic compounds in ripened fruit tissues.

Among the currently available 256,217 ESTs of apple in the NCBI EST database (Table 1), 151,687 were collected by Newcomb et al. [24]. That research group has sequenced transcripts with an average edited length of 468 bp, which were taken from 43 different cDNA libraries representing 34 different tissue types, treatments, and cultivars. The majority of these sequences are from Royal Gala (78.9%) with others coming from such cultivars as M9 (9.7%), Pinkie (3.8%), Braeburn (3.7%), Pacific Rose (1.9%), Aotea (1.1%), and Northern Spy (0.8%). About 54,000 ESTs have been created from developing and ripening "Royal Gala" fruits, including the flower, whole fruit, fruit cortex, skin, and seed tissues. Over 76,000 ESTs have also been collected from buds, shoots, leaves, roots, phloem, and xylem. That group has also collected 21,595 ESTs from fruits stored at low temperature and under altered conditions, as well as from leaves exposed to fungal pathogen and high temperature and from fruit cell lines treated with various chemicals, e.g., boron.

By clustering the EST sequences with a higher threshold (95%), Newcomb et al. [24] have reduced this number to 43,938 nonredundant (NR) sequences, comprising 17,460 tentative consensus sequences and 25,478 singletons. Considering the results of protein-coding gene prediction and homology to *Arabidopsis*, this number of NR sequences likely represents about half the number of expressed genes found in apple. Although only 50% of those genes are represented, Newcomb et al. [24] have proven their EST dataset to be of high quality by showing that this collection contains many genes involved in important traits in apple, such as fruit ripening, flavor and aroma production, and the biosynthesis of color and health-related compounds.

Because these ESTs are now available to the public, it is possible to identify genes with expression that is altered by physiological changes, such as fruit development and stress responses. Park et al. [27] have analyzed over 200,000 apple ESTs released from at least 20 contributors, collected from more than 70 cDNA libraries, and have sampled from at least nine cultivars. Using statistical algorithms that calculate the frequency of each gene in the EST datasets, they have identified apple genes that are likely to be highly expressed in fruit, expressed uniquely or preferentially in fruit, or regulated temporally or spatially during fruit development. Generally, gene-expression studies based on EST frequency have various artifacts due to the differential amplification of cDNAs during library preparation and contamination of abundant organellar DNA, highly repetitive nuclear DNA, and microbial nucleic acids [3]. However, after processing these data with rigorous standardization and normalization, Park et al. [27] have made a relatively accurate estimation of development- and tissue-associated gene expression. In particular, they have used these results to predict a specific lipoxygenase (*LOX*) gene and have suggested its potentially important role in the biosynthesis of volatile esters during fruit ripening. Therefore, they have demonstrated that informative expression data can be produced from EST sequences of heterogeneous sources if they are plentiful enough for statistical analysis.

Recently, Han et al. [16] have analyzed the proportion and frequency of EST genes collected from fully ripened "Fuji" fruits to elucidate the molecular mechanisms for gene regulation during the ripening process. They have found that 11.0% ESTs are related to protein synthesis and degradation. Among them, ten genes related to the ubiquitination pathway are detected at high frequency in the cDNA library of mature fruits. Considering the fact that a number of proteins are degraded during fruit ripening as a feature of senescence [5, 31], these results suggest that the degradation of specific proteins, such as photosynthetic proteins, cell wall and membrane component proteins, and signal transduction pathway proteins, is probably carried out through that pathway. This theory is supported by the fact that transcript of *MdFBCP1* (F-Box-containing protein 1) is uniquely expressed at the climacteric stage and that the MdFBCP1 protein physically interacts with MdSkp1 (apple Skp1 protein) [16]. In addition, based on the specific induction of *MdFBCP1* by ethylene, it is possible that the SCF E3 ubiquitin ligase complex, which is composed of MdFBCP1 and MdSkp1, acts downstream of ethylene, as is the case with $SCF^{EBF1/EBF2}$ E3 ubiquitin ligases [12, 15, 28]. That

Table 1 Publicly available resources from crop species

Species	Common name	Total ESTs[a]	Total unique genes	Reference of microarrays
Ananas comosus	Pineapple	5,649	n/a	
Capsicum annuum	Pepper	33,311	4,685	[20]
Citrus spp.	Orange	232,098	30,171	http://www.affymetrix.com
Citrullus lanatus	Watermelon	7,891	832	[35]
Coffea Arabica	Coffee	1,577	n/a	
Cucumis melo	Melon	22,683	3,068	http://www.icugi.org
Cucumis sativus	Cucumber	6,347	713	[23]
Fragaria ananassa	Strawberry	5430	1,701[b]	[2]
Solanum lycopersicon	Tomato	258,830	13,019	http://ted.bti.cornell.edu
Malus domestica	Apple	256,217	13,000	[18]
Persea americana	Banana	16,558	265	[36]
Prunus armeniaca	Apricot	15,105	n/a	
Prunus avium	Sweet cherry	21	n/a	
Prunus domestica	Plum	54	n/a	
Prunus dulcis	Almond	3,864	n/a	
Prunus persica	Peach	79,023	4,806	[34]
Pyrus communis	Pear	244	1,364[b]	[11]
Vitis spp.	Grape	380,674	15,700	http://www.affymetrix.com

n/a not available
[a] Data from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/dbEST, November 28, 2008)
[b] EST clone numbers on microarrays

complex then participates in the selective degradation of proteins in response to climacteric ethylene.

As described above, EST collections can be used for both gene identification and transcriptome analysis. However, expression studies that rely on EST collections are limited because of their heterogeneous sources. To overcome this, microarrays are now being used for large-scale profiling.

Microarray Analysis of Apple Gene Expression During Fruit Ripening

Microarray analysis provides a powerful means for high-throughput gene expression studies in temporal stages or various tissues under specific physiological and environmental conditions [3]. Because microarrays take advantage of existing EST collections and genome sequence data, custom chips on which cDNAs are printed can be easily made. These have been used for organisms whose genomes have not yet been sequenced, such as tomato, hot pepper, strawberry, and pear [2, 3, 11, 17, 20]. However, fabrication and analysis of cDNA microarrays with apple genes have not been possible until recently because of a lack of EST collections.

Lee et al. [21] have generated cDNA microarrays with 6,253 EST clones that were collected from young and mature "Fuji" fruits. Among these, 3,124 were from the cDNA libraries of young fruits (5 to 8 mm long and 3 weeks after full bloom), while 3,129 were selected from the cDNA libraries of mature fruits (80 to 90 mm long and 25 weeks after full bloom). To investigate differential gene expression in early-fruit development for that cultivar, they hybridized two labeled cDNA probes synthesized from mRNAs of leaves, flowers, young fruits, and mature fruits (Fig. 1). By comparing the hybridization of young fruits versus other organs, they selected 192 cDNA clones as young fruit-preferential genes. These are expressed over twofold higher in young fruits than in all other tissue types and have been assigned to 138 NR sequences that consist of 88 known-function genes and 50 unknown-function genes. A large percentage (26.1%) of the former type encodes photosynthesis-specific protein homologues, while the latter gene class shows homology to proteins related to the biosynthesis of protein building blocks, cell division and enlargement, metabolism, and stress responses. That research group also has found that transcripts of these young fruit-preferential genes are detected in leaf and flower tissues. These results suggest that this gene-expression profile of young fruit resembles the molecular portrait of photosynthetic tissue and, consequently, that the early formation of apple fruit is probably a common process in developing organs.

Based on "Fuji" fruit cDNA microarrays, Lee et al. [21] have reported that the transcripts for 14 genes, selected from 192 young fruit-preferential genes, are not detected in mature fruit and that the expression levels of 46 young fruit-preferential genes are eight times lower in mature fruits. Their results are consistent with those from a more

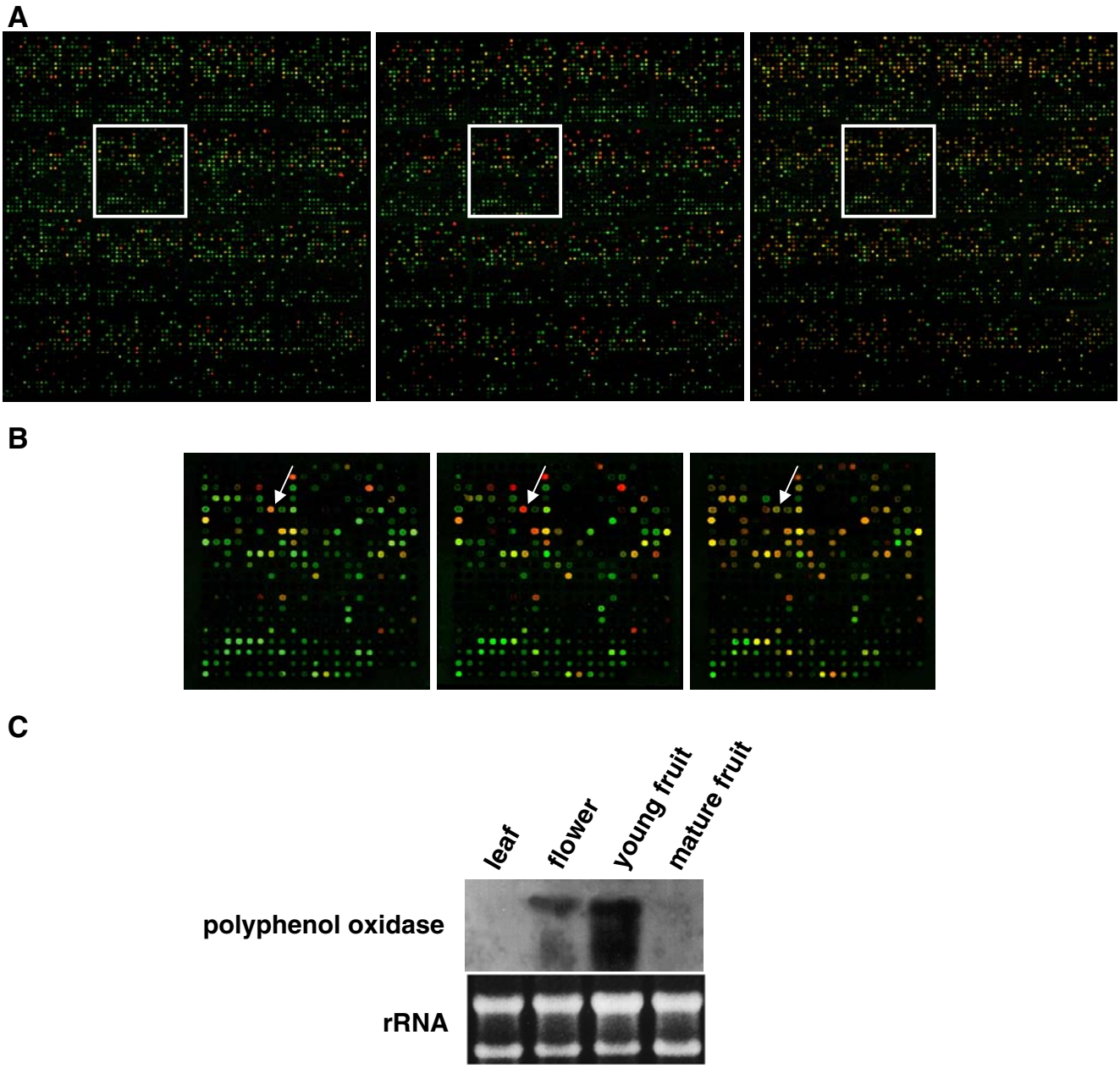

Fig. 1 "Fuji" apple cDNA microarrays. **a** In all, 6,253 EST clones were derived from cDNA libraries of young fruits (stage 1) and mature fruits (stage 4). Three array images were obtained from hybridization of common reference transcripts versus flower (*left panel*), young fruits (*middle panel*), and mature fruits (*right panel*) transcripts. **b** Enlarged image of sub-array boxed in **a**. *Arrows* indicate spots corresponding to apple polyphenol oxidase. *Orange* (*left panel*), *red* (*middle panel*), and *green* (*right panel*) color spots imply slightly higher, strongly higher, and lower transcript amounts, respectively, than reference transcript. **c** RNA gel blot analysis of expression by apple polyphenol oxidase gene in leaf, flower, young fruit, and mature fruit. Microarray expression data of polyphenol oxidase corresponded to this northern analysis result

recent analysis of plastid transcriptome for tomato fruit development [19]. This comparative plastid transcriptome analysis of green leaves and developmental series of fruits (green, turning, light-red, and ripe red tomatoes) using oligonucleotide microarrays has revealed that most plastid genes, especially those that are photosynthesis-related, are strongly down regulated in the tomato fruits.

From experiments with over 15,700 apple ESTs, Schaffer et al. [30] and Janssen et al. [18] have reported that their microarrays consist of 45- to 55-mer oligonucleotides representing approximately 15,100 NR genes of cultivar Royal Gala, described by Newcomb et al. [24]. A comparison with the *Arabidopsis* predicted protein set, via BLASTx, has shown that these uni-genes correspond to about 13,000

different genes [18]. These oligonucleotide microarrays have been used by Schaffer et al. [30] to identify genes that likely play important roles in aroma production during ripening. Because ethylene directly controls the synthesis of volatile compounds, especially esters and α-farnesene in apple cultivar Greensleeves [6], Schaffer et al. [30] used transgenic "Royal Gala" apple, containing an antisense ACC oxidase gene, to find ethylene-regulated transcriptional control points of aroma production. One advantage of using transgenic apple, which has lost its ability to synthesize ethylene, is that the effects of internal ethylene are excluded, and the sampling stage of ripening is synchronized by the simultaneous application of ethylene to fruits. In that investigation, 537 oligonucleotides were selected because they displayed significantly altered expression in response to ethylene treatment in skin tissue. After those genes were compared with 186 potential candidates, 17 were finally identified as aroma-related genes that are differentially expressed in response to such ethylene application. This result indicates that the genes involved in the biosynthesis of aromatic compounds are not coordinately regulated by ethylene, and only one or a couple of genes from multigene families that catalyze the same enzymatic step in fact respond to ethylene. However, the most significant feature of that report has been that all the final steps and some of the initial steps in the pathways for aroma biosynthesis are catalyzed by enzymes whose expressions are regulated by ethylene. Therefore, this biosynthesis occurs at ethylene-regulated transcriptional control points during apple fruit ripening.

The analysis by Janssen et al. [18] of apple oligonucleotide microarrays was conducted to understand the regulation of molecular events required for producing apple fruit. For this purpose, eight time points based on physiological and morphological conditions of apple fruit development were selected (0, 14, 25, 35, 60, 87, 132, and 146 days after anthesis). These were then compared with genomic DNA as a common reference on the apple microarray. In all, 1955 genes were identified as having significant alterations in expression during apple fruit development. They were subsequently classified into four groups according to their pattern of coordinated gene expression (full-bloom, early-fruit development, mid-development, and ripening cluster). When their functional classifications were compared, Janssen et al. [18] found some interesting changes in the assignment of genes within each category. For example, the proportion of metabolism and energy categories increases in ripening fruit, reflecting a high demand for expression related to secondary metabolites for producing volatile compounds during fruit ripening. In contrast, a high proportion of the function of cellular transport and organization in the early-fruit and mid-development stages correspond to the physiological condition of the fruit cells, which take up nutrients and water most rapidly and undergo dramatic structural modifications. Finally, the proportion of genes involved in cell cycle and DNA processing is higher in the early-fruit development cluster than at the ripening stages.

Perspective

A wealth of information concerning the expression of plentiful genes and their functions in apple fruit development has been recently reported. From these large-scale investigations, a number of molecular events associated with biochemical and physiological changes in apple fruit development have been identified [16, 18, 21, 27, 30]. However, researchers are just beginning to understand apple fruit ripening and how to control that process at the genomics level. Using these resources, efforts for elucidating the regulatory mechanisms of biochemical pathways in fruit development will continue. Transgenic apple plants, in which the expression of genes selected via these genomics approaches is altered, will not only promise fruits with higher economic value but also allow us to identify the in vivo functioning of particular genes. As the genomics resources related to fruit development expand, a comparative analysis of microarrays associated with various treatments, transgenic plants, and even different species will provide more comprehensive knowledge about climacteric fruit ripening in apple. Additionally, bridging the genomics approach to proteomic and metabolomic analyses will lead to an entire picture of apple fruit ripening, including gene networks, regulatory proteins, and metabolic processes.

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References

1. Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, Kerlavage AR, McCombie WR, Venter JC (1991) Complementary DNA sequencing: expressed sequence tags and human genome project. Science 252:1651–1656
2. Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen AMML, de Vos RCH, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP (2000) Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12:647–662
3. Alba R, Fei Z, Payton P, Liu Y, Moore SL, Debbie P, Cohn J, D'Ascenzo M, Gordon JS, Rose JK, Martin G, Tanksley SD, Bouzayen M, Jahn MM, Giovannoni J (2004) ESTs, cDNA

microarrays, and gene expression profiling: tools for dissecting plant physiology and development. Plant J 39:697–714
4. Alba R, Payton P, Fei Z, McQuinn R, Debbie P, Martin GB, Tanksley SD, Giovannoni JJ (2005) Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. Plant Cell 17:2954–2965
5. Alonso JM, Granell A (1995) A putative vacuolar processing protease is regulated by ethylene and also during fruit ripening in citrus fruit. Plant Physiol 109:541–547
6. Dandekar A, Teo G, Defilippi B, Uratsu S, Passey A, Kader A, Stow J, Colgan R, James D (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Res 13:373–384
7. Eriksson EM, Bovy A, Manning K, Harrison L, Andrews J, De Silva J, Tucker GA, Seymour GB (2004) Effect of the colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening. Plant Physiol 136:4184–4197
8. Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant J 49:414–427
9. Ewing RM, Ben Kahla A, Poirot O, Lopez F, Audic S, Claverie JM (1999) Large-scale statistical analyses of rice ESTs reveal correlated patterns of gene expression. Genome Res 9:950–959
10. Fei Z, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni J (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. Plant J 40:47–59
11. Fonseca S, Hackler L Jr, Zvara A, Ferreira S, Bald A, Dudits D, Pais MS, Pusk LG (2004) Monitoring gene expression along pear fruit development, ripening and senescence using cDNA microarrays. Plant Sci 167:457–469
12. Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, Yanagisawa S, Vierstra RD (2004) *Arabidopsis* EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. Proc Natl Acad Sci USA 101:6803–6808
13. Giovannoni J (2001) Molecular biology of fruit maturation and ripening. Annu Rev Plant Physiol Plant Mol Biol 52:725–749
14. Giovannoni J (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:S170–180
15. Guo H, Ecker JR (2003) Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell 115:667–677
16. Han SE, Seo YS, Heo S, Kim D, Sung SK, Kim WT (2008) Structure and expression of *MdFBCP1*, encoding an F-box-containing protein 1, during Fuji apple (*Malus domestica* Borkh.) fruit ripening. Plant Cell Rep 27:1291–1301
17. Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G (2001) A transcriptional roadmap to wood formation. Proc Natl Acad Sci USA 98:14732–14737
18. Janssen BJ, Thodey K, Schaffer RJ, Alba R, Balakrishnan L, Bishop R, Bowen JH, Crowhurst RN, Gleave AP, Ledger S, McArtney S, Pichler FB, Snowden KC, Ward S (2008) Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. BMC Plant Biol 8:16
19. Kahlau S, Bock R (2008) Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein. Plant Cell 20:856–874
20. Lee S, Kim SY, Chung E, Joung YH, Pai HS, Hur CG, Choi D (2004) EST and microarray analyses of pathogen-responsive genes in hot pepper (*Capsicum annuum* L.) non-host resistance against soybean pustule pathogen (*Xanthomonas axonopodis* pv. glycines). Funct Integr Genom 4:196–205
21. Lee YP, Yu GH, Seo YS, Han SE, Choi YO, Kim D, Mok IG, Kim WT, Sung SK (2007) Microarray analysis of apple gene expression engaged in early fruit development. Plant Cell Rep 26:917–926
22. Lelievre J-M, Latche A, Jones B, Bouzayen M, Pech J-C (1997) Ethylene and fruit ripening. Physiol Plant 101:727–739
23. Mercke P, Kappers IF, Verstappen FWA, Vorst O, Dicke M, Bouwmeester HJ (2004) Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. Plant Physiol 135:2012–2024
24. 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
25. Nishiyama T, Fujita T, Shin IT, Seki M, Nishide H, Uchiyama I, Kamiya A, Carninci P, Hayashizaki Y, Shinozaki K, Kohara Y, Hasebe M (2003) Comparative genomics of *Physcomitrella patens* gametophytic transcriptome and *Arabidopsis thaliana*: Implication for land plant evolution. Proc Natl Acad Sci USA 100:8007–8012
26. Ogihara Y, Mochida K, Nemoto Y, Murai K, Yamazaki Y, Shin IT, Kohara Y (2003) Correlated clustering and virtual display of gene expression patterns in the wheat life cycle by large-scale statistical analyses of expressed sequence tags. Plant J 33:1001–1011
27. Park S, Sugimoto N, Larson MD, Beaudry R, van Nocker S (2006) Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. Plant Physiol 141:811–824
28. Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. Cell 115:679–689
29. Ronning CM, Stegalkina SS, Ascenzi RA, Bougri O, Hart AL, Utterbach TR, Vanaken SE, Riedmuller SB, White JA, Cho J, Pertea GM, Lee Y, Karamycheva S, Sultana R, Tsai J, Quackenbush J, Griffiths HM, Restrepo S, Smart CD, Fry WE, Van Der Hoeven R, Tanksley S, Zhang P, Jin H, Yamamoto ML, Baker BJ, Buell CR (2003) Comparative analyses of potato expressed sequence tag libraries. Plant Physiol 131:419–429
30. Schaffer RJ, Friel EN, Souleyre EJ, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma JH, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD (2007) A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiol 144:1899–1912
31. Seymour GB, Taylor JE, Tucker GA (1993) Biochemistry of fruit ripening. Chapman & Hall, London
32. Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16: S181–189
33. Theologis A (1992) One rotten apple spoils the whole bushel: The role of ethylene in fruit ripening. Cell 70:181–184
34. Trainotti L, Bonghi C, Ziliotto F, Zanin D, Rasori A, Casadoro G, Ramina A, Tonutti P (2006) The use of microarray μPEACH1.0 to investigate transcriptome changes during transition from pre-climacteric to climacteric phase in peach fruit. Plant Sci 170:606–613
35. Wechter WP, Levi A, Harris KR, Davis AR, Fei Z, Katzir N, Giovannoni JJ, Salman-Minkov A, Hernandez A, Thimmapuram J, Tadmor Y, Portnoy V, Trebitsh T (2008) Gene expression in developing watermelon fruit. BMC Genom 9:275
36. Xu B, Su W, Liu J, Wang J, Jin Z (2007) Differentially expressed cDNAs at the early stage of banana ripening identified by suppression subtractive hybridization and cDNA microarray. Planta 226:529–539